Contaminated sediments: Methods to assess release and toxicity of organic chemical mixtures

Lukas Mustajärvi

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Abstract
Bottom sediments around the world store large amounts of legacy hydrophobic organic contaminants (HOCs), forming mixtures of unknown chemical composition. Primary emissions to the environment of many HOCs have been reduced as a consequence of regulation. However, HOCs may be released from the sediments to water and biota, and there is therefore a risk of negative effects on local ecosystems. The activity of benthic organisms can enhance the sediment-to-water flux of HOCs, a process called bioturbation. Few in situ assessments of the sediment-to-water flux are available in the scientific literature, and the effect of bioturbation on the sediment-to-water flux of HOCs has not been studied in the field. Thus, there is a need to improve in situ methods for direct determination of sediments as a source of HOCs to water, and thereby include the effect of bioturbation. In Paper I, a benthic flow-through chamber was developed for environmentally realistic in situ assessments of the sediment-to-water flux. In Paper II, the sediment-to-water flux of polycyclic aromatic hydrocarbons (PAHs) was assessed using the flow-through chamber at four sites on the Swedish Baltic Sea coast. The sediments at all four sites acted as sources of PAHs to water. In the same study, potential effects of bioturbation, with an increase of the sediment-to-water flux by up to one order of magnitude, were observed at sites with bioturbating organisms. In the past, assessing the toxicity of HOCs has been challenging due to difficulties in maintaining stable exposure concentrations of the test chemical. In Paper III, a passive dosing method, where the test chemical partitions from a polymer (silicone) to the aquatic exposure medium, was developed and tested for chronic exposure. A stable exposure concentration could be maintained, and the chronic toxicity to the sediment-dwelling harpacticoid Nitocra spinipes of chronic exposure to triclosan was assessed in a 6-week population development test. In Paper IV, a passive sampling and dosing method was developed and used to assess the toxicity of an environmental chemical mixture of bioavailable sediment-associated HOCs transferred from a contaminated sediment to the laboratory-based bioassay. The passive sampling and dosing method can be used to assess the toxicity of environmental mixtures of chemicals at environmentally realistic concentrations to which ecosystems are constantly exposed.

Keywords: Sediment, Hydrophobic organic contaminants, Flux, Bioturbation, Passive sampling, Passive dosing, Mixture toxicity.

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OBS!

Denna avhandling innehåller olika artiklar.
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Author’s contribution to paper

I. I was in charge of the validation tests of the flow-through chamber. I performed the fieldwork, the laboratory work and chemical analyses. I performed the data evaluation and I took the lead in writing the manuscript.

II. I planned the study in collaboration with the co-authors and I performed pre studies to select the sampling sites. I performed the field work, the laboratory work and chemical analyses. I performed the data evaluation and I took the lead in writing the manuscript.

III. I tested and validated the methodology. I had an assisting role in the execution of the experiment. I contributed to data evaluation and to the manuscript.

IV. I planned the study in collaboration with the co-authors. I performed the sediment sampling. I tested and validated the sampling and dosing method. I performed the toxicity test and I took the lead in writing the manuscript.
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>Black carbon</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstracts Service</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GF/F</td>
<td>Glass microfiber filter</td>
</tr>
<tr>
<td>HCH</td>
<td>Hexachlorocyclohexane</td>
</tr>
<tr>
<td>HOC</td>
<td>Hydrophobic organic contaminant</td>
</tr>
<tr>
<td>K_{ow}</td>
<td>Octanol-water partition coefficient</td>
</tr>
<tr>
<td>K_{p-w}</td>
<td>Polymer-water partition coefficient</td>
</tr>
<tr>
<td>K_{POM-w}</td>
<td>POM-water partition coefficient</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
</tr>
<tr>
<td>PAH</td>
<td>Polycyclic aromatic hydrocarbon</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated biphenyl</td>
</tr>
<tr>
<td>PCDD/F</td>
<td>Polychlorinated dibenzo-p-dioxin and furan</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>POM</td>
<td>Polyoxymethylene</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>PUF</td>
<td>Polyurethane foam</td>
</tr>
<tr>
<td>REACH</td>
<td>Registration, Evaluation, Authorization and Restriction of Chemicals</td>
</tr>
<tr>
<td>SQG</td>
<td>Sediment quality guideline</td>
</tr>
<tr>
<td>TOC</td>
<td>Total organic carbon</td>
</tr>
</tbody>
</table>
Introduction

Sediment contamination

On a global scale, sediments store large amounts of hydrophobic organic contaminants (HOCs), due to the association of HOCs to organic carbon.\textsuperscript{1, 2} Many of these contaminants, (e.g. polychlorinated biphenyls (PCBs)) are regulated, and as a consequence, emissions from primary sources have been reduced or completely ceased.\textsuperscript{3, 4} The concentrations of PCBs and Polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) in air and water have decreased since the 1970s, illustrated by for instance declining concentrations in Baltic Sea herring,\textsuperscript{5} and sediment.\textsuperscript{6} However, as a consequence of decreasing concentrations in the surrounding environment, the chemical gradient between sediment and the water may increase, making HOCs once accumulated in sediments more prone to be released to water and biota.

For more than 100 years, the semi-enclosed Baltic Sea has received emissions of contaminants such as polycyclic aromatic hydrocarbons (PAHs), PCBs and PCDD/Fs from industries along the coastline as well as from atmospheric transport and deposition. Due to the long water residence time (> 30 years) in the Baltic Sea, contaminants can accumulate in the water body and in sediments. Sediments in several areas of the coastal Baltic Sea contain high amounts of PAHs, PCBs and PCDD/Fs.\textsuperscript{7-9} Sobek et al.\textsuperscript{10} demonstrated that the sediments along the Swedish coast of the Gulf of Bothnia are potential sources of PCDD/Fs and PCBs to water and stationary aquatic organisms such as juvenile perch. The release of HOCs from sediments is governed by several processes such as sorption to sediment organic matter, diffusion through chemical gradients between pore water and bottom water,\textsuperscript{11} resuspension of particle-associated HOCs due to waves,\textsuperscript{12} upwelling ground water,\textsuperscript{13} gas ebullition\textsuperscript{14} as well as bioturbation.\textsuperscript{15, 16} Several studies have demonstrated that the sediment-to-water flux of dissolved and particle-associated HOCs is enhanced by bioturbation, which occurs by two processes.\textsuperscript{17-21} One process is bioirrigation, which implies that the exchange between pore water and bottom water is increased due to flushing of the burrows created by the benthic organism. This effect is associated with burrowing animals such as oligochaetes and polychaetes.\textsuperscript{22} The other process is particle reworking, which implies that sediment particles are moved.
horizontally and vertically within the sediment. Through this process, particle-associated HOCs in deeper, more contaminated sediment layers, can be moved to the sediment surface, and thus increasing the chemical concentration gradient between the surface sediment pore water and bottom water. Practically all sediment-dwelling organisms that are capable to move may rework sediment particles. Moreover, benthic organisms may increase resuspension of sediment particles, either as the organism moves within the sediment, eject ingested particles, or indirectly by destabilizing the sediment, thus making it more susceptible to resuspension by physical factors such as wave action and advective water flow.

Equilibrium passive sampling

Sediment pore water concentrations of HOCs can be determined by using equilibrium passive samplers. As a relationship exists between the freely dissolved concentrations, captured by equilibrium passive samplers, and uptake in biota, equilibrium passive sampling can be used to estimate exposure levels of HOCs in sediment. Equilibrium passive sampling is performed by bringing a polymer in contact with the matrix of interest, such as sediment, soil and water. Organic contaminants in the matrix partition, by diffusion from the matrix to the sampler, until chemical equilibrium is reached between the polymer and the sampled matrix. The sampler polymer thereby attains only the freely dissolved fraction of the chemicals, which is the fraction available for biological uptake, compared to for instance solvent extraction that includes the total amount of chemical in the sample matrix.

