Noël J. Diepens

Sediment

toxicity testing and prospective risk assessment of organic chemicals

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Sediment toxicity testing and prospective risk assessment of organic chemicals

Noël J. Diepens

Thesis

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"*Nothing is more deceptive than the quiet surface of sediments. The world beneath that surface defies the imagination, with millions of species at work in an astonishing variety of ways, biological, physical and chemical.*"

- **Gretchen C. Daily** -
2004

Contents

Chapter 1

General introduction

"Virtually all of the materials we use and the luxuries we enjoy rely in some way on remarkable science. But there is a dark side to our chemical dependency; we have produced such an extraordinary variety and quantity of chemicals so rapidly and with so few controls that they have now spread unseen into every corner of our increasingly contaminated environment."

- **John Replogle** -

"We need regulation to remove chemicals from the supply chain"

The Guardian, 2013

We do not have to look far to see products that would not exist without manmade chemicals. The chemical industry is, not surprisingly, one of the biggest industries in the world. The world chemical products turnover was valued at 3,127 billion euro in 2012 with Asia dominating the market followed by Europe.[1] Every year, thousands of new chemicals are brought onto the market that can be used for many different purposes such as pesticides, human and veterinary medicines, personal health care products, plastics, cleaning products and oils. Hydrophobic organic compounds (HOC) are a group of major concern particularly when they are persistent, toxic and bioaccumulative. HOCs may enter the environment by various pathways. Depending on their chemical characteristics they may be found in the atmospheric, terrestrial and aquatic compartments. Aquatic sediments are an important part of the aquatic ecosystem and constitute a major sink for HOCs where they can stay for a very long time. For example, polychlorinated biphenyls (PCBs), banned in the late 1970s, and the pesticide dichlorodiphenyltrichloroethane (DDT), banned in 1970s in most parts of the world, are still found in sediments. Aquatic sediment is a complex heterogeneous matrix that covers a large part of earth's surface (freshwater 0.5%, marine 74%)[2] and provides critical ecosystem services such as water purification and decomposition of organic matter.[3] HOCs may pose long term risks to benthic organisms, accumulate in the food chain and affect the services provided by aquatic ecosystems.[4-6] This does not only lead to ecological degradation but can also have a major impact on humans and human communities e.g. those living from fisheries.

In historical perspective, application of DDT is a good illustration of how the perception of a chemical product changed over time. DDT was brought onto the market as an effective and safe-to-use pesticide (Figure 1) and had a maximum usage of 36,000 tonnes in the United States in 1959.[7] However, considering the current requirements, the chemical was not properly tested and/or information was not shared. When Rachel Carlson's book Silent Spring (1962)[8] about the effects of DDT reached the general public, awareness increased and questions were raised about the safe use of DDT. Although negative effects such as acute kills of aquatic invertebrates and fish, adverse effects on growth, reproductive failure and shell thinning were known, it took until 1970s for DDT to be banned in most of the world.[9] However, there is still an ongoing debate about the use of DDT in malaria control[10] as is currently done in e.g. South Africa[11], indicating that a cost-benefit analysis may have different, context dependent, outcomes.

Figure 1. Pesticide advertisement in Time Magazine (June 30, 1947).

The DDT example is just one of the many examples of chemicals being introduced and commercially used without understanding the full array of what the environmental consequences may be. There is thus a need for a comprehensive analysis of potential environmental risks before being introduced and used, which is the primary goal of prospective environmental risk assessments (ERAs). However, it will not be possible to predict all potential environmental consequences with 100% certainty before marketing a new product, particularly for chemicals with unknown physicochemical and/or toxicological properties. To address possible flaws in prospective ERA it is realized that feedback mechanisms between prospective and retrospective ERA approaches are required (e.g. Burton et al.[12], Brock[13]).

Historically, retrospective ERA, which evaluates the causal linkages between observed ecological effects and chemical stressors already present in the environment (definition by Environmental Protection Agency (EPA)), received most attention. However, to prevent future ecological effects of chemicals, prospective ERA, transparent data provision and risk communication are needed in the context of market authorization of existing and new chemicals. Prospective ERA evaluates the future risks of a chemical stressor not yet released into the environment (definition by EPA). The first stage of a prospective ERA consists of the problem formulation after which exposure and effect assessments are performed. The exposure assessment predicts exposure patterns and concentrations in environmental media such as sediment. The effect assessment describes the relationship between exposure concentration and effects of the assessed ecological endpoints. The effect assessment is often performed in a tiered way starting with simple laboratory tests in the lower tiers (Figure 2). Test complexity and ecological realism increase when moving up tiers.[14-16] Lower tiers are less data and resource demanding and are more conservative than higher tiers. To provide an estimate of the environmental risk the outcomes of the two assessments are compared in the risk characterization phase. Risk is often expressed as a risk quotient or as a risk probability. A constant evaluation of the risk of the chemical through monitoring and data gathering is needed, including feeding those data back into the risk assessment and re-evaluation of chemicals already approved for the market. For a large number of potentially toxic substances used commercially, prospective risk assessment is already mandatory in many industrialized countries.

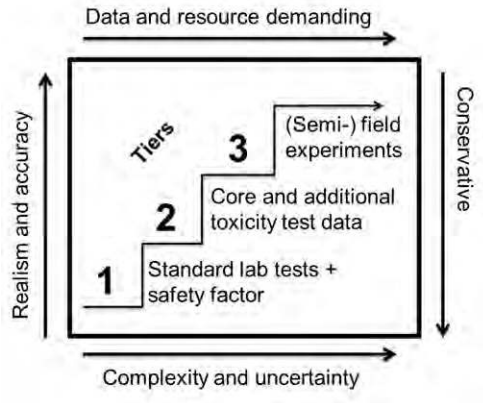

Figure 2. Conceptual framework of the tiered effect assessment used in prospective environmental risk assessment. Redrafted after Solomon et al.[16].

Prospective ERA is relatively well established for the terrestrial and the aquatic compartment. ERA in the aquatic compartment is often only based on effect data for pelagic organisms living in the water column and ignores the additional exposure pathways via the sediment and thus risk of sediment-bound chemicals to benthic organisms.[17-20] A proper conceptual prospective sediment ERA framework for organic chemicals is currently lacking.[20,21] Such a framework requires clearly defined protection goals, evidence-based concepts for linking exposure and effects and a transparent tiered effect assessment procedure for sediment organisms and processes. Moreover, harmonization of data requirements, test protocols and ERA frameworks between regulations/directives would be beneficial.[20]

Within the aquatic ERA, sediment tests are required for those chemicals that meet the triggers i.e. criteria for sediment testing that are mainly based on the chemical fate characteristics and toxicity. However, only for a few benthic species, mainly for invertebrates, standard test protocols are available. Cost effective and widely accepted test methods for microorganisms, macrophytes, vertebrates and invertebrates other than a few arthropods and oligochaete/ polychaete worms, as well as tools for the translation of results between levels of biological organization are in their infancy. Tests with sediment organisms and sediment-associated chemicals typically call for chronic testing as sediment exposure is characterized by low concentrations and long duration, whereas aquatic exposure is typically characterized by higher concentrations and shorter duration. A chronic toxicity test is generally defined as a study in which the species is exposed to the toxicant for at least one full life-cycle or the species is exposed to the toxicant during one or more critical and sensitive life stages. These tests focus on lethal and sub-lethal endpoints (e.g. related to reproduction and growth). There is thus a need to develop chronic sediment tests, which cover different taxonomic groups, trophic levels and exposure pathways and that allow for extrapolation of results between levels of biological organization. Moreover, extrapolation of single species test results into ecological threshold concentrations for sediment communities and processes needs to be improved. A mechanistic understanding of exposure and uptake pathways in the effect assessment is crucial for the development of sediment toxicity tests method in prospective ERA and for the interpretation and extrapolation of test results. It is thus essential to understand exposure in a sediment test by assessing the relative importance and characteristic time scales of exposure pathways and the differences in bioaccumulation for a range of species with different traits. Important questions therefore remain about the uptake routes of sediment-bound chemicals in sediment-rooted macrophytes and benthic invertebrates and how these can be parameterized.

Single species tests use worst case exposure scenarios and have a low ecological realism as they cannot capture processes at the population and/or community level. Community level tests (microcosm and mesocosm, “cosm” experiments) have been developed to increase ecological realism.[22] However, there is insufficient knowledge about the impact of sediment-bound contaminants in cosm tests and the causal relationships between effects in single-species tests and cosm tests, which hampers the data interpretation and calibration of the effect assessment. Cosm tests cannot fully account for all ecological processes present in the natural complexity of ecosystems such as predation by top predators and

recolonization by species that only have an aquatic life stage.[23] These processes, however, can be accounted for in mathematical population models. Yet, present population models addressing toxicity by sediment-bound chemicals are inadequately developed, as they lack the link between species-specific uptake at individual level and effects at population level and often ignore exposure via the sediment. Therefore, development of prospective population models for spatial-temporal extrapolation that include sediment exposure is needed.

In both experimental and model studies, it is important to define the ecotoxicological relevant concentration (ERC), which is the exposure concentration that gives an appropriate correlation to ecotoxicological effects.[14,24,25] Since sediment is often heterogeneous both in horizontal and vertical direction and since the biologically relevant sediment layer is species specific, an additional question is where in the sediment matrix the ERC should be measured.

In summary, four major issues can be identified that are essential for the development of cost-effective and ecologically relevant sediment toxicity tests and methodologies for regulatory sediment risk assessment frameworks. First, current sediment toxicity test methods are limited to a small number of benthic species. It is not clear whether the current benthic standard test species are representative of the broader range of benthic species potentially at risk. This includes the question whether sediment toxicity data for freshwater, estuarine and marine species can be combined and whether the standard tests results are relevant for effects at the community and ecosystem level. Second, current knowledge about toxicant- and species-specific exposure mechanisms in sediment toxicity tests is fragmentary and needs to be expanded in order to obtain a unifying and overarching conceptual basis. Third, population models for prospective ERA of sediment-bound chemicals are hardly developed for typical benthic species. Fourth, an improvement is required of the conceptual risk assessment framework. This framework should be based on clearly defined specific protection goals and unify the different types of test results in a transparent tiered risk assessment procedure for sediment organisms and processes.

Objectives

The overall aim of this thesis was to support the development of whole sediment toxicity tests and the prospective risk assessment of sediment-bound chemicals. This included providing recommendations for improved test methods for macrophytes, invertebrates and microorganisms, across different taxonomic groups and levels of biological organization in freshwater, estuarine and marine ecosystems and increase mechanistic understanding to assess potential effects of organic chemicals in sediments on species and populations.

The main objective resulted in the following specific research objectives:

1. To critically review the state of science with regard to protocolized sediment toxicity testing of single organic compounds in the context of prospective ERA and to provide recommendations for improved test methods.

2. To assess the relative importance and characteristic time scales of exposure pathways and the differences in bioaccumulation for a range of sediment-rooted submerged aquatic macrophytes and freshwater and marine benthic invertebrates with different taxonomy and traits.
3. To model processes and assess parameters that describe bioaccumulation in sediment-rooted macrophytes and benthic invertebrates.
4. To assess the development of bacterial communities and environmentally important microbial functions by analysing microbial gene pools, during pre-equilibration and exposure stages of a whole-sediment test using artificial sediment.
5. To assess the importance of the sediment exposure pathway for population dynamics and recovery of a typical benthic invertebrate in response to pulsed exposure.
6. To providing guidance for establishing a prospective ERA framework for organic chemicals in sediments of freshwater, estuarine and marine ecosystems by integrating the foregoing.

Outline

This thesis starts with a critical review of the state of the art with respect to protocolized sediment toxicity testing of single organic compounds in the context of prospective ERA (**Chapter 2**). This includes discussing the knowledge gaps, providing recommendations for optimum sediment toxicity test designs for microorganisms, macrophytes, benthic invertebrates and benthic communities and identifying new research priorities. Although the focus is on ecosystems in the temperate zone, a comprehensive view of other climate zones is also given. Finally, a first outlook is provided on how the recommendations could be used in the framework of prospective ERA in a regulatory context.

The next three chapters (**3**, **4** and **5**) discuss bioaccumulation and exposure pathways in sediment-rooted macrophytes and marine and freshwater benthic invertebrates. **Chapter 3** assesses the relative importance and characteristic time scales of uptake, translocation and elimination pathways of organic chemicals in sediment-rooted, submerged aquatic macrophytes, in order to assist the development of whole sediment toxicity tests. Parameters that describe bioaccumulation in macrophytes were assessed with a multi-compartment sediment bioaccumulation model that describes the chemical flows in the test systems. **Chapter 4** and **5** assess the differences in bioaccumulation for marine (**Chapter 4**) and freshwater (**Chapter 5**) benthic invertebrates with different traits and processes that drive these differences were modelled. The use of a novel approach to whole-sediment testing of benthic invertebrates was explored. Effect of aging and composition of artificial sediment on the bioavailability of the chemicals was investigated.

Chapter 6 takes a closer look at the development of bacterial communities and assesses potential changes in microbial functions (i.e. in N and S cycling), using functional gene copy numbers as proxies, during the pre-equilibration and exposure stages of the whole-

sediment test. Moreover, potential implications are provided for sediment bioaccumulation and toxicity testing (**Chapter 4**).

Chapter 7 moves from experimental to model approaches and makes a link between the different levels of biological organization. An individual-based population model is presented that couples chemical fate in the sediment, toxicokinetics and toxicodynamics of the chemical within individuals and propagates individual-level effects to the population level. The sediment compartment and particle ingestion were explicitly incorporated in the model. The model was used to assess the importance of chemical uptake routes on the impact and recovery rates of a *Chironomus riparius* population after pulsed exposure to chlorpyrifos.

Chapter 8 provides guidance to establish a prospective ERA framework for organic chemicals in sediments of freshwater, estuarine, and marine ecosystems. This chapter has a focus on European regulations and underlying data requirements for sediment ERA. A synthesis of existing approaches and new scientific insights and data is provided that shows how a rational and cost-effective prospective assessment can be performed. Our analysis starts with defining specific protection goals using the ecosystem services concept, which in turn is based on the ecological role and functions provided by benthic organisms. Then, trigger values for sediment testing and data requirements between current European risk assessment frameworks are presented and discussed. Current procedures for exposure and effect assessment including the use of models are presented and recommendations are given. Finally, several case studies are provided as 'proof of concept' and to illustrate the general features of the framework.

In the last **chapter (9)** a synthesis and general discussion is provided, which summarizes all important findings of this thesis and puts them into a broader perspective. Recommendations for further research that supports the development of prospective sediment ERA are provided.

Chapter 2

Sediment toxicity testing of organic chemicals in the context of prospective risk assessment: A review

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Abstract

Sediment toxicity tests play an important role in prospective risk assessment for organic chemicals. This chapter describes sediment toxicity tests for microorganisms, macrophytes, benthic invertebrates and benthic communities. Current approaches in sediment toxicity testing are fragmentary and diverse. This hampers the translation of single species test results between freshwater, estuarine and marine ecosystems and to the population and community levels. A more representative selection of species and endpoints as well as a unification of dose metrics and exposure assessment methodologies across groups of test species constitutes a first step towards a balanced strategy for sediment toxicity testing of single organic compounds in the context of prospective risk assessment.

2.1 Introduction

2.1.1 History of sediment toxicity testing

Chemical contamination of aquatic sediments is a worldwide issue and may lead to toxic effects in aquatic organisms.[4-6] Sediment is a complex heterogeneous matrix, in which biota may be exposed to sediment-associated contaminants by a number of routes.[26] Historically, toxicity testing mainly used aquatic animals, whereas aquatic plants were used only occasionally.[18,27] It has been recognised, however, that by testing animals in the aquatic phase, the role of sediment as an exposure route is neglected and these tests are not sufficient to assess environmental hazards to benthic invertebrates, plants and microorganisms.[17,18] Consequently, there is an urgent need to evaluate the role of toxicity tests with benthic species in sediment risk assessment procedures including toxicity tests with macrophytes[28] and microorganisms.

Early sediment toxicity testing methods and regulatory instruments were developed in North America,[4] due to dredging concerns and the recognition of widespread contamination of sediments.[29] The development of whole-sediment tests with sediment-related test species has gone through many changes (Figure 1). Originally, aquatic species (e.g. *Daphnia* sp.) were tested in the aqueous phase. These species, which predominantly dwell in the water column, cannot be used to test the toxicity of the solid phase directly, which is why they have been used as a surrogate measure of the toxicity to benthic species by testing them in pore water and elutriate. Pore water contains the bioavailable fraction and therefore is important for exposure to infaunal species.[30,31] Elutriate tests provide information on the leaching capacity of sediment-associated contaminants[26] and were used to mimic the open water disposal of dredged material,[32] thus representing the potential adverse effects to aquatic organisms due to sediment disturbance.[33,34] Nevertheless, simulation of in situ exposure of organisms to contaminated sediments is most realistic when whole-sediment samples are used.[35,36] Whole-sediment tests allow different exposure routes (e.g. via pore water or ingestion of particles)[30] and can be conducted under more realistic sediment physicochemical conditions.[37] Hence, sediment was introduced as an extra compartment. The existence of multiple exposure routes, however, increases the complexity and unpredictability of exposure, which may differ for different chemicals tested, sediment types and species with different living and feeding strategies.

After the early phases of sediment toxicity testing, benthic organisms were introduced in pore water, elutriate or sediment tests with and without an overlying water phase. Macrophytes and soil species (e.g. earthworms) were mainly tested in sediment without overlying water.[38] The first standard protocols for whole sediment tests with benthic invertebrates were developed in the 1990s.[19] So far, however, no standard protocols are available for sediment-rooted macrophytes and sediment-related microorganisms.[39-41] This raises the main question addressed in this chapter: which test species and test methodologies should be recommended to fill this gap? Compared to freshwater sediment tests, marine and estuarine tests have received much less attention.[19,37] Furthermore, sediment tests have

been developed mainly in North America and Europe, indicating a need to develop test methods that are suitable for subtropical, tropical and Australasian organisms.[38] For aquatic microorganisms, the focus today is on how they degrade organic contaminants rather than on how natural microbial populations in water and sediment could be impacted.[40]

Despite the level of sophistication that single-species whole-sediment laboratory tests may have reached, they cannot capture all processes at the population and/or community level. To some extent, community level tests (micro- and mesocosm experiments) have been developed to increase ecological realism.[22] Still, they cannot fully account for the natural complexity of ecosystems. Micro- and mesocosm experiments typically lack the presence of top predators and realistic recolonisation by certain species, for instance semivoltine or univoltine species that lack insensitive life-stages (e.g. eggs) and/or well-developed dispersal abilities (e.g. aerial stages).[23]

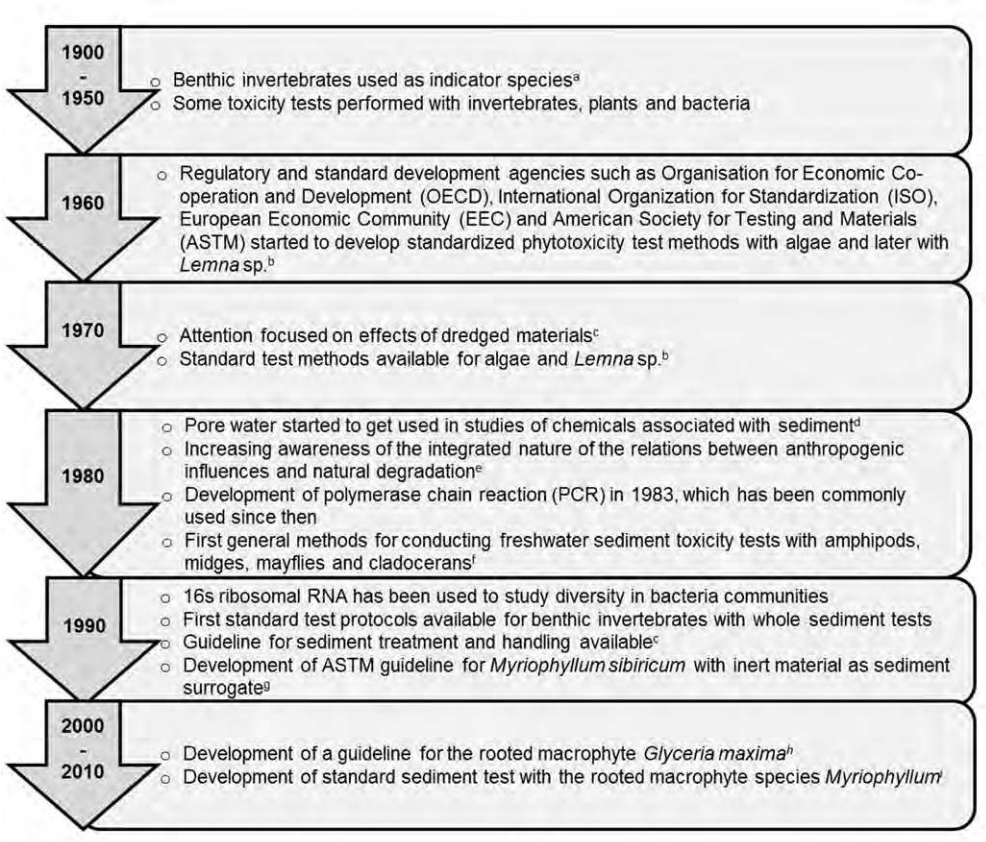

Figure 2. Timeline of the development of sediment toxicity testing with microorganisms, animals and plants. [a,42] [b,27] [c,19] [d,30] [e,39] [f,43] [g,44] [h,45] [i,28]

2.1.2 Regulatory frameworks

Contaminated sediment testing has received most attention within the framework of retrospective risk assessment (RRA). RRA is defined by the Environmental

Protection Agency (EPA) as an evaluation of the causal linkages between observed ecological effects and a stressor in the environment. In, RRA, sediment toxicity tests are used to identify the cause of adverse effects of a stressor already present in the environment and has been used to screen contaminated field sites and rank contaminated sediments, and plan and monitor remedial actions.[15,38] Less effort has been invested in the development of sediment toxicity tests in the framework of criteria setting and prospective risk assessment (PRA) in the context of market authorisation of existing and new chemicals.[19] PRA is defined by the EPA as an evaluation of the future risks of a stressor(s) not yet released into the environment or of future conditions resulting from an existing stressor(s). Prospective risk assessment schemes are mandatory in many industrialised countries for a large number of potentially toxic substances used commercially. This has resulted in a number of regulatory instruments such as the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Control Act (FIFRA) in the United States, the Canadian Environmental Protection Act (CEPA) and the Pest Control Products Act (PCP Act) in Canada, Australian Pesticides and Veterinary Medicines Authority (APVMA) in Australia and Regulation EC No 1907/2006, commonly known as REACH (Registration, Evaluation, Authorisation and Restriction of Chemical Substances) and Regulation EC/1107/2009 (plant protection products) and Directive 98/8/EC (biocides) in the European Union. In all of these laws and regulations, lower-tier effect assessment procedures should be based on protocol tests, but standard protocols are not widely available for sediment toxicity testing. Ideally, such standard protocols would be used in the context of a risk assessment scheme that unifies exposure metrics, enables read-across between freshwater, estuarine and marine environments, as well as read-across between different species and trophic levels, and accounts for interactions at the community level.

2.1.3 Aim of the review

The present chapter critically reviews the state of science with regard to protocol sediment toxicity testing of single organic compounds in the context of PRA. This includes discussing the aforementioned knowledge gaps providing recommendations for optimum sediment toxicity test designs for microorganisms, macrophytes, benthic invertebrates, and benthic communities, and identifying new research priorities. Although our focus is on freshwater, estuarine and marine systems in the temperate zone, we also offer a comprehensive view of other climate zones. Finally, a first outlook is provided on how the recommendations could be used in the framework of PRA in a regulatory context. The fact that this review focuses on organic chemicals implies that metal testing is not covered. Moreover, literature on assessment of effects in the field or on testing with natural or field contaminated sediments is considered only if relevant for chemical test development in the context of PRA.

2.2 Prospective sediment toxicity testing

2.2.1 The tiered approach in prospective risk assessment

Tiered approaches often form the basis of environmental effect assessment schemes that support prospective effect assessments. In this context, a tier is defined as a complete effect assessment resulting in an appropriate assessment endpoint, e.g. the predicted no effect concentration (PNEC). The concept of tiered approaches involves starting with a simple conservative assessment and only doing additional, more complex work when necessary for refinement of the risk assessment (Figure 2). Within a tiered effect assessment scheme all tiers aim to assess the same well-defined specific protection goal, but going from lower to higher tiers the problem is addressed with higher accuracy and precision. Consequently, lower tiers are more conservative than higher tiers.[14-16] The first tier of the effect assessment usually starts with toxicity data from standard tests and assessment factors (AFs) that are prescribed by the relevant legislation. The next tier usually is based on the combination of laboratory toxicity data from standard and additional test species. The highest effect tiers may comprise model ecosystem experiments and ecological models.

A logical consequence of the principles of the tiered approach is that higher tiers can be used to calibrate the lower tiers. In the prospective effect assessment for toxic chemicals in sediments the PNECs derived from appropriate micro-/mesocosm tests may be the most appropriate tier to calibrate the other effect assessment approaches (Figure 2). Note that in the prospective risk assessment, the toxic chemical may not yet be placed on the market so that effect assessments based on field monitoring programs are not an option as a reference. Furthermore, the advantage of microcosm-/mesocosm studies over field monitoring studies is that due to increased control over confounding factors, causality between exposure to a sediment-bound contaminant and effects is easier to demonstrate. In addition, micro-/mesocosms with artificially contaminated sediments allow to study different contaminant levels, replication and real controls (contaminant not present), which normally is not possible in a field study. It is, however, important to note that the biological and environmental conditions in a specific micro-/mesocosm test represent only one of the many possible conditions for sediment communities. This variability should be accounted for in the effect assessment, e.g. by applying an appropriate AF for spatiotemporal extrapolation of the concentration-response relationships observed in micro-/mesocosm tests. The height of this AF may be based on the observed variability in threshold concentrations for effects on sediment organisms derived from different micro-/mesocosm tests and of which the sediment was polluted with the same chemical. Whether in these tests, multiple stressors should be investigated to derive an appropriate AF depends on the specific protection goals defined by risk managers.

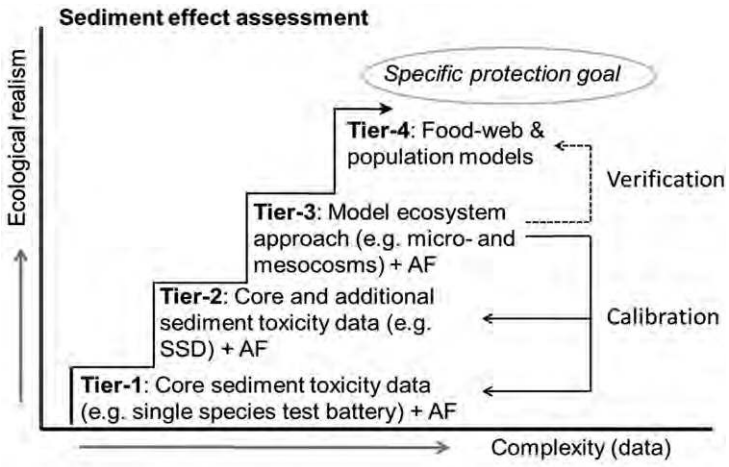

Figure 2. Schematic overview of a tiered approach as used in prospective risk assessment. In each tier an assessment factor (AF) may be necessary to derive a predicted no effect concentration (PNEC). The higher tiers could be used to calibrate the lower tiers (adapted from Brock et al.[46]).

2.2.2 General guidelines from a regulatory perspective

For an optimal toxicity test, both in the lower as higher tiers, many factors need to be considered (Figure 3). This section reviews the recommendations on these factors described in the literature. The ideal sediment toxicity test provides accurate and reproducible results. This requires standardised tests with well-defined endpoints that are linked to the related protection goals. Hence, test guidelines produced by international (e.g. OECD, ISO) and national (e.g. US EPA, ASTM) bodies are highly appreciated. These test guidelines are preferably ring tested. In a ring test, the performance of a method is evaluated across different laboratories and countries. Such a ring test is required, e.g. by the OECD, to approve the test as a guideline. Another regulatory requirement is that the standard sediment species to be used should be easy to obtain or culture, should be ecologically and ecotoxicologically relevant and should represent specific trophic levels or taxonomic groups that allow extrapolation to the wider array of sediment organisms occurring in the field. Battery testing, using species that differ in biological traits and taxonomy should be used to get a more complete view of a compound's toxicity.[19,47-50] A read across can be used, with the data from the test battery, as a method to fill data gaps for a substance or species by extrapolating data from one substance or species to another substance (usually with a similar toxic mode of action) or species (that are usually taxonomically related or have similar traits with respect to sensitivity). A fundamental assumption in every sediment toxicity test conducted for prospective purposes is that an exposure-response curve can be derived. In addition, sediment toxicity tests should be designed in such a way that the measurement endpoints can be evaluated with sufficient statistical power. Ideally, the statistical power of the test should be known.

The duration of the test should be long enough to allow the relevant effect to be fully expressed. Ideally, the incipient should be reached or it should be possible to extrapolate responses observed in time by means of an appropriate assessment factor or model (e.g.[16]). For sediment organisms and sediment-associated chemicals, the relevant exposure regime is usually chronic, which calls for chronic testing. Chronic toxicity tests with sediment-dwelling organisms focus not only on lethal endpoints but also on sub-lethal endpoints (e.g. related to reproduction and growth). Endpoints must be as sensitive and ecologically relevant as possible to allow the effects to be extrapolated from the individual level to the population level. A chronic toxicity test is generally defined as a study in which the species is exposed to the toxicant for at least one full life- cycle, or the species is exposed to the toxicant during one or more critical and sensitive life stages. Assessing potential effects of endocrine-disruptive contaminants in the sediment may require multi-generation tests. Consequently, what is considered chronic or acute depends on the species and endpoint considered.[51,52]

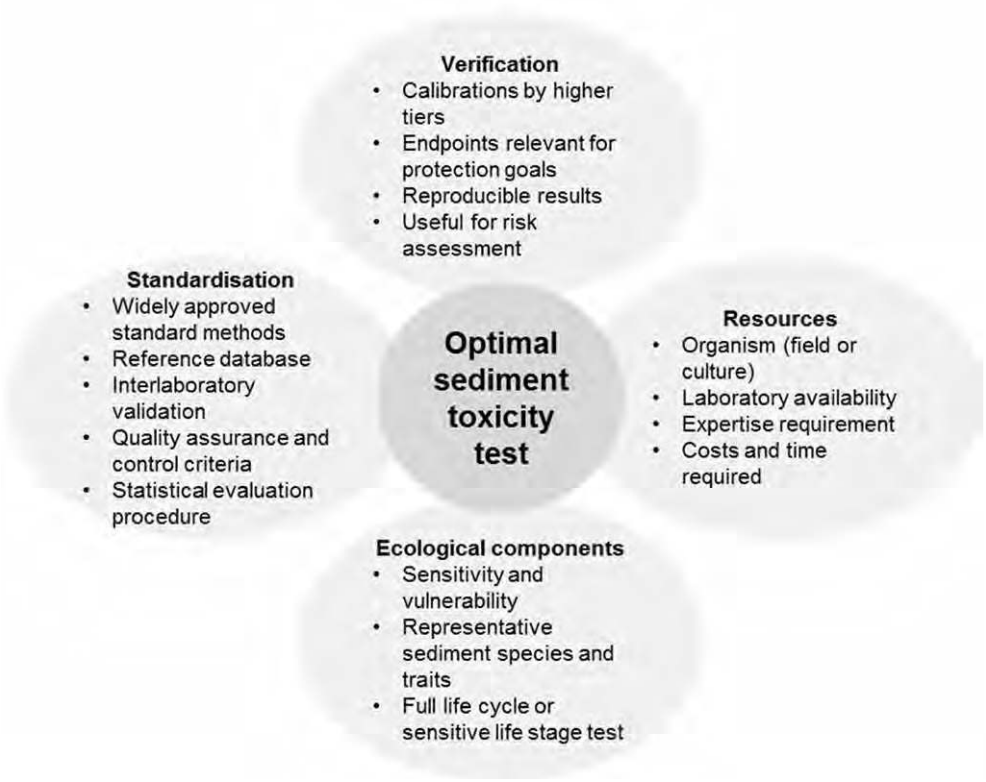

Figure 3. Factors to be considered in designing an optimal sediment toxicity test (modified from Burton and Scott[19] and Chapman[47]).

In the ideal case, the time-to-onset of the effect and preferably the maximum effect should be recorded, however this may be difficult in practice. Ideally, the effect estimates derived from the toxicity test avoid NOECs[53,54] and include EC_x values (e.g. EC_{10}; EC_{50}). Since external exposure is only a surrogate for internal dose, the exposure concentration in the course of

the laboratory test should be well controlled and characterised, either by measurements or by exposure modelling (more details in the next section). This allows maximum flexibility in selecting the best dose metric (the ecotoxicologically relevant concentration, i.e. the C in EC_x), such as the mean or time-weighted average bioavailable concentration during the toxicity test.[14,55] As the effects observed may be modified by intrinsic factors, the history, origin and life-history stage of the test species/individuals should be appropriately described. As exposure in the lab test may also be modified by extrinsic factors, it is important to appropriately describe sediment properties (e.g. organic carbon content, clay content, pH, cation-exchange capacity, grain size), ambient test conditions (e.g. temperature, salinity, light conditions) and exposure duration (including changes in exposure concentrations during the test).

2.2.3 Sediment preparation and exposure

This section briefly reviews the key mechanisms determining the exposure of organisms to sediment-bound organic chemicals in a sediment toxicity test. Subsequently, we provide recommendations for exposure assessment in such a test (overview is given in Table 1). This has substantial links and overlap with recommendations for bioaccumulation testing, on which a review was recently published.[56]

Exposure in a sediment accumulation or toxicity test is best understood using a mass balance approach where the time-course of the concentration in the organism is the net result of chemical uptake and depuration fluxes between the organism and its environment.[57-61] Uptake may take place through fluxes from pore water, overlying water and particle ingestion.[59,62] Transport to water takes place through desorption from the bulk sediment. If uptake through particle ingestion takes place, particle or diet composition is important. Depuration may include passive elimination, defecation, transformation and exudation. Organism concentration may also be reduced by growth dilution. Uptake is a complex time-dependent process, as the relative importance of the individual processes differs among chemicals and organisms, and vary with environmental and life-stage changes over time.[59]

It is impossible to obtain accurate dose-response relationships in the kinetic phase of uptake, or if exposure varies due to non-equilibrium between sediment and water. Test results may also be obscured by mixture toxicity or other stress responses during exposure. Consequently, prospective sediment toxicity tests should be designed to a) sufficiently approach steady state in exposure, b) be in a state of sediment-water sorption (pseudo-) equilibrium, and c) avoid mixture toxicity, unless testing a mixture is required for other reasons. Finally, d), actual exposure should be monitored throughout the test. Subsequently, we describe how this can be achieved at the bench.

a) Steady state can be achieved using prolonged exposure times, which is also the concept of chronic testing. Existing guidelines for invertebrates usually prescribe exposure periods of 28 days, which should suffice to achieve >80% of steady state for hydrophobic organic chemicals (EPA/OECD).[63-66] Ionised chemicals can be assumed to reach steady state earlier, because their adsorption to the sediment surface is generally faster than retarded intraparticle diffusion driving hydrophobic organic chemicals sorption kinetics. Although

some scattered information is available on the uptake kinetics of aquatic macrophytes in water-only test systems without bed-sediment,[67] guidelines are not yet available for this functional group.

b) The requirement of sorption equilibrium relates to the bioavailable fraction only, that is, the pore water concentration and/or the concentration of fast desorbing compounds from sediment.[68-70] These concentrations will remain more or less constant during a 28 day test, once the first (fast) stage of adsorption of the (spiked) test compound has passed and turned into a much slower stage of further adsorption. This second stage should be so slow that its effect on exposure is expected not to occur during the 28 days of the actual test, or at least to stay below a predefined difference between the start and end of exposure. In practice, this can be achieved by pre-equilibrating the sediment for at least three to four times the adsorption half-life.[71] Based on known kinetic data for hydrophobic organic chemicals, a pre-equilibration time of up to 28 days in suspension is recommended, followed by two weeks of incubation in bed sediment.[50,72] However, this time may need to be shorter for rapidly degradable compounds. Furthermore, the biomass should not exhaust the concentration of rapidly desorbing compounds from the sediment in the test.[69] This can be roughly achieved by keeping the total lipid mass below 5% of the amorphous sediment organic matter. Pre-equilibration in suspension also causes the pore water and overlying water to have identical electrolyte compositions at the start of the test.

c) The problem of multiple causation of effect (i.e. mixture toxicity) should be avoided by using a standardised water composition and standardised sediments, spiked with the (single) chemical of interest. Toxic macro-constituents (ammonium, hydrogen sulphide) should be avoided. Natural sediments would be less suitable because effects of unknown background chemicals or differences due to food quality should be ruled out first.[73,74] This is why current protocols generally recommend artificial, formulated sediments for testing (e.g.[64,75]). Guidelines for the preparation of freshwater sediment and the provision of food throughout the test have been provided by the OECD,[76-78] however, similar guidelines for artificial marine sediments are not yet available. The OECD suggests that food can best be mixed in with the sediment and co-equilibrated with the test chemical prior to exposure[76-78], an approach also applied in recent method development studies (e.g.[72]). The OECD guidelines, however, do not yet recommend including condensed carbon[79,80] in the standardised sediment, although such condensed carbon (e.g. black carbon, 'BC') has been shown to be a sediment component with crucial effects on the bioavailability of organic compounds.[59,79-82] Two types of effects of BC have been suggested; a reduction of exposure due to strong sorption of BC[80] and a reduction of exposure due to a lower absorption efficiency of chemicals bound to ingested BC particles.[59,83] The question whether sediment toxicity tests should include a standardised, non-toxic BC phase still needs to be addressed. Improvements with respect to other carbon phases also need to be considered. The *Sphagnum* moss particles generally recommended might not adequately represent the organic matter found in field sediment, whereas dissolved organic carbon is often poorly taken into account. In general, the quality of sediment toxicity testing would be improved if a sediment standard would be developed that best

represents natural sediment. This could be either an artificial sediment prepared in the laboratory from standardised components, or a non-contaminated natural sediment which is made available to all users as a certified reference material. Different sediments may be developed to represent different habitats, like high or low organic content or freshwater versus marine sediment.

d) There are three categories of methods to assess the exposure of hydrophobic organic chemicals. The first method is to estimate exposure from chemical concentration in the bulk sediment and to calculate the available fraction based on sediment parameters, like organic carbon content. This approach uses equilibrium partitioning theory (EPT) and is considered inaccurate due to the fact that state of equilibrium and magnitude of the equilibrium partition coefficient are unknown or uncertain.[80] The second category measures the freely dissolved concentration in the pore water or overlying water using direct solvent extraction, or passive samplers in the case of very low aqueous concentrations.[72,79,82,84,85] Frequently used samplers are POM-SPE (PolyOxyMethylene Solid Phase Extraction)[82] and SPME (Solid Phase Micro Extraction).[84] The samplers are often equilibrated with the water phase in a suspension of the sediment.[79,82] In the framework of a toxicity test with bed sediment, this would mean that exposure conditions could be substantially altered. Alternatively, samplers can be inserted into the sediment.[72,85] This may require equilibration times of days to weeks. Consequently, the use of sediment-inserted passive samplers in 28-day sediment tests is not straightforward. The third category uses mild sediment extraction to measure the concentration in the sediment that is available for uptake, the so-called fast desorbing concentration.[57,69,70] These mild extractions with XAD, Tenax, or cyclodextrin are also used in a suspension of the sediment. Fast desorbing concentrations, however, are not assumed to change when the sediment is taken into suspension. Consequently, exposure may best be assessed by a stirred passive sampler in the overlying water layer, close to the sediment water interface, and by passive samplers inserted into the sediment, which are analysed at regular time intervals. This may be complemented by mild extractions of sediment sampled at 0 and 28 days. To accurately determine fast desorbing concentrations, these mild extractions should be based on at least four time points.

Table 1. General recommendations for standard prospective sediment toxicity testing under laboratory conditions.

Recommended principles for prospective sediment testing under laboratory conditions
• Test single chemicals.
• Use artificial sediment and artificial test water, matching habitat of test organism (salinity). Consider including a 'black carbon' surrogate.
• Mix sediment, food and spiked test compound in suspension in test water.
• Pre-equilibrate 3-4 times the adsorption half-life.
• Allow two weeks for settling and incubation prior to exposure.
• Keep biomass <5% of mass of sediment organic matter plus food.
• Use static exposure with high water-to-solids ratio and minimum periodic water renewal.
• Monitor exposure at start and end of test using passive sampling and/or mild extractions.
• Monitor oxygen, pH, ammonium, sulphide (redox electrodes).