Equilibrium passive dosing

In assessment of the toxicity of chemicals, maintaining and confirming stable exposure concentrations are crucial. Traditionally, test chemicals are spiked to the aqueous exposure media using an organic solvent. However, maintaining a stable exposure concentration in assessments of the toxicity of chemicals with low water solubility, such as HOCs, is challenging due to losses of the test chemical by sorption to vessel walls and organic matter, or due to losses by degradation and volatilization of the test chemical. Data on effects generated in tests where hydrophobic test chemicals are spiked to the aqueous exposure media can thus be
highly uncertain. Over the last two decades, a method has been developed that allows for stable exposure concentrations of HOCs to be maintained by equilibrium partitioning between a dosing polymer and water in toxicity tests. The method is referred to as passive dosing, but is also known from earlier studies as partitioning-driven administration, partition-controlled delivery, or partitioning-based dosing. The method allows for stable exposure concentrations of HOCs in toxicity tests lasting days to weeks. The equilibrium concentration of the test chemical in the aqueous phase in the dosing system is controlled by the concentration in the polymer and the polymer-water partition coefficient ($K_{p-w}$), thus a range of exposure concentrations up to the water solubility level of the chemical can be tested. Single HOCs, and chemical mixtures have been dosed mainly from silicone (polydimethylsiloxane, PDMS)-based polymers, in various formats, such as silicone-cast vials, o-rings, silicone rods and sheets, but also polytetrafluoroethylene (PTFE) stir bars have been used as the dosing polymer phase.

**Risk assessment of chemicals and contaminated sediments**

Chemical regulation is today mainly built around the assessment of single chemicals, as in REACH (Registration, Evaluation, Authorization and Restriction of Chemicals; EC 1907/2006) and the EU regulation on the use of Plant Protection Products (EC 1107/2009). However, there is an increasing body of evidence of mixtures cause toxic effects caused by chemical mixtures, even though the individual chemicals in the mixture are present below the no-observed effect concentration. For instance, Smith et al. assessed the toxicity of artificial mixtures of PAHs using passive dosing. Significant effects were observed on immobilization of *Daphnia magna*, even though the individual PAHs in the mixture had no or limited toxic effect.

Internationally, sediment risk assessments have traditionally been based on sediment quality guidelines (SQGs), which have been derived using either an empirical or a mechanistic approach. The empirical approach, is based on probabilities of which sediment total concentration [$\mu g \, kg^{-1} \, dw$] that causes a toxic effect in benthic organisms. The mechanistic approach builds on equilibrium partitioning theory, which assumes chemical equilibrium between the sediment organic matter and pore water, and the concentration of a certain chemical in the pore water
can therefore be derived from organic carbon-water partition coefficients in combination with organic carbon-normalized total sediment concentration. Pore water concentrations, are then compared with water quality guideline values. Predicting a toxic effect based on total sediment concentrations is highly uncertain. For instance, Kreitinger et al. showed that toxic effects to *Hyalella azteca* after exposure to sediment containing PAHs occurred over a large range of sediment total concentrations, and in some cases no effect occurred despite high total concentrations of PAHs in the sediment. The erroneous predictions of toxic effects are mainly explained by large variations in the sorption properties of sediments as sorption to organic carbon affects bioavailability of HOCs in sediment. The freely dissolved concentration in pore water has shown to be a good predictor for bioavailability of HOCs in sediment, the equilibrium partition approach is therefore theoretically sound, assuming that the pore water and sediment organic carbon is in equilibrium. However, use of generic partition coefficients may result in over or under predictions of pore water concentrations, due to site-specific sorption properties of the sediment.
Aim of thesis

This thesis encompasses both field studies and laboratory experiments that address two aspects of contaminated sediments; the release and toxicity of sediment-associated HOCs. The overarching aims of the thesis were to develop methods for in situ assessment of the release of HOCs from sediments (Paper I and Paper II), and to assess the toxicity of environmental mixtures of bioavailable chemicals in sediment (Paper III and Paper IV).

The objectives of the four papers presented in the thesis were:
I. To develop a benthic flow-through chamber in order to allow for more environmentally realistic in situ measurements of sediment-to-water flux of legacy HOCs.
II. To assess the effect of bioturbation on the sediment-to-water flux of PAHs in the Baltic Sea.
III. To develop the applicability of an equilibrium passive dosing system to maintain stable exposure concentrations of semi-hydrophobic chemicals in chronic bioassays.
IV. To develop a combined equilibrium passive sampling and passive dosing method for assessment of the toxicity of bioavailable environmental mixtures of semi-hydrophobic to hydrophobic chemicals in sediment.
Experimental section

Table 1. Chemicals analyzed in this thesis. Chemical abstract service (CAS) number, molecular weight (MW) and octanol-water partition coefficient (Log Kow). Log Kow for PAHs are from ref. for PCBs from ref. for triclosan from ref. for chlorophenols and γ-HCH from ref.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>CAS</th>
<th>MW</th>
<th>Log Kow</th>
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<tr>
<td>Naphthalene</td>
<td>91-20-3</td>
<td>128.17</td>
<td>3.40</td>
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<tr>
<td>Acenaphthyene</td>
<td>208-96-8</td>
<td>152.19</td>
<td>3.85</td>
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<tr>
<td>Acenaphthene</td>
<td>83-32-9</td>
<td>154.21</td>
<td>3.95</td>
</tr>
<tr>
<td>Fluorene</td>
<td>86-73-7</td>
<td>166.22</td>
<td>4.11</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>85-01-8</td>
<td>178.23</td>
<td>4.47</td>
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<tr>
<td>Anthracene</td>
<td>120-12-7</td>
<td>178.23</td>
<td>4.57</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>206-44-0</td>
<td>202.25</td>
<td>4.97</td>
</tr>
<tr>
<td>Pyrene</td>
<td>129-00-0</td>
<td>202.25</td>
<td>5.01</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>56-55-3</td>
<td>228.29</td>
<td>5.83</td>
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<tr>
<td>Chrysene</td>
<td>218-01-9</td>
<td>228.29</td>
<td>5.67</td>
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<tr>
<td>Benzo(b)fluoranthene</td>
<td>207-08-9</td>
<td>252.31</td>
<td>5.86</td>
</tr>
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<td>207-08-9</td>
<td>252.31</td>
<td>5.86</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>50-32-8</td>
<td>252.31</td>
<td>6.05</td>
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<tr>
<td>Ideno(1,2,3.cd)pyrene</td>
<td>193-39-5</td>
<td>276.33</td>
<td>6.57</td>
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<tr>
<td>Dibenz(a,h)anthracene</td>
<td>53-70-3</td>
<td>278.35</td>
<td>6.61^</td>
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<tr>
<td>Benzo(ghi)perylene</td>
<td>191-24-2</td>
<td>276.33</td>
<td>6.63</td>
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<td></td>
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<td>PCB 28</td>
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<td>5.92</td>
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<td>PCB 53</td>
<td>41464-41-9</td>
<td>291.99</td>
<td>6.26</td>
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<td>PCB 52</td>
<td>35693-99-3</td>
<td>291.99</td>
<td>6.76</td>
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<td>PCB 101</td>
<td>37680-73-2</td>
<td>326.44</td>
<td>7.08</td>
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<td>PCB 118</td>
<td>31508-00-6</td>
<td>326.44</td>
<td>7.31</td>
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<td>PCB 153</td>
<td>35065-27-1</td>
<td>360.88</td>
<td>7.70</td>
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<td>PCB 138</td>
<td>35065-28-2</td>
<td>360.88</td>
<td>7.66</td>
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<tr>
<td>PCB 180</td>
<td>35065-29-3</td>
<td>395.33</td>
<td>8.27</td>
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<tr>
<td>Triclosan</td>
<td>3380-34-5</td>
<td>289.54</td>
<td>4.76</td>
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<td>4-monochlorophenol</td>
<td>106-48-9</td>
<td>128.54</td>
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<tr>
<td>2,6-dichlorophenol</td>
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<td>163.00</td>
<td>2.75</td>
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<tr>
<td>3,5-dichlorophenol</td>
<td>591-35-5</td>
<td>163.00</td>
<td>3.62</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>58-89-9</td>
<td>290.81</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*a Log K\text{ow} predicted from a regression between MW and Log K\text{ow} for the PAHs in the ref.52*
Development of benthic flow-through chamber