2.2.4 Benthic invertebrates

This section provides an overview of current approaches for benthic invertebrate tests. It discusses which species are used most often, the selection of a set of recommended species, and recommendations for preferred endpoints, origin and density of test animals, feeding during the test and test apparatus.

Benthic invertebrate species are often highly abundant in ecosystems and differ in morphological, physiological, behavioural and ecological characteristics (i.e. traits). These traits influence the uptake potential, metabolic capacity, exposure routes and bioaccumulation, and thus the sensitivity of invertebrate species to contaminants.[86] Moreover, benthic invertebrates provide important ecosystem functions,[87,88] which underlines the importance of protecting the biodiversity and functionality of benthic communities. As the sensitivity of species is determined by the biological and ecological traits of taxa, a test battery should be developed that takes into account the trait range within a community.[49]

Many retrospective tests are available in which contaminated field sediments are tested with single species in the laboratory or *in situ*, and a large variety of prospective tests have also been described. Prospective tests are generally conducted with freshwater or marine species, leaving true estuarine species underrepresented.[37] Tests mainly focus on single species and short-term effects, with exposures of 4-10 days,[19] which seems insufficient to detect effects at the population level[89,90] (and references therein), and to reach a steady state in exposure. Tests regarding long-term effects, full life-cycles, multiple generations or their implications at population level are less well developed.[89] Full life-cycle and multi-generation tests are more useful for risk assessment and setting quality standards for sediment-dwelling organisms, since they include all sensitive life stages of an organism. However, these tests are time-consuming and expensive.[52,90] Various short- and long-term standard methods have been validated using ring tests and are internationally accepted (Table 2). Standard methods may vary in terms of test conditions, such as water renewal versus static condition, exposure time, amount of food and the use of sediment and endpoints (Table S1).

A survey of currently available test species for freshwater, estuarine and marine sediments is presented as supplemental information (Table S1). Based on the available information, we have selected species by following the guidelines presented in the above section on general guidelines from a regulatory perspective. Criteria were a) presence in freshwater, estuarine and/or marine environment; b) diversity of feeding modes; c) direct contact with sediment; d) global distribution; and e) availability of standard methods. The selection (Table 3) is intended as a proposed test battery to compare and read across sensitivities of freshwater, estuarine and marine species for chemicals in prospective testing in European countries. Chronic test protocols are available for most of the selected test species, either as standard protocol (Table 2) or in the scientific literature (see Table S1). The selection includes where possible (internationally) standardised tests and involves representatives of three taxonomic groups of freshwater and estuarine / marine test species with similar feeding modes, behaviour and exposure pathways to enable a read across of results and sensitivity to chemicals from freshwater to estuarine and marine environments.

Table 2. Overview of test species used in internationally accepted sediment toxicity tests with benthic invertebrates (ASTM, EPA, ISO and OECD).

Taxonomic group	Guideline	Species	F/E/M[a]	Test type	Endpoints[b]	Ref
Insecta (ephemeroptera)	ASTM E1706	*Hexagenia spp.*	F	Short term (10d)	S, G	91
Insects (diptera)	OECD 218	*Chironomus riparius*	F	Long term (28 d)	E	76
		Chironomus dilutus	F	Long term (65 d)	E	76
		Chironomus yoshimatsui	F	Long term (28 d)	E	76
	OECD 233	*Chironomus riparius*	F	Life-cycle (44 d)	E, TE, SR, No ER, F	78
		Chironomus dilutus	F	Life-cycle (100d)	E, TE, SR, No ER, F	78
		Chironomus yoshimatsui	F	Life-cycle (44 d)	E, TE, SR, No ER, F	78
	EPA 2000	*Chironomus dilutus*	F	Short term (10d)	S, G	64
		Chironomus dilutus	F	Life-cycle (50-56 d)	S, W, E, No E, HS	64
	ASTM E1706	*Chironomus dilutus*	F	Short term (10d)	S, G	91
		Chironomus riparius	F	Short term (10d)	S, G	91
		Chironomus dilutus	F	Life-cycle	S, G, R, E	91
Annelida (oligochaeta)	OECD 225	*Lumbriculus variegatus*	F	Long term (28 d)	S, R, W	77
	EPA 2000	*Lumbriculus variegatus*	F	Bioaccumulation (28d)	B	64
	ASTM E1688	*Lumbriculus variegatus*	F	Bioaccumulation (28d)	B	66
	ASTM E1706	*Tubifex tubifex*	F	Short term (10d)	S, G	91
	ISO 10872:2010	*Caenorhabditis elegans*	F	Short term (4d)	F, G, R	92
Annelida (polychaeta)	ASTM E1611	*Neanthes arenaceodentata*	E/M	Short term (10d)	S	93
		Neanthes arenaceodentata	E/M	Long term (20-28d)	S	93
		Nereis (Neanthes) virens	E/M	Short term (10d)	S	93
Crustacean (amphipoda)	ASTM E1706	*Diporeia spp.*	F	Short term (10d)	S, G	91
	EPA 2000	*Hyalella azteca*	F/E	Short term (10d)	S, G	64
		Hyalella azteca	F/E	Long term (42d)	S, G, R, RFM	64
	ASTM E1706	*Hyalella azteca*	F/E	Short term (10d)	S, G	91
		Hyalella azteca	F/E	Long term (42d)	S, G, R	91
	EPA 1996	*Eohaustorius estuarius*	E	Short term (10-28d)	S, Reb	94
	ASTM E1367	*Eohaustorius estuarius*	E	Short term (10d)	S, Reb	95
	EPA 1996	*Leptocheirus plumulosus*	E	Short term (10-28d)	S, Reb	94
	EPA 2001	*Leptocheirus plumulosus*	E	Long term (28d)	S, G, R	94
	ASTM E1367	*Leptocheirus plumulosus*	E	Short term (10d)	S	95
		Leptocheirus plumulosus	E	Long term (28d)	S, G, R	95
	EPA 1996	*Ampelisca abdita*	M	Short term (10-28d)	S	94
	ASTM E1367	*Ampelisca abdita*	M	Short term (10d)	S	95
	EPA 1996	*Rhepoxynius abronius*	M	Short term (10-28d)	S, Reb	94
	ASTM *E1367*	*Rhepoxynius abronius*	M	Short term (10d)	S, Reb	95
	ISO 16712	*Corophium volutator*	E/M	Short term (10d)	S	96

[a] F=Freshwater, E=Estuarine, M=Marine; [b] B=Bioaccumulation, E =Emergence, F=Fertility, G=Growth, HS=Hatching success, No E=No of Eggs, No ER=No of Egg Ropes, R=Reproduction, Reb=Reburial, RFM=Female/Male ratio, S=Survival, SR=Sex Ratio, TE=Time to Emergence, W=Weight or biomass

This results in a read across for crustaceans (*Hyalella azteca* [fresh] – *Corophium* sp [estuarine/marine]), annelids (*Lumbriculus variegatus* [fresh] – *Arenicola marina* [estuarine/marine]), and a preliminary suggestion for bivalves (*Pisidium* sp [fresh] – *Macoma balthica* [estuarine/marine]). The selected estuarine and marine species possess a high salinity tolerance, which implies they can be used for estuarine and marine prospective testing systems by adaptation of the salinity in the tests. Additionally for freshwater, a representative species of the taxonomic groups of insecta (*Chironomus riparius*) was selected to be able to assess the sensitivity of a predominantly fresh water taxonomic group. This was also done for an exclusively marine species with the taxonomic group of echinoderms (*Echinocardium cordatum*). By selecting both similar and specific species for a certain environment covering different taxonomic groups, we feel that a sufficient assessment of the sensitivity of benthic invertebrates to chemicals in fresh, estuarine, and marine environments can be made. Concerning the group of bivalves, standardised tests have been developed focused on the embryonic development of bivalves, bioaccumulation and for field situations using caged bivalves. However, acute and chronic standard protocols for laboratory toxicity tests are still lacking for freshwater, estuarine and marine bivalves.[97] A suitable bivalve species for estuarine and marine environments appears to be *Macoma balthica*, in view of its wide salinity tolerance, extensive distribution in the northern hemisphere and easy use in handling for instance in sediment bioaccumulation testing.[98] A suitable freshwater species may be *Pisidium sp.*, based on its comparable place in the sediment, its distribution and feeding mode. Sediment-dwelling nematodes are currently not selected as test species, however, they do show a high potential. Nematodes are widely spread in the environment. They are easy to culture, have a short generation time[99] and may tolerate a high salinity range.[100] However, single-species experiments with spiked sediments have been scarce. *Caenorhabditis elegans*, which is well known for its use in soil toxicity tests, has also been used for sediment toxicity testing.[101,102] If additionally standardized estuarine and marine tests with nematodes can be developed, this group may complement the currently selected benthic invertebrates for freshwater, estuarine, and marine environments (*L. variegatus* and *A. marina*).

Most single-species tests focus on alterations within organisms (e.g. biomarkers), their physiology, life history variables, behaviour and mortality.[38] Current chronic tests focus on survival, growth,[109-111] reproduction,[64,106,109,111] behaviour[112,113] and for *Chironomus* species, emergence and male:female ratio.[52,75,78,90] Ideally, endpoints for prospective testing focus on parameters that allow extrapolation from single species to populations and communities, such as reproduction, taking into account a full life-cycle of a species. However, such a full life-cycle often takes too long to complete to be used as a cost-effective test. Therefore, if coverage of a full life-cycle is not feasible, other sub-lethal parameters are recommended, such as emergence, changes in burrowing behaviour and growth, the latter providing more time integrated information on the conditions of an organism during exposure. Even though bioaccumulation does not give information on an effect level at the organism level, it does provide information on the bioaccumulation potential of a chemical. Suitable endpoints for the test species selected in prospective testing are given in Table 3.

Table 3. Selection of benthic invertebrate species and endpoints in freshwater, estuarine and marine habitats to compare sensitivity of species along a salinity gradient. This selection focuses mainly on temperate species. A similar selection can be made for other regions (e.g. *Chironomus yoshimatsui* for Asia). The endpoints mentioned are additional to survival.

Fresh	*Chironomus riparius* (insect)	Emergence[76]
	Hyalella azteca (crustacean)	Reproduction[64,91]
	Lumbriculus variegatus (annelid)	Reproduction,[77,103] growth,[6,77] bioaccumulation[63,66]
	Pisidium sp. (mollusc)	Reburial (to be developed based on[104,105]) bioaccumulation and feeding rate (to be developed)
Estuarine	*Corophium volutator* (crustacean)	Reproduction[106]
	Arenicola marina (annelid)	Growth and/or bioaccumulation (to be developed based on[77])
	Macoma balthica (mollusc)	Reburial (to be developed based on[104,105]) bioaccumulation and feeding rate (to be developed)
Marine	*Corophium volutator* (crustacean)	Reproduction[106]
	Arenicola marina (annelid)	Growth and/or bioaccumulation (to be developed based on[77])
	Macoma balthica (mollusc)	Reburial (to be developed based on[104,105]) bioaccumulation and feeding rate (to be developed)
	Echinocardium cordatum (Echinoderm)	Burrowing activity and/or bioaccumulation[107,108]

Test organisms are most commonly collected from clean local sites. Certain species (e.g. *Chironomus riparius, Hyallella azteca, Corophium volutator*[114] and *Echinocardium. cordatum*[107]), can be cultured in the laboratory. However, there may be differences in the sensitivity of cultured and field organisms. For instance, Schipper et al.[107] found that field urchins showed higher sensitivity than cultured urchins. Cultured organisms are more favourable for prospective testing, as the origin of the test species is known and their quality is more standardised as long as proper protocols are applied to prevent inbreeding. If no cultures exist, organisms should be collected from clean field sites. In all cases, the chemical to be tested should be analysed, prior to testing, to establish the background concentration of the specific chemical in the test organism. Another laboratory-field issue is the animal density since toxic effects can be density-dependent. Laboratory tests may overestimate effects in natural environments since they use low densities, while field populations often have high densities. This could have important consequences for risk assessment.[115] Hence, it is important to use optimum densities – depending on the organisms – for lab conditions. For practical reasons, these do not necessarily equal field conditions. An actual comparison with the field situation is more suitable for mesocosm studies and/or field experiments.

The ingestion of contaminated sediment may be an important exposure pathway especially for highly sorptive substances.[59,62,80] Long-term tests without food are possible for some species, but only with sediments having a high organic carbon content.[116] Usually, food is added either as fresh food or mixed with the sediment at least 48 h prior to spiking.[75,117] Adding food however also adds organic carbon to the system affecting bioavailability of

the chemical and hence the uptake of chemicals through sediment ingestion.[117] On the other hand, fresh food addition is more ecologically relevant for certain species but might exclude the exposure route through the sediment (due to food avoidance or preference). Food source and feeding regime may influence organic carbon, ammonium concentrations and physicochemical parameters.[50,116,117] In static systems, water quality could decrease to unacceptable levels in the course of the test, while maintaining constant exposure conditions is also difficult with a semi-static system (recommended by OECD and EPA).[50,116] As an alternative, Borgmann and Norwood[118] recommended a static test with larger water-to-sediment ratio (67:1, as compared with the normal 4:1 ratio). For practical reasons, static systems are recommended for prospective testing. However, the water-to-sediment ratio used should be as high as possible to keep the water quality at an acceptable level and reduce the need to change the water on a regular basis. Additionally, ammonium (especially unionised ammonia) needs to be measured regularly during the test to avoid toxic effects. These recommendations are summarised in Table 4.

Table 4. General recommendations for standard prospective sediment toxicity testing with benthic invertebrates under laboratory conditions.

Recommended principles for prospective sediment testing of benthic invertebrates under laboratory conditions
• Focus on full life-cycle tests and multi-generation tests or tests that cover the most sensitive life stage.
• Select species based on traits (e.g. ingesters, facultative suspension feeders).
• Source of test species: preferably cultured, if not possible from field.
• If food is needed for the test, mix it into the sediment for a period of 48 h prior to spiking.
• Mix organic carbon into the sediment simultaneously with food to a standardised percentage, prior to spiking the sediment.
• Test with sufficient densities for laboratory conditions.
• Use a static system with water-to-sediment ratio as high as possible.
• Monitor water quality.

2.2.5 Aquatic macrophytes

This section reviews the literature on testing with macrophytes and discusses current types of tests, species used, choice of medium and sediment, chemical spiking method and endpoints.

Aquatic macrophytes fulfil several critical structural and functional roles in aquatic ecosystems.[119] They are at the base of the aquatic food web, and may accumulate and translocate chemicals and enhance or decrease their bioavailability.[41,71,120] Consequently, these organisms and the ecosystem services that they provide must be protected at both local and global scale.[121] The availability of standardised methodologies to assess the environmental risks of organic chemicals to non-target freshwater plants is currently limited. Test guidelines are only available as water-only tests for algae and *Lemna* (duckweed) (e.g. guidelines from ASTM, EPA, and OECD), while the existing ASTM *Myriophyllum* protocol without sediment was never officially accepted. A new *Myriophyllum*-sediment protocol has

recently been ring-tested.[28] In risk assessment, submerged rooted macrophytes are not addressed in any standard procedure. Sediment-testing guidelines for sediment-rooted macrophytes have not been standardised (Table S2). Limited literature is available on sediment toxicity testing of rooted freshwater macrophytes[28,122] and rooted estuarine and marine macrophytes.[41,120,123] As rooted aquatic macrophytes are mostly tested over a period of 14 to 28 days (Table S2), these tests are considered long-term. Macrophytes are usually tested as vegetative shoots in their growth phase, while tests covering a full life-cycle and seed emergence tests have not been reported for aquatic macrophytes within the context of environmental risks of toxicants.

The standard freshwater test species, *Lemna*, is a free-floating, non-sediment-rooted macrophyte and therefore is not representative of sediment-rooted emergent and submerged macrophyte species, especially when chemicals partition to the sediment.[28,124,125] Where sediment exposure is a concern, Maltby et al.[28] proposed to test a sediment-rooted macrophyte species. This approach takes into account the different pathways by which rooted macrophytes take up chemicals, viz. by roots and shoots.[126,127] The considerable current knowledge about and experience gained with *Myriophyllum* sp.[44,128] and its physiological properties as a sediment-rooted and dicot species were reasons to recommend it as an additional test species.[28,129] *Elodea* sp. and *Glyceria maxima* are used for toxicity testing especially when monocot species are required[130,131] For the estuarine and marine environment, coastal wetland species (emergent species including mangrove species) or submerged macrophytes (mainly sea grass species) have been recommended[41,120,132,133] (Table S2). The estuarine species cover a broad salinity range, from low to high values. Table 5 gives an overview of recommended test species, suitable to be used in a test battery in the laboratory. No standardised methods are available for any of the rooted macrophytes, as these are only available for the floating macrophyte *Lemna sp.*. Instead, the literature was screened for available but not standardised test protocols. Selected macrophyte test species are widely distributed in the northern hemisphere. Moreover, they are representative of different sediment-rooted growth forms (submerged and emergent), are specific for different habitats (freshwater and marine) and allow comparison between freshwater, estuarine and marine habitats to determine whether sensitivity to tested chemical may differ between these habitats and vice versa. An important question is to what extent such a read-across is feasible.

For prospective risk assessment, protocols are available for testing rooted freshwater macrophyte species[44] but these tests include the water medium only. An adapted test approach based on this protocol[44] has recently been ring-tested for *Myriophyllum spicatum*. [140] As such tests might suffer from microbial and algal development, they are mostly performed as axenic tests (which is further discussed subsequently). In order to sustain macrophyte growth, the test medium in these tests includes sucrosis.[140-142] Test protocols including sediments and water medium are under development.[28] The test protocols proposed by Maltby et al.[28] are currently being ring-tested for the sediment-rooted macrophytes *Myriophyllum aquaticum* and *M. spicatum*. Protocols for estuarine and marine sediment tests have neither been standardised nor involved in a ring-testing procedure.[133]

Consequently, experimental techniques are varying considerably.[41] Sediment toxicity tests with estuarine and marine macrophyte species have rarely been conducted.[123]

Table 5. Suggested selection of macrophyte species and endpoints in freshwater, estuarine and marine habitats to compare sensitivity of species along a salinity gradient. This selection focuses mainly on temperate species. A similar selection could be made for other regions (e.g. *Zostera capricorni* or *Thalassia testudinum* as a tropical marine submerged species). The endpoints mentioned are additional to biomass based on growth.

Fresh	*Myriophyllum spicatum.*[a]	Shoot length, shoot weight (updated protocol from [28]), total fresh weight[122]
	Elodea sp.	Total length main shoot, weight[131]
	Glyceria maxima	Shoot length, shoot weight, shoot number[45]
Estuarine	*Scirpus sp.*	Growth, peroxidase activity, peroxidation products, chlorophyll[134], length, germination
	Vallisneria (sp. or *americana)*	Leaf to root ratio[135,136]
	Ruppia (sp. or *maritima)*	Rel. growth rate, oxygen production[123]
	Stuckenia pectinatus (previously *Potamogeton pectinatus)*	Weight, rhizome tips,[137] length
Marine	*Scirpus sp.*	Growth, peroxidase activity, peroxidation products, chlorophyll,[134] length, germination
	Ruppia (sp. or *maritima)*	Rel. growth rate, oxygen production[123]
	Zostera (sp. or marina)	Photosynthesis,[138,139] chlorophyll, pigments[133]

[a] Tests are under development as standard test for the OECD.

In retrospective risk assessments, standard protocols are available.[122,143,144] They include contaminated sediments, but lack the overlying water layer.[122,143-146] These methods are not directly applicable to sediment toxicity testing where a water layer is included in the test set-up.

The advantage of sediment tests is that nutrients can be mixed through the sediment, thereby limiting nutrient-availability in the water layer and therefore limiting algae growth. Non-axenic tests do include microorganisms. If this is not desired, axenic, artificial sediments may be used to overcome this problem. However, axenic tests are time-consuming. Therefore, in general, the addition of sediment obviates the need for axenic cultures[147] and offers many other advantages, such as increased macrophyte growth[131,148], decreased endpoint coefficients of variation and increased ecological realism. Artificial[28,136,149,150] as well as natural sediments[136,151,152] have been used in macrophyte toxicity tests. From Table S2 it can be concluded that the available information is scattered and applied test protocols are very different in all kind of aspects including growth media, test duration, macrophyte species, assessed endpoints and chemicals considered. Only the artificial sediments, if applied, were similar in their composition. Sediment spiking is not common practice in macrophyte toxicity tests that include sediment and an overlying water layer. It has been applied by Burešová et al.[126] (herbicide) and is currently part of the *Myriophyllum* sediment ring-test.[28]

A wide range of endpoints is used, and these do differ considerably between tests (Table 5). A combination of morphological and physiological endpoints represents macrophyte fitness better than biomass and growth only.[153] Although macrophyte length and biomass endpoints are characterised by low coefficients of variation,[131,148] macrophyte main shoot length is not a sensitive indicator in all cases, but should be replaced by total shoot length. Total shoot length also takes into account the length of the newly formed side shoots. Root endpoints (e.g. root length) on the other hand are sensitive endpoints both in water-only tests and in sediment tests, although they show high intrinsic variability.[146,153,154] The leaf-to-root surface area has been suggested as a sensitive and robust endpoint in macrophyte tests with sediment and water medium.[135] In general, growth based on biomass can be used as an indication of effects on macrophytes, which can easily be linked to the population level, where a decreased biomass might directly influence the survival potential of a macrophyte population. Appropriate endpoints combine toxicological sensitivity with low coefficients of variation and ecological relevance.[153] For sediment tests, these include belowground and aboveground macrophyte endpoints. It should be noticed that hormesis could stimulate growth in the lower concentration range and should, therefore, be taken into account in the calculation of effect concentrations.[155] An overview is given of the above-mentioned recommendations in Table 6.

Macrophytes can take up organic compounds by roots and shoots.[67] Uptake and elimination studies and sorption models with aquatic macrophytes, and *Myriophyllum* in particular, often disregard the sediment compartment.[127,156-159] However, sediment is an integral part of experiments and models, which describe accumulation of sediment-bound chemicals in aquatic food webs.[69,71,160,161]

Table 6. General recommendations for standard prospective sediment toxicity testing with sediment-rooted macrophytes under laboratory conditions.

Recommended principles for prospective sediment testing of sediment-rooted macrophytes under laboratory conditions
• Use artificial sediment. • Add nutrients to the sediment to avoid algae growth in the water. • Add growth medium to the water layer to support maximum photosynthesis. • Optimise light conditions for the different test species. • Choose experimental conditions to support exponential/steady growth in the controls. • Use field or culture stock populations, which can easily be grown from vegetative cuttings and acclimatised, in the laboratory. • Use macrophyte endpoints that combine toxicological sensitivity, low coefficients of variation, and ecological relevance. • Take account of hormesis in the evaluation of effects. • Mimic natural conditions as closely as possible for marine and estuarine species.

2.2.6 Microorganisms

This section presents an overview of current approaches to microorganism tests, including endpoints and methods for single-species tests and a wide variety of molecular methods that can be used at the community level.

Sediment microbial communities, including benthic bacteria, archaea, algae, fungi and protozoans, perform crucial ecosystem functions like nutrient cycling, primary production and decomposition[162] and form an important food source for many sediment-dwelling organisms.[163] Interactions between different microorganisms and with higher organisms range from mutually beneficial symbiosis to purely antagonistic (pathogenic) relationships, all of which contribute to shaping the ecosystem functioning at different trophic levels. Hence, microbial communities constitute a relevant endpoint in sediment quality assessment. Depending on the regulatory framework, the specific protection goal for microorganisms may concern the population, functional group or community level.[121] The majority of bacteria grows in biofilms on surfaces of submerged substrata or sediments, rather than in suspension, although it should be noted that suspended microorganisms are especially important in degrading highly soluble chemicals.[164] Biofilms are complex communities that besides bacteria, comprise algae, protozoa and fungi embedded in a matrix of extracellular polymeric substances,[165] and are consumed by deposit-feeding invertebrates.[166] Various compounds are effectively adsorbed into the matrix, resulting in increased or decreased bioavailability. However, their role in the bioaccumulation of organic contaminants has been poorly investigated,[167] and most tests focus on suspended microbial cultures. It should be noted that considerable work has been done on the evaluation of biocides on biofilms, however, focussing largely on systems relevant to the prevention of growth of microbial pathogens such as those found associated with medically relevant environments as well as drinking water distribution systems.[168,169] Furthermore, biofilms have been studied with respect to their role in the degradation of environmentally adverse pollutants.[170,171]

The uptake of chemicals from the sediment by microorganisms is more direct than that by higher organisms. Uptake is diffusion-driven and fast due to the much higher surface-to-volume ratio of microbial cells, implying that freely dissolved pore water concentrations are the most relevant dose metric for microbial testing. Some bacteria (e.g. *Bacillus cereus*) have a hydrophobic surface, which further facilitates the direct uptake of chemicals and may enhance bioavailability.[36]

Various microorganisms have the capability to accumulate, detoxify or metabolise chemicals,[40,163,172,173] and are therefore used for bioremediation in polluted soils and sediments. Hence, many studies have focused on microbial degradation of contaminants rather than on impact on the composition and functioning of natural microbial communities. Toxicity data in the open literature on organic contaminants involving microorganisms, however, are limited[40,174] although it should be noted that freshwater protozoans such as *Tetrahymena pyriformis* have been extensively used in toxicity testing.[175] Only a few studies have addressed the effects of chemicals on structural and/or functional responses of microbes.[40,176,177] The wide variety of size classes, morphology, reproductive strategies,

growth rates and metabolism results in a wide range of sensitivities of microorganisms to chemicals.[40] Nevertheless, if the metabolism of a bacterial cell is disturbed, this may also indicate potential toxicity to other organisms.[178] Effects on microorganisms, both negative and positive, may have direct and indirect impacts at higher trophic levels and therefore may change ecosystem functions.[40,167]

Many different methods are available to test effects of sediment-bound chemicals on microorganisms.[179] However, although some are commercially available, none of them have so far been ring-tested and described as standard tests. Ecologically relevant community assessments have been used in RRA, where characteristics of contaminated field sediment have been correlated to microbial activity.[180] In PRA, mixed communities can be much more easily exposed to spiked, artificial, sediments than single species.[17] To improve the microbial component of artificial sediments, it has been suggested[167] that a microbial extract from natural sediment could be added in the sediment preparation procedure. However, it is also possible to introduce pure cultures of microbes into spiked field or artificial sediment. Such tests are relatively cheap and easy to perform, use species that can be easily cultivated and are useful for rapid screening. As they represent principal functions, they relate to an integral part of the ecosystem and are more sensitive than animal and plant tests for a number of compounds.[17,36,167,181] However, very few studies have investigated the microbial communities of artificial sediments and compared these with natural sediments. Hence, further knowledge is needed to assess how microbes govern the fate of test compounds in standardised tests and ultimately affect toxicity test results.[167]

The available microbial tests can be divided into single-species tests; community-level assessments based on functionality, biomass or processes[17] and molecular methods (Figure 4). Tests depending on single-species microbial culture fall into the following categories: population growth, substrate consumption, respiration, adenosine triphosphate (ATP) luminescence, and bioluminescence inhibition assays. Species used for bioluminescence inhibition assays include *Vibrio fischeri* (formerly *Photobacterium phosphoreum*), *Vibrio harveyi* and *Pseudomonas fluorescens*. Although bioluminescence inhibition assays were originally applied to aqueous or extracted samples, a modified solid phase assay has been developed for the analysis of soil and sediment toxicity.[182] Metabolic inhibition tests use the species *Escherichia coli* and *Pseudomonas putida*.[181] Test duration usually varies from 24 to 96 hours. [40] The solid phase bioluminescence inhibition test with *Vibrio fischeri* (marine gram-negative bacterium) is one of the most commonly used single-species tests.[17] It is an acute toxicity test with a sub-lethal endpoint. Several commercial test kits, i.e. Microtox, LUMIStox and ToxAlert are based on this strain.[183] This is the most sensitive microbial test available, is cost-effective, easy to operate[184-186] and takes 5 to 30 min. Other single-species tests are associated with higher costs (ATP luminescence) or low investment cost but high operational costs (nitrification inhibition assay).[185] An inter-laboratory precision study of the solid phase Microtox test showed that the method has acceptable precision and can be developed as a standard method.[187,188] Despite its easy operation, however, there are several pitfalls in interpreting the test results. Direct sediment

contact increases the exposure to potential toxicants. Moreover, sediment composition can affect the test response since bacteria can bind to sediment particles, which results in a reduction of the intensity of luminescence and/or a loss of bacteria by sediment extraction for the test suspension.[189-191] For example, a high proportion of silt or clay in the sediment samples is found to reduce the EC_{50} values, thereby indicating higher toxicity than expected. Moreover, it remains difficult to distinguish between inherent chemical sensitivity and mediating sediment factors. This issue could be circumvented by the use of sediment correction. Bioluminescence tests require normalisation to account for the adsorption of the bacteria to the sediment particles.[190,192] Additionally, sediment properties such as pH, sulphide content, redox potential and oxygen saturation play an important role and may interfere with toxic effects. Consequently, it has been recommended to match organisms with appropriate sediment as well as associated physico-chemical conditions.[17]

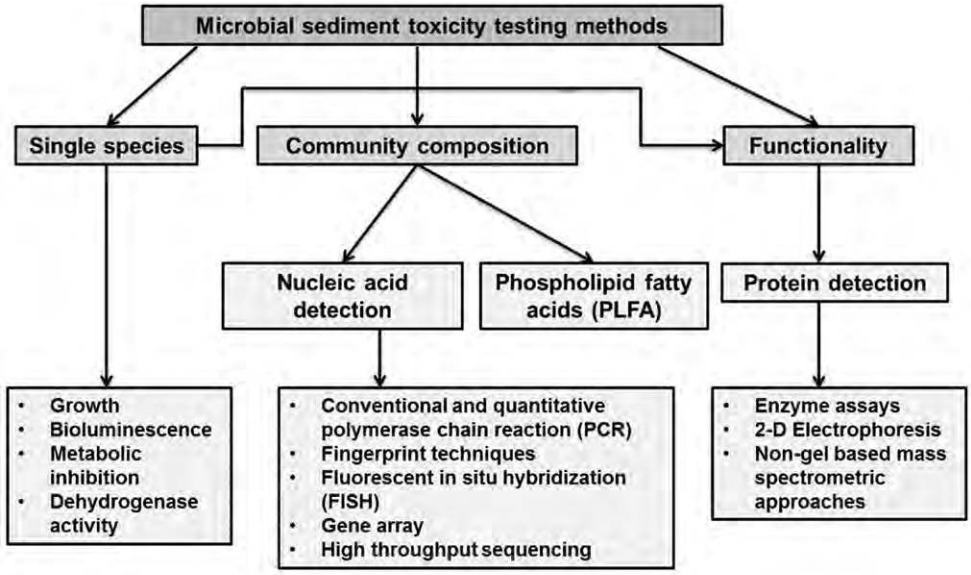

Figure 4. Overview of methods for prospective sediment toxicity testing with microorganisms.

Communities can be used to assess growth inhibition[181] and loss of functionality or processes, the latter of which can be measured either by activity tests or by means of biomolecular proxies (see subsequent discussion). However, measuring functionality alone may cause shifts in microbial composition to be overlooked because tolerant microbes could compensate for the loss of functions of the more sensitive groups (i.e. functional redundancy).[163]

The development of culture-independent molecular methods to analyse microbial communities provides new opportunities to detect pollutant-induced changes in the composition of natural communities.[193,194] To this end, it is important to realise that, to date, the overwhelming majority of microorganisms cannot be cultured as pure culture isolates by routine methodology in the laboratory, but rather can only be maintained in the context of more or less complex defined or natural microbial communities.[195,196] Molecular methods target a range of cellular

biomarkers that provide information with respect to microbial identity and function, and have been developed especially to allow for the analysis of complex mixed microbial communities. Biomarkers that are frequently used include proteins, phospholipid fatty acids (PLFA),[197] and nucleic acids. Whereas proteins can be assessed using enzyme activity assays, as well as proteomics methods such as 2-D gel electrophoresis and non-gel based mass spectrometric techniques, nucleic acids are the biomarkers of choice in most applications. Microbial identity and community composition are routinely determined by targeting ribosomal RNA (rRNA) or its encoding gene, using fluorescent in situ hybrisation (FISH), DNA oligonucleotide microarrays, conventional and quantitative polymerase chain reaction (PCR) and a number of different fingerprint techniques.[198,199] Information about metabolic potential as well as activity can be obtained by analysing functional genes, their transcripts and/or corresponding proteins, largely using the previously mentioned approaches.[196,200]

The three categories of tests show that endpoints for microorganisms are primarily in terms of functions (e.g. nitrogen fixation), processes (e.g. luminescence) or quantitative data (e.g rRNA) (Figure 4 and Table S3). However, most reported EC_{50} values relate to the endpoint of growth rate (e.g. cell counts or optical density).[40] A combination of endpoints relating to functioning (enzyme activity, functional genomics) and microbial composition (rRNA) will offer a more complete overview of the toxicity effects. Single-species tests can be used for rapid screening, whereas higher-tier testing should focus on the level of functions, processes and communities. Hence, a test battery for microorganisms should be focused on the functional diversity of a community rather than on tests with various single species. Therefore, proposed selected methods for prospective sediment toxicity testing with microorganisms on community level are 1) high throughput sequencing for community composition and 2) quantitative PCR assays targeting selected functions for specific functions. Recommended test principals are summarised in Table 7. Moreover, combining test outcomes in a species sensitivity distribution (SSD) would significantly improve the PRA.[17]

Table 7. General recommendations for standard prospective sediment toxicity testing with microorganisms under laboratory conditions.

Recommended principles for prospective sediment testing of microorganisms under laboratory conditions
• Focus on community functionality, using culture-independent proxies. • Include solid surfaces in test systems, allowing biofilm testing. • Use field communities to mimic complex interactions in situ. • Inoculate the artificial sediment with microorganisms from natural sediments. • Use proper oxic state. • Use sediments with low clay content and sediment correction for the loss of microbes, when artificial sediments are used • Use static/dynamic systems, depending on target ecosystem (e.g. lake vs. stream). • Use a combination of endpoints on microbial composition and functioning.

2.2.7 Community level tests

Micro- and mesocosm experiments are carried out to study the effects of chemicals at the population level, the recovery of affected species, and to include interactions between species and/or evaluate more realistic exposure patterns than those used in single-species laboratory tests.[22,201] Only a few micro- and mesocosm studies were found that had evaluated the effects of single, organic contaminants on sediment-associated macroinvertebrates or macrophytes in multi-species test systems (Table 8). Twelve studies were retrieved, six of which had been performed in Europe, five in North America and one in Australia (Table 8).

Although the difference between micro- or mesocosms is often based on their size (a criterion used rather loosely by different authors) both should comprise bounded systems that are constructed artificially with samples from, or portions of, natural ecosystems, or consisting of enclosed parts of natural ecosystems. Although these model ecosystems are usually characterised by reduced size and complexity when compared with natural ecosystems, they have to include an assemblage of organisms representing several trophic levels to allow realistic food-web interactions. Moreover, the micro-/mesocosms require an acclimatization period long enough to allow the establishment of a community that is recovered from the construction-stress and adapted to the conditions in the test system.[202]

Table 8. Characteristics of the micro- and mesocosm studies evaluated in this review. For further details, see Table S4.

References	Invertebrates or Macrophytes	Size (m^3)	F/E/M[a]	Geographic region	Chemical
Fletcher et al.[203]	Inv.	(25x25cm)	F	North America	Pesticide
Rand[204]	Inv.	31	F	North America	Pesticide
Brock et al.[124]	Inv.	60	F	Europe	Pesticide
Pablo and Hyne[205]	Inv.	1.05	F	Australia	Pesticide
Roessink et al.[206]	Inv./Macr.	0.84	F	Europe	Pesticide
Bouldin et al.[207]	Inv./Macr.	0.047	F	North America	Pesticide
Roessink et al.[71]	Macr.	0.847	F	Europe	PCB/PAH
Tessier et al.[208]	Macr.	0.144	F	Europe	Antifouling
Thorsson et al.[209]	Inv.	0.0025	E	Europe	PCB
Cunningham et al.[210]	Macr.	0.7	E	North America	Pesticide
Farke et al.[211]	Inv.	13	M	Europe	Oil
Frithsen et al.[212]	Inv.	13	M	North America	Oil

[a] F=Freshwater, E=Estuarine, M=Marine

Out of the 12 studies, eight had been performed in freshwater, two in a marine and two in an estuarine setting (Table 8). Regardless of system size, experimental studies show a preference for block designs involving, for instance, control, low and high exposure conditions, instead of a regression design. Eight of the 12 studies had evaluated the impact

of a pesticide on benthic communities, with the test compound actively added to the systems, while the other studies were based on oil, PCBs, or PAHs that were usually already present in the sediment. All 12 studies used natural sediment for testing. With the exception of the studies by Fletcher et al.[203] and Brock et al.[124], all studies include analytical verification of the contaminants of interest in the sediment compartment (Table S4).

The invertebrate organisms studied comprised mostly benthic invertebrates and nematode meiofauna. Test organisms were always chronically exposed to the contaminants and endpoints studied always included abundance and, in the case of PCBs and PAH, also biomass and bioaccumulation (Table 8 and Table S4). Most studies performed with macrophytes monitored the bioaccumulation of these chemicals after spiking them to the water or the sediment, and sometimes evaluated mediation of effects on invertebrates by the presence of macrophytes ([207], see also Table 8 and Table S4). If effects were studied, threshold concentrations were only expressed as concentrations in sediment in those studies examining the effects of oil addition.[211,212]

It is clear that if micro- and mesocosms are to be used more routinely in the higher-tier risk assessment of sediment-mediated exposure of chemicals, further standardisation is needed. Therefore, further guidelines need to be developed on the conduct (i.e. which standard sediment to use and in which matrix to measure the used compound), interpretation of micro-/mesocosm tests that focus on sediment effect assessment as this is not sufficiently addressed in guidance documents.[213,214] Moreover, it would be helpful to gain more experience in the use of spiked artificial sediment, to study the bioaccumulation and biomagnification of the chemicals through the food web and direct and indirect biological effects on the various biological levels of organisation (e.g.[69,71]).

2.3 Use of standardised sediment toxicity tests in risk assessment

While the previous sections reviewed the technical details of single chemical tests for single species and communities of species, this section describes how such tests (with different species and environments) could be integrated in one risk assessment framework, and which research priorities would emerge from this integration.

Depending on the protection goals in legislation, results of laboratory toxicity tests with benthic organisms may be used in a regulatory context for deriving predicted no effect concentrations and setting sediment quality standards in both retrospective and prospective effect assessments.[215-219] Currently, the protection goals for benthic organisms are defined in general terms only (e.g. no unacceptable effects). These protection goals could be made operational by using the ecosystem services concept to derive specific protection goals.[121] To date, however, this remains a research objective for benthic organisms in freshwater, estuarine and marine sediments. Note that specific protection goals may differ for different types of benthic organisms. For example, the European Food Safety Authority[220] defined specific protection goals for microorganisms at the functional group level to assess environmental risks of pesticides, whereas they were defined at the population level for

invertebrates and macrophytes. A future dialogue between stakeholders is required to define which specific protection goals should be adopted for benthic organisms, depending on the regulatory context. Whatever the outcome of this dialogue will be, a separate tiered decision scheme may be necessary for each specific protection goal that will be defined for sediment key drivers (i.e. main taxonomic groups relevant for a specific ecosystem service) in order to derive sediment quality standards. This derivation usually follows a hierarchy depending on the amount of data available (see Figure 2).