A benthic flow-through flux chamber (Figure 1) was developed and tested in order to assess the sediment-to-water flux of dissolved HOCs. The chamber has a cylindrical design, a chamber area of 0.049 m² and a total volume of 3.4 L. Ambient oxygen levels in the water within the chamber are maintained by pumping water (1 L h⁻¹) through the chamber. A battery to power the water pump is placed on top of the chamber. Dissolved HOCs released from the enclosed surface sediment are collected on a sorbent (polyurethane foam, PUF) at the chamber outlet. A glass microfiber filter (GF/F), placed before the PUF sorbent, prevents suspended particles to enter the PUF. Before the pump starts, suspended sediment is allowed to settle by using a delay function (10 h) on the pump. It is assumed that steady state is reached quickly within the flow-through chamber, where the net flux of HOCs from the enclosed sediment inside the chamber (Figure 1, orange arrows) equals the flux of HOCs out of the chamber (Figure 1, blue arrow at 2nd sorbent). Incoming water is cleaned by passing through a PUF sorbent at the chamber inlet (1st sorbent). The experimental setup of the flow-through chamber is analogous to methods previously used to measure sediment-to-water fluxes in several ex situ studies. The ambient oxygen levels in the flow-through chamber design allows for assessment of effects of bioturbation on the sediment-to-water flux. In an earlier (closed) chamber design, the activity of benthic organisms was reduced due to depletion of the oxygen, and the effect of bioturbation on sediment-to-water flux of HOCs was thereby compromised.

The following parameters were tested and determined in laboratory experiments prior to the field validation of the flow-through chamber:

1) The amount of PUF sorbent needed to clean incoming water of its content of HOCs and to collect the HOCs released from the sediment within the chamber. The sorption capacity of the PUF was determined by pumping water (2 L, 2 L h⁻¹) spiked with PAHs (0.3-0.7 μg L⁻¹) and PCBs (9-12 ng L⁻¹) through various amounts (48-124 g) and densities (93-124 g PUF L⁻¹) of PUF and analyzing the concentrations of PAHs and PCBs in the water that passed through the sorbent.

2) The hydraulic conductivity of incoming and outgoing PUF sorbents was determined to ensure that there was no risk for drawing pore water out of sediment into the chamber, and thus affecting the sediment-to-water flux of HOCs. The hydraulic conductivity of the sorbent is also important as there is a tradeoff between increasing the sampling
duration, by reducing the flow resistance, and having enough PUF to sorb all HOCs in the incoming and outgoing water. The hydraulic conductivity of the sorbent, tested for two densities of PUF (0.48 g L\(^{-1}\) or 0.93 g L\(^{-1}\)), was derived from the hydraulic head (i.e. distance [m] between water level in a water reservoir and inlet of the sorbent) and the water flow [L h\(^{-1}\)] through, the length of the sorbent holder [m] and the cross section area of the sorbent holder [m\(^2\)].

3) The sediment oxygen demand during sampling. The water flow needed to maintain ambient oxygen concentrations inside the chamber during sampling was determined by measuring the oxygen concentration in the water above the sediment surface in incubated sediment cores (17\(^{\circ}\) C, for 6 days). The oxygen demand was derived from a linear regression between (declining) oxygen concentrations and time.

4) The water flow rate and sampling duration. The water flow rate through the chamber was assessed using the optimized amount of sorbent (point 1 and 2 above). A chamber was placed in a tank with sediment on the bottom and filled with water to cover the chamber in- and outlets. The flow of water was measured by timing how long it took to fill up 20 mL in a 100 mL measuring cylinder at several time points, until the chamber had stopped pumping. The maximum sampling duration was determined from the time the batteries could sustain pumping of water.

![Figure 1. Benthic flow-through chamber developed in Paper 1. A) Side view, orange arrows indicate sediment-to-water flux of HOCs and blue arrows indicate direction of the water flow. B) Illustration of the water flow (blue dashed arrows) through the chamber. (Figure from Paper 1.)](image)
Field validation of flow-through chamber

The field testing of the benthic flow-through chamber (Paper 1) was done in a Baltic Sea bay (Ålöfjärden, ÅF), with sediment contaminated with legacy HOCs. The sediment-to-water flux of PCBs and PAHs measured with the flow-through chamber were compared with fluxes measured in parallel using closed benthic flux chambers.

The measured sediment-to-water flux is calculated from the mass of HOCs on the 2nd sorbent (PUF), the enclosed sediment area and the sampling duration [ng m\(^{-3}\) d\(^{-1}\)] (Figure 2). The closed chamber collects HOCs released from the enclosed sediment on an infinite sink sorbent (semi-permeable membrane device, SPMD) that is placed inside the chamber.\(^{11}\) Passive uptake of HOCs in the SPMD is slow relative to active sampling (pumping of water) over the PUF sorbent in the flow-through chamber, and the deployment time for the closed chamber therefore exceeds one month. The concentration of PCBs and PAHs in pore water (\(C_{pw}\)) and bottom water (\(C_{bw}\)) was determined using polyoxymethylene (POM) passive samplers \([C_{pw} and C_{bw} in ng L^{-1}]\). The concentration in pore water and bottom water was used to determine the ratio between the \(C_{pw}\) and \(C_{bw}\) (i.e. activity ratio) and were also used for calculating flux using Fick’s first law of diffusion (F\(_{calculated}\), Figure 2), which describes diffusive transfer of solutes along a chemical gradient \(C_{pw}-C_{bw}\). The transfer of chemicals from sediment to water is governed by the thickness of the diffusive boundary layer (\(d_x\)) [m] at the sediment-water interface and the diffusion coefficient (\(B_w\)) [m\(^2\) d\(^{-1}\)], which is solute specific. The diffusive boundary layer at the sediment-water interface is constantly varying in thickness\(^{27}\) from μm to mm,\(^{58, 59}\) due to e.g. water turbulence and bioturbation. \(B_w\) was determined from molecule weight\(^{11, 60}\) and \(d_x\) was estimated based on reported thicknesses in the literature.\(^{11, 58, 59, 61-63}\)
Figure 2. Setup during field deployment of benthic chambers. Measured sediment-to-water flux ($F_{\text{measured}}$ [ng m$^{-2}$ d$^{-1}$]) determined by the mass of HOCs on the sorbent (PUF or SPMD), and the sampling duration (days) and chamber area [m$^2$]. Concentration in bottom water ($C_{bw}$ [ng L$^{-1}$]) and pore water ($C_{pw}$ [ng L$^{-1}$]) were determined from equilibrium concentrations in POM and POM-water partition coefficients ($K_{\text{POM-water}}$). $F_{\text{calculated}}$ is the calculated flux [ng m$^{-2}$ d$^{-1}$] using Fick's first law of diffusion, where $d_x$ is the thickness of the diffusive boundary layer [m], at the sediment-water interface and $B_w$ is the diffusion coefficient [m$^2$ d$^{-1}$]. Number of replicate chambers and sorbent type for respective chamber, as well as sampling duration for the chambers and POM is given in the boxes.

Mass balance model

A mass balance model was developed and used to evaluate the possible effect of the flow-through chamber design on the sediment-to-water flux measurements (Paper I). Placing the chamber on the sediment bed can affect the flux in two ways: 1) The water flow through the chamber can change the thickness of the diffusive boundary layer ($d_x$) at the sediment-water interface. 2) Pumping clean water into the chamber can affect the chemical concentration gradient within the chamber and thus the sediment-to-water flux of HOCs. The mass balance model predicts the diffusive sediment-water exchange of HOCs based on the measured concentration gradient between pore water and bottom water as well as
the advective transport of HOCs via water out of the chamber. The effect of chamber design on the sediment-to-water flux of HOCs was assessed by comparing the modeled net flux of PAHs and PCBs from sediment per area and time at the beginning of sampling, with the modeled mass of HOCs captured on the PUF sorbent by the end of sampling, per area and sampling duration. A larger discrepancy between the modeled net flux out of the sediment compared to the advective transport out of the chamber indicates a larger effect of the chamber on the sediment-to-water flux. A sensitivity analysis was performed using the mass balance model, to evaluate to what extent the flow-through chamber potentially could affect the sediment-to-water flux. The chemical concentration gradient between pore water and bottom water (ratio between C_{pw} and C_{bw} ranging 1 to 1000), thickness of the diffusive boundary layer (100-2000 μm), and water flow through the chamber (0.1-10 L h^{-1}), were varied one at a time and the discrepancy between the modeled net flux out of the sediment and the modeled mass collected on the PUF, per sediment area and sampling duration was assessed.

Field measurements of sediment-to-water flux of PAHs

The sediment-to-water flux of PAHs, and the impact of bioturbation on flux, was determined at four Swedish coastal sites in the Baltic Sea (Paper II) (Figure 3) using the same set up as in Figure 2. At each site, the total concentration of PAHs as well as the content of total organic carbon (TOC) and black carbon (BC) was determined in surface sediment. The effect of bioturbation on the sediment-to-water flux of HOCs was assessed by comparing the fluxes from the parallel measurement using the flow-through chamber and the closed chamber, as the activity of the bioturbating organisms is maintained in the flow-through chamber and ceased in the closed chamber. The four sites were selected to ensure elevated concentrations of HOCs in the sediment, and to have benthic species composition with various degree of surface dwelling organisms such as bivalves and crustaceans, as well as deep burrowing organisms such as polychaetes and oligochaetes. The density of benthic organisms was determined in sediment (0-20 cm sediment depth) sieved on a 1 mm mesh. The northern most site, Rundvik (RV), was located in the Gulf of Bothnia adjacent to an area with a long (> 100 year) history of industrial activity related to forestry. Two sites, Lilla Värtan (LV) and Lidingöbron (LB) were located in the city of Stockholm (Baltic Proper), where LV was located close to a leisure boat marina and a few km from
an active industrial harbor, and LB was located in a former coal harbor of a closed gasworks. The southernmost site (ÅF, Baltic Proper), was located approximately 100 km south of Stockholm, in a harbor of an active steelworks.

![Map of the Baltic Sea sampling sites](image)

*Figure 3. The four Baltic Sea sampling sites, from north to south, Rundvik (RV, Gulf of Bothnia), Lilla Värtan (LV, Baltic Proper), Lidingöbron (LB, Baltic Proper) (4 km apart), and Ålojarden (ÅF, Baltic Proper). (Figure from paper II.)*

**Chemical analysis**

PAHs and PCBs were analyzed in the sorbent matrices (PUF, SPMD and POM, Figure 2), and in surface sediment. Prior to extraction, a mixture of $^{13}$C-labeled PCBs (3-7 Ci) and deuterated (dc-d14, 2-5 rings) PAHs was added to each sample as internal surrogate standard. PUFs were extracted with Soxhlet (toluene). POM samplers and sediment samples were extracted (n-hexan:acetone 1:1) by ultrasonication or accelerated solvent extraction (ASE), respectively. Clean up for PAHs, and PCBs in POM samples, was done by liquid-liquid extraction using dimethylformamide (DMF), followed by a silica gel open column. The PUF extract (in n-hexane) containing the PCB fraction was cleaned with H$_2$SO$_4$, followed by a silica gel open column. Clean up for PCBs in sediment was done on a 3-layer silica gel open column (from the bottom: SiO$_2$/H$_2$SO$_4$, SiO$_2$/KOH and SiO$_2$/H$_2$O). All extracts were analyzed on GC-MS.
The recoveries of the mass labeled internal surrogate standards (\(^{13}\text{C}_{12}\text{-PCBs, and d}_{8}\text{-d}_{14}\text{-PAHs}\)) were 36 ± 15% and 49 ± 22% in the PUF samples, 77 ± 8.2% and 47 ± 19% in the SPMD samples, 92 ± 7.8% and 68 ± 20% in the POM samples; and 91 ± 2.1% and 62 ± 27% in the sediment samples. The limit of detection (LOD) was defined for each matrix (PUF, SPMD, POM and sediment) as three times the average mass in the blank samples. If no PCB or PAH were detected in the blank samples, the LOD was defined as 3 times the signal to noise ratio (Paper I) or three time the mass in the lowest calibration standard (Paper II). Further details can be found in respective paper (I-IV).

**Evaluation of passive dosing method for chronic exposure in a bioassay**

An equilibrium passive dosing method was evaluated in a chronic exposure test with triclosan, a semi-hydrophobic biocide. The effect was evaluated in a 6-week population development test with the sediment dwelling harpacticoid \(N.\ spinipes\), using three exposure levels of triclosan and a control (Paper III). The dosing silicone (500 mg or 1000 mg) was cast into the bottom of the test vials (10 mL or 24 mL). The silicone was loaded with triclosan dissolved in methanol, by forcing triclosan into the silicone by step-wise adding small amounts of Milli-Q water to the methanol solution (Figure 4). The concentration of triclosan in both silicone and water (10 mL) was determined after 16 hours of shaking at 150 rpm, when equilibrium between silicone and water had been established (Figure 4).
Figure 4. Passive dosing vials with silicone cast at the bottom. To the left, loading of dosing silicone with triclosan from a methanol solution. Water is added step-wise to force triclosan into the silicone. To the right, equilibrating the dosing system by partitioning of triclosan between the silicone and the water phase during horizontal shaking.

A certain amount of organic test chemicals will be sorbed by organic carbon that build up in the test vial during exposure (due to detritus, added food and growth of the test organism population). Thus, there is a risk that the silicone is depleted of its content of test chemical and that the exposure concentration therefore decrease during test duration, despite passive dosing. Two sets of vials for each tested concentration, were therefore used during the population development test in order to allow for periodical exchange of the water, and thus minimizing the buildup of organic carbon in the vials. Once a week during the population development test the *N. spinipes* population was counted and transferred to the other set of vials, which had been equilibrated (silicone and water). The vials in the first set were cleaned, and new exposure medium was equilibrated with the silicone. The procedure was repeated weekly throughout the 6-week test.