In prospective effect assessments, the basic dossier requirements may comprise chronic toxicity data for a limited set (e.g. 3 to 4) of standard sediment organisms that represent different taxonomic/trophic groups (e.g. benthic arthropod; benthic annelid; rooted macrophyte) and the application of an appropriate assessment factor. Although in chronic Tier-1 effect assessments usually an AF of 10 is applied to derive a PNEC, the height of this AF needs to be scientifically underpinned (e.g. on the basis of comparisons with SSD curves or micro-/mesocosm tests for a sufficient number of sediment contaminants). Ideally, candidate standard sediment test species, for which internationally accepted test protocols are available, should be selected as soon as possible, to harmonise the lower-tier effect assessment procedure across different laws and regulations. Note that the same benthic test species (e.g. *Lumbriculus variegatus* or *Echinocardium cordatum*), which are recommended for toxicity assessment, may be used as well to assess risks due to bioaccumulation and subsequent transfer of the chemical to higher trophic levels (e.g. to assess risk due to secondary poisoning of predators that have sediment organisms on their diet). Laboratory tests that include a full life cycle of the test species are considered most suitable, as these cover all sensitive life stages. In addition, results of full life-cycle tests are more appropriate to extrapolate to the field.[221] Examples of full life-cycle tests are chronic protocol tests with *C. riparius*, *H. azteca*, *L. variegatus* and *C. volutator* (Table 3). Often, however, the life cycle of test species takes too long to complete in order to design a cost effective full life-cycle laboratory test, e.g. for macrophytes and some macroinvertebrates. Therefore, a good alternative are tests that include the most sensitive part of the life cycle and/or the most sensitive parts or tissues (e.g. new shoots) of the sediment test species and focus on the endpoints survival and growth (e.g. tests with *E. cordatum*, *Myriophyllum sp. and Stuckenia pectinatus*). Tests exclusively focusing on activity (of the sensitive life stage) and/or functional endpoints, such as burrowing activity or feeding rate, (e.g. *L. variegatus*), photosynthesis, (e.g. macrophytes), and luminescence (e.g. *Vibrio fischeri*) may be sensitive (and useful for early-warning) but harder to extrapolate to community-level effects.

The data and recommendations presented in this review suggest that the invertebrate and macrophyte taxa presented in Table 9 are the most promising. Note, however, that harmonised test protocols are available or under development only for the set of freshwater taxa mentioned in Table 9. Consequently, an important future activity is the development of such test protocols for candidate estuarine and marine standard test species. For chemicals with a specific toxic mode of action, e.g. pesticides and biocides with an insecticidal or herbicidal mode-of-action, it is the taxonomic group rather than the place in the food chain or food web (trophic level) that determines sensitivity.[222] Therefore, an important

research question is whether specific taxonomic groups that exclusively occur in the marine environment (e.g. Echinodermata) are sufficiently covered by the traditional taxonomic groups tested (Annelida, Crustacea, Insecta). As this information is lacking, a comparative study that evaluates the relative sensitivity of different taxonomic groups of sediment dwelling organisms to a suit of chemicals that differ on mode-of-action is a research priority. Such a comparative study may trigger the development of a standard test protocol for relevant sediment organisms not yet covered by the traditional taxonomic groups tested. For example, this might theoretically be the case for marine Echinodermata and if so *E. cordatum* might be a candidate test species. Furthermore, cross-linking results from sediment toxicity tests, such as those for microorganisms, invertebrates, macrophytes and sediment micro- and mesocosm tests, requires a unification of dose metrics and exposure assessments in these tests, such as those summarised in Table 1. This involves development of artificial or standardised sediment, to better represent natural sediment. Current standardized test protocols recommend the use of artificial sediments and aim at the closest possible match with natural conditions in the field. If sediment toxicity assessment is to be as realistic as possible in terms of exposure, test designs may need to include condensed carbon phases (i.e. black carbon) as a part of artificial sediment,[80,223] particularly if the chemical becomes bioavailable when sediment particles are ingested, (i.e. increased bioavailability of the chemical in the gastrointestinal tract).[59] Omitting a condensed carbon phase such as BC from artificial sediment could lead to an overestimation of the bioavailability and risk.

Table 9. Possible suitable species for the first-tier assessment. Species were selected from the species recommended for a test battery (Tables 3, 5 and Figure 4).

		Species	Motivation
Fresh	Insecta	*Chironomus riparius* or *C. dilutus*	Specific for freshwater, OECD test
	Crustacea	*Hyalella azteca*	Comparable across environments, ASTM test
	Annelida	*Lumbriculus variegatus*	Comparable across environments, OECD test
	Dictoyledonous	*Myriophyllum spicatum*	Wide distribution, standard test is being developed
	Proteobacteria	*Pseudomonas fluorescens*	Rapid and cheap test
Estuarine	Crustacea	*Corophium volutator*	Comparable across environments, ISO test
	Annelida	*Arenicola marina*	Comparable across environments
	Monocotyledonous	*Stuckenia (pectinatus) / Ruppia (sp or maritima) / Vallisneria (americana)*	Wide distribution, easy to culture
	Proteobacteria	*Vibrio fischeri*	Rapid and cheap test
Marine	Crustacea	*Corophium volutator*	Comparable across environments
	Echinodermata	*Echinocardium cordatum*	Specific for marine water
	Monocotyledonous	*Zostera sp. (noltii)*	Wide distribution
	Proteobacteria	*Vibrio fischeri*	Rapid and cheap test

The uncertainties and possible risks indicated by the first-tier assessment can be used by risk assessors and risk managers to decide which organisms and methods they should focus on in the higher-tier effect assessment. Appropriate intermediate tiers may be developed based on additional toxicity data for potentially sensitive sediment organisms. Suitable additional test species may be selected from the species mentioned in Tables 2, 5 and 7. It is anticipated that the test conditions for additional test species will not fully comply with the specific testing guidelines for standard test species. Any deviations in terms of test conditions and the properties of the test organisms should, however, be documented in detail. If this leads to additional toxicity data becoming available for the relevant taxonomic groups of sediment organisms, an approach might be to calculate the geometric mean of the chronic toxicity values (e.g. EC_{10} values addressing the same measurement endpoint) within taxonomic groups and to apply the assessment factor (e.g. 10) that is also used in the first tier when the basic set of standard test species is complete. This approach was suggested by the European Food Safety Authority Panel on Plant Protection Products and their Residues, as an intermediate effect assessment tier for pesticides and water organisms[220] and may also be an option for the effect assessments for a wider array of chemicals and sediment organisms. Note, however, that the predictive value of this "Geomean" approach needs to be calibrated e.g. with focussed micro- and mesocosm tests.

If enough chronic toxicity data for sediment-dwelling organisms become available, the SSD concept may be used for prospective risk assessment by using the HC_5 (hazardous concentration to 5% of the species tested) to derive the sediment quality standard (e.g. by applying an appropriate assessment factor). For aquatic species, at least toxicity data on 8 to 10 different taxa are usually recommended to apply the SSD approach within a regulatory context.[215,224] Toxicity data used in the SSD need to be expressed in terms of equivalent exposure conditions and dose metrics, as was discussed previously. To date, this number of appropriate chronic toxicity data is usually not available for sediment organisms and one particular chemical. If future research demonstrates that the chronic toxicity data for freshwater, estuarine and marine sediment organisms could be combined in a single SSD, there might be an increased scope for effect assessment based on the SSD approach.

As discussed already, appropriate community-level (micro-/mesocosm) experiments that address the concentration-response relationship for sediment organisms may in the near future be used as an appropriate higher-tier test (e.g. by selecting the most sensitive endpoint for sediment-dwelling organisms and an appropriate assessment factor or modelling approach for spatio-temporal extrapolation) and to calibrate the risk assessment on the basis of laboratory toxicity tests with sediment organisms. Current guidance documents[213,214] focus on effect assessment and water exposure. Consequently, guidance for conducting and interpreting sediment micro- and mesocosm tests is required. Another research need is to study the possible variability in threshold concentrations of population and community-level effects for sediment organisms in different model ecosystem experiments in order to derive an appropriate AF for spatio-temporal extrapolation if only one appropriate micro-/mesocosm test is available for the sediment contaminant under evaluation.

Note that the prospective effect assessment tiers described previously can also be evaluated and verified by means of the extensive information gained from the development of sediment quality guidelines in North America and Europe within the context of retrospective risk assessment.[4,225-230]

2.4 Conclusions

In this chapter, we have summarised the technical literature on whole-sediment toxicity tests for microorganisms, benthic invertebrates, macrophytes and benthic communities. We have presented recommendations based on earlier papers and reviews, and have identified knowledge gaps and priorities for further research. All in all, despite the observed progress in individual fields of sediment toxicity testing over the past two decades, the approaches are currently still too heterogeneous to allow unification in risk assessment frameworks. Consequently, we have proposed a balanced selection of species that seem to be most suitable for future frameworks for the prospective assessment of risks associated with single chemicals. Together with optimised standard test protocols, these selected species could form the basics of the first tier of sediment toxicity risk assessment. Consequently, the formal selection and approval of species and tests in regulatory contexts is an important priority. Within this domain of prioritised protocol development, a second distinct priority is the development of standardised test protocols for estuarine and marine species, microorganisms and macrophytes, as these are still less well developed than freshwater benthic invertebrate tests. A further question is whether specific taxonomic groups that exclusively occur in the estuarine and marine environment are sufficiently covered by the traditional test species, which may call for the development of tests for species that characteristically occur in the estuarine/marine environment. In addition, guidance for conducting and interpreting higher-tier sediment micro- and mesocosm tests needs to become available in the near future, as such tests are crucial for the calibration of tests in lower tiers of the risk assessment.

Ultimately, results from sediment toxicity tests focusing on microorganisms, invertebrates, macrophytes and communities (in micro- and mesocosm tests) may be combined in higher tiers of prospective risk assessment such as the SSD approach. This, however, requires unification of dose metrics and exposure assessment methodologies across the groups of test species. We have therefore proposed recommendations for exposure assessment and sediment preparation.

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Supporting information

Table S1. Overview of available literature and guidelines for prospective sediment toxicity tests with invertebrates.

Nr.	Taxonomic group	Species	F/E/M	salinity range (g/l)	Feeding mode	Geographic region
1	amphipoda	*Ampelisca abdita*	M	20-32	deposit, suspended particles	temp, NA
2		*Amphiporeia virginica*	M			
3		*Bathyporeia sarsi*	M			
4		*Corophium insidiosum*	M		sediment licker	temp, NA
5		*Corophium mutisetosum*	F/E	0-35	sediment licker	temp EU
6		*Corophium spinicorne*	E/M			
7		*Corophium volutator*	E/M	7.5-47.5	sediment licker	temp EU
8		*Diporeia affinis*	F		deposit feeder	temp
9		*Diporeia hoyi*	F		algae and bacteria	temp, NA
10		*Diporeia spp*	F		detritivore	temp, NA
11		*Eohaustorius estuarius*	E	2-34		temp, NA
12		*Eohaustorius sencillus*	E/M			
13		*Foxiphalus xiximeus*	M			
14		*Gammarus duebeni*	E			
15		*Gammarus fasciatus*	F	0-8	gatherer/collector, carnivore, filter feeder	temp, NA, EU
16		*Gammarus lacustris*	F	0-5	omnivore	temp, NA
17		*Gammarus pulex*	F	0-11	shredder, predator	Temp, EU, Asia
18		*Grandidierella japonica*	E/M			
19		*Hyalella azteca*	F/E	0-30	sediment licker	temp, NA
20		*Lepidoctylus dytiscus*	E			
21		*Leptocheirus plumulosus*	E	1.5-32		temp, NA
22		*Melita nitida*	E	3-20		
23		*Paraphoxus epistomus*	M			
24		*Rhepoxynius abronius*	M	25-32	detritivore + predator	temp, NA
25	copepoda	*Amphiascus tenuiremis*	M		sediment ingestor	temp, EU, Atlantic
26		*Enhydrosoma propinquum*	M			
27		*Microarthridion littorale*	M			
28		*Nannopus palustris*	E/M			
29		*Paranychocamtus wilsoni*	M			
30		*Pseudobrady pulchella*	E			
31		*Tisbe battagliai*	M		grazer	EU Atlantic
32	decapoda	*Asellus communis*	F		detritivores	temp NA, EU
33		*Carcinus maenas*	M	5-41	Opportunistic feeder	N-Atlantic
34		*Crangon crangon*	M	7-40		temp EU
35		*Crangon septemspinosa*	M			arctic
36		*Diastylis alaskensis*	M			trop
37		*Diastylopis dawsoni*	M			
38		*Lamprops quadriplicata*	M			
39		*Mysidopis bahia*	M	9-29		Mexico indigenous, indo pacific

40		*Orconectes virilis*	F		omnivore	Temp, native NA, introduced EU
41		*Palaemonetes pugio*	M	1-55		NA, EU
42		*Penaeus duoarum*	M			cosmopolitan
43		*Sicyonia ingentis*	M			
44	bivalve	*Abra alba*	M			
45		*Anodonta imbecillis*	F		filter feeder (algae, phytoplankton)	temp, NA
46		*Cerasoderma edule*	M			
47		*Corbicula fluminea*	F	0-13	filter feeder (sandy or muddy bottoms)	cosmopolitan
48		*Crassostrea gigas*	E		filter-suspension	invasive in EU
49		*Crassostrea virginica*	M		filter-suspension	
50		*Macoma balthica*	E/M	5-30	Facultative suspension feeder	temp, arctic
51		*Macoma inquinata*	M			
52		*Macoma nasuta*	M			
53		*Mercenaria mercenaria*	M			
54		*Mulinia lateralis*	M			
55		*Mya arenaria*	E			
56		*Mytilus edulis*	M		suspension-feeding	
57		*Protothaca staminea*	M			
58		*Yoldia limatula*	M			
59	gastropoda	*Littorina littorea*	M			
60	oligochaete	*Limnodrilus claparedeanus*	F	0.2-3	sediment ingestors (detritiherbivore)	cosmopolitan
61		*Limnodrilus hoffmeisteri*	F	0-10	sediment ingestors (detritiherbivore)	cosmopolitan
62		*Limnodrilus udekemianus*	F		sediment ingestors (detritiherbivore)	cosmopolitan
63		*Lumbriculus variegatus*	F		Deposit feeder (Sediment ingester)	temp
64		*Monopylephorus cuticulatus*	M			
65		*Potamothrix hammoniensis*	F/E	0.5-5	sediment ingestors (detritiherbivore)	Temp, EU
66		*Pristina leidyi*	F		sediment ingestors (detritiherbivore, algea)	cosmopolitan
67		*Stylodrilus heringianus*	F		sediment ingestors (detritiherbivore subsurface)	Temp, NA, EU
68		*Tubifex tubifex*	F	<7	sediment ingestors (detritiherbivore)	cosmopolitan
69	polychaete	*Arenicola marina*	M	18-40	Sub-surface deposit feeder	Temp (N/W Europe)
70		*Capitella capitata*	E	18-40	deposit feeder	cosmopolitan
71		*Dinophilus gyrocilatus*	M			
72		*Glycinde picta*	M			
73		*Nephtys incisa*	M			

74		*Nereis arenaceodentata*	E/M		omnivorous deposit feeder	temp, pacific NA
75		*Nereis diversicolor*	E/M	18-40	opportunistic feeder	temp EU
76		*Nereis virens*	E/M	25-30	omnivorous deposit feeder	temp EU
77		*Scoloplos armiger*	M			
78		*Streblospio benedicti*	E			
79	nematoda	*Panagrellus redivivus*	F		bacteriophagous	cosmopolitan
80		*Caenorhabditis elegans*	F			temp
81		*Chromadorina germanica*	E/M			temp
82		*Diplolaimella punicea*	E/M			temp
83	echinodermata	*Echinocardium cordatum*	M	28-33	deposit feeder	temp EU, NA, AU
84		*Lytechinus pictus*	M			
85	diptera	*Chironomus plumosus*	F		filter feeder, rare cases scraper of sediment	temp
86		*Chironomus prasinus*	F			temp
87		*Chironomus riparius*	F/E		Deposit feeder (Detritus (< 1mm))	temp
88		*Chironomus tentans*	F		filter feeder	temp
89	ephemeroptera	*Hexagenia bilineata*	F		gatherer/collector	temp, NA
90		*Hexagenia sp*	F			

Table S1 (Continued).

		Endpoints								
Nr.	**Species**	**Mortality**	**Reproduction**	**Growth**	**Behaviour**	**Avoidance**	**(re)Burrowing**	**Emergence**	**Bioaccumulation**	**Others**
1	*Ampelisca abdita*	x			x		x	x		
2	*Amphiporeia virginica*	x								
3	*Bathyporeia sarsi*	x			x		x			
4	*Corophium insidiosum*	x								
5	*Corophium mutisetosum*	x		x						
6	*Corophium spinicorne*	x								
7	*Corophium volutator*	x		x	x		x			
8	*Diporeia affinis*	x			x					
9	*Diporeia hoyi*	x								
10	*Diporeia spp*	x			x	x				
11	*Eohaustorius estuarius*	x				x	x	x		
12	*Eohaustorius sencillus*	x			x					
13	*Foxiphalus xiximeus*	x								
14	*Gammarus duebeni*									pleodod beat frequency; swimming endurance
15	*Gammarus fasciatus*	x	x	x					x	
16	*Gammarus lacustris*	x							x	
17	*Gammarus pulex*	x		x	x					feeding rate
18	*Grandidierella japonica*	x		x	x		x	x		
19	*Hyalella azteca*	x	x	x					x	development

20	*Lepidoctylus dytiscus*	x		x						
21	*Leptocheirus plumulosus*	x	x	x						morphological development
22	*Melita nitida*	x	x							abnormal brood pouch setae; intermolt period
23	*Paraphoxus epistomus*	x								
24	*Rhepoxynius abronius*	x			x	x	x	x		
25	*Amphiascus tenuiremis*	x	x							age structure
26	*Enhydrosoma propinquum*	x								
27	*Microarthridion littorale*	x	x							
28	*Nannopus palustris*	x	x							
29	*Paranychocamtus wilsoni*	x	x							
30	*Pseudobrady pulchella*	x	x							
31	*Tisbe battagliai*	x								development
32	*Asellus communis*	x								
33	*Carcinus maenas*					x				
34	*Crangon crangon*	x				x			x	
35	*Crangon septemspinosa*	x								
36	*Diastylis alaskensis*	x								
37	*Diastylopis dawsoni*	x								
38	*Lamprops quadriplicata*	x								
39	*Mysidopis bahia*	x								
40	*Orconectes virilis*	x								
41	*Palaemonetes pugio*	x								
42	*Penaeus duoarum*	x								
43	*Sicyonia ingentis*	x								
44	*Abra alba*				x					
45	*Anodonta imbecillis*	x	x	x		x		x		
46	*Cerasoderma edule*	x					x			
47	*Corbicula fluminea*	x	x	x		x		x		
48	*Crassostrea gigas*			x						embryo larval development, spermiotoxicity
49	*Crassostrea virginica*									
50	*Macoma balthica*				x		x		x	
51	*Macoma inquinata*	x								
52	*Macoma nasuta*	x							x	
53	*Mercenaria mercenaria*						x			
54	*Mulinia lateralis*	x		x						
55	*Mya arenaria*						x			
56	*Mytilus edulis*	x							x	
57	*Protothaca staminea*	x							x	
58	*Yoldia limatula*						x		x	
59	*Littorina littorea*	x							x	imposex; intersex
60	*Limnodrilus claparedeanus*	x	x	x						
61	*Limnodrilus hoffmeisteri*	x	x	x	x				x	weight
62	*Limnodrilus udekemianus*	x	x	x						
63	*Lumbriculus variegatus*	x	x	x	x				x	
64	*Monopylephorus cuticulatus*									respiration rate
65	*Potamothrix hammoniensis*	x	x	x						
66	*Pristina leidyi*	x	x							
67	*Stylodrilus heringianus*	x			x	x			x	weight
68	*Tubifex tubifex*	x	x	x						

69	*Arenicola marina*	x						x	feacal (cast) production
70	*Capitella capitata*	x	x	x					
71	*Dinophilus gyrocilatus*	x	x						
72	*Glycinde picta*	x							
73	*Nephtys incisa*	x		x	x				
74	*Nereis arenaceodentata*	x		x					fecundity
75	*Nereis diversicolor*					x		x	
76	*Nereis virens*	x							
77	*Scoloplos armiger*					x			
78	*Streblospio benedicti*			x					colonization
79	*Panagrellus redivivus*	x	x						maturation
80	*Caenorhabditis elegans*		x	x					
81	*Chromadorina germanica*	x	x						
82	*Diplolaimella punicea*	x	x						
83	*Echinocardium cordatum*	x							
84	*Lytechinus pictus*	x		x	x			x	gonadal production; spermiotoxicity
85	*Chironomus plumosus*						x	x	
86	*Chironomus prasinus*						x		oviposition success
87	*Chironomus riparius*	x	x	x			x		
88	*Chironomus tentans*	x	x	x			x	x	
89	*Hexagenia bilineata*								
90	*Hexagenia sp*	x		x	x				enzyme inhibition; molting

Table S1 (Continued).

				Sediment				
Nr.	**Species**	**Standard guidelines**	**Test duration range**	**Natural**	**Artificial**	**Spiked**	**See references within these reviews**	**Additional or original references**
1	*Ampelisca abdita*	Y, ASTM	4-10d	x	x		37,38,231	
2	*Amphiporeia virginica*	Y, Environment Canada	10d		x		38,231	
3	*Bathyporeia sarsi*	Y	10d	x			38,231	106,112
4	*Corophium insidiosum*	Y	10d		x		38	
5	*Corophium mutisetosum*	N					37	
6	*Corophium spinicorne*	N	10d	x			37,38	
7	*Corophium volutator*	Y, Environment Canada, ISO, OSPAR	1h-47d	x	x	Y	38,231	
8	*Diporeia affinis*	N	1-2d	x			38	
9	*Diporeia hoyi*	Y, protocol after Dernott	7d	x			38	
10	*Diporeia spp*	Y	4-28d	x		Y	38	
11	*Eohaustorius estuarius*	Y, ASTM; EPA; Environment Canada	10d		x		37,38,231	
12	*Eohaustorius sencillus*	N	72h	x		Y	38	
13	*Foxiphalus xiximeus*	Y, Environment Canada	10d		x		38,231	
14	*Gammarus duebeni*	N					37	
15	*Gammarus fasciatus*	N	10-70d	x	x		38	

16	*Gammarus lacustris*	N	10-28d		x		38	232
17	*Gammarus pulex*	N	14h-70d				38	233
18	*Grandidierella japonica*	Y, ASTM; Environment Canada	10-28d	x	x		38,231	
19	*Hyalella azteca*	Y, EPA; ASTM; Envrironment Canada	10-30d	x	x		37,38	
20	*Lepidoctylus dytiscus*	N					37	
21	*Leptocheirus plumulosus*	Y, ASTM; Environment Canada	10-40d	x	x		37,38,231	
22	*Melita nitida*	N					37	
23	*Paraphoxus epistomus*	N	10d	x			38	
24	*Rhepoxynius abronius*	Y, ASTM; EPA; Environment Canada	72h-10d	x		Y	19,37,38,231	
25	*Amphiascus tenuiremis*	N	96h - 7w		x	Y	38	234
26	*Enhydrosoma propinquum*	N	7d	x			38	
27	*Microarthridion littorale*	N	7d	x			38	
28	*Nannopus palustris*	N	7d	x			38	
29	*Paranychocamtus wilsoni*	N	7d	x			38	
30	*Pseudobrady pulchella*	N	7d	x			38	
31	*Tisbe battagliai*	N	4d			Y	38,231	
32	*Asellus communis*	N	4d	x			38	
33	*Carcinus maenas*	N	10min	x			38	
34	*Crangon crangon*	N	10min-60d	x			38,231	
35	*Crangon septemspinosa*	N	4d		x	Y	38,231	
36	*Diastylis alaskensis*	N	10d	x			38	
37	*Diastylopis dawsoni*	N	10d	x			38	
38	*Lamprops quadriplicata*	N	10d	x			38	
39	*Mysidopis bahia*	Y, ASTM	4-10d		x	Y	38,231	235
40	*Orconectes virilis*	N	30-100d				38	
41	*Palaemonetes pugio*	N	4d		x	Y	38	236
42	*Penaeus duoarum*	N	4-10d		x	Y	38	
43	*Sicyonia ingentis*	N	4d				38	
44	*Abra alba*	N	20h-5d	x	x	Y	38	
45	*Anodonta imbecillis*	N					19	
46	*Cerasoderma edule*	N	20h-10d	x			38	
47	*Corbicula fluminea*	N					19	
48	*Crassostrea gigas*	N	10 min-24h				37,38,231	237
49	*Crassostrea virginica*	N	4d				38,231	238
50	*Macoma balthica*	N	20 - 28d	x			38,231	98,239
51	*Macoma inquinata*	N	10d	x			38	97
52	*Macoma nasuta*	N	4-28d				38,231	97
53	*Mercenaria mercenaria*	N	4d	x			38,231	
54	*Mulinia lateralis*	N	7d	x			38,231	
55	*Mya arenaria*	N	4h				37,38	
56	*Mytilus edulis*	N	4 - 60d	x	x		38,231	
57	*Protothaca staminea*	N	10d	x			38	
58	*Yoldia limatula*	N	4-28d	x			231	
59	*Littorina littorea*	N	2-10d	x			38,231	

60	*Limnodrilus claparedeanus*	N	500d	x			38	
61	*Limnodrilus hoffmeisteri*	N	4-500d	x	x	Y	38	
62	*Limnodrilus udekemianus*	N	500d	x			38	
63	*Lumbriculus variegatus*	Y, OECD, ASTM	1-28d	x	x	Y	38,231	62
64	*Monopylephorus cuticulatus*	N	4-8h	x			38	
65	*Potamothrix hammoniensis*	N	500d	x			38	
66	*Pristina leidyi*	N	2-18d	x			38	
67	*Stylodrilus heringianus*	N	10min-55d	x	x	Y	38	
68	*Tubifex tubifex*	Y, ASTM	28d-500d	x	x	Y	38	
69	*Arenicola marina*	N	10-100d			Y	38,231	112,240,241
70	*Capitella capitata*	N	4-50d	x			38	
71	*Dinophilus gyrocilatus*	N	7d				38	
72	*Glycinde picta*	N	10d	x			38	
73	*Nephtys incisa*	N	4-10d	x			38	
74	*Nereis arenaceodentata*	Y, EPA	4-153 d	x			38,231	
75	*Nereis diversicolor*	N	20h - 28d	x			38,231	
76	*Nereis virens*	N	12d		x	Y	38	
77	*Scoloplos armiger*	N	20h	x			38	
78	*Streblospio benedicti*	N	7d	x			37,38,231	
79	*Panagrellus redivivus*	N	4d	x			38	
80	*Caenorhabditis elegans*	Y, ISO	3d -4d	x			38	
81	*Chromadorina germanica*	N	14d	x			38	
82	*Diplolaimella punicea*	N	14d	x			38	
83	*Echinocardium cordatum*	N	14d				38,231	107,242
84	*Lytechinus pictus*	N	10min-60d	x	x	Y	231	
85	*Chironomus plumosus*	N	10d		x	Y		243
86	*Chironomus prasinus*	N	16d		x	Y		90
87	*Chironomus riparius*	Y, OECD; EPA; ASTM	1-60 d	x	x		38,231	52
88	*Chironomus tentans*	Y, OECD; ASTM	10-100 d	x	x		38	
89	*Hexagenia bilineata*	N					19	
90	*Hexagenia sp*	Y, ASTM	1.5h-14d	x	x			

Table S2. Overview of available literature and guidelines for prospective sediment toxicity tests with macrophytes.

Nr.	Taxonomic group	Species	F/E/M	Growth form	Geographic region	Standard protocol	Chemical group			Test duration
							Oil	PCBs	Pest-icides	
1	monocotyledonous	*Cymodocea serrulata*	M	submerged	trop	N			x	5d
2	monocotyledonous	*Elodea canadensis*	F	submerged	temp	N			x	21 d
3	monocotyledonous	*Elodea nuttallii*	F	submerged	temp	N			x	21 d
4	monocotyledonous	*Glyceria maxima*	F	emerged	temp	N			x	21 d
5	monocotyledonous	*Halodule wrightii*	M	submerged	trop	N	x		x	5-100h, 22 d
6	monocotyledonous	*Halophila ovalis*	M	submerged	trop	N			x	5d
7	monocotyledonous	*Hydrilla verticillata*	F	submerged	temp - trop	N			x	192 h
8	monocotyledonous	*Juncus roemerianus*	E/M	emerged	temp - sub trop	N		x	x	
9	monocotyledonous	*Lagarosiphon major*	F	submerged	temp - trop	N			x	21 d
10	monocotyledonous	*Najas sp.*	F/E/M	submerged	temp - subtrop	N			x	14 d
11	monocotyledonous	*Potamogeton crispus*	F	submerged	temp	N			x	21 d
12	monocotyledonous	*Potamogeton perfoliatus*	F	submerged	temp	N			x	21 d
13	monocotyledonous	*Ruppia maritima*	F/E/M	submerged	cosmopolitan	N			x	3-4 w
14	monocotyledonous	*Scirpus olneyi*	E/M	emerged	Temp- trop	N			x	
15	monocotyledonous	*Scirpus robustus*	E	emerged	temp - sub trop	N				21 d
16	monocotyledonous	*Spartina alterniflora*	E/M	emerged	temp - sub trop	N			x	28 d
17	monocotyledonous	*Stuckenia pectinatus*	F/E/M	submerged	temp - trop	N			x	21 - 28d
18	monocotyledonous	*Syringodium filliforme*	M	submerged	trop	N	x			5-100h
19	monocotyledonous	*Thalassia testudinum*	M	submerged	trop	N	x		x	5-100h
20	monocotyledonous	*Typha latifolia*	E	emerged	temp	N			x	7 - 10 d
21	monocotyledonous	*Vallisneria americana*	F/E/M	submerged	temp - trop	N				7 d
22	monocotyledonous	*Zostera capricorni*	M	submerged	trop	N			x	10h - 5d
23	monocotyledonous	*Zostera marina*	M	submerged	temp	N			x	3d
24	dictoyledonous	*Myriophyllum aquaticum*	F	submerged/ emerged	temp	Y (ASTM and ISO test on wet sediment)			x	7 - 14 d
25	dictoyledonous	*Myriophyllum sibiricum*	F	submerged	temp	N			x	14 d
26	dictoyledonous	*Myriophyllum spicatum*	F	submerged	temp	N (in development)				14 d
27	dictoyledonous	*Ranunculus aquaticum*	F	submerged	temp	N			x	21 d
28	dictoyledonous	*Ranunculus circinatus*	F	submerged	temp	N			x	21 d

Table S2 (Continued).

					Nutrient medium						
Nr.	Species	Sediment	Spiked sediment	Water	Barko and Smart	M4	Andrew's	Hoagland	Steinberg	No nutrients	Light/ dark period (h)
1	*Cymodocea serrulata*	natural sediments	N	seawater							
2	*Elodea canadensis*	natural clay			x	x					16/8
3	*Elodea nuttallii*	OECD, natural clay			x						16/8
4	*Glyceria maxima*	sandy-loam with osmocote	N								16/8
5	*Halodule wrightii*	quartz sand, bay sediment	N	seawater							14/10
6	*Halophila ovalis*	natural sediments	N	seawater							
7	*Hydrilla verticillata*	fine sand	Y					x			
8	*Juncus roemerianus*	quartz sand	Y	estuarine water							natural
9	*Lagarosiphon major*	sandy-loam with osmocote	N								
10	*Najas sp.*	silty-clay loam					x				16/8
11	*Potamogeton crispus*	natural clay			x						16/8
12	*Potamogeton perfoliatus*	natural clay			x						16/8
13	*Ruppia maritima*	reference sediments	Y	seawater							12/12
14	*Scirpus olneyi*										
15	*Scirpus robustus*	artificial, slightly different from OECD						x			
16	*Spartina alterniflora*	quartz sand	Y	estuarine water							natural, 16/8
17	*Stuckenia pectinatus*	natural clay			x						16/8
18	*Syringodium filliforme*	quartz sand	N	seawater							natural
19	*Thalassia testudinum*	quartz sand, sand	N	(artificial) seawater							natural
20	*Typha latifolia*	artificial, slightly different from OECD						x		x	16/9, natural
21	*Vallisneria americana*	natural sediment								x	natural
22	*Zostera capricorni*	natural sediments	N	seawater							16/8
23	*Zostera marina*	glass beads	N	filtered seawater						x	
24	*Myriophyllum aquaticum*	OECD, turface			x		x	x	x		16/8
25	*Myriophyllum sibiricum*	turface, glass beads					x				16/8
26	*Myriophyllum spicatum*	OECD			x						16/8
27	*Ranunculus aquaticum*	natural clay			x						16/8
28	*Ranunculus circinatus*	natural clay			x						16/8

Table S2. (Continued).

Nr.	Species	Endpoints: Biomass	Length/ Growth	Side shoots	Roots	Leafs	Seedling survival	Chlorophyll/ Pigments	Others	Reference
1	*Cymodocea serrulata*							x	photosynthesis	[138]
2	*Elodea canadensis*	x	x	x						[131]; pers. comm. Jonas
3	*Elodea nuttallii*	x	x	x	x					[41] and refs within; pers. comm. Jonas
4	*Glyceria maxima*	x	x			x			number of shoots	[45,130]
5	*Halodule wrightii*								mortality, number and weight new ramets, above ground biomass	[244,245]
6	*Halophila ovalis*							x	photosynthesis	[138,246]
7	*Hydrilla verticillata*	x			x				bioaccumulation, dry weight root and shoot	[67]
8	*Juncus roemerianus*		x					x	(lipid) peroxidase activity	[247]
9	*Lagarosiphon major*	x	x						number of shoots	[130]
10	*Najas sp.*	x								[151]
11	*Potamogeton crispus*	x	x	x	x					pers. comm. Jonas
12	*Potamogeton perfoliatus*	x	x	x	x					pers. comm. Jonas
13	*Ruppia maritima*								oxygen production	[123]
14	*Scirpus olneyi*		x					x	(lipid) peroxidase activity	[134]
15	*Scirpus robustus*	x			x	x	x			[149]
16	*Spartina alterniflora*	x	x		x	x	x	x	(lipid) peroxidase activity	[41,149,247]
17	*Stuckenia pectinatus*	x	x		x					pers. comm. Jonas
18	*Syringodium filliforme*								mortality	[245]
19	*Thalassia testudinum*							x	mortality, photosynthesis: respiration ratio	[245,248]
20	*Typha latifolia*		x				x	x	glutathione S-transferase activity	[249]
21	*Vallisneria americana*								leaf: root ratio	[135,136]
22	*Zostera capricorni*							x	photosynthesis	[138,139]
23	*Zostera marina*								total leaf length, RNA:DNA ratio	[250]
24	*Myriophyllum aquaticum*	x	x	x	x			x		[28,44,143,144,146]
25	*Myriophyllum sibiricum*	x	x	x						[44]
26	*Myriophyllum spicatum*	x	x							[28,130]
27	*Ranunculus aquaticum*	x	x	x	x					pers. comm. Jonas
28	*Ranunculus circinatus*	x	x	x	x					pers. comm. Jonas

Table S3. Overview of available literature for prospective sediment toxicity tests with single species microorganisms.

Nr.	Species	Method	Taxonomic group	Environment	Distribution	Endpoint	Test medium	Spiked	Reference
1	*Vibrio fischeri*	Solid phase tox test	Bacteria	Marine	Cosmopolitan	Bioluminescence	Sediment/ porewater/ elutriate	Y	190,251,252
2	*Vibrio harveyi*		Bacteria	Marine	Tropic	Bioluminescence			
3	*Pseudomonas fluorescens*		Bacteria	Fresh water/ soil		Bioluminescence			
4	*Pseudomonas putida*		Bacteria			Metabolic inhibition			181
5	*Escherichia coli*	Toxi-ChromoPad, MetPAD	Bacteria	lower ingesting		Metabolic inhibition, B-galactosidase	Sediment/ porewater	Y	26,181
6	*Bacillus cereus**	solid phase DHA test, FDA assay	Bacteria	soil/ sediment	Cosmopolitan	dehydrogenase activity	Freshwater sediment/ porewater/ elutriate	Y	36
7	*Cytophaga johnsonae**		Bacteria	Marine					253
8	*Selenastrum capricornutum**		Algae				elutriate		254
9	*Skeletonema costatum**		Diatom	Marine	Cosmopolitan	growth	inter-tidal sediment porewater		26

*Studies were done with metals, though this is not the scope of this review the studies were includes for their methods

Table S4. Overview of available literature and guidelines for prospective sediment toxicity tests with micro- and mesocosms.

Nr.	References	Types/size Micro- or mesocosm	Size (m3)	Invertebrate or Macrophyte	F/E/M	Geographic region	Chemical group Oil	PCBs	PAKs	Pesticides
1	211	mesocosm	13	Inv.	M	Europe	y			
2	203	mesocosm	? (25x25cm)	Inv.	F	North America				atrazine
3	204	mesocosm	31	Inv.	F	North America				chlorfenapyr
4	124	mesocosm	60	Inv.	F	Europe				lufenuron
5	205	mesocosm	1.05	Inv.	F	Australia				endosulfan
6	209	microcosm	0.0025	Inv.	E	Europe		y		
7	206	microcosm	0.84	Inv./Macr.	F	Europe				triphenyltin acetate
8	212	mesocosm	13	Inv.	M	North America	y			
9	207	microcosm	0.047	Inv./Macr.	F	North America				atrazine and lambda-cyhalothrin
10	71	microcosm	0.847	Macr.	F	Europe		y	y	
11	210	microcosm	0.7	Macr.	E	North America				atrazine
12	208	microcosm	0.144	Macr.	F	Europe				tributyltin

Table S4. (Continued).

	Sediment				Water		Exposure				Duration
Nr.	Natural	Artificial	Nutrients added	Spiked	Depth of layer (cm)	Natural	Ground/ Drinking water	Acute	Multiple appl.	Chronic	Analytical verification water/Sediment	Test duration (d)
1	y			y	15	y			y		Y/Y	38
2	y		y	y	10	y				y	N/N	42
3	y				28-35	y		y			Y/Y	14
4	y				unknown	y		y			Y/N	114
5	y				0.7-1	y		y			Y/Y	14
6	y		y	y	30	y				y	N/Y	34
7	y				7	y				y	Y/Y	280
8	y				30	y				y	Y/Y	232
9	y			y	10		y			y	Y/Y	56
10	y			y	7	y				y	Y/Y	105
11	y			y	12	y				y	Y/Y	28
12		y		y	6		y			y	Y/Y	23

Table S4. (Continued).

	Communities sampled												Endpoints
Nr.	Fish	Zooplankton	Benthic inv.	Meiofauna	Other inv.	Periphyton	Phyto-plankton	Macro-phytes	Microbes	Bio-accumulation	Abundance/ Cover	Biomass	Other
1			y	y		y			y		y		Microphytobenthos = photosynthetic activity; bivalves and worm =feeding rate
2		y	y	y							y		
3	y	y	y	y			y				y		
4			y		y						y		
5	y										y		
6			y							y		y	
7		y	y	y			y	y			y		
8		y		y							y		
9								y		y			
10	y	y	y			y	y	y		y			
11								y			y	y	O2 production, length
12			y					y		y			

Chapter 3

Uptake, translocation and elimination in sediment-rooted macrophytes: A model-supported analysis of whole sediment test data

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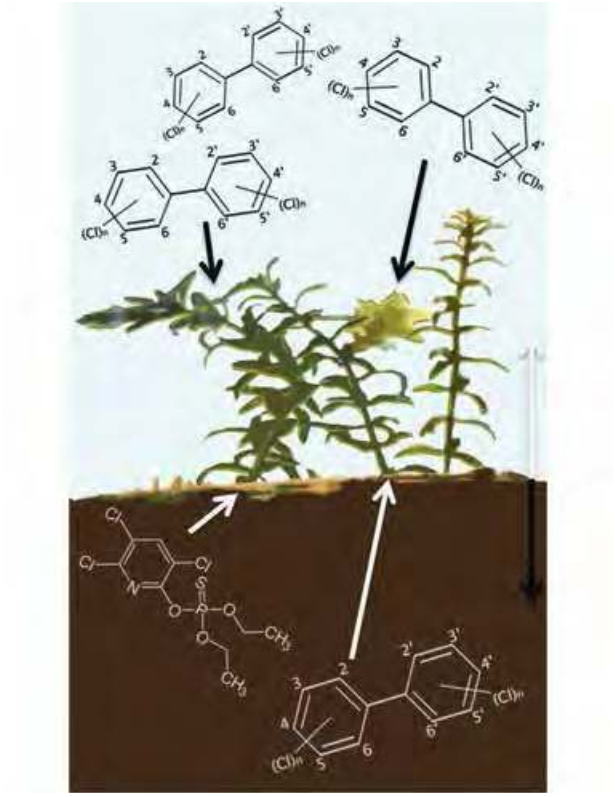
Abstract

Understanding bioaccumulation in sediment-rooted macrophytes is crucial for the development of sediment toxicity tests using macrophytes. Here we explore bioaccumulation in sediment-rooted macrophytes by tracking and modelling chemical flows of chlorpyrifos, linuron, and six PCBs in water-sediment-macrophyte systems. Chemical fluxes across the interfaces between pore water, overlying water, shoots, and roots were modelled using a novel multicompartment model. The modelling yielded the first mass transfer parameter set reported for bioaccumulation by sediment-rooted macrophytes, with satisfactory narrow confidence limits for more than half of the estimated parameters. Exposure via the water column led to rapid uptake by *Elodea canadensis* and *Myriophyllum spicatum* shoots, followed by transport to the roots within 1-3 days, after which tissue concentrations gradually declined. Translocation played an important role in the exchange between shoots and roots. Exposure via spiked sediment led to gradual uptake by the roots, but subsequent transport to the shoots and overlying water remained limited for the chemicals studied. These contrasting patterns show that exposure is sensitive to test set up, chemical properties, and species traits. Although field-concentrations in water and sediment will differ from those in the tests, the model parameters can be assumed applicable for modelling exposure to macrophytes in the field.



3.1 Introduction

Macrophytes play a key role in the ecological functioning of aquatic ecosystems[119] and form an important pathway for redistribution of organic chemicals among plant material, water column[71] and the food web and therefore should be considered in environmental risk assessment. Historically, most ecotoxicological plant research concerned terrestrial and emergent plants, and focused on agricultural crops or phytoremediation. Submerged macrophytes, however, cannot be compared with terrestrial and emergent plants as they lack transport processes driven by transpiration.[255] For submerged macrophytes, research has been limited to uptake and elimination kinetics from the overlying water (e.g. refs [127,156,157,159,256-258]), lacking the presence of a sediment phase. Absence of sediment in toxicity tests for sediment-rooted macrophytes is not ecologically realistic. Recently, the importance of developing whole sediment toxicity tests for sediment-rooted macrophytes has been recognized (Chapter 2).[28,259] For instance, the Aquatic Macrophyte Risk Assessment for Pesticides (AMRAP) workshop identified a lack of knowledge regarding the relative importance of sediment exposure for uptake of toxicants by rooted macrophytes.[28] To date, uptake of organic chemicals in sediment-inclusive test systems has been described only for *Hydrilla verticillata* with three insecticides,[67] for *Myriophyllum spicatum* with one herbicide,[126] and *Myriophyllum elatinoides* for a metabolite of pyrethroids.[260] The last two studies also considered elimination to the water column.

Aquatic macrophytes may accumulate, translocate, and eliminate organic chemicals by roots, shoots or by both (Table S1), thereby enhancing or decreasing chemical bioavailability in a complex manner.[41,71,120] The importance of exposure from sediment, water, or air depends on macrophyte traits such as growth form, e.g. free floating, emerged, or submerged. In prospective risk assessment, *Lemna* is the standard freshwater test species. *Lemna* is, however, a free-floating, non-sediment-rooted macrophyte that might not represent other growth forms like sediment-rooted macrophytes, especially if the chemical test concerns sediment-bound chemicals.[28,125] For sediment-bound chemicals, tests with sediment-rooted macrophyte species, such as the dicot *M. spicatum*, are recommended to account for different exposure routes.[28] As monocot or dicot species might differ in their uptake and elimination traits and sensitivity, it is recommended to use both types (e.g. *M. spicatum* and *Elodea canadensis*) in the risk assessment.[28] In addition to macrophyte traits, chemical properties influence uptake, translocation, accumulation, and elimination of organic compounds.[159,261-263] This implies that studies on chemical bioavailability and exposure should account for a range of chemical properties.

Bioaccumulation models are very useful to generalise bioaccumulation data. However, we are not aware of modelling studies with sediment-rooted macrophytes that consider chemical exchange across all relevant compartments such as pore water, overlying water, sediment, macrophyte shoots, and roots, while also accounting for translocation.

The aim of the present study was to assess the relative importance and characteristic time scales of uptake, translocation, and elimination pathways of organic chemicals in

sediment-rooted, submerged aquatic macrophytes, in order to assist the development of whole sediment toxicity tests in the context of prospective risk assessment. The second aim was to assess the parameters that describe bioaccumulation in macrophytes, which also is relevant for modelling these processes in the field.

To achieve these aims, laboratory experiments were performed in which concentrations in sediment, overlying water, shoots, and roots were measured as a function of time for two freshwater sediment-rooted macrophytes; *E. canadensis* and *M. spicatum*, representing different macrophyte anatomy and physiology. Test chemicals included six polychlorinated biphenyls (PCBs) and the insecticide chlorpyrifos (CPF). Our previously published data on the herbicide linuron (LIN)[126] were included in the modelling. The experimental design included spiking of six PCBs in three couples that were practically identical based on hydrophobicity. Per couple, one PCB was spiked in the overlying water phase and the other PCB in the sediment phase. CPF was also spiked in the sediment phase. Experimental data were used to parameterise a multicompartment sediment bioaccumulation model that describes the chemical flows in the test systems.

3.2 Materials and Methods

3.2.1 Chemicals and materials

Experimental test chemicals were PCB couples 2 and 3, 28 and 29, 149 and 155 and CPF. LIN data were obtained from our earlier study.[126] Further details are provided as Supporting Information (SI).

3.2.2 Sediment and test medium

Sediment was prepared following OECD 218,[76] with a small modification described in ISO 16191.[122] Shortly, peat (5%), calcium carbonate powder (2%), and an aqueous nutrient medium of 0.36 g P/L and 0.30 g N/L were mixed to obtain a homogeneous slurry. The slurry was spiked with PCBs and CPF, and thoroughly mixed with quartz sand (75%), and kaolin clay (18%). Barko and Smart medium[147] was used as the overlying water phase. Further details and sediment characteristics are provided as supporting information (Table S2).

3.2.3 Spiking procedure

Overlying water was spiked with PCBs 2, 28, and 149, whereas the sediment was spiked with almost identical PCBs 3, 29, 155 and CPF (see Table 1 for chemical characteristics). PCB pairs (2 and 3; 28 and 29; 149 and155) were selected on the basis of their very similar K_{ow} values (Table 1), whereas the pairs represented a range of log K_{ow} between 4.63 and 6.67. Furthermore, log K_{ow} of CPF matches with PCBs 2 and 3. This setup allows for a direct comparison of chemical flows from water versus sediment as source compartments in the test systems. CPF was chosen to see potential differences between a pesticide and PCBs e.g. with respect to uptake, degradation, and metabolism.

Table 1. Chemical characteristics.

Chemical	Log K_{ow} [a]	Solubility at 25 °C (mol/L)[c]	K_{oc} (L/kg)[f]	$K_{P,SED}$ (m^3/kg)[h]	K_{POM} (L/kg)[i]
Linuron	3.00[b]	$2.56*10^{-4}$ [d,e]	406[g]	$8.12*10^{-3}$	269[j]
Chlorpyrifos	4.66[b]	$2.99*10^{-6}$ [d,e]	8151[d]	$1.63*10^{-1}$	12782[j]
PCB 2	4.60	$2.54*10^{-5}$	15136	$3.03*10^{-1}$	45352
PCB 3	4.60	$7.13*10^{-6}$	15136	$3.03*10^{-1}$	45352
PCB 28	5.58	$6.12*10^{-7}$	143582	$2.87*10^{0}$	268835
PCB 29	5.58	$3.50*10^{-7}$	143582	$2.87*10^{0}$	268835
PCB 149	6.66	$1.18*10^{-8}$	1737801	$3.48*10^{1}$	1932235
PCB 155	6.50	$7.45*10^{-9}$	1202264	$2.40*10^{1}$	1443777

[a]van Noort et al.[264], [b]Tomlin[265], [c]Paasivitra and Sinkkonen[266], [d]The pesticide properties database[267], [e] Solubility was measured at 20 °C, [f]Seth et al.[268], [g]Burešová et al.[126], [h]$K_{P,SED}$ was as calculated with K_{OC} values and an organic carbon fraction of 0.02, [i]Hawthorne et al.[269], [j]Endo et al.[270]

Spiking of Sediment

Sediment was spiked with PCBs 3, 29, 155 and CPF in acetone to reach target concentrations of 20 µg/kg dry weight (DW) for these PCBs and 40 µg/kg for the more degradable CPF. After spiking, pre-equilibration was 4 weeks for PCBs and 2 weeks for CPF. Polyoxymethylene (POM) passive samplers[82] were added to the sediment in order to acquire in situ pore water concentrations at start of exposure.

Spiking of overlying water

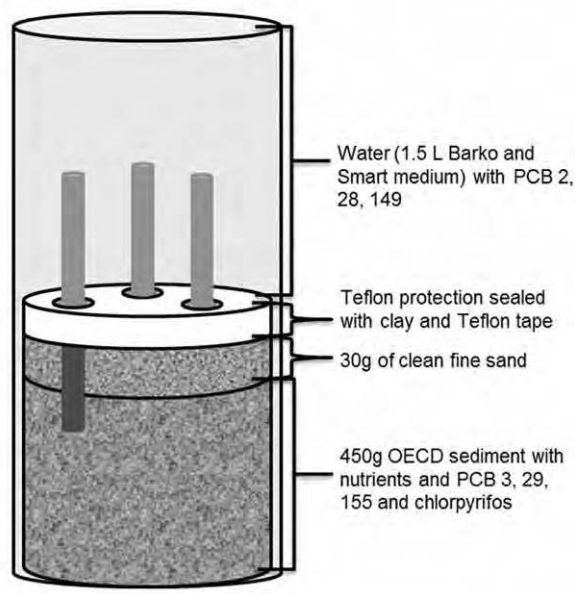
Under gentle stirring, the overlying water of each test system was spiked with a solution of PCB 2, 28, and 149 in acetone in three portions of 25 µL to reach target concentrations of 10,000 µg/m^3 for PCB 2 and 28, and 1000 µg/m^3 for PCB 149. These initial concentrations were at least 75% below the aqueous solubility of the compounds. Further details on spiking are provided as supporting information.

3.2.4 Macrophytes

Myriophyllum spicatum (Linnaeus 1753) (Eurasian water milfoil, dicotyledonous) and *Elodea canadensis* (Michx) (water pest, monocotyledonous) (Table S3), were collected from uncontaminated ditches at the experimental station The Sinderhoeve in Renkum, The Netherlands. A random selection of pregrown healthy macrophytes of similar size was used for the experiment. A subsample of 10 individuals per species was used for chemical analyses of background concentrations. Ten individuals were used for the determination of lipid content,[271] which was expressed as percentage based on wet weight. Lengths of main and side shoots, number of side shoots and roots, wet and dry weight of roots and shoots, concentrations in shoots and roots were determined at start and at the end of the experiment. (further method details provided as SI).

3.2.5 Macrophyte bioaccumulation test

The experiment followed the test protocol accepted by OECD for *Myriophyllum spicatum*,[272] with modifications regarding sediment layering and spiking (see SI for details). The 28-day test was conducted in a climate room at 18 ºC under white fluorescent light with an average (standard deviation (SD)) light intensity of 156 (16) µE m^{-2} s^{-1} and a photoperiod of 16 h light:8 h dark. Tested treatments were (A) "capped" system i.e. with a polytetrafluoroethylene (Teflon) impermeable layer at the sediment-water interface modified from Hinman and Klaine[67] and with the macrophyte stem penetrating the Teflon layer, and (B) "open" system i.e. without impermeable layer (Figures 1, S2). The Teflon cap in treatment (A) separated the sediment from the overlying water to specifically detect translocation by the macrophytes. Treatment (B) represented conditions in a standard toxicity test set up and accounted for all naturally occurring pathways. Non-spiked control treatments were (n=3): capped without acetone, open without acetone, open with acetone (to detect acetone spike effects), and Teflon layer cap penetrated by a stainless steel rod instead of the macrophytes stems to check on leakage through the barrier (details provided as SI). After 1, 3, 7, 14, and 28 d, three pots per treatment were sacrificed for chemical analysis. Chlorophyll, dissolved oxygen (DO) concentrations, pH, and temperature were recorded weekly (see SI for details).



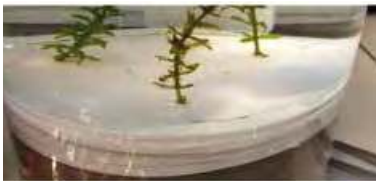


Figure 1. Test set up showing the Teflon impermeable layer to prevent direct sediment-water exchange.

3.2.6 Extraction and analyses

For details on extraction, detection, and quality assurance, the reader is referred to the SI (Tables S4-S6). In short, overlying water samples were hexane extracted, macrophyte samples were acetone extracted, and sediment samples were extracted with accelerated solvent extraction (ASE). POM samplers were Soxhlet extracted. Extracts were analysed

on a gas chromatograph with a μ-electron capture detector. Data were corrected for blanks. Analytical recoveries for macrophyte samples ranged between 75.6% and 101.8%, and for sediment between 77.3% and 96.6%.

3.2.7 Data analyses

Root and shoot relative growth and chemical concentrations were calculated on the basis of DW. Relative growth rate data were checked for normality with Q-Q plots and for equality of variances with Levene's test and tested with an ANOVA with a significant level α=0.05 using IBM SPSS Statistics version 19.

Sediment-water partition coefficients ($K_{P,SED}$) were calculated as the ratio of DW based concentration in sediment (C_{SED}) and POM based concentration in pore water (C_{PW}), after pre-equilibration. Macrophyte-water partition coefficients (m^3/kg) after 28 days of bioaccumulation were calculated for shoots ($K_S = C_S/C_{OW}$) and roots ($K_R = C_R/C_{PW}$) based on concentration in shoots (C_S), roots (C_R), overlying water (C_{OW}), and pore water (C_{PW}) and biota sediment accumulation factors (BSAF; -) were calculated as $BSAF=C_R/C_{SED}$.

3.2.8 Modelling chemical flows in sediment systems with rooted macrophytes

Model definition. Following first order mass balance modelling concepts[273,274], a model was developed that accounts for mass transfer across overlying water, shoot, root, and sediment interfaces and translocation (Figure 2, Table S7). The concentration in overlying water (C_{OW}; μg/m^3) as a function of time (t) can be described by transport between overlying water and pore water, between overlying water and shoots, and by a lumped first order loss (volatilization, degradation, photolysis) rate constant (k_{LOSS}; d^{-1}):

$$\frac{dC_{OW}}{dt} = \frac{K_L A_{SED}}{V_{OW}}\left(C_{PW} - C_{OW}\right) + \frac{P_S A_{S,t}}{V_{OW}}\left(\frac{C_S}{K_S} - C_{OW}\right) - k_{LOSS} C_{OW} \quad (1)$$

with K_L (m/d) the benthic boundary layer (BBL) mass transfer coefficient, A_{SED} (m^2) sediment water interface surface, V_{OW} (m^3) volume of overlying water, P_S (m/d) shoot chemical permeability coefficient, $A_{S,t}$ (m^2) shoot surface in overlying water, C_{pw} (μg/m^3) concentration in pore water, C_S (μg/kg) concentration in shoots, and K_S (m^3/kg) shoot-water partition coefficient. Time dependent parameters carry subscript 't' and are calculated with auxiliary functions (see below).

The concentration in pore water can be described by transport between overlying water and pore water, and pore water and roots:

$$\frac{dC_{PW}}{dt} = \frac{K_L A_{SED}}{V_{PW}}\left(C_{OW} - C_{PW}\right) + \frac{P_R A_{R,t}}{V_{PW}}\left(\frac{C_R}{K_R} - C_{PW}\right) \quad (2)$$

with P_R (m/d) root chemical permeability coefficient, $A_{R,t}$ (m^2) root surface, V_{PW} (m^3) *apparent* pore water volume[273-275], and K_R (m^3/kg) root-water partition coefficient. The apparent pore water volume is defined as:

$$V_{PW} = V'_{PW} + K_{P,SED} M_{SED} \quad (3)$$

with V'_{PW} (m^3) sediment interstitial pore water volume, $K_{P,SED}$ (m^3/kg) sediment-water partition coefficient (Table 1), and M_{SED} (kg DW) sediment mass.

The concentration in shoots can be described by transport between overlying water and shoots, and transport between shoots and roots (translocation):

$$\frac{dC_S}{dt} = \frac{P_S A_{S,t}}{M_{S,t}}\left(C_{OW} - \frac{C_S}{K_S}\right) + \frac{P_{TR} A_{TR,t}}{M_{S,t}}\left(\frac{C_R}{K_R} - \frac{C_S}{K_S}\right) \quad (4)$$

with P_{TR} (m/d) translocation mass transfer coefficient, $A_{TR,t}$ (m^2) time dependent stem cross-sectional area, and $M_{S,t}$ (kg DW) mass of shoots.

The concentration in roots (C_R; µg/kg) can be described by transport between pore water and roots, and transport between shoots and roots:

$$\frac{dC_R}{dt} = \frac{P_R A_{R,t}}{M_{R,t}}\left(C_{PW} - \frac{C_R}{K_R}\right) + \frac{P_{TR} A_{TR}}{M_{R,t}}\left(\frac{C_S}{K_S} - \frac{C_R}{K_R}\right) \quad (5)$$

The time dependent masses $M_{R,t}$ and $M_{S,t}$ were modelled assuming first order growth with rate constants (d^{-1}) for root ($k_{G,R}$) or shoot ($k_{G,S}$), calculated from measured data. Growth made a relevant contribution to modelling uptake as neglecting it resulted in different modelled concentrations, e.g. about 30% in macrophytes for PCB 28 (pilot simulations not shown). Surface areas $A_{R,t}$ and $A_{S,t}$ were calculated as the product of these masses ($M_{R,t}$ and $M_{S,t}$) and the specific surface areas 25 m^2/kg for *E. canadensis* and 40 m^2/kg for *M. spicatum*.[276] Stem cross-sectional area (A_{TR}) was calculated from measured stem biomass and length over time, assuming a cylindrical shape and constant density of the stem. Equations for these calculations are provided as SI.

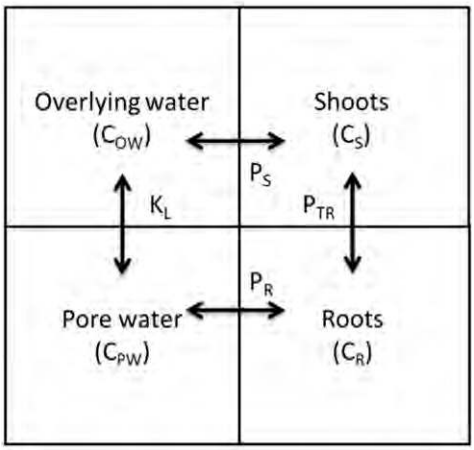


Figure 2. Schematic model description for uptake, translocation and elimination in a sediment, water, and submerged macrophyte system with C_{OW} the concentration in overlying water, C_S the concentration in shoots, C_R the concentration in roots, C_{PW} the concentration in pore water, K_L the benthic boundary layer mass transfer coefficient, P_S the shoot chemical permeability coefficient, P_{TR} the translocation mass transfer coefficient, and P_R the root chemical permeability coefficient.

Parameter estimation

The above model equations were implemented in Mathematica 8.0 (Wolfram Research). Parameters for PCBs and CPF were estimated accounting for experiment-specific boundary conditions. Additional optimizations were done with data from the open and capped

systems combined. For LIN, data from five exposure concentrations[126] were combined. As many parameters as possible were set at independently measured or estimated values (e.g. K_S and K_R). If a value was not available for a PCB, the value of the partner PCB within the chemically identical congener pair was used. This assumes that PCBs with (practically) identical log K_{ow} have identical K_S or K_R within error limits. This assumption is supported by earlier evidence for bioconcentration of hydrophobic organic chemicals to macrophytes being driven by hydrophobic partitioning into lipids.[161] For LIN, a value for K_S was calculated from earlier data.[126] The LIN K_R value was estimated using a significant regression between log K_R and log K_{oc}[268] (log K_R=(0.892 ± 0.118 log) K_{oc} – (0.372 ± 0.239) (R^2=0.80) (Figure 3), constructed with K_R values for CPF, PCB 3, 29, and 155 measured for *E. canadensis* and *M. spicatum*. For PCBs and CPF in capped systems, K_L was set to 0, because mass transfer across the cap was negligible. For open systems, K_L was set to the established literature value of 0.025 m/d, which was assessed in similar systems.[275] The previously published LIN systems[126] used a sand bed as a layer of limited permeability. The mass transfer coefficient in this layer was estimated to be 2.38×10^{-4} m/d based on an in-bed and BBL dual mass transfer resistance model, which is detailed in the SI (Table S8). Because LIN mass transfer across the sand bed was very limited, uncertainty in this parameter was of marginal importance. Initial pore water and overlying water concentrations were based on measured concentrations. Remaining parameters were optimized i.e. shoot and root permeability coefficients (P_S and P_R) and translocation mass transfer coefficient (P_{TR}). For volatile PCBs 2 and 3, also the loss rate constant (k_{LOSS}) was optimized. Model input parameters are summarized in Table S9. Equations 2-5 were solved with the Mathematica function NDSolve. Goodness-of-fit of the model was calculated using Pearson's Chi^2 statistic. Confidence intervals of 90% (α = 0.90) for the parameters were calculated using the likelihood-profiling method as described previously.[277] Confidence limits wider than two orders of magnitude were not reported. For further details, the reader is referred to the SI.

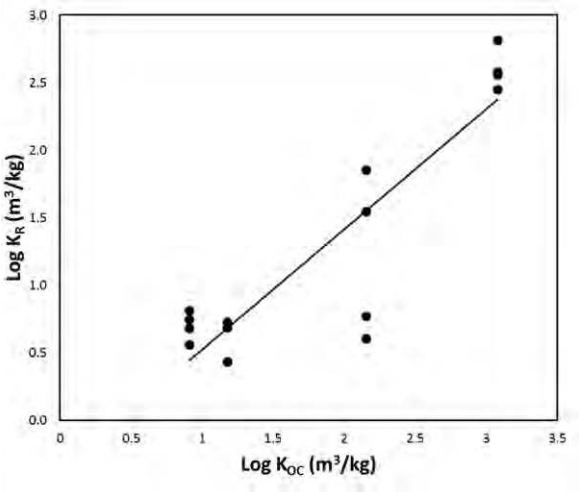


Figure 3. Relation between the root-water partition coefficient (K_R; m^3/kg) and organic carbon-water partition coefficient (K_{oc}; m^3/kg). Regression line: log K_R=(0.892 ± 0.118) log K_{oc} – (0.372 ± 0.239) (R^2=0.80).

3.3 Results and discussion

3.3.1 General evaluation and features of the test

Macrophyte performance

During the test, a good water quality was attained with an average (SD) water temperature of 21.5 (1.7) ºC, pH 9.14 (0.86), 13.01 (3.44) mg/L dissolved oxygen, and conductivity 396 (98) µS/m for all treatments (Table S10). For the control treatments, coefficients of variation for measured total length and weight were below 35% and in most cases below 25% at day 28 (Table S11), which meets the validity requirements for macrophyte tests.[272,278] Macrophytes grew better in open systems then in capped systems, although for *M. spicatum* differences were small and within the range of experimental variation and published values.[153] In open systems, measured macrophyte endpoints total length and biomass met the requirement of a doubling within a period of 14 days.[272] For *E. canadensis*, measured (ANOVA p=0.003 for specific growth rate for total length) and modelled specific growth rates were higher in open systems compared to capped systems (Table S12). Main stem growth for *E. canadensis* was less than the growth in total length, which can be explained by its growth strategy that is to invest more in side shoots. Moreover, development of water roots might have hampered root development in the capped systems, where roots could not penetrate into the sediment. For *M. spicatum*, the specific growth rates were similar between capped and open systems.

Average (SD) lipid content was 2.1 (0.54)% for *E. canadensis*, and 0.2 (0.09)% for *M. spicatum* at the start of the experiment. The value for *M. spicatum* is very similar to the 0.2 (0.02)% reported by Gobas et al.[159].

Efficiency of the impermeable layer

In the capped control treatment, none of the PCBs spiked in the overlying water layer were found in the sediment after 28 days. For PCBs and CPF spiked in the sediment layer, average concentrations in overlying water were below detection limit. Based on these data we conclude that the Teflon cap was practically impermeable during the test.

Spiking losses and mass balance

Concentrations in overlying water measured 20 min after spiking ranged between 27% (PCB 2) and 62% (PCB 28) of nominal concentrations. Concentrations in sediment measured after 28d pre-equilibration ranged from 66% (PCB 3) to 95% (PCB 155) of nominal concentrations at t=0 (Table S6). Thus, in both compartments the more volatile PCBs deviated more from the nominal concentration, suggesting volatilization losses for these PCBs, as expected. Total mass for chemicals spiked in overlying water decreased rapidly and was between 24% and 0% of initial mass after 28 d. Total mass for chemicals spiked in sediment was stable for the first 14 d, after which the mass started to decrease slightly. CPF losses were highest, which is in accordance with the shorter half-life of CPF, observed earlier in sediment water systems.[267] Other than volatilization, loss of chemicals might also be due to chemical or biological degradation e.g. metabolism by macrophytes or microorganisms. Not much is known, however, about sequestering and transformation of hydrophobic chemicals in macrophytes. These

processes are often assumed to be of minor importance compared to loss of chemicals in the sediment-water compartments e.g. due to macrophyte-induced pH changes or to dissolved organic carbon (DOC) exudates absorbing the chemicals.[159,161,279] Moreover, chemical uptake by the Teflon layer (<0.4%) and algae (<0.1%) showed a negligible contribution in the mass balance (calculations not shown). It must be noted that conservation of mass was not aimed for in these systems that were designed to mimic actual open systems as used in toxicity tests with macrophytes. For details on measured concentrations, see Tables S13-S18 and for details on mass balances, see Tables S19-S22.

Bioconcentration factors

Measured K_S and K_R values (Table S23) were in general higher for PCBs with a higher hydrophobicity. This pattern for root partitioning was in agreement with literature.[261,280,281] Shoot partitioning, however, differs from the typical patterns for terrestrial and emerged plants[157,280-282] and the theory stating that uptake and translocation diminishes with increasing K_{ow}.[283] For more hydrophobic PCBs, K_R values were much higher than K_S, reflecting differences in shoot and root tissue composition. BSAFs range between 0.6 and 2.9 for *E. canadensis* (Table S23) after lipid and OM normalization, which agrees well to values between 1.35 and 3.05 reported for *Elodea nuttallii* after 4 months of equilibration[71], and which is also close to the range of 1-2 suggested by equilibrium partitioning theory.[284]

3.3.2 Chemical flows in sediment-water macrophyte test systems

In general, similar exposure patterns over time were observed for *E. canadensis* and *M. spicatum* in capped and open systems for both water and sediment spiked chemicals (Figures 4, S2). Below, we discuss water and sediment spiked chemicals separately.

Water spiked PCBs

In the first 3 days, concentrations of PCBs 2, 28, and 149 in overlying water decreased rapidly whereas concentration in shoots and roots rapidly increased (Figures 4A, S2 and Tables S13, S14, S17, S18). After a maximum was reached, concentrations in water, shoots, and roots gradually decreased, a decrease that was less for more hydrophobic PCBs. Concentrations in overlying water decreased more rapidly during the first day in capped systems than in open systems. Although we cannot provide a conclusive explanation, this might be caused by higher DOC concentrations being present in open systems, leading to higher concentrations of DOC-associated PCBs in the water layer. No steady state was reached within 28 days, although water and shoot concentrations had a constant ratio after 7-14 days, confirming that equilibrium had been reached.

Sediment spiked PCBs, CPF, and LIN

Concentrations of PCBs 3, 29, 155, and CPF in sediment decreased slightly, whereas the concentration in the roots increased rapidly during the first days and then increased slowly during the remaining days of exposure (Figures 4B, S2 and Tables S15, S18). Chemicals in shoots were detected mainly in open systems compared to only a few cases in capped systems. Concentrations in overlying water were a factor 10-100X higher for open systems than in capped systems. CPF concentrations in overlying water were 10-100X higher than PCB concentrations,

and PCB 3 concentrations were slightly higher than PCB 29 and 155 concentrations (Figures 4B, S2). Both observations can be explained by the lower hydrophobicity and higher solubility of CPF and PCB 3. For capped systems, chemical concentrations in overlying water increased earlier in time than in shoots, or even when no chemicals were detected in the shoots at all. This could be caused by some incidental leakage through the impermeable layer, although the control showed that the layer worked well. Another explanation could be that translocation was initially high and decreased over time while elimination to overlying water occurred very fast, decreasing concentrations in shoots below the limit of detection. In previous experiments in our lab where OECD sediment was spiked with LIN, similar patterns were observed[126]: an initial rapid increase of LIN in roots and shoots of *M. spicatum* during the first week, after which an equilibrium was reached. Steady state was reached only in open systems on day 28, where the overlying water concentrations appeared to approach pore water concentrations.

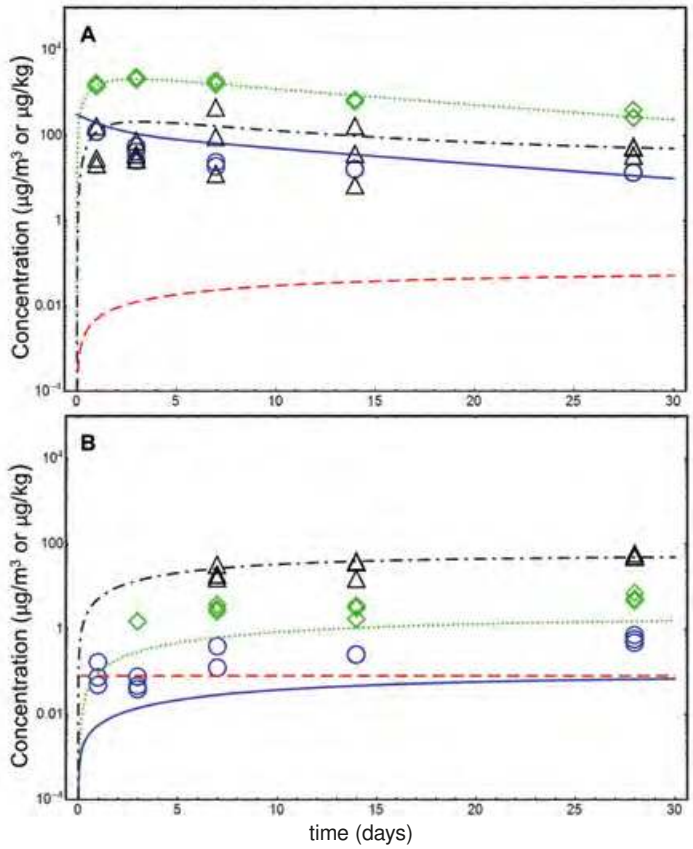


Figure 4. Measured (symbols) and modelled (curves) concentrations in overlying water (blue circles ○, solid line; µg/m³), pore water (red dashed line; µg/m³), shoots (green diamonds ◊, dotted line; µg/kg), and roots (black triangle Δ, dash dot line; µg/kg) for water spiked PCB 149 (**A**) and sediment spiked PCB 155 (**B**) for *Elodea canadensis* in open systems.

3.3.3 Modelling flows between water, macrophytes, and sediment

The multicompartment model provided good fits to the observed data for most chemicals (Figures 4, S2). More non-detects occurred in sediment-spiked systems than in water spiked systems, which affected the number of data available for parameter estimation and therefore affected the precision of parameter values and confidence limits (Figure 5, Table S24). For PCB 3, the number of meaningful confidence limits was lowest, which is explained from the low number of data. Although chemical concentrations in roots were unavailable for LIN, parameter estimates were quite accurate because multiple experimental data sets were combined in the optimization.

To our knowledge, the modelling yielded the first mass transfer parameter set reported for bioaccumulation in macrophytes in sediment-water systems (Figure 5, Table S24). For more than half of the estimated parameters, satisfactory narrow confidence limits were found (Figure 5), which allow for further interpretation. Overall, the chemical permeability coefficients seem to vary across macrophyte species and chemicals (Figure 5, Table S24), yet seem to be similar for capped and open systems. This confirms that the process descriptions (Eqs 1-5) and parameterisations are valid in both capped and open systems and that parameter estimation can also be done with combined capped and open system data (Table S25).The latter combined estimations yielded similar parameter values and ranges as the separate sets, albeit that the number of estimated confidence limits was slightly higher, at the cost of losing experiment-specific (i.e. capped vs. open systems) information. Therefore, the separate parameter sets are discussed here.

Shoot chemical permeability coefficients (P_S) were fairly similar across species and chemicals (Figure 5) implying that the resistance of cell walls in the shoots does not substantially change with hydrophobicity.[261] The root chemical permeability coefficients (P_R) were in general higher than shoot chemical permeability coefficients, which imply that root permeation is easier than shoot permeation (Figure 5). P_R values for water-spiked chemicals were higher than the sediment spiked chemicals especially for *E. canadensis*. It can be hypothesized that this overall slower permeation of chemicals from the sediment phase can be explained from a fraction of total chemical concentration being bound to DOC, which therefore is less bioavailable. P_R value for LIN was lower compared to the other chemicals, possibly due to the lower log K_{ow} that might cause LIN to be transported more easily by the water stream into the roots.

Translocation might occur from roots to shoots when the chemical passes the endodermis[282] and enters the xylem and from shoots to roots when the chemical passes the cuticle and enters the phloem. Translocation coefficients (P_{TR}) were much higher than P_S and P_R (Table S24). Note, however, that the translocation values were calculated relative to stem cross sectional area, which was very small, yielding a much smaller difference when fluxes are compared (discussed below). Furthermore, we hypothesize that the higher values might reflect the result of water flows in the stem including some DOC facilitated transport of hydrophobic test chemicals.[274,285] It is expected that transport trough the phloem and xylem depends on solubility and hydrophobicity, thus both a soluble and a hydrophobic chemical

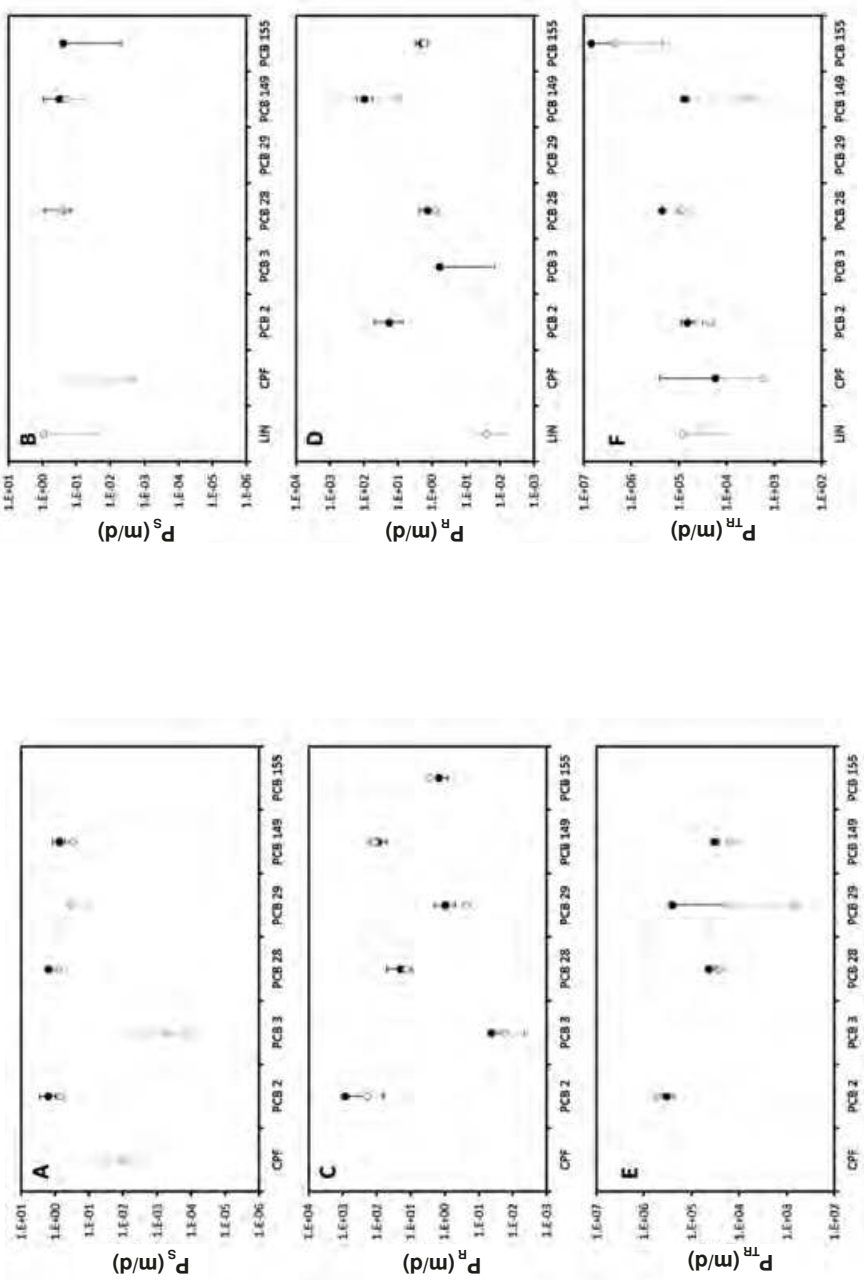


Figure 5. Optimized model parameters and 90% confidence limits (CL) for: shoot chemical permeability coefficient (P_S m/d) (**A**, **B**), root chemical permeability coefficient (P_R; m/d) (**C**,**D**), and translocation mass transfer coefficient (P_{TR}; m/d) (E,F) for capped system (black circles ●) and open systems (grey open circles ○) and *Elodea canadensis* (**A**, **C**, **E**), and *Myriophyllum spicatum* (**B**, **D**, **F**). LIN, CPF, PCB 3, PCB 29 and, PCB 155 are sediment spiked. PCB 2, PCB 28 and PCB 149 are water spiked. Parameter values are only included if a 90% confidence limit could be assessed in at least one direction.

might have a high translocation potential by either water or DOC. For *M. spicatum,* this trend of increasing translocation coefficients with increasing $\log K_{ow}$ was observed whereas for *E. canadensis* a slightly decreasing trend was observed. CPF is expected to quickly cross biomembranes but then might sorb to lipid membranes of the inner root tissue[286] while translocation of CPF to shoot biomass is low.[282] Our results for CPF did not show different P_{TR} values compared to those for PCBs with similar $\log K_{ow}$. Also P_{TR} for LIN was similar to other chemicals (Figure 5). In capped sediment spiked systems, concentrations in shoots did not align with model predictions as most values were below detection limits except for the first few days for *M. spicatum*. High translocation values might account for the overestimation.

3.3.4 Relative importance of transport pathways in whole-sediment test systems with sediment-rooted macrophytes

Knowledge on the chemical transport and exposure pathways is important for the development and interpretation of sediment-rooted macrophyte tests such as proposed by the OECD[272]. Therefore, chemical transport fluxes (µg/d) were calculated across the interfaces between the four compartments: sediment, overlying water, shoots, and roots (Eqs S13-S16), using the parameters from Table S24.

In general, initial fluxes are high and directed towards the compartments with lower fugacity as the system strives for equilibrium (Figures 6, S3, S4). After this initial phase, fluxes decrease and might even change direction, for instance like for water to shoot exchange of PCB 149 in the water spiked systems (Figure S3, panels 26-29). In the capped systems, macrophytes can take up, translocate, and eliminate organic chemicals both from overlying water to pore water and vice versa via roots and shoots (Figures S3, S4). This confirms that macrophytes can act as a chemical pump and thus can contribute to the redistribution of chemicals in aquatic ecosystems as was proposed earlier by Roessink et al.[71]. However, the relative importance of this pathway depends on the role of direct BBL transfer, which can only be assessed by analysing the open systems. Therefore, below, we mainly discuss the open systems, as these are most relevant for test development and field situations.

Water spiked PCBs in open systems

Water spiked systems seem to approach a state with low fluxes faster than sediment spiked systems (Figures 6A, S3). The major pathway was from overlying water to shoots, then from shoots to roots and then from roots to pore water. For PCB 2, translocation was more important than direct transport from overlying water to pore water (Figure S3, panels 10-13). With increasing hydrophobicity, the flux from overlying water to pore water became more important. For PCB 149 (Figure 6A), the flux across the BBL dominated the translocation flux at start and became less important over time.