The stability of the exposure concentration of triclosan was validated in four tests (Table 2). In the first test, the stability of the water concentration of triclosan over 5 weeks was investigated and validated using passive dosing. 15 vials were cast with silicone (500 mg) and loaded with triclosan, and allowed to equilibrate. Once a week during 5 weeks, a set of vials \((n=3)\) was removed and the concentration of triclosan was analyzed in the water phase. The second method validation step was to investigate whether the passive dosing system could maintain the exposure concentration of triclosan in the presence of a growing population of test organisms and buildup of organic carbon in the vials. A 2-week
test was therefore performed, twice, with a population of *N. spinipes*. The population growth is the largest during the first weeks of this test and the buildup of organic carbon in the dosing vials during the 2-week test was therefore considered to mimic carbon buildup in the vials during the 6-week test. The 2-week test was first performed with three exposure concentrations, in triplicate, and two sets of vials (10 mL, 500 mg silicone), which allowed for periodical exchange of water. In the second 2-week validation test one exposure concentration was tested using two sets of 10 mL vials containing 500 mg silicone and two sets of 24 mL vials containing 1000 mg silicone. This was done in order to study the effect of silicone mass on its ability to maintain stable exposure concentrations. During both the 2-week validation tests, the water concentration in the vials was analyzed at three time points, to monitor any potential decrease in concentration over time. Lastly, the exposure concentration was validated before and after the 6-week population development test (24 mL vials, 1000 mg silicone). The triclosan in the water (10 mL) was liquid-liquid extracted twice using *n*-hexane, and triclosan in the silicone phase was extracted twice, using methanol. Analysis was done on GC-MS and quantification was done using $^{13}$C-labeled triclosan as internal standard.

Table 2. Tests for validation of stable exposure concentration of triclosan in the water phase of passive dosing vials.

<table>
<thead>
<tr>
<th>Test #</th>
<th>Mass silicone in vials [mg]</th>
<th>Test duration [weeks]</th>
<th><em>N. spinipes</em> in vials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>5</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>500 and 1000</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>6</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Population development test**

The population development test ([Paper III](#)) was carried out in 24 mL clear glass vials with silicone as passive dosing polymer cast (1000 mg) in the bottom of the vials. At the start of the test, three individuals from each life stage (nauplii, juvenile, adult and female with egg sack) were added to each dosing vial. Once a week the abundance of each life stage was recorded, and at the same time the organisms were transferred to the second set of pre-equilibrated vials. The test organisms were fed...
with *Rhodominas salinas* once a week, as well as at the start of the test. The continuous exposure lasted for 6 weeks (2-3 generation cycles) until the development of the population had reached steady-state. The effect of both triclosan concentration and exposure time was evaluated on the abundance of the age classes (nauplii, copepodite and adult), and total population size. The effect of exposure was also evaluated using a development index,\(^{67,68}\) which shows if the development of any copepod stage is retarded due to chemical exposure. The sex ratio was used to determine if either females or males were more sensitive to triclosan exposure.

**Development and validation of equilibrium passive sampling and dosing method for transferring chemical mixtures from sediment to a bioassay**

In paper IV, a method for transferring a chemical mixture of bioavailable HOCs from sediment to a bioassay was developed and tested using the green algae *Tetraselmis suecica* as test organism. The method consisted of 3 experimental sections (Figure 5): 1) Equilibrium passive sampling of an environmental mixture of bioavailable HOCs in sediment, 2) loading the mixture of HOCs, retrieved from the sediment by passive sampling, into silicone of a passive dosing system and equilibrating the loaded passive dosing silicone and the aquatic exposure medium, 3) assessing the toxicity of the mixture of HOCs in a live/dead bioassay using the green algae *T. suecica.*
Figure 5. The general experimental setup for the passive sampling of environmental mixtures of bioavailable HOCs in sediment and transfer of the chemical mixture to passive dosing vials for live/dead bioassay (Paper IV). (1) Passive sampling of an environmental mixture of HOCs in sediments using silicone-coated jars, and extraction of the mixture of HOCs partitioned into the silicone. (2) Loading passive dosing vials (cast with silicone at the bottom) with the mixture of HOCs and equilibrating with the aquatic exposure media (3). Exposure of algae (T. suecica) to a mixture of HOCs for 72 h, sample preparation, staining of cells and live/dead analysis. (Figure from paper IV.)

Validation of the equilibrium passive sampling and passive dosing method

The method based on equilibrium passive sampling and dosing was validated on the aspects of equilibrium sampling of the environmental mixture of HOCs from sediment into a silicone polymer, quantitative loading of HOCs into the dosing silicone polymer, stability of exposure levels of HOCs and the absence of toxic effects associated to the test system in the bioassay (Paper IV).
Amber glass jars, with a range of silicone thicknesses (11 to 30 μm, 110-290 mg) coated on the inside vertical wall, were used to validate the time to reach equilibrium between sediment and sampling silicone. The coated jars were filled with sediment and were rolled on the side for 3 weeks as previously done for sampling of PAHs, PCBs and hexachlorobenzene in soil and sediment. A linear relationship between the mass of chemical in the sampling silicone and mass of silicone indicates equilibrium, which is what we aimed for. To load HOCs into silicone used for passive dosing, HOCs dissolved in an organic solvent were added to the silicone-cast dosing vials. The HOCs partition into the dosing silicone as the solvent evaporated. We used the evaporative loading method instead of the method based on dissolving the chemical mixture in methanol with subsequent addition of water to force the chemicals into the silicone, as precipitation in methanol of HOCs in environmental mixtures was observed in initial experiments. We validated the loading method quantitatively by loading PAHs and PCBs into silicone and after evaporation of solvent (n-hexane) and equilibration with water, concentrations of PAHs and PCBs were determined in both silicone and water.

The stability of the exposure concentration of the chemical mixture was evaluated in order to determine any depletion of the dosing silicone after repeated use of the vials, which may be useful in any situation where the exposure test needs to be repeated, for testing of additional toxicity end-points, or for assessment of chronic exposure. Dosing vials, cast with 30 mg silicone and loaded with a PAHs, PCBs, hexachlorocyclohexane, triclosan and chlorophenols, were used twice in a 72 h algae assay with the green algae *Tetraselmis suecica*. After the first 72 h assay the *T. suecica* and the test medium were discarded. New exposure medium was equilibrated (horizontal shaking 170 rpm, 18 h) and a second 72 h algae assay using the same vials was performed. The mass of the PAHs, PCBs, hexachlorocyclohexane, triclosan and chlorophenols in the dosing silicone was determined before, after the first, and after the second 72 h assay.

**Assessing the toxicity of a mixture of chemicals using passive dosing**

The applicability of the combined passive sampling and passive dosing method to transfer environmental chemical mixtures from sediment to
a bioassay followed by assessment of the toxicity of the mixture was validated using sediment from a contaminated Baltic Sea bay (Ålöfjärden ÅF; Experimental section 1, Figure 5) (Paper IV). For this, amber glass jars, coated with 300 mg silicone on the inside vertical walls were filled with sediment from the ÅF site and were rolled on the side for three weeks. The mixture of HOCs in the sampling silicone after equilibrium with sediment was solvent extracted and the extracts were transferred to dosing vials (Experimental section 1 and 2, Figure 5). Dosing vials (1.5 mL) were cast with 30 mg silicone (Figure 5). A range of exposure scenarios in the live/dead assay were achieved by adapting the amount of silicone in the passive dosing vials to the amount of silicone in the passive sampling jars (concentration factors: 1:1 (ambient), 1:10, 1:50 and 1:200, dosing:sampling silicone) (Table 3). The ambient concentration factor was achieved by using jars coated with 30 mg silicone, the 1:10 concentration factor was achieved by transferring extracts from one jar coated with 300 mg to one vial. Extracts from the jars (coated with 300 mg silicone) were combined to achieve the concentration factors 1:50 and 1:200.

Table 3. Concentration factors from the sampling polymer to the dosing polymer. The 1:1 (ambient concentration) and the 1:10 concentration were achieved by loading dosing vials from jars with 30 mg and 300 mg silicone polymer, respectively. The concentration factors 1:50, 1:200 were achieved by loading dosing vials with extracts from 5 and 20 jars coated with 300 mg silicone, respectively.

<table>
<thead>
<tr>
<th>Concentration factor in live/dead assay</th>
<th>Mass sampling polymer in jar [mg]</th>
<th>Number of extracts from jars combined</th>
<th>Mass dosing polymer in vial [mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>30</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>1:10</td>
<td>300</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>1:50</td>
<td>300</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>1:200</td>
<td>300</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

The toxicity of the environmental chemical mixture of bioavailable HOCs was assessed in a 72 h live/dead bioassay using the marine green algae *T. suecica* (Experimental section 3, Figure 5). Four exposure scenarios were tested, and each exposure scenario had a control loaded with extracts from clean sampling jars. The dosing vials were shaken horizontally (150 rpm) during exposure. After 72 h exposure, the algae
were collected and stained using TO-PRO-1 iodine. The stain penetrates damaged cell membranes and is visible under UV-light. Live/dead cells were manually counted (200 cells, 3 times per sample) in a fluorescence microscope. The live/dead bioassay allows for assessment of narcosis (baseline toxicity) and was chosen as hydrophobic chemicals that penetrate the lipid bilayer of the cell membrane accumulate in sediment. The stability and repeatability of the bioassay was validated by evaluating the percentage of mortality and variability of mortality in the controls.
Results and discussion

Validating the flow-through chamber

The initial tests of the benthic flow-through chamber (Paper I) showed that HOCs are effectively removed from incoming and outgoing water by the PUF sorbents, and that the PUFs used (48-65 g) can efficiently sorb 1000-1500 ng of individual PAHs and 20 ng of individual PCBs. The hydraulic conductivity of the PUFs was $10^{-2}$ m s$^{-1}$ which is 3-7 orders of magnitude higher than in the sediment at ÅF. The pressure drop inside the chamber is therefore assumed to be negligible, and as a consequence the risk of drawing pore water into the chamber during sampling is low. The oxygen level inside the chamber is maintained as the water flow through the chamber during sampling (3 days) provides 18000 μmol O$_2$, which is 2-7 times the amount of oxygen expected to be consumed in the chamber during sampling (2500-9000 μmol O$_2$). Thus benthic organisms inside the chamber will not suffer from depletion of oxygen during sampling. The maximum sampling duration, limited by the battery capacity, is currently 4-5 days.

The mass balance model demonstrated that the flow-through chamber is applicable for measuring sediment-to-water fluxes of legacy HOCs that have a high chemical concentration gradient ($C_{pw}/C_{bw} > 10$) between sediment pore water and bottom water. For these chemicals and situations, the net flux out of the sediment and the advective flow out of the chamber is similar and the effect of the chamber design on the measured sediment-to-water flux will be 10% at the most. In the flow-through chamber, there is a tradeoff between maintaining the oxygen levels inside the chamber (governed by the water flow) and adjusting the water flow to reduce the discrepancy between the advective transport out of the chamber and the net flux out of the sediment, for HOCs with a weak gradient between pore water and bottom water. For instance, in order to not affect the flux of HOCs with weak chemical concentration gradients ($C_{pw}/C_{bw} < 10$), the water flow through the chamber needs to be reduced, however, reducing the water flow with more than a factor of 2 may result in depletion of the oxygen in the chamber and as a consequence reduce the activity of bioturbating organisms.
Field validation of the flow-through chamber

Sediment-to-water flux at the site ÅF of individual PAHs and PCBs ranged between 62-2300 ng m\(^{-2}\) d\(^{-1}\) and 5.5-150 ng m\(^{-2}\) d\(^{-1}\), respectively (Paper I). These fluxes of individual PAHs and PCBs were 3-23 and 12-74 times higher than the sediment-to-water flux determined using the closed chamber, deployed in parallel in the same area. The discrepancies in flux of PCBs and PAHs from sediment to water measured with the two chamber types (expressed as ratio [flow-through chamber:closed chamber]) indicate a possible effect of bioturbation on the sediment-to-water flux, as the discrepancies were larger than the modeled effects of the flow-through chamber on the net flux. The ratio in flux between the two benthic chamber types, that is potential effect of bioturbation on sediment-to-water flux agrees with the effect of bioturbation on sediment-to-water flux reported in previous laboratory and modeling studies (Table 4).17-21, 73, 74

Sediment-to-water flux measurements using the benthic flow-through chamber

The sediments at all the investigated sites (RV, LV, LB, and ÅF, Figure 3) were acting as sources of PAHs to water, and as a source of PCBs at site ÅF (Paper II). It is possible that sediments at RV, LV and LB act as sources of PCBs to the water, however, PCBs were mainly below the detection limit at these sites. Fluxes of the ∑PAH\(_{15}\) measured using the flow-through chamber were 1100, 1100, 21000 and 7000 ng m\(^{-2}\) d\(^{-1}\) at sites RV, LV, LB and ÅF, respectively and the fluxes of individual PAHs ranged between 21-510, 11-370, 3.0-9700 and 62-2300 ng m\(^{-2}\) d\(^{-1}\) at the same sites (Figure 6). The contribution of individual PAHs to the total flux from sediment varied between the sites. For instance, at sites RV, LV and LB, the flux of ∑PAH\(_{15}\) was dominated by the lighter 2-3 ring PAHs, such as naphthalene, acenaphthene, fluorene, and phenanthrene, while at the site ÅF, the 4-ring PAHs fluoranthene and pyrene, were the main contributors to total flux. The different congener patterns between the sites may be explained by the different emission sources and sorption capability of the sediment organic matter at each site.
Figure 6. Sediment-to-water flux (μg m⁻² d⁻¹) of 15 PAHs at the four sites (RV, LV, LB, AF) on the Swedish Baltic Sea coast measured using the flow-through chamber. The range of the fluxes for individual congeners is presented in paper II. Naphthalene (Naph.), acenaphthene (Acen.), fluorene (Fluo.), phenanthrene (Phen.), anthracene (Anth.), fluoranthene (Flu.), pyrene (Pyrene), benzo(a)anthracene (B(a)A.), chrysene (Chrys.), benzo(b)fluoranthene (B(b)Flu.), benzo(k)fluoranthene (B(k)Flu.), benzo(a)pyrene (B(a)Pyrene), indeno(1,2,3-cd)pyrene (Ind.), dibenzo(a)anthracene (Dib(a)A.), benzo(ghi)perylene (B(ghi)Per.).