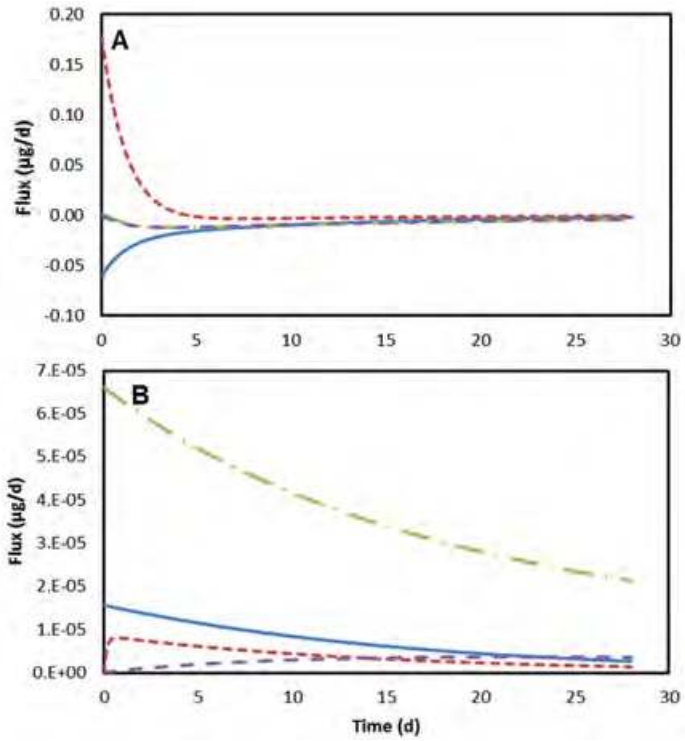


Figure 6. Chemical fluxes (µg/d) from pore water to overlying water (blue solid line), from overlying water to shoots (red dotted line), from pore water to roots (green dash dot line), and from roots to shoots (purple dash line) for water spiked PCB 149 (A) and sediment spiked PCB 155 (B) for *Elodea canadensis* in open systems. Note the differences in scale on the y-axes.

Sediment spiked PCBs, CPF, and LIN in open systems

Patterns for sediment-spiked chemicals were less clear than for water-spiked chemicals, probably due to the lower confidence of the parameters. For LIN, the increased concentrations in the shoots were explained from translocation by the roots as concentrations of LIN in overlying water were 1000 times lower than those in pore water.[126] The fluxes calculated for LIN (Figure S3, panels 1-5) supports this interpretation. At the start, both pathways from pore water to overlying water and from pore water to roots played an important role. Later, the contribution of the flux from pore water to overlying water decreased while at the same time translocation fluxes from roots to shoots increased followed by a flux of LIN from shoots to overlying water. For CPF and *E. canadensis* (Figure S3, panel 6, 7), there was an initial flux from overlying water to pore water, which after 9 days switched from pore water to overlying water due to the initial CPF concentration in overlying water. Uptake in the macrophyte was from pore water to roots and overlying water to shoots. Translocation and elimination did not occur. For CPF for *M. spicatum* and PCB 3 (Figure S3, panels 8, 9 and14-17), the flux from pore water to overlying water was higher than the translocation flux for *E. canadensis* whereas for *M. spicatum* these two fluxes were similar. In all cases,

except for PCB 3 accumulating in *M. spicatum*, the flux was directed from overlying water to shoots. For PCB 29 accumulating in *E. canadensis*, the BBL flux dominated over the translocation flux (Figure S3, panel 23) and uptake was by shoots from overlying water and by roots from pore water. For *M. spicatum*, chemical fluxes were difficult to interpret although it appeared that the BBL and the translocation fluxes were similar. For PCB 155 accumulating in *E. canadensis*, the BBL flux dominated the translocation flux at start while later the dominance switched whereas for *M. spicatum* translocation dominated the BBL flux during the entire 28 d test period. In general, the BBL flux dominated translocation for *E. canadensis* whereas the opposite was observed for *M. spicatum*. This might explained by the higher water flow in *M. spicatum* compared to *E. canadensis* (Table S3). The presently modelled fluxes indicated that translocation from roots to shoots occurred. Previous reports on exposure from sediment spiked systems showed translocation for atrazine (log K_{ow}=2.7), and to some extent for lindane (log K_{ow}=5.2) and chlordane (log K_{ow}=5.6) in *H. verticillata*[67], for 3-phenoxybenzoic acid (log K_{ow}=3.91) in *M. elatinoides*[260], for linuron (log K_{ow}=3.0) in *M. spicatum*[126], and for polycyclic aromatic hydrocarbons (logK_{ow}=3.4-6.2) in *Zostera marina*[287]. PCBs, however, were not measured in shoots of *Z. marina* apart from low concentrations after 32 weeks, indicating that no translocation occurred. Terrestrial plants and emerged macrophytes show more translocation for PCBs (e.g.[288,289]), probably because of transport induced by evaporation. Suppression of translocation, however, might occur as an active process in submerged macrophytes, as has been shown for *Myriophyllum aquaticum* and 2,4 D.[290]

In summary, for flux- and concentration temporal patterns of water spiked chemicals in open systems the major pathway was from overlying water to shoots to roots and then to pore water. With increasing hydrophobicity, the direct overlying water to pore water exchange became more important. For sediment spiked chemicals in open systems, the major pathway was parallel transport from pore water to roots and to overlying water, followed by translocation from roots to shoots. Depending on the chemical, shoots could take up from or release to overlying water. For *E. canadensis*, BBL transfer was more important than translocation for all sediment spiked chemicals whereas for *M. spicatum* translocation was more important expect for CPF.

3.4 Implications

This work showed that an exposure period of 28 days might not be sufficient for sediment spiked toxicity tests with sediment-rooted macrophytes as the uptake from sediment and translocation to shoots is a slow chemical- and species specific process and equilibrium is only reached after 28 days. For macrophyte toxicity tests with a spiked water layer, 28 days are sufficient as chemicals were more rapidly translocated from shoot to root than the other way around. In both cases however, the chemical transport processes are highly dynamic and assessing exposure in the test would require sufficiently frequent sampling of macrophyte biomass for chemical analysis.

This work further showed that chemical flows in macrophyte-sediment-water test systems can be understood using first order mass balance modelling concepts. Using this type of parameterised models, optimum test duration and conditions can be designed a priori as part of a prospective risk assessment framework. Vice versa, actual exposure in a test can be assessed using the modelled concentration profiles. Furthermore, the model parameters can be applied for modelling hydrophobic organic chemical fate under natural conditions in the field, especially for stagnant systems, where the relative importance of root to shoot transfer compared to sediment to water to shoot transfer also depends on the macrophyte density. The model can be applied to other species when accounting for differences in parameter values and required process formulations (e.g. exposure routes). When linking chemical exposure to effects this model can be used as input for population effect models, which could serve as a tool in environmental risk assessment.

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Supporting information

Table S1. Overview of chemical uptake, translocation and elimination routes in macrophytes, and possible mechanisms that might prevent this.

		Transport routes	Mechanisms
Roots	Mucigel	Apoplastic	Accumulation
	Epidermis	Apoplastic; Symplastic	Accumulation; blockage
	Cortex	Apoplastic; Symplastic	
	Endodermis	Apoplastic; Symplastic	Blockage by casparian strip and suberin deposition
	Pericycle	Apoplastic; Symplastic	
Shoot	Xylem	Symplastic from roots to shoots	
	Phloem	Symplastic from shoots to roots	Blockage by sieve-tube elements
	Cell walls	Apoplastic	
Leaves	Lower or upper epidermis	Apoplastic; Symplastic	Accumulation; blockage
	Cutile	Apoplastic; Symplastic	Accumulation; blockage
	Stoma		Blockage

Materials and Methods

Chemicals and materials

PCBs standards IUPAC numbers 2, 3, 28, 29, 143 (internal standard), 149, 155, CPF (purity 98.0 %) and CPF-D10 (internal standard) were obtained from Dr. Ehrenstorfer. Other chemicals used were n-hexane and acetone (Promochem; picograde), methanol (Mallinckrodt Baker, Deventer, The Netherlands; HPLCgradient grade), acetonitrile (Lab-Scan, Dublin, Ireland; HPLC grade), Barnstead Nanopure water (Sybron-Barnstead, Dubuque, IA, USA), and calcium chloride (Merck; p.a), sodium azide (Merck; p.a.). Polyoxymethylene sheets (POM; thickness 76 µm) were obtained from CS Hyde Company, Lake Villa, IL, USA. For OECD sediment peat from Klasmann Deilmann Benelux BV, $CaCO_3$ powder from Sigma Aldrich, Germany, quartz sand from Geba 0.06-0.25 mm, Eurogrid, The Netherlands and kaolin from Sigma Aldrich, German was used.

Sediment and water medium

Standard sediment (OECD 218[76] with small modification described in ISO 16191) was prepared, in four batches of 10 kg dry weight, by mixing peat (5%), $CaCO_3$ powder (2%), and an aqueous nutrient (Na_3PO_4•$12H_2O$ and NH_4Cl) medium of 0.36 g P/L and 0.30 g N/L to obtain a homogeneous slurry. After three batches were spiked with PCBs and CPF, and thoroughly mixed, quartz sand (75%), and kaolin clay (18%) were added to each of the four batches. Barko and Smart medium[147] consists of 91.7mg/L $CaCl_2.2H_2O$, 69.0mg/L $MgSO_4.7H_2O$, 58.4 mg/L $NaHCO_3$, 15.4 mg/L $KHCO_3$.

Sediment samples were taken at start and end of the experiment to determine wet weight, dry weight (24h at 105°C), organic matter (OM) (3h at 550°C) and organic carbon (OC) (2h at 950°C) content. Sediment had an average (standard deviation (SD)) pH of 6.12 (0.03), and OM content of 6.46 (0.14)% at the start of the experiment. The moisture content was 33.7 (0.7)%.

Table S2. Sediment characteristics at start of equilibrium period, start of the experiment, and per species end of the experiment.

	Water content (%)		OM (%)	
	average	**SD**	**average**	**SD**
t=-28 (n=12)	33.73	0.68	6.46	0.14
t=0 (n=12)	33.85	0.69	6.30 (n=11)	0.11
t=28 M (n=27)	31.72	1.95	5.59	0.71
t=28 E (n=27)	30.20	0.65	6.12	0.32

Spiking procedure

Sediment was spiked with PCBs 3, 29, 155, and CPF in acetone to reach target concentrations of 20 µg/kg for these PCBs and 40 µg/kg for CPF. All concentrations are expressed on a sediment dry weight (DW) basis. These target concentrations have been shown to yield detectable concentrations in macrophytes.[69] The CPF spike target concentration was higher to compensate for possible degradation. PCB spike solution was added to the sediment in five portions of 1 mL with 30 minutes of vigorous agitation in between. The volume of acetone was less than 0.098% (v:v), well below the recommended level of ISO.[122] Polyoxymethylene (POM) passive samplers[82] were added to the sediment to acquire in situ pore water concentrations at start of exposure (see below). To assure a state of (pseudo-)equilibrium between chemicals and sediment prior to exposure (discussed in Chapter 2),[259] sediment with POM samplers were agitated for four weeks on a roller bank in the dark. After seven days, the solvent was allowed to evaporate in a fume hood. After two weeks, CPF stock solution was spiked into the sediment, thoroughly mixed, and the solvent was allowed to evaporate seven days later, after which CPF was equilibrated for one more week. Consequently, PCBs had a pre-equilibration of four weeks and CPF, which equilibrates faster, two weeks.

Macrophytes

Preparation of macrophytes

The macrophytes were gently rinsed with demineralized water, then cut off at 8 cm, and planted with three nodes in an aquarium (40 x 64 cm) containing a 7 cm sediment layer consisting of potting soil, and natural clay in a 1:1 ratio, and 25 L of Barko and Smart medium.[147] Macrophytes were pre-grown for seven days under conditions mimicking the experimental conditions. Afterwards, macrophytes were taken from the aquaria and carefully cleaned with demineralized water.

Table S3. Test species characteristics.

Species	Specific leaf area (mm^2/mg)[a]	Average lipid content (SD) (%)[b]	Water flow plant (SD) (µl H_2O g^{-1} plant DW h^{-1})[291]	Water flow shoot (SD) (µl H_2O g^{-1} shoot DW h^{-1})[291]	Water flow root (SD) (µl H_2O g^{-1} root DW h^{-1})[291]
Myriophyllum spicatum (Dicotyledonous)	15-33	0.2 (0.09)	16 (3)	18 (3)	165 (30)
Elodea canadensis (Monocotyledonous)	25.7-59	2.1 (0.54)	11 (4)	12 (4)	146 (12)

[a] TRYdatabase[276], [b] measured in this experiment, values are based on wet weight.

Macrophyte bioaccumulation test

Test set up. Glass pots (370 mL) were filled with OECD sediment of 450 g (corresponding to 298 g dry weight) (160 g of each container in case of spiked sediment). A thin layer (30 g) of fine quartz sand was put on the top of the sediment in order to reduce suspension of sediment into the water. Three shoots were carefully planted in each pot. Pots were placed in 2 L beakers filled with 1.5 L Barko and Smart medium. Pot locations were randomly varied during the test to prevent influence from the light conditions. Water loss was compensated by adding demineralized water weekly. Lamps used were Philips 400 W HPI-T.

Impermeable layer

The impermeable layer existed of a Teflon plate with three holes (diameter of 2 mm). From each hole to the edge, a small incision was made, which enabled us to place the main stem into the hole without damaging the macrophyte. To cover the hole, sulphur free plasteline (NPS non-drying modelling clay medium Chavant™) was used on the sediment side of the Teflon. The layer was sealed onto the glass pot with Teflon tape.

Control treatments

To check if the test system (e.g. the Teflon layer) had any influence, a control and a solvent control spiked with appropriate amount of acetone were used. In order to quantify any potential leakage by the Teflon layer a control with spiked water and sediment but without macrophytes was used. An 8 cm stainless steel bar replaced each macrophyte.

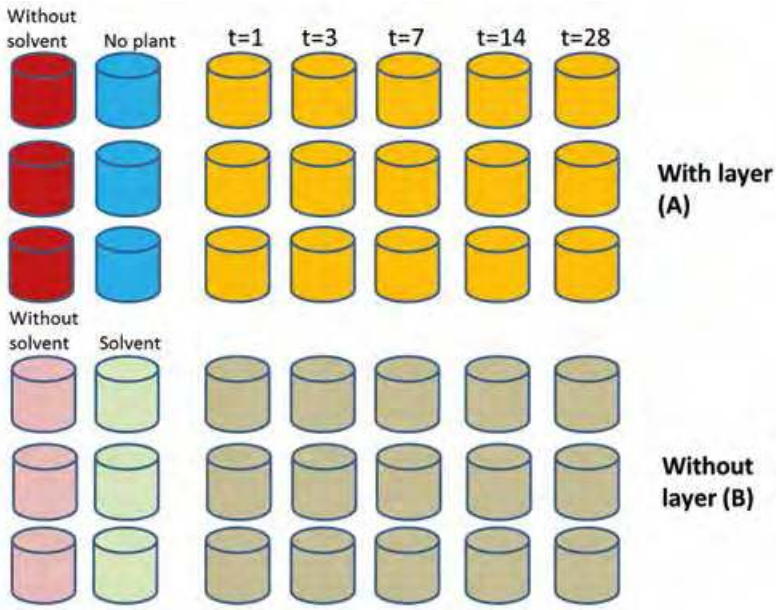

Figure S1. Overview of the experimental design

Water quality

Oxygen (Wissenschaftlich-Technische Werkstätten (WTW) Oxi 330), pH and temperature (WTW pH 323) and conductivity (WTW Cond 315i) were recorded weekly and each time when pots were taken out for chemical analyses. Algae growth (brown, green, blue) was determined by chlorophyll measurements (Phyto-pam, WAL2 mess and Regeltechiek) in a mixture of three separate samples (each 2 mL) from one test unit when pots were taken out for chemical analyses.

Extraction and analyses

Analytical verification

Fifteen minutes after spiking the water phase, a water sample was taken and extracted. Samples of 25 mL with 2 mL n-hexane were shaken, and vortexed. At the end of each treatment, 750 mL of water was transferred into a dark green bottle, 50 ml of n-hexane was added and shaken for at least 45 minutes. In some bottles, no clear separation of water and hexane was achieved. Bottles, therefore, were sonicated for at least 15 minutes and stored at 4°C. The n-hexane was carefully transferred to a glass tube and evaporated under a gentle flow of nitrogen until approximately 200 µL. Then, 50 µL of internal standard (PCB 143) was added, mixed, and stored in an insert vial for subsequent analyses.

Macrophyte shoots and roots from one treatment were pooled (i.e. 3 shoots or roots from 1 pot) to obtain sufficient material for analysis. *M. spicatum* root samples at t=1, and t=3 were pooled (i.e. 9 roots from 3 pots). Samples were transferred into a mortar and liquid

nitrogen was added. The macrophytes were crushed to almost powder and the sample was transferred into a 100 mL centrifuge tube. A known volume of ± 30 mL acetone was added to the sample and the tubes were vigorously shaken for 30 minutes on a shaking apparatus at a speed of 175 r.p.m.. A known amount of acetone was transferred into a 25 ml test tube and the extract was evaporated to just dryness. The samples were redissolved in approximately 1.5 mL of hexane followed by a clean-up procedure on florisil according to Brock et al.[292]. After chemical analysis, plant material was dried in an oven (70 °C for 24 h) to determine dry weight.

Sediment was extracted with ASE (accelerated solvent extraction) technique. Sediment samples were intensively homogenized with a small spoon. A subsample was transferred in a mortar and mixed with a sufficient amount of diatomeous earth. This mix was transferred into a 100 mL ASE cell and was extracted at a temperature of 100 °C with ± 75 mL hexane:acetone (6:1 v/v) mixture. The test tubes were evaporated to a volume between 10 and 20 ml by placing them without stopper in the fume hood. The extract was transferred into a graduated test tube of 25 mL and it was evaporated to a known volume of ± 2 mL. The samples were analysed without any clean-up.

Pore water concentrations at t=0 were measured using passive sampler polyoxymethylene sheets (POM; thickness 76 µm). POM was prepared by cutting sheet into pieces (approx. 400 mg), and cleaned with hexane (30 min) and methanol (3 times 30 min), following previously published procedures.[82] Air dried pieces were directly added to the spiked sediment (3 pieces to 10 kg DW). After equilibration, POM strips were dried with a tissue, and Soxhlet-extracted. Concentrations were calculated from concentrations in POM and POM-water equilibrium partition coefficients (Kpom).[269] Kpom values for CPF were calculated from the regression of the SP-LFER model provided by Endo et al.[270].

Samples were analysed on a Hewlett Packard 6890N gas chromatograph equipped with a µ-ECD detector. Splitless injections were done at 225°C on a HP5MS column with a 0.25µm film thickness. The following temperature program was run: Initial temperature: 70°C; Initial time: 1 min; Rate A: 25°C/min; Final temperature A: 250°C; final time A: 0 min; Rate B: 3°C/min; Final temperature B: 280°C; Final time B: 5 min. The LOD for PCB's is 0.1 ng/l.

Quality assurance

Limit of quantification (LOQ) depended upon sample intake, typically this was <10 ng/L for water and pore water and <50 ng/L for shoots, roots and sediment for PCBs and < 200 ng/L for chloropyrifos.

Background concentrations in macrophytes were below detection limit except for PCB 28 with an average (SD) of 5.0 (1.2) µg/kg DW, and PCB 149 of 0.4 (0.4) µg/kg DW for *E. Canadensis*, and PCB 28 of 2.0 (0.4) µg/kg DW, and PCB 149 of 0.4 (0.1) µg/kg DW for *M. spicatum*. Macrophytes concentrations were corrected for background concentrations. Background concentrations in sediment were below detection limit except for PCB 28, 29, 149 and 155 and CPF. The concentrations ranged between 0.04 µg/kg DW for PCB 29

and 2.9 µg/kg DW for PCB 28. Background concentrations in water were below detection limit except for PCB 2, 28 and CPF. The concentrations ranged between 0.43 ng/L for PCB 2, and 6.25 ng/L for CPF. Overlying water concentrations in controls were mainly below detection limit or very low with maximal concentration of 49 ng/L for CPF.

Table S4. Average (SD) recovery percentage per test chemical for sediment and macrophytes.

Average (SD) %	PCB 2	PCB 3	PCB 28	PCB 29	PCB 149	PCB 155	CPF (n=2)
Sediment (n=2)	78 (8)	77 (8)	93 (12)	97 (12)	88 (13)	83 (12)	92 (8)
Macrophytes (n=5)	76 (10)	76 (10)	96 (9)	90 (9)	92 (11)	87 (8)	102 (36)

Table S5. Limit of quantification per test chemical for water, shoots, roots, sediment, and pore water in µg/kg after correction with internal standard.

Average (SD)	PCB 2	PCB 3	PCB 28	PCB 29	PCB 149	PCB 155	CPF (n=2)
Water	0.01	0.01	0.01	0.01	0.01	0.01	0.2
Shoots	0.05	0.05	0.05	0.05	0.05	0.05	0.2
Roots	0.05	0.05	0.05	0.05	0.05	0.05	0.2
Sediment	0.05	0.05	0.05	0.05	0.05	0.05	0.2
Pore water	0.01	0.01	0.01	0.01	0.01	0.01	0.2

Table S6. Nominal and measured chemical concentrations in overlying water (µg/L) (n=60) and sediment (µg/kg) (n=3) at start of the experiment (t=0).

	Chemical	Nominal concentration	Measured concentration	% of nominal
Overlying Water (µg/L)	PCB 2	10	2.8 (0.7)	27
n=60	28	10	6.2 (2.6)	62
	149	1	0.6 (0.1)	59
Sediment (µg/kg)	3	20	13.1 (1.2)	66
n=3	29	20	15.2 (1.3)	76
	155	20	18.9 (1.6)	95
	Chlorpyrifos	40	27.1 (11.1)	68

Modelling chemical flows in sediment systems with rooted macrophytes

Table S7. Overview of model parameters.

Symbol	Parameter	Unit
A_{SED}	surface of sediment water interface	m^2
$A_{S,t}$	surface of shoot in overlying water	m^2
$A_{R,t}$	surface of root in pore water	m^2
$A_{TR,t}$	stem cross-sectional area at the sediment-water interface	m^2
$A_{SP,S}$	shoot specific surface area	m^2/kg
$A_{SP,R}$	root specific surface area	m^2/kg
C_{OW}	chemical concentration in overlying water	$\mu g/m^3$
C_{PW}	chemical concentration in pore water	$\mu g/m^3$
C_S	chemical concentration in shoot	µg/kg
C_R	chemical concentration in root	µg/kg
K_L	benthic boundary layer mass transfer coefficient	m/d
K_S	shoot-water partition coefficient	m^3/kg
K_R	root-water partition coefficient	m^3/kg
$K_{P,SED}$	sediment-water partition coefficient	m^3/kg
k_{LOSS}	lumped first order loss (volatilization, degradation, photolysis) rate constant	d^{-1}
$k_{G,l}$	first order growth rate constant for growth of main stem	d^{-1}
$k_{G,R}$	first order growth rate constant for growth of root	d^{-1}
$k_{G,S}$	first order growth rate constant for growth of shoot	d^{-1}
l_t	length of the main stem	m
$M_{R,t}$	mass of roots	kg DW
$M_{S,t}$	mass of shoots	kg DW
M_{SED}	mass of sediment	kg DW
P_R	root chemical permeability coefficient	m/d
P_S	shoot chemical permeability coefficient	m/d
P_{TR}	translocation mass transfer coefficient	m/d
t	time	d
V_{OW}	volume of overlying water	m^3
V_{PW}	apparent pore water volume	m^3
V'_{PW}	sediment interstitial pore water volume	m^3

Model equations macrophyte growth

Macrophyte growth (Eq. 6, 7) and change of shoot and root surface areas (Eq. 8, 9) over time were accounted for through the following auxiliary functions. Mass (kg DW) of root ($M_{R,t}$) and shoot ($M_{S,t}$) were modelled exponentially, using first order growth rate constants (d^{-1}) for root ($k_{G,R}$) or shoot ($k_{G,S}$), which were based on measured data.

$$M_{R,t} = M_{R,t=0} e^{k_{G,R} t} \quad \text{(S1)}$$

$$M_{S,t} = M_{S,t=0} e^{k_{G,S} t} \quad \text{(S2)}$$

Surface area (m^2) for root ($A_{R,t}$) in pore water and shoot ($A_{S,t}$) in overlying water was determined by macrophyte growth, and specific surface area (m^2/kg) of root ($A_{SP,R}$) or shoot ($A_{SP,S}$). Specific surface area for roots and shoots were defined as 25 m^2/kg for *E. canadensis* and 40 m^2/kg for *M. spicatum*.[276]

$$A_{R,t} = A_{SP,R} M_{R,t} \quad \text{(S3)}$$

$$A_{S,t} = A_{SP,S} M_{S,t} \quad \text{(S4)}$$

Stem cross-sectional area ($A_{TR,t}$; m^2) was calculated from relative stem biomass growth, assuming a cylindrical shape and constant density of the stem:

$$A_{TR,t} = A_{t=0} \frac{l_{t=0}}{l_t} e^{k_{G,R} t} \quad \text{(S5)}$$

Length of the main stem (l_t; m) was modelled exponentially, with the first order length growth rate constant ($k_{g,l}$) deduced from measured data.

$$l_t = l_{t=0} e^{k_{g,l} t} \quad \text{(S6)}$$

Parameter estimation

For the optimisation of parameters, the Mathematica function *NMinimize* was used with the *SimulatedAnnealing* optimisation algorithm to find for each of the experiments a parameter set for which the value of Pearson's Chi^2 statistic was minimal. Options for *SimulatedAnnealing* included "*PerturbationScale= 3, SearchPoints =25, MaxIterations=200*".

Rough initial parameter estimates were used as starting values for the optimisation in order to take into account that various orders of magnitude of the parameter values are expected from theory.

Goodness-of-fit of the model was calculated using the Pearson's Chi2 statistic defined as:

$$Chi^2(Y,\Theta) = \sum_{Y \in (C_W, C_R, C_S)} \sum_{i=1}^{n} \frac{\left(Y_i - S_i(\Theta)\right)^2}{Y_i} \quad \text{(S7)}$$

where there are n observations in time, Yi are the measured concentrations in overlying water (CW), in roots (CR), and shoots (CS), and Si are the corresponding model simulations at time points i using the parameter vector θ.

Calculation of the overall sediment-water mass transfer coefficient for linuron

The transfer parameters for linuron across the sediment bed can be a priori calculated based on established mass transfer theory.[293] In the linuron experiments, the contaminated sediment and overlying water phase were separated by a 0.5 cm clean layer of sediment. This means that this transfer experiences a resistance from the benthic boundary layer (BBL) as well as from the transfer through the sediment bed. Transfer across the sediment bed can be modelled as molecular diffusion retarded by sorption to the organic matter in the sediment, with corrections for the diffusion path of linuron in the sediment based on porosity and tortuosity.

The overall resistance to mass transport $1/K_L$ is:

$$\frac{1}{k_L} = \frac{1}{k_{L,BBL}} + \frac{1}{k_{L,SED}} \quad \text{(S8)}$$

in which $k_{L,BBL}$ is the BBL mass transfer coefficient (0.025 m/d) and $k_{L,SED}$ is the mass transfer coefficient in the sediment bed. The value for $k_{L,SED}$ can be calculated as:

$$k_{L,SED} = \frac{D_{eff}}{\delta} \quad \text{(S9)}$$

with D_{eff} is the effective diffusion coefficient for linuron in the sediment bed (m^2/d) and δ is the thickness of the sediment layer (m).

The effective diffusion coefficient D_{eff} can be calculated as:

$$D_{eff} = \frac{D_m \Phi}{(1-\Phi)\sigma K_D \tau} \quad \text{(S10)}$$

with D_m is the molecular diffusion coefficient for linuron in water (m^2/d), Φ is sediment porosity (-), σ is the density of the sediment (kg/L), K_D is the equilibrium distribution coefficient for sorption of linuron to the sediment (L/kg) and τ (-) is the tortuosity of the diffusion path.

$$K_D = K_{oc} f_{oc} \quad \text{(S11)}$$

The overall value for k_L (eq 1) was calculated by substitution of eq 2 and 3 in eq 1 and using the parameters as indicated in Table S9, which yields a value of $5.86*10^{-4}$ m/d.

Table S8. Parameters used for the calculation of the overall sediment-water mass transfer coefficient for linuron.

Parameter	Value	Unit	Reference
Density of the sediment (σ)	1.208	kg/L	126
Fraction of organic carbon (f_{oc})	0.02	-	126
Organic carbon - water equilibrium distribution coefficient (K_{oc})	406	L/kg	126
Equilibrium distribution coefficient (K_D)	8.12	L/kg	126
Tortuosity (τ)	1.5	-	294
Sediment porosity (Φ)	0.464	-	126
Thickness of the sediment layer (δ)	0.005	m	126
Molecular diffusion coefficient (D_m)	$5.90*10^{-6}$	cm^2/s	295
Benthic boundary layer mass transfer coefficient ($k_{L,BBL}$)	0.025	m/d	275

Calculation of confidence intervals

The calculation of the confidence intervals of 90% (α = 0.90) for the parameters was processed using the likelihood-profiling method as described previously.[277] In short, for one of the parameters (i.e. the one for which the confidence intervals should be calculated), values were changed in steps starting at the optimal parameter value. For each changed parameter value, all other parameters were optimised resulting in a new optimal parameter set , and values for the Pearson's Chi^2 statistic were calculated for this changed parameter set. The procedure of changing the values of the parameter was repeated until either the condition:

$$\frac{X'}{X^*} = \frac{Chi(Y,\Theta')}{Chi(Y,\Theta^*)} \geq \frac{p}{n-p} F(p,n-p,90\%) \qquad \text{(S12)}$$

was fulfilled or the parameter was varied to a value of more than two orders of magnitude below or above the optimal parameter value. In equation 13, *n* is the number of data used in the optimization (n=XX), *p* is the number of fitted parameters (3 or 4), and *F* (*p,n-p*,90%) is the *F* distribution.

Table S9. Overview of model parameters for the macrophyte model. Abbreviations are: A to E are different concentration levels, EC *Elodea canadensis* capped system, EO *Elodea canadensis* open system, MC *Myriophyllum spicatum* capped system, MO *Myriophyllum spicatum* open system, BDL below detection limit, PF parameter was fitted.

		LIN					CPF			
	Unit	**A**	**B**	**C**	**D**	**E**	**EC**	**EO**	**MC**	**MO**
A_{SED}	m^2	0.0050	0.0050	0.0050	0.0050	0.0050	0.0079	0.0079	0.0079	0.0079
M_{SED}	kg DW	0.200	0.200	0.200	0.200	0.200	0.298	0.298	0.298	0.298
V_{OW}	m^3	0.002	0.002	0.002	0.002	0.002	0.0015	0.0015	0.0015	0.0015
V'_{PW}	m^3	0.00170	0.00170	0.00170	0.00170	0.00170	0.04868	0.04868	0.04868	0.04868
K_L	m/d	0.00024	0.00024	0.00024	0.00024	0.00024	0	0.025	0	0.025
$A_{SP,S}$	m^2/kg	40	40	40	40	40	25	25	40	40
$A_{SP,R}$	m^2/kg	40	40	40	40	40	25	25	40	40
$M_{R,init}$	kg DW	2.36E-06	2.90E-06	4.76E-06	3.85E-06	3.4E-06	1.44E-05	1.22E-05	1.58E-05	2.25E-05
$M_{S,init}$	kg DW	3.27E-04	3.58E-04	2.51E-04	3.17E-04	2.48E-04	5.50E-05	7.76E-05	1.66E-04	2.30E-04
$k_{G,R}$	d^{-1}	1.62E-01	1.46E-01	1.40E-01	1.38E-01	9.82E-02	2.47E-02	5.42E-02	4.53E-02	1.88E-02
$k_{G,S}$	d^{-1}	5.02E-02	4.06E-02	7.02E-02	4.49E-02	3.25E-02	2.70E-02	4.14E-02	3.32E-02	3.09E-02
l_{init}	m	1.19E-01	1.03E-01	1.09E-01	1.01E-01	1.24E-01	6.24E-02	6.88E-02	1.01E-01	1.34E-01
$k_{G,l}$	m/d	2.32E-02	2.97E-02	3.00E-02	3.14E-02	4.07E-03	5.53E-03	3.41E-04	2.56E-02	1.15E-02
$A_{TR,init}$	m^2	1.13E-08	1.13E-08	1.13E-08	1.13E-08	1.13E-08	7.85E-09	7.85E-09	1.13E-08	1.13E-08
$C_{OW,init}$	$\mu g/m^3$	0	0	0	0	0	3.97	4.57	0	0
$C_{PW,init}$	$\mu g/m^3$	BDL	2545	33650	447000	1079500	3.22	3.22	3.22	3.22
K_S	m^3/kg	0.4	0.4	0.4	0.4	0.4	3	4	2	1
K_R	m^3/kg	0.19	0.19	0.19	0.19	0.19	5.5	6.4	3.6	4.7
k_{LOSS}	d^{-1}	0	0	0	0	0	0	0	0	0

Table S9 (Continued).

		PCB 2				PCB 3				PCB 28			
	Unit	**EC**	**EO**	**MC**	**MO**	**EC**	**EO**	**MC**	**MO**	**EC**	**EO**	**MC**	**MO**
A_{SED}	m^2	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079
M_{SED}	kg DW	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298
V_{OW}	m^3	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015
V'_{PW}	m^3	0.09027	0.09027	0.09027	0.09027	0.09027	0.09027	0.09027	0.09027	0.85504	0.85504	0.85504	0.85504
K_L	m/d	0	0.025	0	0.025	0	0.025	0	0.025	0	0.025	0	0.025
$A_{SP,S}$	m^2/kg	25	25	40	40	25	25	40	40	25	25	40	40
$A_{SP,R}$	m^2/kg	25	25	40	40	25	25	40	40	25	25	40	40
$M_{R,init}$	kg DW	1.44E-05	1.22E-05	1.58E-05	2.25E-05	1.44E-05	1.22E-05	1.58E-05	2.25E-05	1.44E-05	1.22E-05	1.58E-05	2.25E-05
$M_{S,init}$	kg DW	5.50E-05	7.76E-05	1.66E-04	2.30E-04	5.50E-05	7.76E-05	1.66E-04	2.30E-04	5.50E-05	7.76E-05	1.66E-04	2.30E-04
$k_{G,R}$	d^{-1}	2.47E-02	5.42E-02	4.53E-02	1.88E-02	2.47E-02	5.42E-02	4.53E-02	1.88E-02	2.47E-02	5.42E-02	4.53E-02	1.88E-02
$k_{G,S}$	d^{-1}	2.70E-02	4.14E-02	3.32E-02	3.09E-02	2.70E-02	4.14E-02	3.32E-02	3.09E-02	2.70E-02	4.14E-02	3.32E-02	3.09E-02
l_{init}	m	6.24E-02	6.88E-02	1.01E-01	1.34E-01	6.24E-02	6.88E-02	1.01E-01	1.34E-01	6.24E-02	6.88E-02	1.01E-01	1.34E-01
$k_{G,l}$	m/d	5.53E-03	3.41E-04	2.56E-02	1.15E-02	5.53E-03	3.41E-04	2.56E-02	1.15E-02	5.53E-03	3.41E-04	2.56E-02	1.15E-02
$A_{TR,init}$	m^2	7.85E-09	7.85E-09	1.13E-08	1.13E-08	7.85E-09	7.85E-09	1.13E-08	1.13E-08	7.85E-09	7.85E-09	1.13E-08	1.13E-08
$C_{OW,init}$	$\mu g/m^3$	2308.07	2137.07	3171.37	3106.15	0	0	0	0	1057	1826.76	3337.32	3542
$C_{PW,init}$	$\mu g/m^3$	0	0	0	0	4.29	4.29	4.29	4.29	0	0	0	0
K_S	m^3/kg	3	4	2	1	3	4	2	1	37	22	22	16
K_R	m^3/kg	2.7	2.7	5.3	4.8	2.7	2.7	5.3	4.8	34.8	71.0	4.0	5.9
k_{LOSS}	d^{-1}	PF	PF	PF	PF	PF	PF	PF	PF	0	0	0	0

Table S9 (Continued).

		PCB 29				PCB 149				PCB 155			
	Unit	**EC**	**EO**	**MC**	**MO**	**EC**	**EO**	**MC**	**MO**	**EC**	**EO**	**MC**	**MO**
A_{SED}	m^2	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079
M_{SED}	kg DW	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298
V_{OW}	m^3	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015
V'_{PW}	m^3	0.85504	0.85504	0.85504	0.85504	10.34702	10.34702	10.34702	10.34702	7.15835	7.15835	7.15835	7.15835
K_L	m/d	0	0.025	0	0.025	0	0.025	0	0.025	0	0.025	0	0.025
$A_{SP,S}$	m^2/kg	25	25	40	40	25	25	40	40	25	25	40	40
$A_{SP,R}$	m^2/kg	25	25	40	40	25	25	40	40	25	25	40	40
$M_{R,init}$	kg DW	1.44E-05	1.22E-05	1.58E-05	2.25E-05	1.44E-05	1.22E-05	1.58E-05	2.25E-05	1.44E-05	1.22E-05	1.58E-05	2.25E-05
$M_{S,init}$	kg DW	5.50E-05	7.76E-05	1.66E-04	2.30E-04	5.50E-05	7.76E-05	1.66E-04	2.30E-04	5.50E-05	7.76E-05	1.66E-04	2.30E-04
$k_{G,R}$	d^{-1}	2.47E-02	5.42E-02	4.53E-02	1.88E-02	2.47E-02	5.42E-02	4.53E-02	1.88E-02	2.47E-02	5.42E-02	4.53E-02	1.88E-02
$k_{G,S}$	d^{-1}	2.70E-02	4.14E-02	3.32E-02	3.09E-02	2.70E-02	4.14E-02	3.32E-02	3.09E-02	2.70E-02	4.14E-02	3.32E-02	3.09E-02
l_{init}	m	6.24E-02	6.88E-02	1.01E-01	1.34E-01	6.24E-02	6.88E-02	1.01E-01	1.34E-01	6.24E-02	6.88E-02	1.01E-01	1.34E-01
$k_{G,l}$	m/d	5.53E-03	3.41E-04	2.56E-02	1.15E-02	5.53E-03	3.41E-04	2.56E-02	1.15E-02	5.53E-03	3.41E-04	2.56E-02	1.15E-02
$A_{TR,init}$	m^2	7.85E-09	7.85E-09	1.13E-08	1.13E-08	7.85E-09	7.85E-09	1.13E-08	1.13E-08	7.85E-09	7.85E-09	1.13E-08	1.13E-08
$C_{OW,init}$	$\mu g/m^3$	0	0	0	0	144.58	308.26	658.98	891.38	0	0	0	0
$C_{PW,init}$	$\mu g/m^3$	0.83	0.83	0.83	0.83	0	0	0	0	0.08	0.08	0.08	0.08
K_S	m^3/kg	37	22	22	16	58	23	36	18	58	23	36	18
K_R	m^3/kg	34.8	71.0	4.0	5.9	375.8	649.8	278.6	360.3	375.8	649.8	278.6	360.3
k_{LOSS}	d^{-1}	0	0	0	0	0	0	0	0	0	0	0	0

Results

Water quality

Average (SD) water quality values were water temperature 21.5 (1.7) ºC, pH 9.14 (0.86), oxygen 13.01 (3.44) mg/L, and conductivity 396 (98) µS/m for all treatments over all time points. Blue green algae were not measured at any time point. Brown algae developed slightly over time. At 28 days, average concentration ranged from 0 to 58 µg/l for *Elodea Canadensis*, and 0 to 25 µg/l for *Myriophyllum spicatum* treatments. Green algea concentrations followed the same trend and increased to max 76.42 µg/l for *M. spicatum* treatments.

Table S10. Average (SD) water quality parameters per treatment and species.

Treatment/species		pH (-)	SD	Temp.(°C)	SD	O_2 (mg/L)	SD	Cond. (µS/cm)	SD
Capped	***Elodea canadenis***								
	Control	8.71	0.35	21.7	0.6	10.37	0.95	334	36
	Spiked	8.36	0.68	20.0	1.3	10.26	2.19	355	75
	Myriophyllum spicatum								
	Control	9.65	0.39	23.3	0.6	14.48	2.86	359	50
	Spiked	9.44	0.54	20.7	1.1	13.75	2.65	322	26
	No Macrophyte								
	Leakage control	8.39	0.48	21.9	1.0	9.84	2.32	342	33
Open	***Elodea canadenis***								
	Control	8.76	1.60	21.4	0.8	12.46	2.77	477	107
	Control solvent	9.23	0.58	21.7	0.8	12.40	2.82	481	92
	Spiked	8.64	0.70	20.1	0.9	11.63	2.45	442	119
	Myriophyllum spicatum								
	Control	9.79	0.32	23.3	0.8	15.44	2.44	464	89
	Control solvent	9.93	0.43	23.5	0.8	15.74	3.12	467	91
	Spiked	9.73	0.48	22.6	1.0	15.71	3.59	419	95

Macrophyte endpoints

Table S11. Total length and biomass for *Elodea canadensis* and *Myriophyllum spicatum* in the control treatments (n=3) at t=28.