Effect of bioturbation on sediment-to-water flux of HOCs

The potential effect of bioturbation on the sediment-to-water flux of HOCs was investigated in situ at the four sites, RV, LV, LB and AF (Paper II). The discrepancy between the measured flux using the flow-through chamber and the closed chamber was used as an indicator of a possible effect of bioturbation. The ratio between the flux measured with the two chamber types (flow-through closed) was on average 11 (3 to 55, min-max) for individual PAHs. The discrepancy in measured flux between the two chamber types was the largest at the sites with the highest density of bioturbating organisms, i.e. RV and AF, where the density of bioturbating organism (e.g. Marenzelleria spp. Macoma balthica and Monoporeia affinis) was 1200 and 860 ind. m⁻², respectively. However, a similar discrepancy between the chambers was observed at
site LV, where the density of bioturbating organisms was more than one order of magnitude lower. At LB, no effect of bioturbation could be detected by comparing the fluxes from the two chamber types, which agrees with the low density (< 150 ind. m\(^{-2}\)) of bioturbating organisms observed at this site. The observed discrepancy between the sediment-to-water flux using the two chamber types at sites RV and ÅF is comparable to previous observations from laboratory-based and modeling studies where the effect of bioturbation on the sediment-to-water flux was investigated (Table 4).\(^{17, 19, 20, 73, 74}\) The effect of bioturbation may thus have a substantial effect on sediment-to-water flux of HOCs in areas with high densities of bioturbation organisms, and the density of benthic organisms should thus be a parameter considered in sediment risk assessments.

Table 4. Increase in sediment-to-water flux of HOCs due to bioturbation reported in the literature and from paper I and II. (Table adapted from paper I.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Increase due to bioturbation</th>
<th>Type of study</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCB, HCH, DDx, PCBs</td>
<td>1-5</td>
<td>Laboratory</td>
<td>(^{19})</td>
</tr>
<tr>
<td>PCB 32</td>
<td>3</td>
<td>Laboratory</td>
<td>(^{20})</td>
</tr>
<tr>
<td>PCBs</td>
<td>4-13</td>
<td>Laboratory</td>
<td>(^{21})</td>
</tr>
<tr>
<td>PCBs</td>
<td>1-25</td>
<td>Laboratory</td>
<td>(^{74})</td>
</tr>
<tr>
<td>Chlorobenzenes and trifluoralin</td>
<td>4-6</td>
<td>Laboratory</td>
<td>(^{17})</td>
</tr>
<tr>
<td>Trichlorophenyl and PCBs</td>
<td>40-190</td>
<td>Model</td>
<td>(^{73})</td>
</tr>
<tr>
<td>ΣPAHs</td>
<td>11 (3-55, min-max)</td>
<td>Field ((in situ))</td>
<td>Paper I and Paper II</td>
</tr>
<tr>
<td>ΣPCBs</td>
<td>42 (12-74, min-max)</td>
<td>Field ((in situ))</td>
<td>Paper I</td>
</tr>
</tbody>
</table>
Validation of exposure concentration in passive dosing system for chronic toxicity assessment

Stable exposure concentrations of triclosan could be maintained throughout the 6-week population development test (Paper III), as confirmed by the four validation tests (Table 2). First, the water concentration was stable during 5 weeks in dosing vials with water but without test organisms (confirmed by linear regression of concentration over time, where the regression line was not significantly different from zero). The exposure concentrations of triclosan were stable in the two 2-week validations tests, using 500 mg and 1000 mg silicone, with the exception for one exposure concentration in one set of vials cast with 500 mg silicone, where the concentration was reduced from 72 ± 4 μg L⁻¹ to 27 ± 1 μg L⁻¹ in the first 2-week validation test. The reduction of the exposure concentration indicates that vials cast with 500 mg may be depleted during a 6-week population development test. In the second 2-week validation test, no significant difference in the exposure concentration could be observed between the second and the third water extraction. The concentration of triclosan in the second and third water extraction were 167 ± 11 μg L⁻¹ and 190 ± 31 μg L⁻¹ and 211 ± 6 μg L⁻¹ and 208 ± 2 μg L⁻¹ in the vials with 500 mg silicone and 1000 mg, respectively. Thus, vials cast with 1000 mg silicone had a satisfactory buffering capacity to compensate for losses of triclosan in the dosing system with a population of N. spinipes similar in size as in the population in the 6-week test. In the subsequent 6-week population development test, vials cast with 1000 mg silicone were therefore used. Concentrations of triclosan were approximately 3 times higher in the first 10 mL of water equilibrated with the dosing silicone compared to the concentration in the second and third 10 mL of water equilibrated with the dosing silicone. The reason for the elevated concentrations is unknown, but one possible explanation is that microcrystals precipitated on the silicone surface and was washed off in the first 10 mL of water equilibrated with the silicone. The first 10 mL of water equilibrated with the dosing silicone was therefore discarded. The exposure concentration was validated before and after the 6 week population development test, in the first 10 mL of water equilibrated with the dosing silicone and in water after the population development test. The concentration in the first 10 mL equilibrated with the dosing silicone was a factor 3 higher, compared to the last 10 mL of water equilibrated with the dosing silicone (Table 5), similar to the observations in the 2-week
validation tests. Thus, the vials cast with 1000 mg silicone could maintain stable exposure concentrations throughout the 6-week population development test.

Table 5. The average exposure concentrations of triclosan in the population development test [μg L⁻¹], for vial set 1 and 2. Concentrations in the water before the development test was measured in the first 10 mL of water equilibrated with the dosing silicone, and is a factor of 3 higher than the concentration in the water in the population development test. The exposure concentrations were confirmed in 10 mL water equilibrated with the dosing vials after the population development test. (Table adapted from paper III.)

<table>
<thead>
<tr>
<th>Set</th>
<th>Before exposure μg L⁻¹ ± S.D.</th>
<th>After exposure μg L⁻¹ ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 ± 2</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>32 ± 3</td>
<td>11 ± 1</td>
</tr>
<tr>
<td></td>
<td>70 ± 13</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Set 2</td>
<td>16 ± 1</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>24 ± 15</td>
<td>6.7 ± 1</td>
</tr>
<tr>
<td></td>
<td>69 ± 8</td>
<td>16 ± 1</td>
</tr>
</tbody>
</table>

Toxicity of chronic exposure to triclosan

The sediment dwelling harpacticoid copepod *N. spinipes* was exposed to triclosan during a 6 week population development test. A significant concentration effect was observed on the abundance of copepodites (juvenile life stage). Results further indicated that the concentration may have an effect on all life stages (nauplii, copepodites, adult, and female) as a significant time-concentration effect was found in all treatments. Increasing triclosan exposure resulted in lower development index values during the first four weeks of the test, which indicates a retardation in the population development. The most influential parameter for the development index was the decline in percentage of copepodites. For instance, the percentage of copepodites in the whole population development test was approximately a factor two lower in the in the highest exposure (40 %) compared to the control treatment (80 %). This study demonstrates that passive dosing is a useful method for assessing chronic toxicity of hydrophobic substances. Passive dosing resolves the challenges of maintaining of stable exposure concentrations in long term toxicity tests of hydrophobic chemicals, which have been difficult using traditional solvent spiking methods.
Validation of the equilibrium passive sampling and dosing method for transferring chemical mixtures from sediment to a bioassay

The passive sampling and dosing method was validated regarding equilibrium sampling, loading efficiency, stability of mixture composition and background mortality of the test organism (Paper IV). Equilibrium between sediment and the passive sampling silicone was reached within 3 weeks of sampling, which was confirmed by a linear relation between the mass of PCBs in the silicone versus mass of silicone in the sampling jars, for chemicals with a log $K_{ow}$ up to 7.3 (Figure 7).