Treatment	Total Length (cm)			Total biomass (g DW)		
	Average	SD	CV (%)	Average	SD	CV (%)
Myriophyllum spicatum						
Capped control	42.1	10.7	25.4	0.1019	0.0134	13.1
Open control	40.1	0.8	2.1	0.1712	0.0575	33.6
Open solvent control	47.7	5.3	11.1	0.2180	0.0286	13.1
Elodea canadensis						
Capped control	14.4	3.5	24.2	0.0561	0.0086	15.3
Open control	16.3	3.6	21.9	0.0914	0.0099	10.8
Open solvent control	16.3	1.8	11.1	0.1151	0.0267	23.2

Table S12. Average (SD) specific growth rates (SGR) at day 28, and modelled growth rates (d^{-1}) based on exponential growth for *Elodea canadensis* and *Myriophyllum spicatum* for capped and open systems.

	Measured		Modelled		
	SGR total length	SGR total biomass	Growth rate main stem length (d^{-1})	Growth rate shoots (d^{-1})	Growth rate roots (d^{-1})
Elodea canadensis					
capped systems	0.025 (0.007)	0.029 (0.006)	0.006	0.027	0.025
open systems	0.053 (0.003)	0.044 (0.008)	0.0003	0.041	0.054
Myriophyllum spicatum					
capped systems	0.048 (0.011)	0.026 (0.008)	0.026	0.033	0.045
open systems	0.051 (0.006)	0.042 (0.013)	0.012	0.031	0.019

Chemical flows in sediment-water macrophyte systems

Table S13. Average overlying water concentrations (ng/L) (n=3) for *Elodea canadensis*.[a]

Elodea canadensis			Average concentrations in overlying water (ng/L) (n=3)						
Treatment		**Time (d)**	**PCB 2**	**PCB 3**	**PCB 28**	**PCB 29**	**CPF**	**PCB 149**	**PCB 155**
Background	B&S	0	0.34	0.00	0.92	0.00	6.25	0.00	0.00
Controls	AC	0	0.00	BDL	0.00	BDL	9.04	BDL	BDL
	BC	0	0.50	BDL	1.66	BDL	49.49	BDL	BDL
	BS	0	0.33	BDL	1.83	BDL	18.35	BDL	BDL
	AC	28	0.05	BDL	0.28	0.03	0.42	BDL	0.04
	BC	28	0.03	BDL	0.35	BDL	0.48	0.05	0.01
	BS	28	0.03	0.02	0.34	1.68	0.69	0.05	0.68
Control layer	AP	0	2305.63	BDL	3330.81	BDL	0.00	457.47	BDL
	AP	28	0.98	0.16	30.17	1.81	0.24	9.59	0.57
Capped(A)	A1	0	2186.10	BDL	4037.88	BDL	3.77	430.08	BDL
	A3	0	2138.76	BDL	4986.71	BDL	3.82	438.94	BDL
	A7	0	2481.49	BDL	3412.32	BDL	5.03	467.16	BDL
	A14	0	2450.29	BDL	3278.02	BDL	1.68	461.80	BDL
	A28	0	2271.50	BDL	5482.16	BDL	5.53	578.40	BDL
	A1	1	563.92	BDL	936.14	0.02	BDL	86.37	0.03
	A3	3	251.91	BDL	307.98	0.03	0.47	24.41	0.03
	A7	7	32.54	BDL	105.34	0.01	0.33	14.84	0.01
	A14	14	6.80	BDL	71.91	0.01	0.39	11.34	0.04
	A28	28	1.07	0.05	39.29	0.04	1.65	9.63	0.03
Open (B)	B1	0	1816.51	BDL	4764.33	BDL	2.97	443.92	BDL
	B3	0	2119.32	BDL	2485.28	BDL	5.35	494.11	BDL
	B7	0	2355.93	BDL	3636.19	BDL	7.55	508.86	BDL
	B14	0	2543.47	BDL	3504.40	BDL	6.67	507.90	BDL
	B28	0	1850.11	BDL	4803.75	BDL	0.31	466.55	BDL
	B1	1	770.92	BDL	1557.96	0.28	5.17	120.03	0.09
	B3	3	161.93	BDL	307.18	0.18	3.13	40.08	0.05
	B7	7	19.60	0.68	167.49	0.47	21.76	20.59	0.25
	B14	14	3.66	0.54	69.95	0.42	6.34	16.01	0.24
	B28	28	0.58	0.35	18.72	0.45	0.76	13.27	0.58

[a]B&S = Barko and Smart medium, BDL = below detection limit, , A=capped, B=open, C=non-spiked control, S=non-spiked solvent control

Table S14. Average overlying water concentrations (ng/L) (n=3) for *Myriophyllum spicatum*[a].

Myriophyllum spicatum			Average concentrations in water (ng/L) (n=3)						
Treatment		**Time (d)**	**PCB 2**	**PCB 3**	**PCB 28**	**PCB 29**	**CPF**	**PCB 149**	**PCB 155**
Controls	AC	0	3.85	BDL	4.90	BDL	0.00	BDL	0.26
	BC	0	3.13	BDL	7.35	BDL	31.41	BDL	0.53
	BS	0	9.93	BDL	12.01	BDL	BDL	BDL	0.50
	AC	28	0.02	BDL	0.12	BDL	BDL	BDL	BDL
	BC	28	0.05	BDL	0.19	BDL	2.71	BDL	BDL
	BS	28	0.05	BDL	0.30	BDL	3.91	BDL	BDL
Capped (A)	A1	0	2982.01	0.17	8428.22	BDL	BDL	711.09	BDL
	A3	0	3820.51	BDL	9813.79	BDL	0.00	818.36	0.13
	A7	0	3431.20	BDL	9469.89	BDL	BDL	734.91	BDL
	A14	0	2894.01	BDL	8651.83	BDL	BDL	704.97	BDL
	A28	0	2666.00	BDL	8117.33	BDL	BDL	702.94	BDL
	A1	1	1453.29	BDL	1640.13	BDL	0.03	127.11	BDL
	A3	3	402.43	BDL	456.80	0.01	0.37	47.72	0.01
	A7	7	117.55	BDL	211.24	0.01	0.97	37.99	0.11
	A14	14	11.10	BDL	171.07	BDL	0.31	26.73	BDL
	A28	28	1.32	BDL	75.87	BDL	BDL	17.04	BDL
Open (B)	B1	0	2726.35	BDL	6529.20	BDL	1.12	631.09	0.40
	B3	0	3066.61	BDL	8354.93	BDL	BDL	680.03	BDL
	B7	0	3321.29	BDL	7832.13	BDL	BDL	606.61	BDL
	B14	0	3222.20	BDL	8385.37	BDL	BDL	735.71	BDL
	B28	0	3194.32	BDL	8581.06	BDL	BDL	700.17	0.16
	B1	1	1723.52	BDL	1855.77	0.04	1.83	139.83	0.02
	B3	3	464.03	BDL	265.37	0.05	3.05	48.84	0.02
	B7	7	68.00	BDL	243.82	0.10	2.51	33.08	0.02
	B14	14	10.90	0.43	169.28	0.31	4.02	27.17	0.10
	B28	28	1.01	0.30	71.50	0.32	0.35	20.29	0.12

[a] BDL = below detection limit, A=capped, B=open, C=non-spiked control, S=non-spiked solvent control.

Table S15. Average sediment (µg/kg DW) (n=3) values spiked OECD sediment[a].

			Average concentration in sediment (µg/kg DW) (n=3)						
Treatment		**Time (d)**	**CPF**	**PCB 2**	**PCB 3**	**PCB 28**	**PCB 29**	**PCB 149**	**PCB 155**
Control (ng/kg)		0	0.93	BDL	BDL	39.84	0.04	0.14	1.07
Spiked		0	26.96	BDL	13.07	0.00	15.14	0.00	18.85
Capped (A)	P	28	30.94	BDL	12.70	0.00	14.05	0.00	16.93
Capped (A)	M	28	25.33	BDL	11.99	0.00	15.55	0.00	17.94
Open (B)	M	28	22.20	0.17	14.66	0.72	17.40	0.06	19.82
Capped (A)	E	28	18.35	0.07	11.32	0.00	14.38	0.00	17.21
Open (B)	E	28	18.00	0.15	11.46	0.47	13.62	0.18	15.87

[a] BDL = below detection limit, A=capped, B=open, P=Cap control without macrophytes, M=*Myriophyllum spicatum*, E=*Elodea canadensis*

Table S16. Average pore water (ng/L) concentrations at t=0 for spiked OECD sediment (n=3).

	CPF	PCB 3	PCB 29	PCB 155
Pore water (ng/L)	12.76	4.29	0.83	0.08
Standard deviation	10.21	1.51	0.45	0.02

Table S17. Average shoot concentrations (µg/kg DW) (n=3) for *Elodea canadensis* and *Myriophyllum spicatum*[a].

		Average concentration in shoots (µg/kg DW) (n=3)						
Treatment	**Time (d)**	**PCB 2**	**PCB 3**	**PCB 28**	**PCB 29**	**CPF**	**PCB 149**	**PCB 155**
Elodea canadensis								
A1	1	1360.80	BDL	14685.73	BDL	BDL	1723.01	BDL
A3	3	266.53	BDL	10388.72	BDL	BDL	1753.61	BDL
A7	7	78.29	BDL	6502.05	BDL	BDL	1673.91	BDL
A14	14	15.56	BDL	5376.12	BDL	BDL	1289.81	BDL
A28	28	3.29	BDL	1490.69	BDL	BDL	560.54	BDL
B1	1	998.72	BDL	15199.19	1.26	BDL	1486.14	BDL
B3	3	207.54	BDL	12307.87	1.88	BDL	2128.59	1.49
B7	7	78.87	BDL	8419.53	5.86	17.35	1746.51	3.06
B14	14	13.18	BDL	3049.24	3.52	12.15	647.60	2.75
B28	28	2.18	0.84	404.43	4.27	5.75	299.67	5.52
Myriophyllum spicatum								
A1	1	2539.09	BDL	12987.17	0.75	8.56	3164.40	0.42
A3	3	1397.08	BDL	13555.18	BDL	BDL	3846.20	BDL
A7	7	306.13	BDL	6570.86	BDL	2.67	2284.70	BDL
A14	14	72.02	BDL	2713.18	BDL	BDL	1198.17	BDL
A28	28	2.70	BDL	1323.22	0.26	BDL	539.17	BDL
B1	1	1878.44	BDL	14899.39	BDL	BDL	3437.88	BDL
B3	3	1249.44	BDL	9997.61	1.72	4.34	2815.42	0.91
B7	7	254.35	BDL	6247.57	1.11	2.73	1995.52	0.60
B14	14	61.62	BDL	3882.01	2.85	5.18	987.43	2.06
B28	28	1.45	0.49	1070.91	1.82	1.66	371.52	2.62

[a] BDL = below detection limit, A=capped, B=open

Table S18. Average root concentrations (µg/kg DW) (n=3) for *Elodea canadensis* and *Myriophyllum spicatum*[a].

		Average concentration in root (µg/kg DW) (n=3)						
Treatment	**Time (d)**	**PCB 2**	**PCB 3**	**PCB 28**	**PCB 29**	**CPF**	**PCB 149**	**PCB 155**
Elodea canadensis								
A1	1	26.77	BDL	321.47	6.56	32.92	32.65	BDL
A3	3	6.76	BDL	256.08	6.76	24.14	39.98	BDL
A7	7	24.44	BDL	224.88	12.71	62.84	24.99	BDL
A14	14	9.25	6.77	187.19	15.74	49.25	12.71	7.04
A28	28	BDL	11.56	415.99	28.89	70.20	80.99	30.06
B1	1	59.49	BDL	950.47	6.21	BDL	69.56	BDL
B3	3	12.65	BDL	445.97	10.67	33.61	44.44	BDL
B7	7	16.46	BDL	1505.42	28.54	89.93	181.83	21.82
B14	14	14.68	9.03	675.30	30.95	69.29	68.73	29.80
B28	28	BDL	11.52	292.21	58.96	81.93	45.58	51.98
Myriophyllum spicatum								
A1	1	714.49	BDL	4767.92	9.01	43.68	776.49	3.45
A3	3	108.11	6.44	458.19	10.81	BDL	51.28	4.38
A7	7	29.43	19.52	301.89	28.51	43.68	16.79	11.17
A14	14	8.48	23.44	275.22	40.52	51.14	18.19	17.13
A28	28	BDL	22.74	317.42	3.33	46.00	22.98	22.29
B1	1	88.94	6.28	226.41	11.16	16.74	23.94	5.58
B3	3	187.85	6.85	3537.00	23.73	28.41	475.13	10.03
B7	7	60.23	16.07	1587.37	29.23	38.66	324.70	14.38
B14	14	17.61	27.01	124.02	48.93	64.10	32.06	18.18
B28	28	BDL	20.61	336.87	4.86	60.57	22.12	28.82

[a] BDL = below detection limit, A=capped, B=open

Mass distribution of test chemicals over the compartments

Mass in overlying water for PCBs spiked in the water layer decreased rapidly with 0.92% (PCB 2 EB) to 5.17% (PCB 149 MA) of initial mass left after 7 days, to 0.03% (PCB 2 E and MB) to 3.02% (PCB 149 MB) of initial mass after 28 days. Mass decreased less for PCBs with a higher hydrophobicity.

Mass in sediment for PCBs and CPF spiked in the sediment layer was stable with 100% of initial mass left after 7 days, to 68% (CPF EA) to 114% (PCB 149 MB) of initial mass after 28 days. Mass decreased most for CPF in systems with *E. canadensis*. PCBs spiked in the water layer increased slowly over time, a maximum of 0.11% (PCB 149 EA) was found in the sediment on day 7, and 7.24% (PCB 149 EB) on day 28.

Table S19. Proportion of initial mass per day for *Elodea canadensis* in capped systems, based on measured concentrations.

A/E		Proportion of initial mass (%)					
	Time (d)	**Water**	**Shoots**	**Roots**	**Sediment**	**Sum**	**Loss**
PCB 2	1	24.46	1.94	0.00	0.00	26.4	73.6
	3	10.93	0.52	0.00	0.00	11.4	88.6
	7	1.41	0.15	0.01	0.00	1.6	98.4
	14	0.29	0.04	0.00	0.00	0.3	99.7
	28	0.05	0.01	0.00	0.61	0.7	99.3
PCB 3	1	0.00	0.00	0.00	100.00	100.0	0.0
	3	0.00	0.00	0.00	100.00	100.0	0.0
	7	0.00	0.00	0.00	100.00	100.0	0.0
	14	0.00	0.00	0.00	100.00	100.0	0.0
	28	0.00	0.00	0.00	86.58	86.6	13.4
PCB 28	1	22.08	11.43	0.09	0.00	33.6	66.4
	3	7.26	10.93	0.06	0.00	18.3	81.7
	7	2.48	6.54	0.04	0.00	9.1	90.9
	14	1.70	6.92	0.08	0.00	8.7	91.3
	28	0.93	2.87	0.17	0.00	4.0	96.0
PCB 29	1	0.00	0.00	0.00	100.00	100.0	0.0
	3	0.00	0.00	0.00	100.00	100.0	0.0
	7	0.00	0.00	0.00	100.00	100.0	0.0
	14	0.00	0.00	0.01	100.00	100.0	0.0
	28	0.00	0.00	0.02	94.99	95.0	5.0
PCB 149	1	18.17	11.92	0.08	0.11	30.3	69.7
	3	5.14	16.63	0.07	0.11	21.9	78.1
	7	3.12	15.38	0.04	0.11	18.6	81.4
	14	2.39	14.76	0.05	0.11	17.3	82.7
	28	2.03	8.99	0.29	0.00	11.3	88.7
PCB 155	1	0.00	0.00	0.00	100.00	100.0	0.0
	3	0.00	0.00	0.00	100.00	100.0	0.0
	7	0.00	0.00	0.00	100.00	100.0	0.0
	14	0.00	0.00	0.00	100.00	100.0	0.0
	28	0.00	0.00	0.00	91.34	91.3	8.7
CPF	1	0.00	0.00	0.00	99.93	99.9	0.1
	3	0.01	0.00	0.00	99.93	99.9	0.1
	7	0.01	0.00	0.01	99.93	99.9	0.1
	14	0.01	0.00	0.01	99.93	99.9	0.1
	28	0.03	0.00	0.00	67.99	68.0	32.0

Mass in shoots for PCBs spiked in the water layer increased first rapidly, then started to decrease again with 0.21% (PCB 2 EB) to 62.87% (PCB 149 MB) of initial mass after 7 days to 0.02% (PCB 2 MA, E and MB) to 19.02% (PCB 149 MB) of initial mass after 28 days.

Mass in shoots was higher and decreased less for PCBs with a higher hydrophobicity. Mass in *M. spicatum* was higher than mass in *E. Canadensis*. For PCBs and CPF spiked in the sediment layer, a maximum of 0.01% for PCBs (PCB 29 E and MB) and 0.02% CPF (EB) was found in shoots on day 7, and 0.02% for PCBs (PCB 2, 29, 155 MA, E and MB) and 0% for CPF on day 28.

Table S20. Proportion of initial mass per day for *Elodea canadensis* in open systems, based on measured concentrations.

B/E	Proportion of initial mass (%)						
	Time (d)	**Water**	**Shoots**	**Roots**	**Sediment**	**Sum**	**Loss**
PCB 2	1	36.07	2.26	0.00	0.00	38.3	61.7
	3	7.58	0.52	0.00	0.00	8.1	91.9
	7	0.92	0.21	0.00	0.00	1.1	98.9
	14	0.17	0.08	0.00	0.00	0.2	99.8
	28	0.03	0.02	0.00	1.41	1.5	98.5
PCB 3	1	0.00	0.00	0.00	100.00	100.0	0.0
	3	0.00	0.00	0.00	100.00	100.0	0.0
	7	0.00	0.00	0.00	100.00	100.0	0.0
	14	0.02	0.00	0.00	100.00	100.0	0.0
	28	0.01	0.00	0.02	87.64	87.7	12.3
PCB 28	1	40.58	19.23	0.31	0.00	60.1	39.9
	3	8.00	16.80	0.14	0.00	24.9	75.1
	7	4.36	12.59	0.46	0.00	17.4	82.6
	14	1.82	9.36	0.23	0.00	11.4	88.6
	28	0.49	1.64	0.30	2.42	4.8	95.2
PCB 29	1	0.01	0.00	0.00	100.00	100.0	0.0
	3	0.01	0.00	0.00	100.00	100.0	0.0
	7	0.02	0.01	0.01	100.00	100.0	0.0
	14	0.01	0.01	0.01	100.00	100.0	0.0
	28	0.01	0.02	0.07	89.91	90.0	10.0
PCB 149	1	24.79	14.85	0.18	0.10	39.9	60.1
	3	8.28	23.22	0.11	0.10	31.7	68.3
	7	4.25	20.75	0.44	0.10	25.5	74.5
	14	3.31	16.10	0.18	0.10	19.7	80.3
	28	2.74	9.58	0.38	7.24	19.9	80.1
PCB 155	1	0.00	0.00	0.00	100.00	100.0	0.0
	3	0.00	0.00	0.00	100.00	100.0	0.0
	7	0.01	0.00	0.01	100.00	100.0	0.0
	14	0.01	0.01	0.01	100.00	100.0	0.0
	28	0.02	0.02	0.05	84.23	84.3	15.7
CPF	1	0.10	0.00	0.00	100.00	100.1	-0.1
	3	0.06	0.00	0.01	100.00	100.1	-0.1
	7	0.41	0.02	0.02	100.00	100.4	-0.4
	14	0.12	0.00	0.02	100.00	100.1	-0.1
	28	0.00	0.00	0.06	66.76	66.8	33.2

Mass in roots for PCBs spiked in the water layer increased first, then started to decrease again with 0% (PCB 2 EB) to 1.51% (PCB 149 MB) of initial mass after 7 days to 0% (PCB 2) to 0.38% (PCB 149 EB) of initial mass after 28 days. Mass in roots was higher and decreased less for PCBs with a high hydrophobicity. For PCBs and CPF spiked in the sediment layer, a maximum of 0.03% for PCBs (PCB 29 MB) and 0.02% CPF (EB) was found in roots on day 7, and 0.07% for PCBs (PCB 29 EB) and 0.06% for CPF (EB) on day 28.

Table S21. Proportion of initial mass per day for *Myriophyllum spicatum* in capped systems, based on measured concentrations.

A/M	Proportion of initial mass (%)						
	Time (d)	**Water**	**Shoots**	**Roots**	**Sediment**	**Sum**	**Loss**
PCB 2	1	46.01	10.32	1.72	0.00	58.0	42.0
	3	12.74	4.80	0.13	0.00	17.7	82.3
	7	3.72	1.45	0.00	0.00	5.2	94.8
	14	0.35	0.37	0.01	0.00	0.7	99.3
	28	0.04	0.02	0.00	0.00	0.1	99.9
PCB 3	1	0.00	0.00	0.00	100.00	100.0	0.0
	3	0.00	0.00	0.01	100.00	100.0	0.0
	7	0.00	0.00	0.00	100.00	100.0	0.0
	14	0.00	0.00	0.03	100.00	100.0	0.0
	28	0.00	0.00	0.03	91.68	91.7	8.3
PCB 28	1	18.44	19.02	4.07	0.00	41.5	58.5
	3	5.13	15.48	0.20	0.00	20.8	79.2
	7	2.37	10.79	0.05	0.00	13.2	86.8
	14	1.92	4.99	0.09	0.00	7.0	93.0
	28	0.85	4.18	0.12	0.00	5.2	94.8
PCB 29	1	0.00	0.00	0.02	100.00	100.0	0.0
	3	0.00	0.00	0.01	100.00	100.0	0.0
	7	0.00	0.00	0.01	100.00	100.0	0.0
	14	0.00	0.00	0.04	100.00	100.0	0.0
	28	0.00	0.00	0.00	102.67	102.7	-2.7
PCB 149	1	17.31	56.71	8.04	0.07	82.1	17.9
	3	6.50	53.55	0.27	0.07	60.4	39.6
	7	5.17	45.87	0.03	0.07	51.1	48.9
	14	3.64	26.56	0.07	0.07	30.3	69.7
	28	2.32	20.25	0.10	0.00	22.7	77.3
PCB 155	1	0.00	0.00	0.01	100.00	100.0	0.0
	3	0.00	0.00	0.00	100.00	100.0	0.0
	7	0.00	0.00	0.00	100.00	100.0	0.0
	14	0.00	0.00	0.01	100.00	100.0	0.0
	28	0.00	0.00	0.02	95.18	95.2	4.8
CPF	1	0.00	0.00	0.06	100.00	100.1	-0.1
	3	0.01	0.00	0.00	100.00	100.0	0.0
	7	0.02	0.00	0.00	100.00	100.0	0.0
	14	0.01	0.00	0.03	100.00	100.0	0.0
	28	0.00	0.00	0.03	93.92	94.0	6.0

Table S22. Proportion of initial mass per day for *Myriophyllum spicatum* in open systems, based on measured concentrations.

B/M		Proportion of initial mass (%)					
	Time (d)	**Water**	**Shoots**	**Roots**	**Sediment**	**Sum**	**Loss**
PCB 2	1	55.49	6.59	0.09	0.00	62.2	37.8
	3	14.94	6.76	0.40	0.00	22.1	77.9
	7	2.19	1.74	0.06	0.00	4.0	96.0
	14	0.35	0.54	0.00	0.00	0.9	99.1
	28	0.03	0.02	0.00	1.11	1.2	98.8
PCB 3	1	0.00	0.00	0.01	100.00	100.0	0.0
	3	0.00	0.00	0.02	100.00	100.0	0.0
	7	0.00	0.00	0.02	100.00	100.0	0.0
	14	0.02	0.00	0.00	100.00	100.0	0.0
	28	0.01	0.01	0.01	112.17	112.2	-12.2
PCB 28	1	23.38	20.63	0.09	0.00	44.1	55.9
	3	3.34	21.38	2.94	0.00	27.7	72.3
	7	3.07	16.63	0.62	0.00	20.3	79.7
	14	2.13	13.09	0.06	0.00	15.3	84.7
	28	0.90	4.75	0.08	1.79	7.5	92.5
PCB 29	1	0.00	0.00	0.01	100.00	100.0	0.0
	3	0.00	0.01	0.05	100.00	100.1	-0.1
	7	0.00	0.01	0.03	100.00	100.0	0.0
	14	0.01	0.03	0.00	100.00	100.0	0.0
	28	0.01	0.02	0.00	114.89	114.9	-14.9
PCB 149	1	20.85	55.91	0.11	0.07	76.9	23.1
	3	7.28	71.03	4.68	0.07	83.1	16.9
	7	4.93	62.87	1.51	0.07	69.4	30.6
	14	4.05	39.28	0.16	0.07	43.6	56.4
	28	3.02	19.02	0.06	1.83	23.9	76.1
PCB 155	1	0.00	0.00	0.00	100.00	100.0	0.0
	3	0.00	0.00	0.02	100.00	100.0	0.0
	7	0.00	0.00	0.01	100.00	100.0	0.0
	14	0.00	0.01	0.02	100.00	100.0	0.0
	28	0.00	0.02	0.01	105.18	105.2	-5.2
CPF	1	0.03	0.00	0.01	100.00	100.0	0.0
	3	0.06	0.00	0.04	100.00	100.1	-0.1
	7	0.05	0.00	0.02	100.00	100.1	-0.1
	14	0.08	0.03	0.04	100.00	100.1	-0.1
	28	0.01	0.00	0.02	82.33	82.4	17.6

Table S23. Shoot and root-water partition coefficient (K_S Kr; m^3/kg) and BSAF (-) normalized on dry weight (DW) as well as on and lipids and organic matter (OM).

		DW normalized			*Lipid and OM normalized*		
		K_S (m^3/kg)	**K_R (m^3/kg)**	**BSAF (-)**	**K_S (m^3/kg)**	**K_R (m^3/kg)**	**BSAF (-)**
E. canadensis	*CPF*		5.5	2.6		47.9	1.4
Capped	*PCB 2*	3			48		
	PCB 3		2.7	0.9		31.9	0.6
	PCB 28	37			510		
	PCB 29		34.8	1.9		337.6	1.1
	PCB 149	58			828		
	PCB 155		375.8	1.6		3268.7	0.8
E. canadensis	*CPF*		6.4	3.0		78.8	2.3
Open	*PCB 2*	4			45		
	PCB 3		2.7	0.9		31.9	0.6
	PCB 28	22			266		
	PCB 29		71.0	3.9		860.8	2.9
	PCB 149	23			276		
	PCB 155		649.8	2.8		7920.2	2.1
M. spicatum	*CPF*		3.6	1.7		221.4	5.9
Capped	*PCB 2*	2			138		
	PCB 3		5.3	1.7		325.5	6.0
	PCB 28	22			1398		
	PCB 29		4.0	0.2		243.8	0.7
	PCB 149	36			2336		
	PCB 155		278.6	1.2		16765.3	4.0
M. spicatum	*CPF*		4.7	2.2		252.8	6.7
Open	*PCB 2*	1			72		
	PCB 3		4.8	1.6		228.3	4.2
	PCB 28	16			742		
	PCB 29		5.9	0.3		336.4	1.0
	PCB 149	18			914		
	PCB 155		360.3	1.5		18023.1	4.3

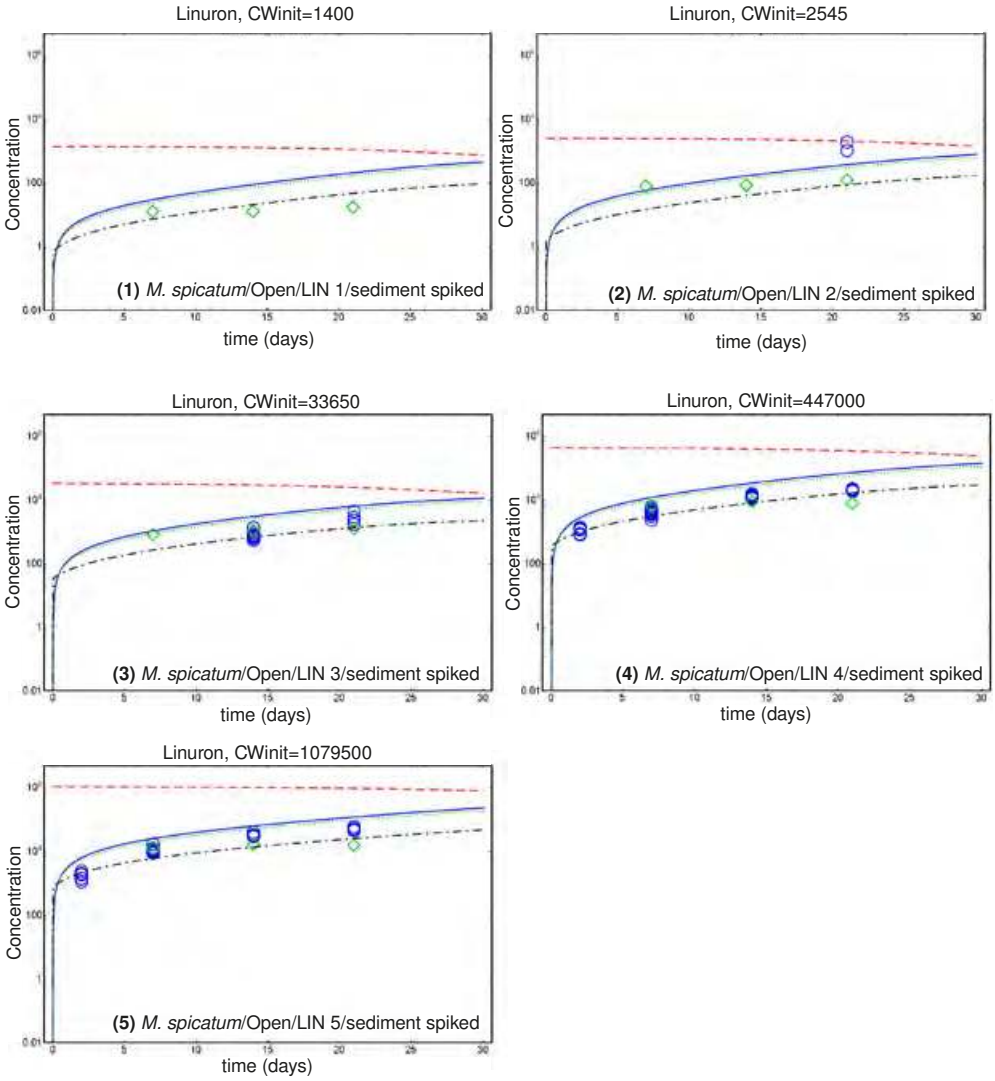

Figure S2. Measured (symbols) and modelled (curves) concentrations in overlying water (blue circles ○, solid line; µg/m^3), pore water (red dashed line; µg/m^3), shoots (green diamonds ◊, dotted line; µg/kg), and roots (black triangle Δ, dash dot line; µg/kg) for water spiked PCBs and sediment spiked PCBs, CPF, and LIN for *Elodea canadensis* and *Myriophyllum spicatum* in capped and open systems. Panels 1-5.

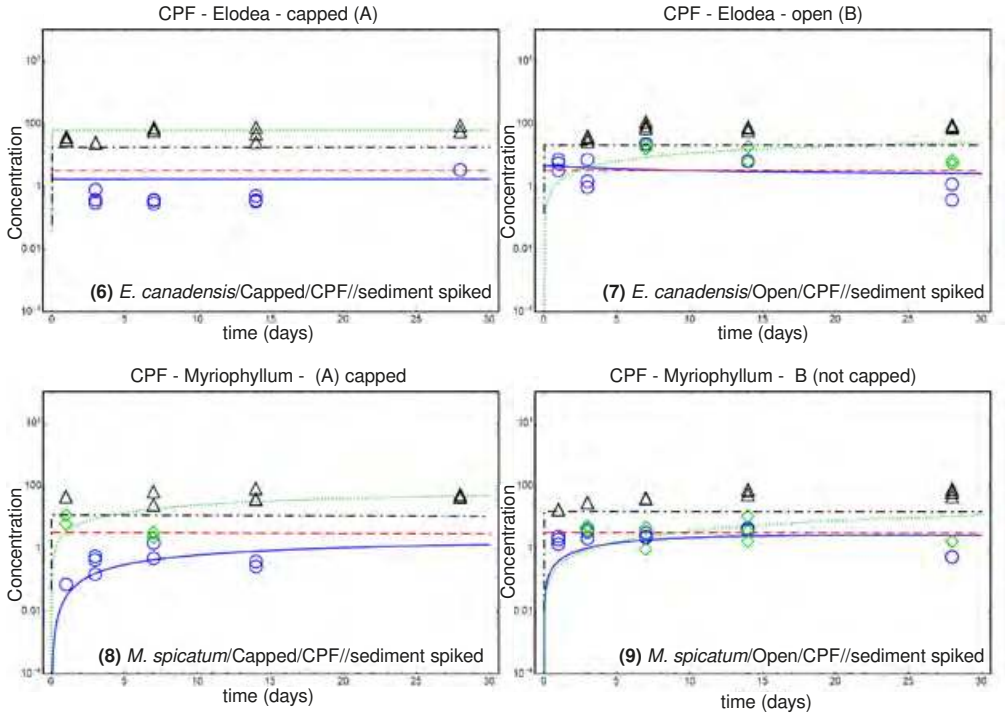

Figure S2 (Continued). Panels 6-9.

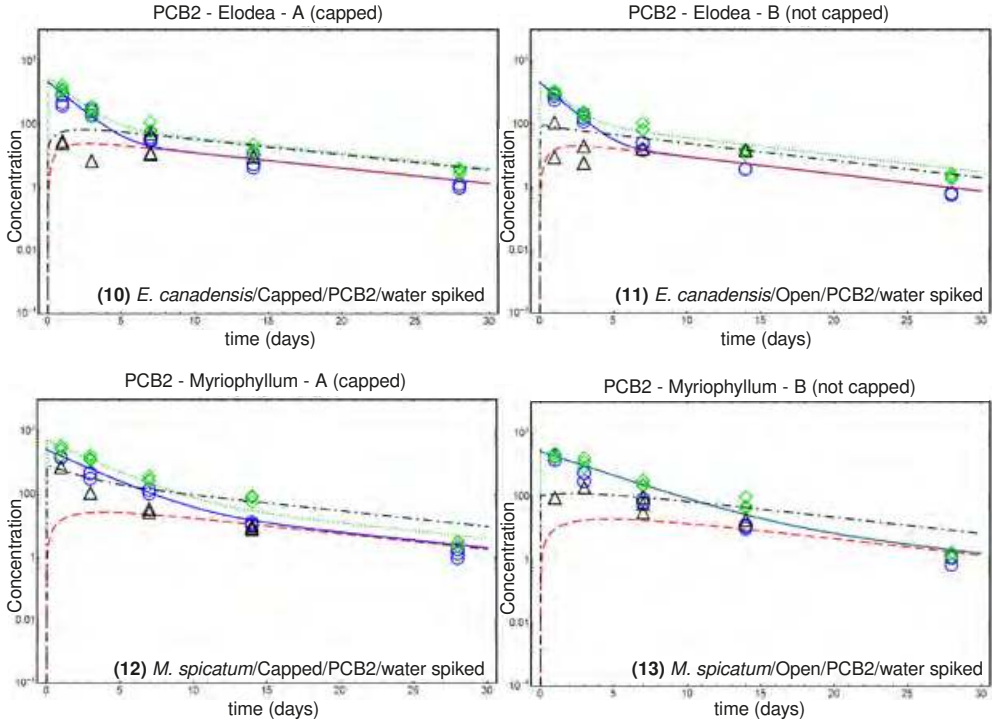

Figure S2 (Continued). Panels 10-13.

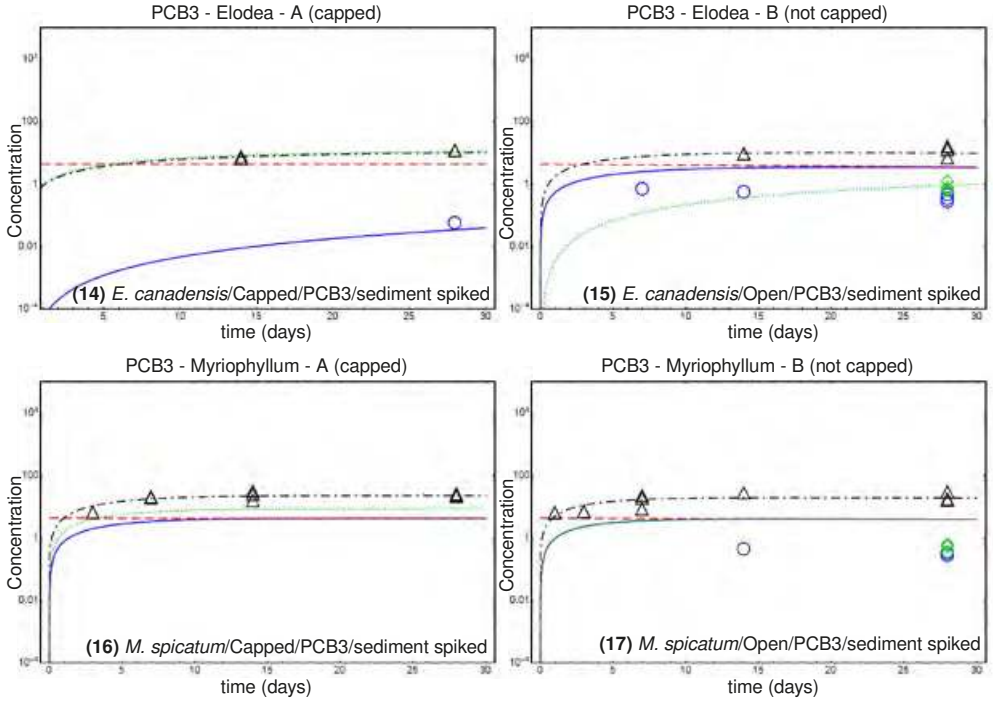

Figure S2 (Continued). Panels 14-17.

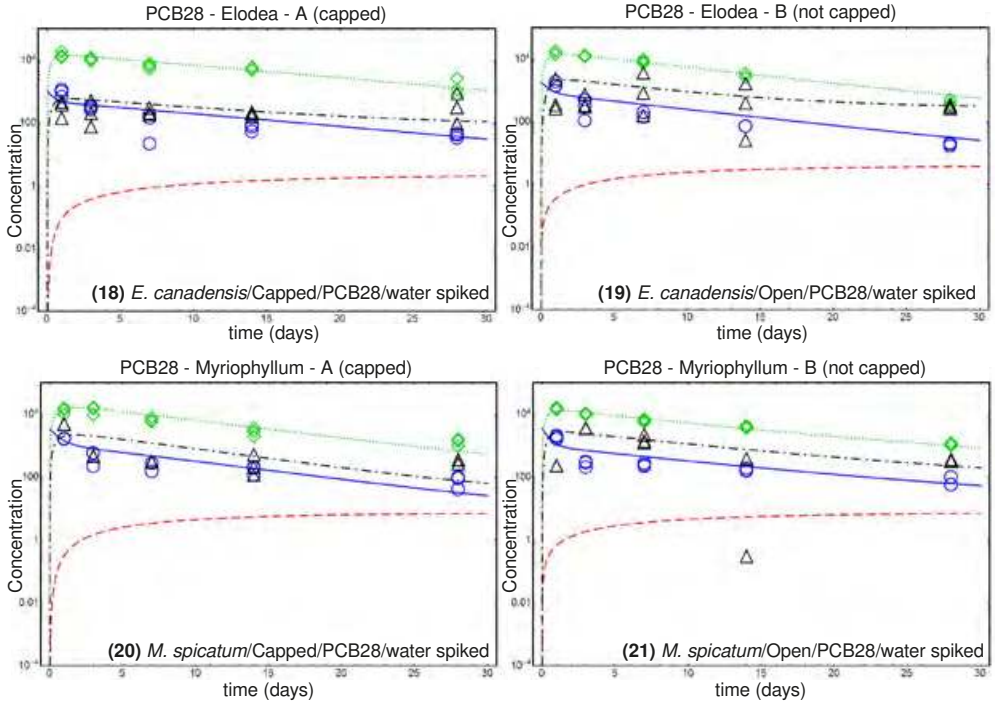

Figure S2 (Continued). Panels 18-21.