![Figure 7. Relationship between mass PCB (PCB 138 and PCB 153) [pg] and mass silicone [g] in jars, confirming equilibrium conditions between the sediment and silicone after 3 weeks of sampling. (Figure from paper IV.)](image)

A quantitative loading of HOCs into the dosing silicone was possible using the evaporative loading method, where the mass of anthracene, PCB 28 and PCB 52 in the silicone was 80-100 % of the nominal mass, on average. For pyrene and benzo(a)pyrene the average mass was 130-140 % of the nominal mass, which is possibly explained by analytical difficulties, but still supports a quantitative loading using evaporative loading. The exposure concentrations of PAHs, PCBs and hexachlorocyclohexane were maintained within 50 % of the concentration before the test, using the system with 30 mg dosing silicone in two consecutive 72 h algae exposure tests (Figure 8).
Furthermore, it was confirmed that the passive dosing method did not exert any measurable stress to the test organism, as shown by a low mortality (0 - 1.6 %, min-max, dead cells; Figure 9) in the controls and that the dosing system provided conditions with high repeatability, shown by a low between run variability in the controls. The combined passive sampling and passive dosing method is thus applicable for assessing the toxicity of environmental mixtures of bioavailable semi-hydrophilic and hydrophilic (approx. log Kow 3–7) chemicals in sediments. Exposure levels up to 200 times higher than the ambient levels were assessed and a significant dose-response relationship was found in the live/dead assays using T. suecica (Figure 9). The average mortality ranged between 0.7 % and 3.5 %, in the lowest and highest exposure level, respectively. This study showed that it is possible to detect a toxic effect from exposure of environmental chemical mixtures of HOCs at ambient levels using the combined passive equilibrium sampling and dosing method. The toxic effect of the environmental chemical mixture of HOCs at ambient exposure would be significantly different from the control treatment if the statistical power was higher, i.e. if 10 replicates had been used instead of 3 replicates, albeit, increasing the number of replicates increases the experimental costs. In a previous study, no inhibitory effects on marine diatom growth (72 h test duration), as a consequence of exposure to environmental mixtures of chemicals from marine water, could be observed using a similar passive sampling and passive dosing method. In order to detect effects from
exposure to ambient levels of environmental mixtures of chemicals, the test end points need to be sensitive and the test duration may need to be extended to last more than 72 h.

Figure 9. Dose-response relationship between the fraction of dead algae cells [%] and concentration factors, 1:1, 1:10, 1:50, 1:200, of an environmental mixture of chemical from a Baltic Sea sediment. (Figure from paper IV)
Conclusions

The benthic flow-through chamber enables for within-days (3d) measurements of sediment-to-water fluxes of legacy HOCs that include the effect of bioturbation on the flux (Paper I). This compare to the closed benthic chamber\textsuperscript{11} or methods based on passive samplers,\textsuperscript{76, 77} where the sampling duration often exceeds 1 month and the effect of bioturbation on the sediment-to-water flux in not included. Thus, the flow-through chamber provides more environmentally realistic \textit{in situ} sediment-to-water flux measurements than previously possible using closed chambers. \textit{In situ} sediment-to-water flux measurements could serve as useful and complementary information for the identification of sediments that are acting as sources of legacy HOCs to water and biota. Obtaining information on which sediments are posing a risk of releasing HOCs should be an important step in the management of contaminated sediments, as remediation actions come with high costs, and prioritizations are necessary. Flux measurements of HOCs may furthermore be used to assess the relative contribution of HOCs from various sources to a water body. For instance, sediments in several areas on the Swedish Baltic Sea coast are releasing PAHs (Paper II) and also potentially PCBs and PCDD/Fs. The release of HOCs may have adverse effects on the local ecosystem, however, the contribution of HOCs from sediment may be minor compared to other sources such as atmospheric deposition. In order to achieve reduced levels of HOCs in the Baltic Sea water body, assessing remote sources of HOCs using methods applicable for chemicals prone to long-range transport may be more cost efficient from a larger perspective. Bioturbation may increase the sediment-to-water flux of legacy HOCs by up to one order of magnitude, at sites with a density of bioturbating organisms of approximately 1000 ind. m\textsuperscript{-2} (Paper II); the effect may be even higher at sites with higher density of bioturbating organisms. Thus it would be meaningful to include the effect of bioturbation in the assessment of sediments as potential sources of HOCs to water.

Passive dosing has previously been demonstrated to allow for stable exposure concentrations of HOCs in acute toxicity tests, and here it was shown that it is applicable also for assessing chronic toxicity during a 6-week population development test (Paper III). Hence, with passive...
dosing, it is possible to address chronic effects of exposure to HOCs on a population scale (Paper III), as it requires exposure times of several weeks.

The combined equilibrium passive sampling and passive dosing method could transfer a chemical mixture from sediment to a bioassay, and was useful in assessing the toxicity of a complex mixture of sediment-associated semi-hydrophobic and hydrophobic contaminants to a marine alga (Paper IV). Reliable methods to determine the toxic effects of HOCs in sediments are crucial for sound assessments of the risk for adverse effects on benthic organisms. The method presented in paper IV has a large potential to help improve assessments of the toxicity of contaminated sediments, as it includes exposure of the environmental chemical mixture, containing also unknown semi-hydrophobic and hydrophobic organic contaminants accumulated in the sediment. Furthermore, the method presented in paper IV may be used for assessing environmental mixtures of HOCs from a range of matrices. Besides sediment, soil is the obvious matrix to retrieve HOCs from, but the methodology should also be applicable, in its current or in a new design, for assessing exposure of mixtures of chemicals from any matrix containing HOCs, for instance, water, air, and biological lipids. The number of chemicals in society is constantly increasing and the potential adverse effects due to constant exposure to complex chemical mixtures is not well known. The method presented in paper IV may be used for gaining valuable insights on the effects of mixtures of chemicals on many types of aquatic organisms.
Future work

The sediment-to-water flux measurements could be used in mass balance modeling, to assess flux between sediment and water on a local or regional scale. In addition to the flux of dissolved HOCs measured with the chambers, information on particle-associated flux is needed in order to assess total flux between sediment and water. Furthermore, measurements of sediment-to-water flux of HOCs could be used for evaluating new sediment remediation strategies. One example is where activated carbon is mixed into contaminated sediments to increase the sorption of HOCs to sediments and thus reducing the pore water concentration and flux from the sediment to water.\(^78\) The flow-through chambers (\textit{Paper I and II}) may be used to determine the efficiency of such remediation methods. In the same context, the passive sampling and dosing method presented in \textit{paper IV} may be used to evaluate if such remediation methods also are successful in reducing the toxicity of the sediment. Furthermore, the years after a sediment has been amended with for instance activated carbon, the benthic communities may be reduced\(^79\) and accordingly the effect of bioturbation decreases. However, as benthic organisms reestablish in amended sediment,\(^80\) the effect of bioturbation on the sediment-to-water flux may need to be assessed in order to determine the longevity of the remediation action. Future work, may also involve investigation of species-specific effects of bioturbation on the HOC flux, using the flow-through benthic chamber. In addition, density of benthic organisms vary substantially over time and the temporal effects of bioturbation could be investigated in order to determine effects of bioturbation on a long-term perspective.

In terms of expanding the potential of the passive sampling and dosing method (\textit{Paper IV}), additional toxicity end points should be used in order to increase the sensitivity to detect toxic effects at low exposure concentrations, which may be found in remote areas, and to determine a potential reduction in the mixture toxicity after sediments have been amended with activated carbon. Additional end points are also needed to detect toxic effects from chemicals that have specific modes of action. To determine which chemicals are contributing the most to the overall mixture toxicity effect, a battery of toxicity end points, in combination with chemical analysis, could be used. Furthermore, it would add extra value to the method if future research was directed to develop
it for use in long-term toxicity tests (Paper III), as this would allow for further assessments of the toxicity of environmental mixtures at low doses that are comparable to the background concentrations found in the environment.
References


22. Kristensen, E.; Penha-Lopes, G.; Delefosse, M.; Valdemarsen, T.; Quintana, C. O.; Banta, G. T., What is bioturbation? The need for a precise


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