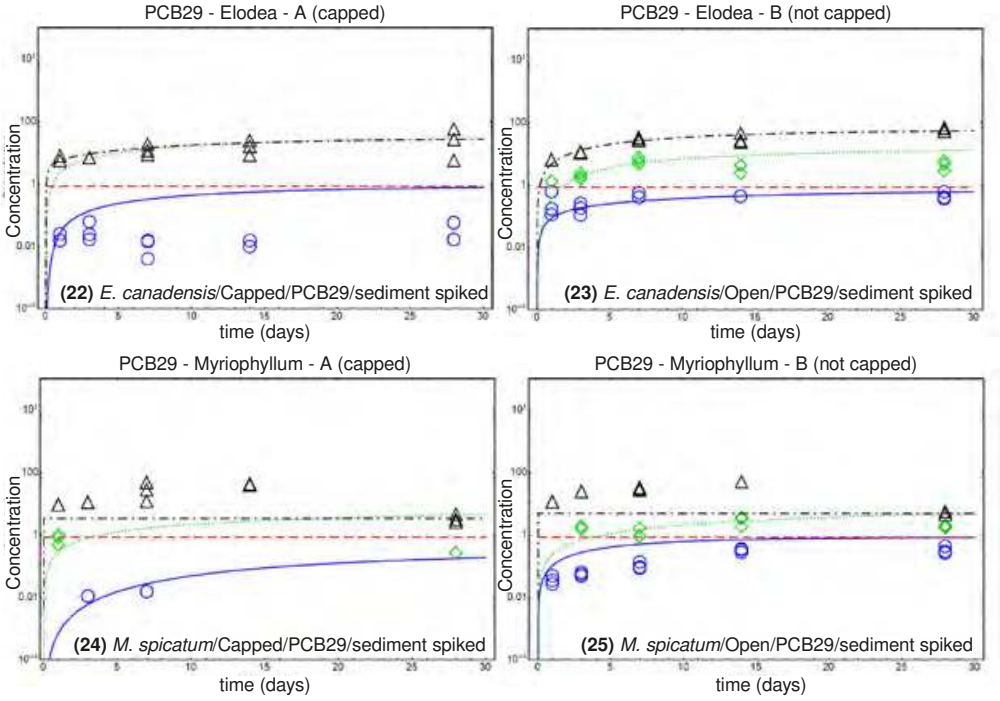

Figure S2 (Continued). Panels 22-25.

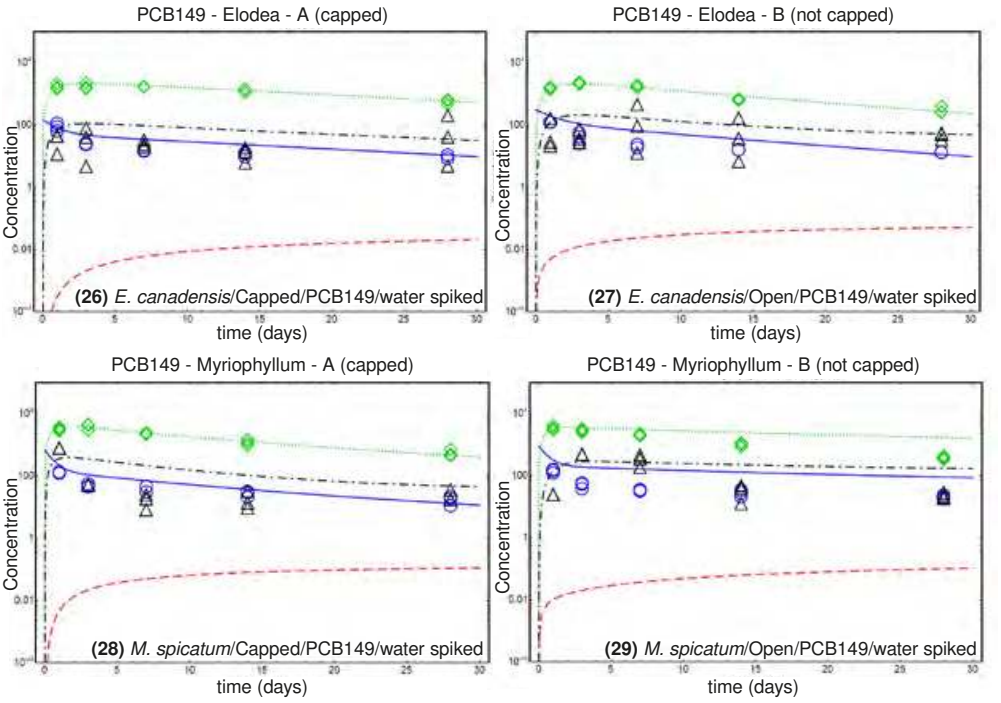

Figure S2 (Continued). Panels 26-29.

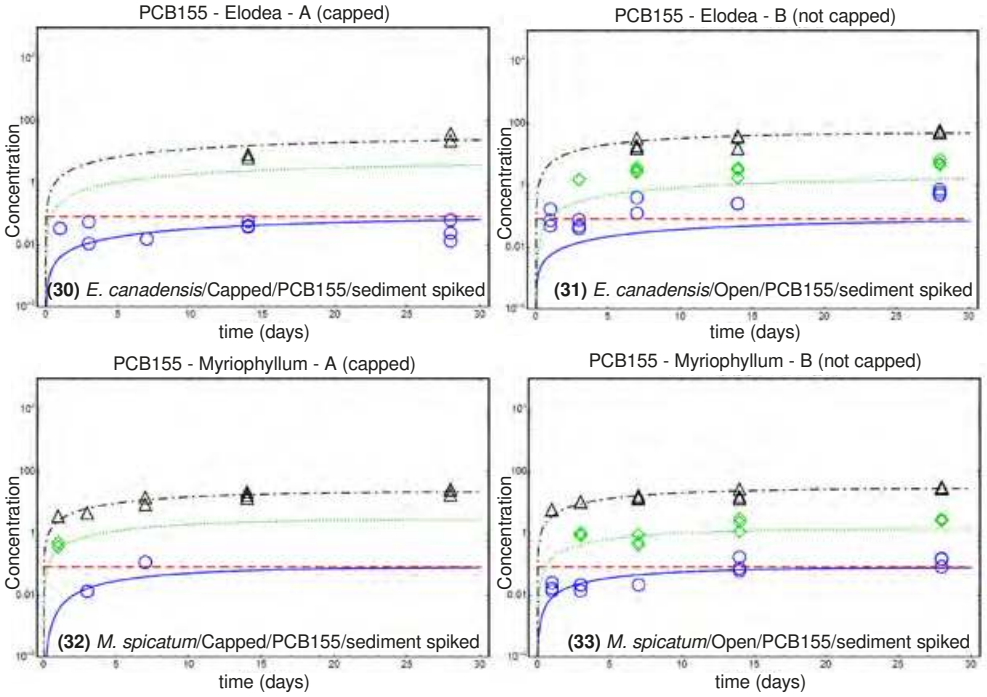

Figure S2 (Continued). Panels 30-33.

Parameter Estimates

Table S24. Parameters and their confidence intervals obtained from fitting with data from the separate experiments for *Elodea canadensis* (E) and *Myriophyllum spicatum* (M) in capped and open systems. * not estimated, parameter fixed at zero, - confidence limit not within two orders of magnitude above or below estimated value, L90= lower boundary of the 90% Confidence Interval, H90= higher boundary of the 90% Confidence Interval.

		CPF				PCB 2				PCB 3			
	CI	E capped	E open	M capped	M open	E capped	E open	M capped	M open	E capped	E open	M capped	M open
	L90					0.05	0.08	0.03	0.01				
k_{LOSS} (m^3/kg)		*	*	*	*	0.11	0.13	0.12	0.12	1.53E-09	0.01	3.05E-09	2.43E-03
	H90					0.19	0.18	0.29	0.29		0.04		0.02
	L90	-	2.56E-03	-	-	0.95	0.49	-	-	-	5.12E-05	-	-
P_S (m/d)		615.37	0.01	0.17	2.14E-03	1.53	0.68	104.75	102.71	3.34E-04	4.76E-04	22.56	26.98
	H90	-	0.11	-	0.18	2.85	0.90	-	-	-	0.01	-	-
	L90	-	-	-	-	63.62	65.63	7.18	-	0.00	0.00	0.01	-
P_R (m/d)		1297.47	2818.15	619.17	135.08	841.77	186.39	17.49	30.45	0.04	0.02	0.60	0.31
	H90	-	-	-	-	-	-	48.11	-	-	-	-	-
	L90	-	-	1756	-	236498	387833	31268	-	-	-	-	-
P_{TR} (m/d)		17	9E-02	17081	1673	327560	510307	65280	20286	738342	8E-04	2604081	1093457
	H90	-	-	246801	18039	433362	642987	90684	43717	-	-	-	-
K_S (m^3/kg)[a]		37	37	37	37	3	4	2	1	3	4	2	1
K_R (m^3/kg)[a]		5.5	6.4	3.6	4.8	2.7	2.7	5.3	4.8	2.7	2.7	5.3	4.8
K_L (m^3/kg)[a]		0	0.025	0	0.025	0	0.025	0	0.025	0.025	0.025	0	0.025
N of experimental data points		22	30	22	34	37	33	35	36	4	12	9	16
F-ratio value		1.259	1.179	1.259	1.155	1.199	1.228	1.212	1.205	161.780	1.938	2.644	1.591

[a] Independently measured value after 28 d.

Table S24 (continued).

		PCB 28				PCB 29				PCB 3			
	Cl	**E capped**	**E open**	**M capped**	**M open**	**E capped**	**E open**	**M capped**	**M open**	**E capped**	**E open**	**M capped**	**M open**
	L90												
k_{LOSS} *(m³/kg)*		*	*	*	*	*	*	*	*	1.53E-09	0.01	3.05E-09	2.43E-03
	H90										0.04		0.02
	L90	0.85	0.47	0.15	0.18	-	0.11	-	-	-	5.12E-05	-	-
P_S *(m/d)*		1.51	0.75	0.24	0.26	1.51	0.35	0.02	5.86E+13	3.34E-04	4.76E-04	22.56	26.98
	H90	-	-	0.86	-	-	-	-	-	-	0.01	-	-
	L90	9.50	8.12	0.82	0.55	0.48	0.18	-	-	0.00	0.00	0.01	-
P_R *(m/d)*		19.83	13.80	1.32	0.81	0.96	0.23	224.46	436.99	0.04	0.02	0.60	0.31
	H90	49.47	23.30	2.34	1.18	2.07	0.33	-	-	-	-	-	-
	L90	31646	17113	107461	53504	20701	-	-	-	-	-	-	-
P_{TR} *(m/d)*		41640	26263	224349	86432	247208	646	5314	5761	738342	8E-04	2604081	1093457
	H90	49968	36484	-	205356	-	15973	-	-	-	-	-	-
K_S *(m³/kg)*[a]		37	22	22	16	37	22	22	16	3	4	2	1
K_R *(m³/kg)*[a]		34.8	71.0	4.0	5.9	34.8	71.0	4.0	5.9	2.7	2.7	5.3	4.8
K_L *(m³/kg)*[a]		0	0.025	0	0.025	0	0.025	0	0.025	0.025	0.025	0	0.025
N of experimental data points		45	42	39	41	24	37	17	35	4	12	9	16
F-ratio value		1.113	1.122	1.133	1.129	1.233	1.141	1.359	1.150	161.780	1.938	2.644	1.591

[a] Independently measured value after 28 d.

Table S24 (continued).

		PCB 149				PCB 155				PCB 3			
	Cl	**E capped**	**E open**	**M capped**	**M open**	**E capped**	**E open**	**M capped**	**M open**	**E capped**	**E open**	**M capped**	**M open**
	L90												
k_{LOSS} (m^3/kg)		*	*	*	*	*	*	*	*	1.53E-09	0.01	3.05E-09	2.43E-03
	H90										0.04		0.02
	L90	0.51	0.21	0.20	0.06	-	-	4.96E-03	-	-	5.12E-05	-	-
P_S (*m/d*)		0.74	0.30	0.31	0.20	269.18	2.82	0.24	6.61	3.34E-04	4.76E-04	22.56	26.98
	H90	1.19	0.43	0.89	-	-	-	-	-	-	0.01	-	-
	L90	53.28	64.52	55.47	4.61	0.84	1.43	1.33	1.19	0.00	0.00	0.01	-
P_R (*m/d*)		90.60	109.71	94.33	11.81	1.43	2.70	1.94	1.65	0.04	0.02	0.60	0.31
	H90	160.63	185.26	159.28	1155.49	3.13	-	2.98	2.29	-	-	-	-
	L90	25906	10400	59826	1621	-	-	220717	161369	-	-	-	-
P_{TR} (*m/d*)		32383	15162	74782	3750	40428891	18082	6889427	2028466	738342	8E-04	2604081	1093457
	H90	38859	20060	89738	73264	-	-	-	-	-	-	-	-
K_S (m^3/kg)[a]		58	23	36	18	58	23	36	18	3	4	2	1
K_R (m^3/kg)[a]		375.8	649.8	278.6	360.3	375.8	649.8	278.6	360.3	2.7	2.7	5.3	4.8
K_L (m^3/kg)[a]		0	0.025	0	0.025	0	0.025	0	0.025	0.025	0.025	0	0.025
N of experimental data points		45	42	39	41	14	31	14	36	4	12	9	16
F-ratio value		1.113	1.122	1.133	1.125	1.468	1.172	1.468	1.145	161.780	1.938	2.644	1.591

[a] Independently measured value after 28 d.

Table S25. Parameters estimated using combined data sets of open and capped systems, per chemical, for *Elodea canadensis* (E) and *Myriophyllum spicatum* (M). * not estimated, parameter fixed at zero, - confidence limit not within two orders of magnitude above or below estimated value.

		LIN		CPF		PCB 2	
	CI	M	E	M	E	M	
k_{LOSS} (m^3/kg)	*L90*	*	*	*	0.08 0.12	0.03 0.09	
	H90				0.17	0.19	
P_S (m/d)	*L90*	0.02	0.00	0.00	0.83	-	
		0.90	0.03	0.02	1.10	182.77	
	H90	-	-	-	1.45	-	
P_R (m/d)	*L90*	4.92E-03	-	-	109.04	7.63	
		0.02	1170.20	1126.66	909.35	18.57	
	H90	0.04	-	-	-	48.64	
P_{TR} (m/d)	*L90*	8670	668	2110	294428	25553	
		82000	6843	5407	368035	45739	
	H90	-	-	28043	463724	60512	
K_S (m^3/kg)[a]		0.8	37 37	37 37	3 4	2 1	
K_R (m^3/kg)[a]		0.14	5.5 6.4	3.6 4.7	2.7 0.5	0.5 0.5	
K_L (m^3/kg)[a]		0.00073	0 0.025	0 0.025	0 0.025	0 0.025	
N of experimental data points		90	52	56	70	71	
F-ratio value		1.054	1.096	1.089	1.070	1.069	

[a] Independently measured value after 28 d.

Table S25 (continued).

		PCB 3		PCB 28		PCB 29	
	CI	E	M	E	M	E	M
k_{LOSS} (m^3/kg)	*L90*	-	-	*	*	*	*
		0.02	0.02				
	H90	0.05	0.05				
P_S (m/d)	*L90*	-	-	0.75	0.17	0.19	-
		3.5E-04	3.5E-04	1.22	0.25	16.06	1.1E-03
	H90	-	-	-	0.52	-	-
P_R (m/d)	*L90*	0.02	0.02	11.59	0.69	0.17	-
		0.10	0.10	17.79	0.96	0.23	1217.26
	H90	-	-	27.25	1.47	0.36	-
P_{TR} (m/d)	*L90*	-	-	28197	89576	495	115
		478627	478627	35247	168777	2117	5484
	H90	-	-	42296	919101	8605	-
K_S (m^3/kg)[a]		3 3	3 3	37 22	22 16	37 22	22 16
K_R (m^3/kg)[a]		2.7 2.7	2.7 2.7	34.8 71.0	4.0 5.9	34.8 71.0	4.0 5.9
K_L (m^3/kg)[a]		0.025 0.025	0.025 0.025	0 0.025	0 0.025	0 0.025	0 0.025
N of experimental data points		16	16	87	79	61	52
F-ratio value		1.390	1.390	1.056	1.062	1.081	1.096

[a] Independently measured value after 28 d.

Table S25 (continued).

		PCB 149				PCB 155			
	Cl	E		M		E		M	
k_{LOSS} (m^3/kg)	*L90*	*		*		*		*	
	H90								
P_S (m/d)	*L90*	0.37		0.21		0.02		0.01	
		0.51		0.35		0.77		0.45	
	H90	0.74		-		-		-	
P_R (m/d)	*L90*	77.57		54.45		1.98		1.41	
		119.05		83.57		3.03		1.77	
	H90	191.45		127.99		5.38		2.22	
P_{TR} (m/d)	*L90*	16897		39866		-		301408	
		21121		49833		11488102		1429720	
	H90	26613		59799		-		-	
K_S (m^3/kg)[a]		58	23	36	18	58	23	36	18
K_R (m^3/kg)[a]		375.8	649.8	278.6	360.3	375.8	649.8	278.6	360.3
K_L (m^3/kg)[a]		0	0.025	0	0.025	0	0.025	0	0.025
N of experimental data points		87		80		45		50	
F-ratio value		1.056		1.061		1.113		1.101	

[a] Independently measured value after 28 d.

Definition of equations used to calculate fluxes across the interfaces between pore water, overlying water roots and shoots

Fluxes (ø; µg/d) were calculated between the four compartments: sediment, overlying water, shoots, and roots (see also schematic representation in Figure 2):

Flux from pore water to overlying water:

$$ø_{pw-w} = K_L A_{SED} \left(C_{PW} - C_{OW} \right) \quad \text{(S13)}$$

Flux from overlying water to shoots:

$$ø_{ow-s} = P_S A_{S,t} \left(C_{OW} - \frac{C_S}{K_S} \right) \quad \text{(S14)}$$

Flux from pore water to roots:

$$ø_{pw-r} = P_R A_{R,t} \left(C_{PW} - \frac{C_R}{K_R} \right) \quad \text{(S15)}$$

Flux from roots to shoots (translocation):

$$ø_{r-s} = P_{TR} A_{TR,t} \left(\frac{C_R}{K_R} - \frac{C_S}{K_S} \right) \quad \text{(S16)}$$

Fluxes were calculated using the parameters from single experiment data (Table S25). Note that fluxes in Figure 6 and Figure S3 are reported as positive if they occur in the direction as indicated in eqs S13-S16.

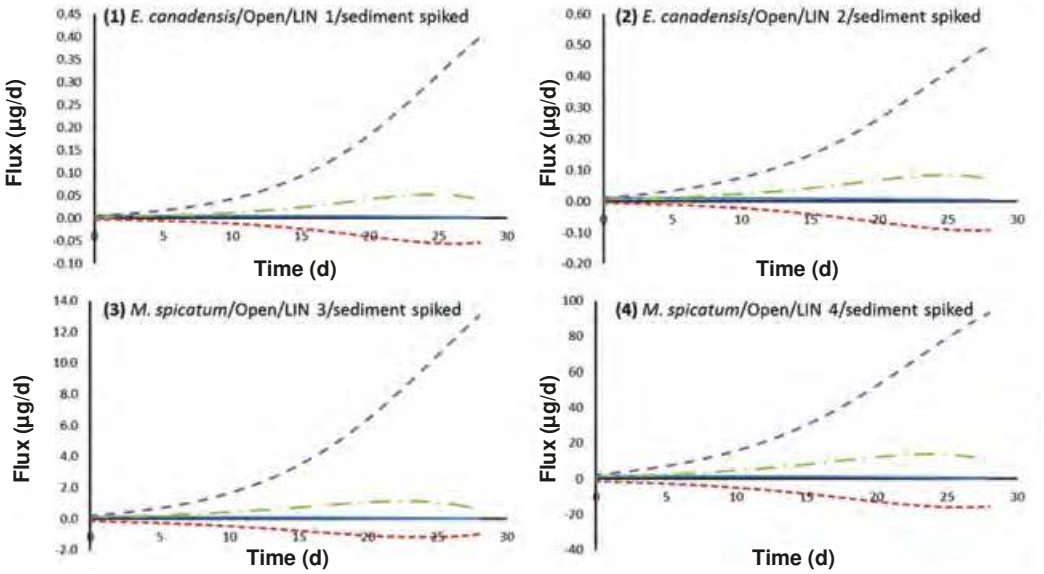

Figure S3. Chemical fluxes (µg/d) from pore water to overlying water (Eq. S7; blue solid line), from overlying water to shoots (Eq. S8; red dotted line), from pore water to roots (Eq. S9; green dash dot line), and from roots to shoots (Eq. S10; purple dash line) for water spiked and sediment spiked PCB, CPF and LIN for *Elodea canadenis,* and *Myriophyllum spicatum* in capped and open systems, as indicated. LIN was only spiked in the sediment. Panels 1-5 Linuron (LIN) data.

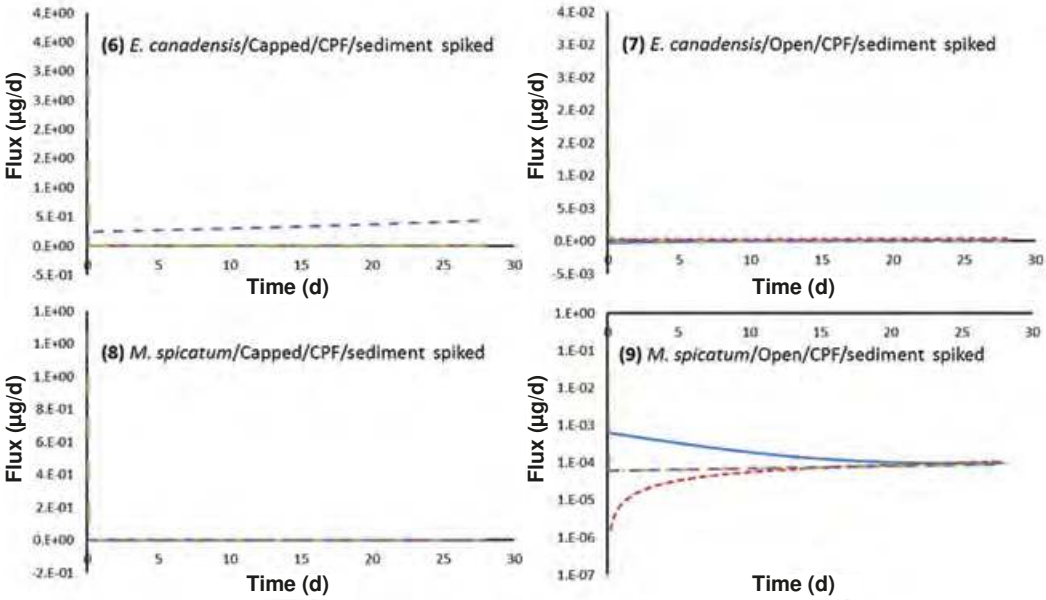

Figure S3 (continued). Panels 6-9, CPF data.

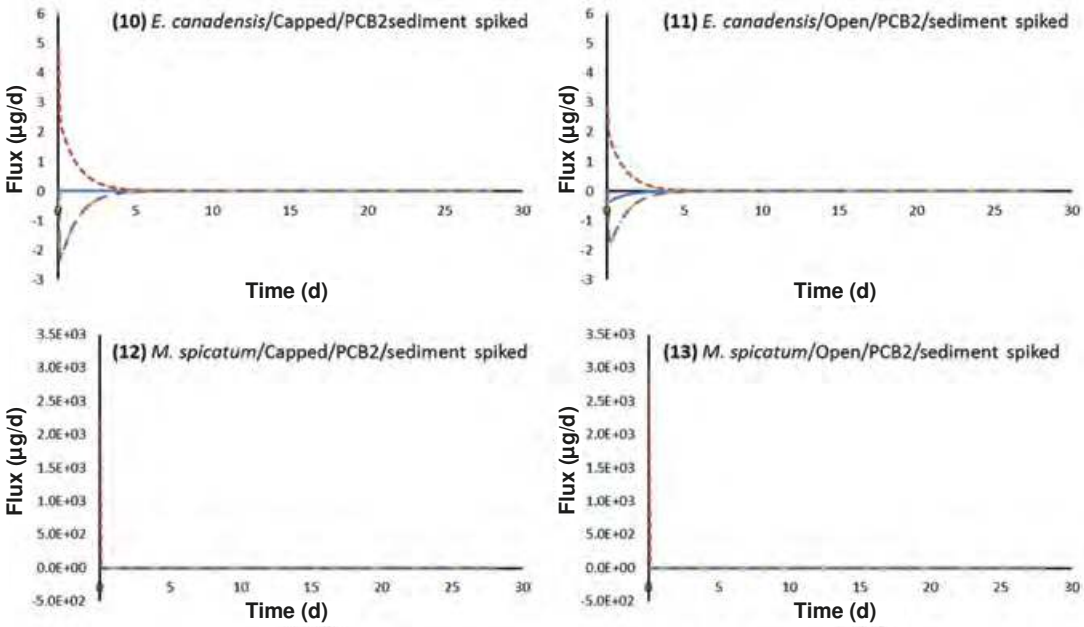

Figure S3 (continued). Panels 10-13, PCB 2 data.

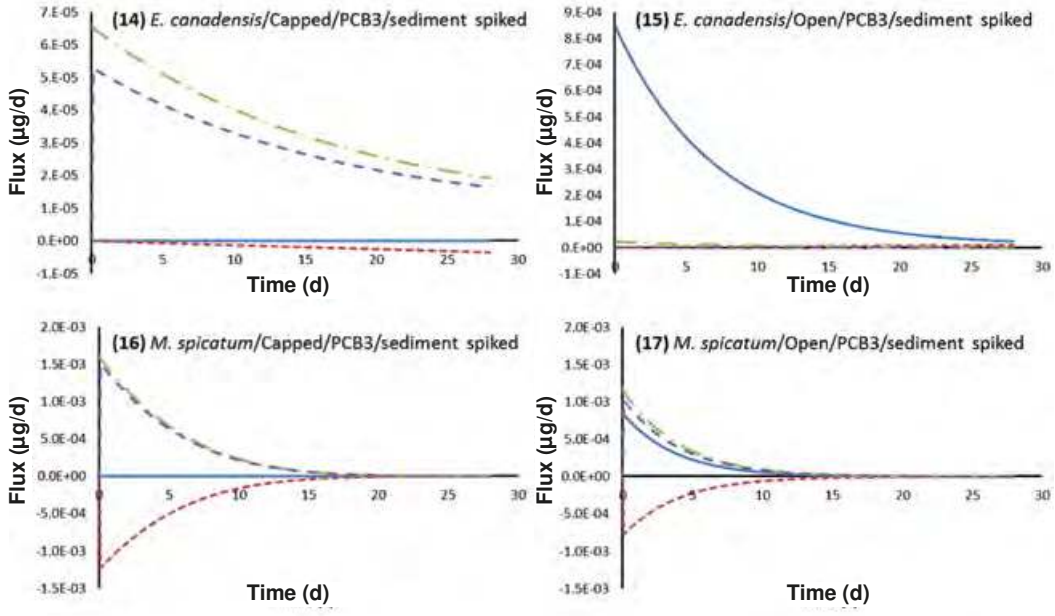

Figure S3 (continued). Panels 14-17, PCB 3 data.

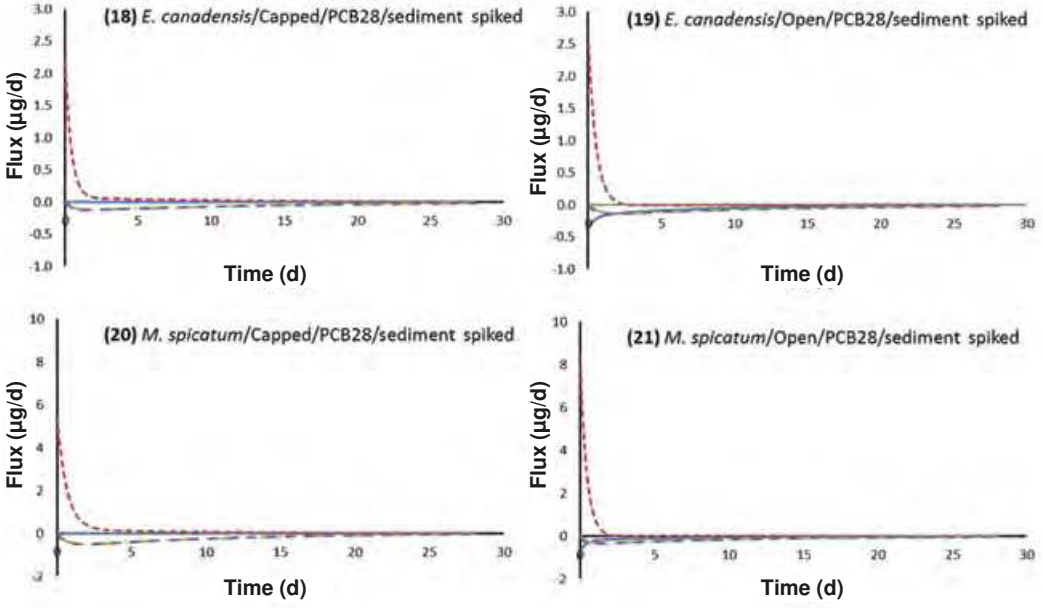

Figure S3 (continued). Panels 18-21, PCB 28 data.

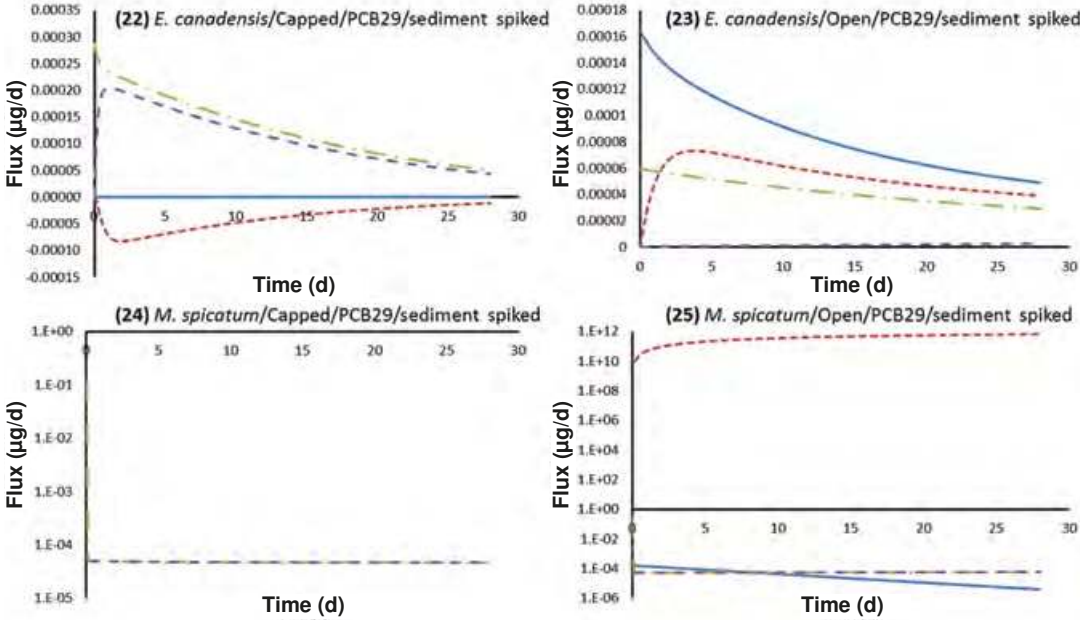

Figure S3 (continued). Panels 22-25, PCB 29 data.

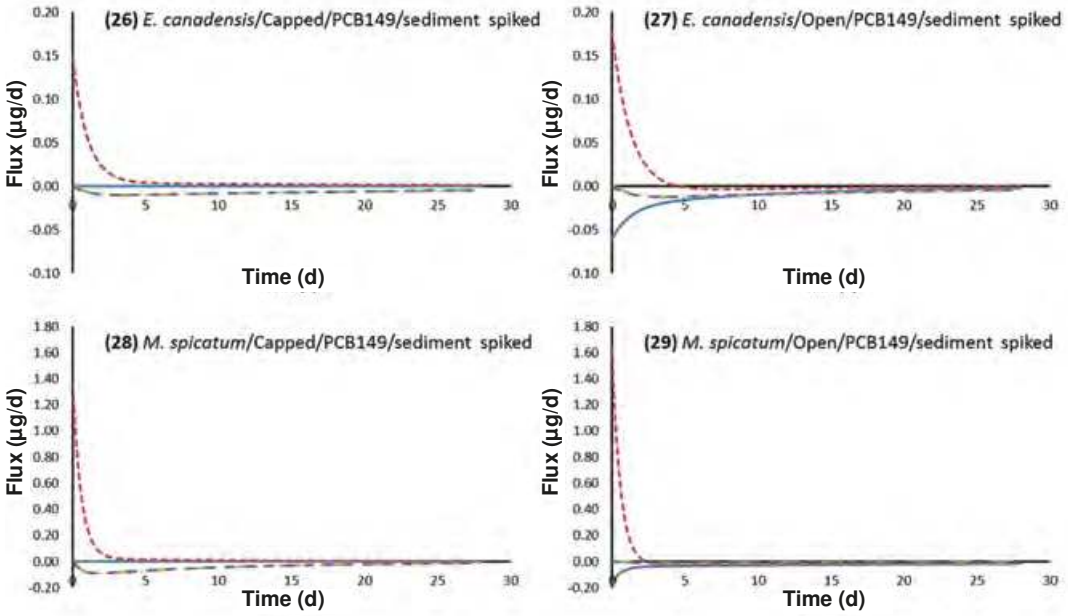

Figure S3 (continued). Panels 26-29, PCB 149 data.

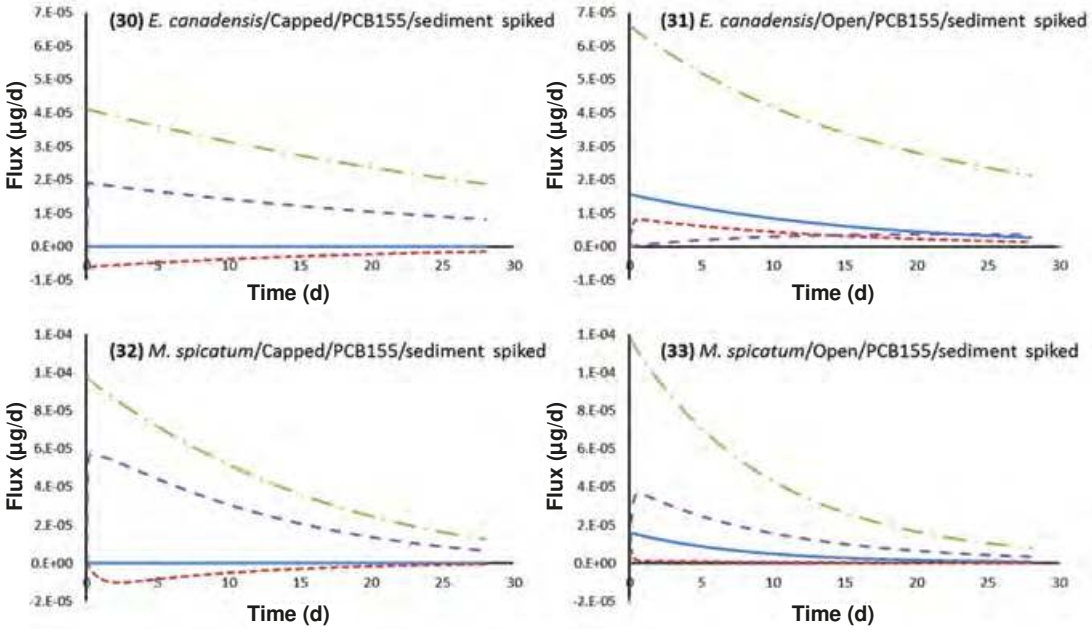

Figure S3 (continued). Panels 30-33, PCB 155 data.

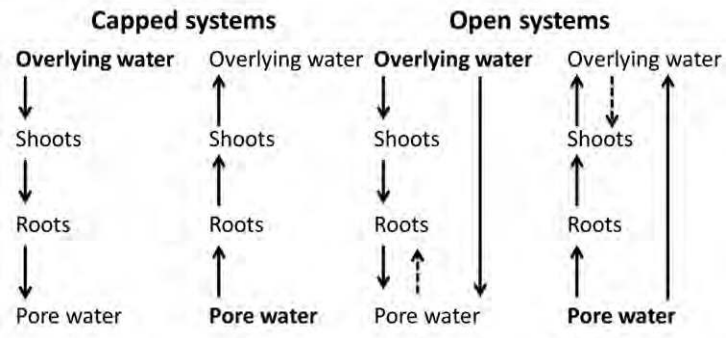

Figure S4. General patterns in the modelled fluxes between pore water and overlying water, overlying water and shoots, pore water and roots, and roots and shoots for water spiked and sediment spiked capped and open systems. Spiked compartments indicated **in bold**.

Chapter 4

Model-supported bioaccumulation assessment by battery testing allows read across among marine benthic invertebrate species

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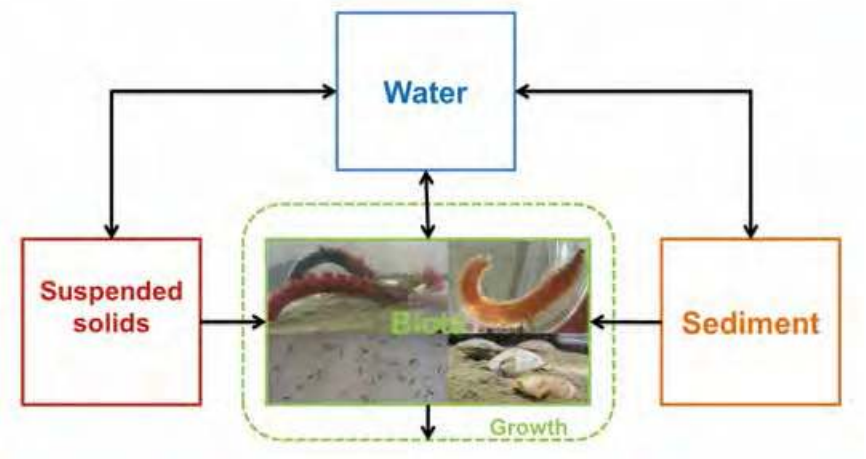
Abstract

The causal links between species traits and bioaccumulation by marine invertebrates are poorly understood. We assessed these links by measuring and modelling polychlorinated biphenyl bioaccumulation by four marine benthic species. Uniformity of exposure was achieved by testing each species in the same aquarium, separated by enclosures, to ensure that the observed variability in bioaccumulation was due to species traits. The relative importance of chemical uptake from pore water or food (organic matter; OM) ingestion was manipulated by using artificial sediment with different OM contents. Biota sediment accumulation factors (BSAFs) ranged from 5 to 318, in the order *Nereis virens* <*Arenicola marina* ≈*Macoma balthica* <*Corophium volutator*. Calibration of a kinetic model provided species-specific parameters that represented the key species traits, thus illustrating how models provide an opportunity to read across benthic species with different feeding strategies. Key traits included species-specific differentiation between (a) ingestion rates, (b) ingestion of suspended and settled OM and (c) elimination rates. The high BSAF values and their concomitant variability across the species challenges approaches for exposure assessment based on pore water concentration analysis and equilibrium partition theory. We propose that combining multi-enclosure testing and modelling will substantially improve exposure assessment in sediment toxicity tests.



4.1 Introduction

In the current Environmental Risk Assessment (ERA) of sediment-bound chemicals, effects are assessed using tests with only a few taxonomic groups, mainly freshwater benthic species, whereas tests with estuarine and marine species are rare.[259] The current set of test species poorly represents the wide range of species dwelling in the sediment compartment. Consequently, improving the assessment of environmental risks posed by chemicals in sediments requires the development of chronic sediment tests that cover different trophic levels, taxonomic groups and exposure pathways (Chapter 2).[259] Although empirical tests are crucial in ERA, they are not sufficient, because the regulatory context also requires mechanistic understanding of exposure pathways and effects of chemicals, as well as prospective models for spatio-temporal extrapolation.[20,259]

Bioaccumulation of chemicals depends on species-specific traits,[86,296] chemical characteristics[59,284,297] and species-species interaction. Species-species interaction can occur directly by activity of neighbouring species leading to changes in behaviour and feeding patterns[298] or indirectly through chemical cues.[299] Important species-specific traits for bioaccumulation include body size, lipid content, diet, digestive processes and dietary assimilation,[59,86,296] all of which can be accounted for in bioaccumulation models.

Previous research addressed effects of sediment type or chemical characteristics on exposure, whereas variability among species with different traits, e.g. regarding ingestion, received less attention.[300] Sediment particle ingestion is a major uptake route for some benthic invertebrates, e.g. *Lumbriculus variegatus,*[62,296] *Arenicola marina*[300] and *Macoma balthica,*[98,300,301] whereas water uptake dominates for other species, e.g. *Ilyodrilus templetoni.*[302] The relative importance of chemical uptake through food ingestion compared to uptake from water is still subject to some debate, especially as to whether ingestion may lead to bioaccumulation exceeding the levels predicted by equilibrium partitioning theory (EPT). Furthermore, it is not clear whether species-specific differences in the relative ingestion of sediment versus suspended particles affect bioaccumulation. Expansion of the current suite of available standard test species (see Chapter 2[259] and Fojut et al.[303] for a summary of species) might help to address such variability in uptake routes and sensitivities among species. Subsequently, bioaccumulation models may capture species traits through their parameterization, and assist in reading across test results of species and freshwater, estuarine and marine ecosystems.

The main objective of the present study was (a) to assess differences in bioaccumulation among a range of marine benthic invertebrate species, (b) to understand the underlying bioaccumulation mechanisms by modelling the processes that drive these differences and (c) to interpret the model parameters in terms of species traits. A secondary objective was to test a novel approach to whole-sediment testing of benthic invertebrates, by testing the species either separately in gauze enclosures or mixed together in an aquarium. In this set-up, all species are exposed to the same sediment layer, which ensures more equal exposure from the sediment and pore water.

Bioaccumulation tests were performed with *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens*, each with different feeding strategy, habitat and sediment contact. Polychlorinated biphenyls (PCBs) were chosen to represent a range of legacy compounds (POPs), which are relatively inert[206,304,305] and have a low direct toxicity for invertebrates, and are therefore ideal tracer chemicals for bioaccumulation. Chlorpyrifos (CPF) was selected as an example of pesticides, which contrast with PCBs in terms of degradability and usage patterns. In addition to using different species, we manipulated the relative importance of uptake from either pore water or particle ingestion by using spiked standard OECD sediment with low, medium or high organic matter (OM) content. At constant total contaminant concentration, differences in OM content cause different relative values of pore water and OM concentrations and thus cause differences in the relative importance of exposure (uptake) pathways. Prior to the bioaccumulation experiment, effects of sediment OM content and multispecies test design on the test species were assessed in two pilot experiments. The pilot tests (coded pilot tests 1 and 2) served to optimize the test conditions that were then used in bioaccumulation test 3, which addressed our main research aim (Figure 1). Data analysis was supported by bioaccumulation modelling and interpretation of trait-specific model parameters.

4.2 Materials and Methods

In this section we describe the materials, chemicals, animals and procedures used for the three tests (Figure 1). Two pilot tests investigated test conditions like OM content (Pilot test 1) and a standard single species (SSS) test design versus a multispecies test design with species separated by gauze (enclosed single species (ESS)) (Pilot test 2). The third main test assessed the effect of OM content and species traits on bioaccumulation in a multispecies test design. Species were either separated by gauze in an aquarium (ESS) (Main test 3a) or mixed together in an aquarium without enclosures (mixed species (MS)) (Main test 3b) (Figure 1).

4.2.1 Chemicals and materials

Test chemicals were PCB 28, 52, 101, 118, 153, 180 and CPF. Further details on chemicals and materials are provided as Supporting Information (SI).

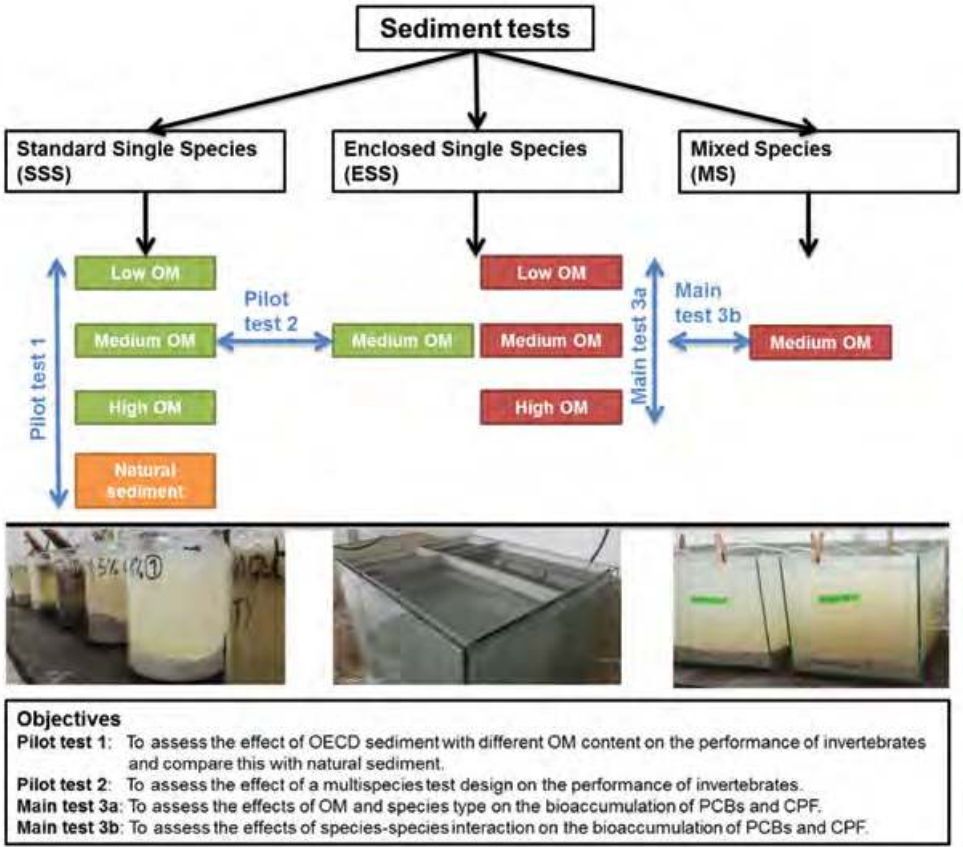


Figure 1. Schematic overview of pilot tests 1 and 2 and main tests 3a and 3b for standard single species, enclosed single species and mixed species. The pilot tests served to optimize the methodologies used in test 3, which addressed the primary research aim. Blue arrows indicate comparisons made in a test. Green boxes stand for non-spiked OECD sediment with different organic matter (OM) contents, orange box for natural sediment and red boxes for spiked OECD sediment with different OM contents. Pictures show the set-up of the different test designs.

4.2.2 Sediment and water medium

Standard sediment was prepared according to OECD guideline 218[76] with small modifications. Peat, calcium carbonate (1%) and natural seawater were mixed to obtain a homogeneous slurry, which was spiked with PCBs and CPF and thoroughly mixed with quartz sand (75%) and kaolin clay (20%). Peat was added to obtain sediment with low (1%), medium (5%; standard OECD) and high (15%) OM content (nominal values). Clean natural sediment (1.2% OM) was collected at the Oesterput, Zeeland, The Netherlands. Dry weight (DW) (24h at 105°C) and OM (3h at 550°C) were measured at the end of the pre-equilibration period and at the end of the experiment. Salinity, pH and temperature were measured just before the peat slurry was spiked with PCBs. Unfiltered natural seawater from the Eastern Scheldt (the Netherlands) was used as pore water and overlying water.

Evaporation was corrected for by carefully replenishing with ultrapure water (Milli-Q). More details are provided in SI.

4.2.3 Spiking procedure

Sediment was spiked with PCB 28, 52, 101, 118, 153, 180 and CPF at non-toxic concentrations. The nominal concentration for total PCBs was 36 µg/kg DW, which is below the threshold effect concentration at which adverse effects are still unlikely to occur (40 µg/kg).[229] For CPF, 3.12 µg/kg DW was used, which is a factor 10 lower than the lowest sediment quality criterion found in the literature.[306] Two stock solutions were made by dissolving the chemicals in acetone: one with PCBs and one with CPF. Following Chapter 3[307], the PCBs were mixed into the agitated sediment in four or five portions of 0 to 1 mL of the spike solution with 20-minute intervals. Acetone additions were such that each spiking chamber, including the controls, had the same volume of 0.11% (v:v) of acetone, a volume that has been shown to yield negligible co-solvent effects[82,293,308-310] and is below the recommended level of ISO[122] and OECD.[311] After 7 days, the acetone was allowed to evaporate for about 30 min by opening the spiking chamber. After 21 days of spiking the PCBs, the first portion of CPF stock solution was spiked to the sediment and thoroughly mixed, and acetone was allowed to evaporate for about 30 min after 7 days. After 66 days, the sediment was spiked for a second time with the CPF stock solution to compensate for degradation losses. This meant that the more hydrophobic and stable PCBs[312] had a longer pre-equilibration than the more degradable CPF.[267] To enable (pseudo-)equilibrium between chemicals and sediment prior to the start of exposure, sediment was agitated for 77 days on a roller bank in the dark (see Figure S1 for overview of spiking procedure and Chapter 3[307]).

Fish food (TetraMin) was grounded, suspended in ultrapure water (Milli-Q) and spiked with PCBs and CPF. Chemical concentrations in food were designed to match the concentrations in the OM in the sediment.

4.2.4 Test species

Four marine benthic invertebrate species were used: *Arenicola marina* (Linnaeus, 1758) (annelida; sub-surface deposit feeder), *Corophium volutator* (Pallas, 1766) (crustacean: detritus feeder), *Macoma balthica* (Linnaeus, 1758) (mollusc; facultative suspension feeder) and *Nereis virens* (Sars, 1835) (annelida; omnivore). Details on species traits are provided in Table S1.

A. marina was collected in the Southern Wadden Sea by professional bait collectors (Rotgans, Hippolytushoef, the Netherlands). *C. volutator* was collected with a 500 µm sieve at low tide in the Wadden Sea near Den Helder, the Netherlands (for Pilot tests 1 and 2) or at a clean reference site at the Oesterput, Zeeland, the Netherlands (for Main test 3). *M. balthica* was collected at low tide at the Oesterdam, Zeeland, the Netherlands. *N. virens* were obtained from a professional bait farm, Topsy Baits, Zeeland, the Netherlands. We used randomly selected healthy individuals with a biomass and length as described in

Table S2. For details on the acclimatization of test species, see the SI.

During the experiments, test species were fed with either non-spiked (Pilot tests 1 and 2) or spiked fish food (Main test 3) three times a week after the first week. To ensure sufficient food supply, 10 mg of dry food for *A. marina*, 1.5 mg for *C. volutator,*[106] 3 mg for *M. balthica* and 10 mg for *N. virens* were added per individual.[313]

4.2.5 Details of the test designs

As mentioned above, three 28-day tests were performed in a temperature-controlled room at 14 °C under average (SD) light conditions of 21 (2) lux with a photoperiod of 16 h light:8 h dark. Here we provide further details.

Pilot test 1: Effect of organic matter on invertebrate performance using a SSS design
To determine the effects of OM content on mortality and growth, four treatments (n=3) were used: non-spiked standard sediments with 1%, 5% and 15% OM contents and natural sediment. The test used available standard or previously published protocols.[83,106,313,314] The sediment-to-water volume ratio was 1:3 for all systems.

Pilot test 2: Effect of a multispecies test design on invertebrate performance
To compare the impact of ESS and SSS test designs on mortality and growth, an ESS pilot test was done with clean standard sediment with 5% OM (n=3). The set-up for the ESS design was the same as that for the bioaccumulation test described below, except for the number of individuals added to each aquarium: pilot test 2 used 5 *A. marina*, 50 *C. volutator*, 10 *M. balthica* and 10 *N. virens* (Table S2).

Main test 3: *Effects of organic matter, species traits and species-species interaction on bioaccumulation*
This test consisted of two subtests: test 3a assessed the effects of OM content and species traits and test 3b the effect of species-species interaction on the bioaccumulation of PCBs and CPF. Test 3a used three treatments in an ESS set-up (n=4): spiked sediment at low OM content, medium OM content and high OM content. Test 3b used the medium OM content of the ESS test 3a, and compared it with an MS set-up with medium OM content (n=4). The different OM contents were chosen such that a constant total contaminant concentration would cause different relative contaminant concentrations in pore water and OM, resulting in a difference in relative importance of exposure pathways. However, since both OM and added (fish) food contribute to the diet of the organisms, varying OM content also affects food abundance, which in turn affects the relative importance of the exposure pathways. Together, this provided a wide range of exposure conditions for each species and chemical, allowing a more rigorous model evaluation (see below).

In the ESS test 3a (Figure 1), the four species were tested in one aquarium (35L×30W×30H cm), but in separate enclosures, to avoid direct species interaction. The enclosures were made from seawater-resistant stainless steel gauze (RVS 316L) with 0.5 or 1 mm mesh. The enclosures were designed such that space was not limiting. Enclosures for *A. marina* and *N. virens* were 24.5L×14.5W×30H cm and for *C. volutator* and *M. balthica*

9.5L×14.5W×30H cm. The water was aerated and could flow freely across the gauze. This ensured a well-mixed water layer and thus equal aqueous exposure. In each aquarium, 5 *A. marina*, 70 *C. volutator*, 25 *M. balthica* and 10 *N. virens* individuals were added in their respective enclosures. In the MS test 3b (Figure 1), the same numbers of individuals per species were put together in an aquarium without enclosures, to test effects of species-species interaction on bioaccumulation.

All aquaria were filled first with 1 kg of spiked and pre-equilibrated sediment. Then, enclosures were added and filled with 7 kg of sediment in total, 2.5 kg for the two big enclosures and 1 kg for the two small enclosures. Aquaria without enclosures were filled with 7 kg of sediment to obtain an equal sediment volume for the animals. In all cases, the biota biomass was less than 0.7% of the mass of the sediment (see Table S2 for detailed overview).The volume of the overlying water was approximately 25 L. The ESS set-up ensured identical chemical concentrations in sediment, pore water and overlying water for all enclosures within each aquarium of each treatment, which implies that observed differences in bioaccumulation can be related to species traits.

4.2.6 Endpoints

At the start of the experiments, subsamples of each of the species were used to determine wet weight, dry weight (24 h at 60°C) and ash-free dry weight (2 h at 600°C). Test units were checked daily for dead animals, which were removed immediately, weighed and stored at -20°C. Death was defined as lack of movement after 30s of gentle stimulation. The feeding rate of *A. marina* was determined daily by counting and flattening faeces heaps. At the end of the experiment, the surviving animals were counted; their wet weights were measured and they were stored at -20°C until further analysis of chemicals and lipids. The sediments from each enclosure were combined and well mixed, and a sediment sample for chemical analysis was taken from the mixture and stored at -20°C until further analysis.

4.2.7 Water quality

To check general water quality, dissolved oxygen (DO), temperature, salinity, conductivity and pH were measured three times a week in one randomly picked enclosure per aquarium. To check the homogeneity of the overlying water, water quality measurements were done in each individual enclosure once a week. Ammonium, nitrate, chlorophyll (cyanobacteria, green algae and diatoms) and turbidity as a measure of dissolved OM were measured weekly in a mixed sample containing an equal volume of water from each enclosure. Further details are provided as SI.

4.2.8 Extraction and analyses

For details on extraction, detection procedures and quality assurance see the SI (Tables S3, S4). Briefly, water samples were extracted using Empore disks, whereas biota, sediment and fish food samples were soxhlet extracted. The extracts were analysed by gas chromatography–mass spectrometry. Recovery was 80-110% for all compounds. Spiked

concentrations ranged from 20% to 65% of the nominal concentrations in the sediment (Table S3) and from 65% to 128% of the nominal concentrations in the food (Table S4). Mass conservation was not aimed for in the open test systems. Lipids were extracted with chloroform:methanol:water and quantified gravimetrically.[271]

4.2.9 Data analyses

Relative growth was calculated as the relative increase in wet weight (%). Biota sediment bioaccumulation factors (BSAF) after 28 d were calculated as $(C_{org,WW}/f_{lip})/(C_{sed,DW}/f_{OM})$ with C_{org} being the chemical concentrations in the organism in wet weight (µg/kg), C_{sed} the chemical concentration in sediment in dry weight (DW; µg/kg), f_{lip} the fraction of lipids in the organism based on wet weight (WW) and f_{OM} the OM fraction in the sediment (DW).

Data were checked for normality with Q-Q plots and Shapiro-Wilk test and for equality of variances with Levene's test. Outliers in the water quality dataset were detected using Grubbs' test with a significance level α=0.05. The endpoints, survival, relative growth rate and BSAF, were tested with one-way ANOVA and least significant difference (LSD) as a post-hoc test, independent t-test, Kruskal-Wallis with pairwise comparison or Mann-Whitney U-tests. All analyses were done with SPSS version 19 and a significance level of α=0.05.

4.2.10 Modelling bioaccumulation

Bioaccumulation of hydrophobic organic chemicals ($dC_{L,t}/dt$; $\mu g \times kg^{-1} \times d^{-1}$) in invertebrate lipids (subscript L) was modelled, following earlier bioaccumulation models[81,301,304,315,316], as a mass balance of uptake and loss processes:

$$\frac{dC_{L,t}}{dt} = k_w C_w + \alpha I\left[\beta C_{OM}^{SED} + (1-\beta) C_{OM}^{SS}\right] - k_e C_{L,t} - k_g C_{L,t} \quad (1)$$

in which C_w ($\mu g \times L^{-1}$) is the concentration in the water, k_w ($L \times kg^{-1} \times d^{-1}$) a first-order rate constant for dermal uptake, k_e (d^{-1}) the rate constants for overall elimination by processes such as faecal elimination and biotransformation, k_g (d^{-1}) the growth dilution, α (-) the chemical assimilation efficiency (assumed to be independent of food source) and I (≥0, $kg_{OM} \times kg_{LIP}^{-1} \times d^{-1}$) the mass of OM ingested per unit of time and organism lipid weight. The ingested OM was assumed to originate partly from suspended solids (SS) in the overlying water or recently settled particles, and partly from the sediment (SED). Two different sources of OM can be distinguished: the sediment and the added fish food. The SS and recently settled particles would mainly consists of fish food OM, which was added to the overlying water, and for a smaller part of sediment OM suspended in the water column e.g. by bioturbation. Ingestion of multiple food items by benthic invertebrates has been modelled in a similar manner by Selck et al.[59]. The concentrations C_{OM}^{SED} and C_{OM}^{SS} ($\mu g\ kg^{-1}$) are the chemical concentrations in sediment OM and suspended solids (seston) OM, respectively, and β ($0<\beta<1$) is the fraction of ingested OM originating from the sediment. In our thoroughly pre-equilibrated sediment test, C_w was constant during 28 d and an analytical solution to Eq. 1 is:

$$C_{L,t} = \frac{k_w C_W + \alpha l\left[\beta C_{OM}^{SED} + (1-\beta) C_{OM}^{SS}\right]}{k_e + k_g} \times \left(1 - e^{-(k_e + k_g)t}\right) \quad (2)$$

Again assuming sediment-water (pseudo-)equilibrium in our pre-equilibrated test, can be written as $C_{OM}^{SED} = K_{OM}^{SED} C_W$ and C_{OM}^{SS} can be written as $C_{OM}^{SS} = K_{OM}^{SS} C_W$. The ratio k_w/k_e equates to an apparent lipid:water partition coefficient K_{lip}. Substitution of these partitioning relationships into Eq. 2 yields an equation for the lipid- and OM-normalized BSAF: $BSAF_t$ ($=C_{L,t}/C_{OM}^{SED}$):

$$BSAF_t = \frac{k_e K_{lip} + \alpha l\left[\beta K_{OM}^{SED} + (1-\beta) K_{OM}^{SS}\right]}{(k_e + k_g) K_{OM}^{SED}} \times \left(1 - e^{-(k_e + k_g)t}\right) \quad (3)$$

Equation 3 shows how the time-dependent BSAF can be calculated from kinetic constants, ingestion rates and partition coefficients. Assuming a constant ratio γ between the sorption affinities for suspended matter OM and sediment OM ($K_{OM}^{SS} = \gamma K_{OM}^{SED}$) Eq. 3 simplifies to:

$$BSAF_t = \frac{\frac{k_e K_{lip}}{K_{OM}^{SED}} + \alpha l\left[\beta + (1-\beta)\gamma\right]}{(k_e + k_g)} \times \left(1 - e^{-(k_e + k_g)t}\right) \quad (4)$$

At infinite time, $BSAF_t$ approaches the steady state $BSAF_{SS}$. For a description of the model and schematic overview see Table S5 and Figure S2.

The percentages uptake through water is calculated based on Eq. 4 as:

$$\%WaterUptake = \frac{k_e}{k_e + \alpha l\left[\beta + (1-\beta)\gamma\right] \frac{K_{OM}^{SED}}{K_{lip}}} \quad (5)$$

The fraction of steady state reached (F_{SS}, $0<F_{SS}<1$) in the 28-day bioaccumulation test (t=28 d) was calculated as:

$$F_{SS} = 1 - e^{-(k_e + k_g)t} \quad (6)$$

Parameterization

Observed bioaccumulation was linked to species traits by parameterizing the above model. The processes incorporated and the parameter values quantifying the relative importance of these processes reflect the species traits that affect bioaccumulation. In our modelling and model parameterization we aimed at balancing model complexity with informed simplifications. The sediment OM-water partition coefficient K_{OM}^{SED} was assumed proportional to $LogK_{ow}$: $LogK_{OM}^{SED} = LogK_{OW} + b$.[268,293] The parameter 'b' reflects the relative affinity of chemical partitioning to peat, which is used in the OECD test set-up, and octanol, which may differ from natural sediments. The suspended solid OM-water partition coefficient K_{OM}^{SS} was estimated assuming a constant ratio γ with K_{OM}^{SED} (i.e. $K_{OM}^{SS} = \gamma K_{OM}^{SED}$). The chemical assimilation efficiency parameter 'α' was estimated according to Thomann et al.[60] with α=0.8 for $LogK_{ow}$ 4.5-6.5 and $\alpha=-0.375LogK_{ow} + 3.24$ in the $LogK_{ow}$ range between

6.5 and 8.5. The food ingestion rate 'I' was fitted using initial values from Thomann et al.[60] The parameter β relates to species-specific feeding habits: with β=1 for species feeding exclusively on sediment particles and β=0 for organisms feeding exclusively on suspended OM particles. The elimination rate constant k_e was assumed to be species-specific, but to decrease linearly with $LogK_{ow}$, and thus was calculated by fitting the parameter 'a' in the relation $Logk_e$=-$LogK_{ow}$ + *a*.[317] The growth rate constant k_g was calculated from the wet weight measurements assuming a first-order growth model. Equation 4 was implemented in Microsoft Excel 2010 and the model was fitted to the experimental BSAF data using the Excel Solver tool with scaling of parameters and a relative least-squares criterion. The 26 parameters were estimated using a two-stage iterative approach. First, the chemical sorption parameters 'b' and γ were set at default literature values,[268,293,318] and the species-specific parameters 'a', 'I' (constrained: I≥0) and β (constrained 0<β<1) were optimized for each species separately by minimizing their individual sum of squares. Subsequently, the parameters 'b' and γ were optimized by minimizing the total sum of squares, after which the parameters 'a', 'I' and β were fitted again for each of the species. This procedure was repeated until all minimum sums of squares had stabilized.

Confidence intervals (90% CI) were calculated according to Draper and Smith[319]:

$$SS_{90} = SS_{min}\left(1+\frac{p}{(n-p)}F(p,n-p,90\%)\right) \quad (7)$$

with SS_{90} the sum of squares at the 90% confidence contour, SS_{min} the minimum sum of squares, n the number of BSAF measurements (n=324), p the number of estimated parameters (p= 26) and F(p,n-p,90%) the F-distribution according to Fisher. Confidence intervals were estimated using n, p and F either for the whole dataset for the general parameters or for the species-specific dataset. Negative confidence limits for 'I' and β were set to zero.

In case of overlapping CIs for OM or species-specific parameters, reduced models were tested and evaluated for statistical relevance. The trade-off between model complexity and statistical rigour of parameter estimates was quantified for four reduced model versions using the F-test criterion:

$$F = \frac{(SS_{min,r} - SS_{min,f})/(p_r - p_f)}{SS_{min,f}/(n-p_f)} \quad (8)$$

in which the subscript 'r' indicates the reduced model and 'f' the full model (Eq. 4). The first reduced model did not differentiate between uptake of sediment particles and suspended solids obtained by setting β=1 and γ=0, reducing the number of parameters from p=26 to p=21. In the full model, β gave overlapping CIs for the species. Hence, the second reduced model used one β for all species (p=23). Ingestion had overlapping CIs for sediments with different OM content, but ingestion differed between *C. volutator* and the other species. Therefore, the third reduced model used one 'I' for each species, regardless of sediment treatment (p=14). The last reduced model used both of the previous simplifications, i.e. one β for all species and one I for each species (p=11).

4.3 Results and Discussion

4.3.1 Pilot test 1: Effect of OECD sediment organic matter content on invertebrate performance

During the test, a good and constant water quality was maintained (Table S6). DO was lower than 5 mg/L in 9 cases, with a minimum of 0.14 mg/L for *N. virens* at the highest OM treatment. Since all replicates of this treatment had low DO concentrations, the water was renewed. Ammonium concentrations were higher for the high OM treatment than for the low and medium OM treatments. Low DO and high ammonium concentrations may have been caused by dead animals and mineralization of OM.

Whereas survival of *A. marina*, *C. volutator* and *M. balthica* was above 70% (Table S8), that of *N. virens* was below 60%, with the lowest value, 33%, recorded in the high OM treatment. *A. marina*, *M. balthica* and *N. virens* lost weight, whereas *C. volutator* gained weight (Table S9). This weight loss may be explained by stress and/or insufficient feeding. Survival and growth were not significantly different between the different OM contents (Tables S10, S11). We conclude that the OM contents were suitable for the subsequent bioaccumulation testing (Tests 2 and 3).

4.3.2 Pilot test 2: Effect of a single vs. multispecies test design on invertebrate performance

Overall water quality was better for the multispecies ESS test than for the SSS test (Table S6). Ammonium concentrations in the ESS test were half of those in the SSS test at medium OM, probably due to the higher water-to-sediment ratio.

In the ESS test, average survival was over 80% for all species (Table S8). *A. marina*, *M. balthica* and *N. virens* lost weight, whereas *C. volutator* gained weight (Table S9). There was no significant difference in survival or growth between the ESS test and the SSS test with medium OM content (Tables S10, S11). For *M. balthica*, however, the average growth based on dry weight was higher in the ESS test than in the SSS test (independent t-test, t(4) -4.993, p=0.015). Since these data meet the OECD criteria for survival in control systems and good water quality in test systems, we conclude that the ESS set-up was suitable for the bioaccumulation test (Main test 3).

4.3.3 Main test 3: Bioaccumulation test (ESS 3a and MS 3b)

Effects of organic matter and multispecies test design on invertebrate performance

Good water quality was maintained during the test (Tables S6, S7), and variations in temperature, pH, DO and conductivity among enclosures were below 0.15% (four outliers removed, 14% based on all data). This implies that the overlying water was homogeneous and that the samples taken from random enclosures were representative for the whole aquarium. Turbidity was lower in the ESS tests than in the MS test, especially during the first week. Direct species interaction in the MS test caused more bioturbation due to individuals competing for space, as was observed especially during the first days.

Survival ranged from 47% for *C. volutator* to 60% and higher for *A. marina*, *M. balthica* and *N. virens* in all OM treatments (Table S8) with and without enclosures, and did not significantly differ with OM content (Table S10). In the MS test, however, no *A. marina* individuals survived, which was significantly different from the ESS test (3a) (independent t-test, t(6) 5.166, p=0.002). We assume that *A. marina* had been consumed by the omnivore *N. virens*, as the weight of *N. virens* increased significantly and substantially (28%) compared to the weight of this species in the ESS test (independent t-test, t(6) -10.890, p=0.000).

OM content only had a significant effect on the growth of *M. balthica* (one-way ANOVA, F(2,11)=5.277, p=0.031). At high OM content, its relative growth was higher or less reduced than at low and medium OM contents (Table S11), which may reflect the higher nutritional value of the sediment and/or a preference for sandy mud over muddy sediment.[320] The average feeding activity of *A. marina* was 0.4 (0.1) heaps per individual per day (Table S12), which is in agreement with recent data provided by Besseling et al.[321] No significant differences were found between OM treatments as regards feeding activity (one-way ANOVA, F(2,11)=0.520, p=0.611), so OM content influenced neither *A. marina's* ingestion rate nor its relative growth. This is in agreement with findings by Cammen[322] that OM ingestion is mainly a function of body weight and is independent of the organic content of the food. Survival and growth of *C. volutator* and *M. balthica* were not significantly different between the ESS (3a) and MS (3b) test set-ups; for *N. virens* this was only the case for survival (Tables S10, S11).

In conclusion, the water quality in the ESS and MS multispecies tests designs was better than in the SSS tests, due to the higher water-to-sediment ratios in the ESS and MS tests. Survival in the MS test, however, may have been influenced by interspecies interactions, as was shown by the disappearance of *A. marina* and concomitant weight gain by *N. virens*.

Effect of organic matter content and species traits on bioaccumulation

The PCB concentrations in the sediment were similar among OM treatments and remained relatively constant during the experiment (Table S13). Some PCBs had higher concentrations at the end of the experiment, which can be explained by PCB-spiked food that was added but not consumed. CPF concentration in sediment, however, was below the detection limit in all treatments at the end of the experiment, which may be explained by degradation and volatilization.[267] In a parallel study described in Chapter 6[323], addressing the detailed microbiology during the bioaccumulation test, we did indeed observe an increased abundance of bacteria with genes encoding for the hydrolysis of organophosphate compounds (*opd* gene) with decreasing CPF concentrations.

BSAF order and ranges for PCBs were *N. virens* (5-19) <*A. marina* (7-37) ≈*M. balthica* (8-36) <*C. volutator* (49-318) (Figure 2, Tables S14, S15). Similar high BSAF values have been reported for other compounds e.g. nonylphenol, for some freshwater[324] and estuarine and marine species.[325] The BSAF range observed for *A. marina* agrees very well with the PCB BSAF range of 10-40 (DW normalized) recently reported by Besseling et al.[321] for the same species in natural sediment. BSAF values for *M. balthica* were within the range

for molluscs (0.03-66, lipid and organic carbon normalized),[297] but higher than the range for the similar species *Macoma nasuta* (0.1-5, lipid and organic carbon normalized)[326] for PCBs. EPT would predict BSAF values of 1-2 for all species and treatments.[59,284,297] However the overall range of BSAF of 5 to 318 across species implies that exposure cannot be accurately assessed from pore water concentration data and EPT, and that species-specific traits, such as ingestion rate and diet, need to be taken into account. Indeed, the ingestion rate and sediment absorption efficiency for *M. balthica* were the most sensitive traits in earlier biodynamic modelling.[301] Additionally, the high BSAFs might be explained not only by ingestion but also by the relatively low $logK_{om}$ values of the artificial OECD sediment (see discussion below).

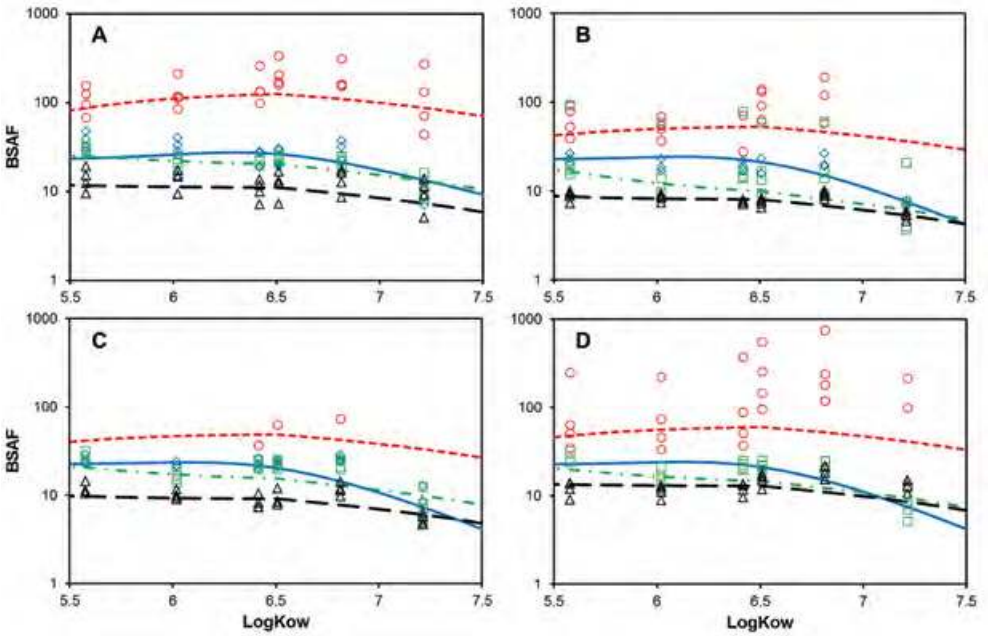


Figure 2. Measured (symbols) and modelled (curves) lipid- and organic matter-normalized BSAF for *Arenicola marina* (blue diamonds ◊, solid line), *Corophium volutator* (red circles ○, dotted line), *Macoma balthica* (green squares □, dash–dot line) *and Nereis virens* (black triangle Δ, dashed line), for the treatments with enclosures: low OM content (**A**), medium OM content as in the OECD test guideline (**B**), high OM content (**C**) and the treatment with mixed species and medium OM content as in the OECD test guideline (**D**).

Effect of organic matter and species traits on BSAF (test 3a)

In general, BSAF differed significantly between OM treatments for the lower hydrophobic PCBs 28 and/or 52, except for *M. balthica*, for which no differences were found (Table S16). BSAF differed significantly between species (Kruskall-Walis test, $0.003 < p < 0.030$, Tables S15, S17). *C. volutator* and *N. virens* differed significantly in the low-OM ESS, medium-OM ESS and medium-OM MS treatments for all PCBs, except for PCB 180 and *A. marina* and *N. virens* differed significantly in the high OM treatment for all PCBs except for PCB 28 and 180 (Figure 2, Tables S15, S17). The observed differences in BSAF can be interpreted in

terms of species traits. The higher BSAFs for *C. volutator* compared to *N. virens* may be explained by *C. volutator*'s smaller body size, its diet and its high growth rate (and thus high ingestion rate) in the test. *C. volutator* feeds on particulate OM, bacteria living freely on the sediment or attached to sediment particles and diatoms,[300,320,327] whereas *N. virens* feeds on mud, sand, detritus, plankton, macrofauna and bacteria.[320] The selective diet of *C. volutator* may explain the higher bioaccumulation, as bacteria increase the bioavailability of sediment-bound chemicals.[328] Diatom abundance in the overlying water (Table S7) and total bacterial abundance in the sediment (as shown in Chapter 6[323]) increased during the experiment, implying that the system is dynamic and that quality and quantity of the food source and consequently bioaccumulation may change over time. The BSAF of *A. marina* was higher than that of *N. virens*. Both are polychaete worms, but have different feeding strategies, *A. marina* being a bulk feeder and *N. virens* a more selective feeder, which may have caused the difference.

Effect of species-species interaction on BSAF (Main test 3b)

BSAF values were significantly higher for *N. virens* for all PCBs, except PCB 28 and 52, in the MS test (3b) compared to the same treatment in the ESS test (3a) (Mann-Whitney U test, p=0.029, Table S18). The higher BSAFs might be explained by biomagnification, because we deduced earlier that *N. virens* may have been predating on *A. marina*. The BSAF of *C. volutator* was higher in the MS test than in the ESS test, but the difference was not significant, which may be caused by an increased availability of detritus from the faeces of *N. virens*. For *M. balthica*, however, competition for space may have led to a decrease in food uptake, leading to lower BSAF values. We conclude that species-species interactions influenced bioaccumulation.

Evaluation of model complexity and parameter accuracy

The mechanistic species-specific model condensed in Eq. 4, with species and OM-specific parameters, provided good fits to the observed BSAF values (Figure 2, Table S19). The model was not over-parameterized, as the reduced model versions did not yield better statistical rigour (Eq. 7) (Table S20). For instance, the change in residual error when neglecting the differentiation between uptake by sediment and suspended solid OM was not significant (F-test, F=1.666, p=0.143) and neither was the effect of reducing four βs to one for all species (Table S20). However, reducing the OM treatment-specific ingestion rates 'I' to one 'I' value for all OM treatments made the model perform significantly worse (Table S20, p<0.001), implying that ingestion is species-specific and that the species-specific model parameterization is to be preferred. The model appeared to be more sensitive to changes in the ingestion rates 'I' than to changes in the proportion of sediment OM ingestion β. Therefore, based on Ockham's razor principle, one could prefer to refrain from distinguishing between sediment and suspended solid OM ingestion (i.e. use β=1). However, since defining β≠1 did not decrease the model's statistical rigour, and in order to be able to interpret the BSAF data in the most species-specific and mechanistic manner, the discussion below uses the full model parameterization.

The full BSAF model as condensed in Eq. 4 provided satisfactorily narrow confidence intervals for most of the parameters (Figure 3; Table S19). *C. volutator* had the most non-detects, and the MS dataset for *A. marina* was missing as this species was most probably consumed by *N. virens*, as argued above, which affected the precision of parameter values and CIs (Figure 3, Table S19). Part of the residual error in the model might be explained by the variability in OM types consumed. We assumed constant assimilation efficiency, as is often done in bioaccumulation models for invertebrates,[59,60,317] whereas in reality assimilation efficiency may depend on the quality of the food source.

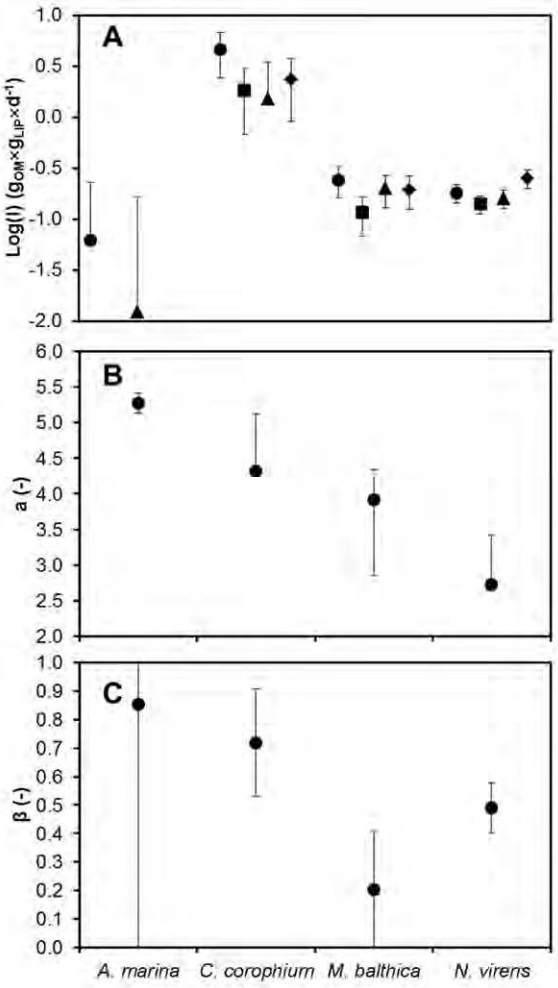


Figure 3. Optimized model parameters and 90% confidence limits (CL) for: Ingestion rate (I; g OM x g lipids x d^{-1}) (**A**), intercept for k_e (a; -) (**B**), and fraction of ingested OM originating from the sediment (β; -) (**C**), for *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens*. Ingestion rate was fitted separately for low (circles ○), medium (squares □), high (triangles Δ) and medium mixed (diamonds ◊) OM content. Parameter values are only included if a 90% confidence limit could be assessed in at least one direction.

$LogK_{ow}$ dependence of BSAF

BSAF values for *A. marina*, *M. baltica* and *N. virens* were constant or increased slightly up to $LogK_{ow}$ 6.5, after which BSAF decreased with increasing $LogK_{ow}$ of the PCBs (Figure 2). This pattern has been observed before[60,329-333] and has been explained by variation in congener lipid solubility, slow desorption from the sediment, biomagnification,[60,329,333] effects of molecular size, inaccurate K_{ow} values, overestimation of bioavailable water concentrations and elimination in faeces.[330] An additional explanation may be that steady state is not always reached in the tests (i.e. Eq. 6; F_{SS}<0), as we found for several species and higher PCB congeners (Table S21). In case of equilibrium partitioning without substantial uptake by food ingestion, BSAFs would remain rather constant over a range of $LogK_{ow}$ values. However, with substantial food ingestion, BSAFs would increase with increasing $LogK_{ow}$ as more PCBs would have partitioned into the food. Thus, the relevance of sediment ingestion route is assumed to increase with increasing hydrophobicity.[59,62,98,300,301,321,334-336]

Species- and OM-specific parameters

The parameter values and mechanistic inferences deduced from the modelling provide the opportunity to interpret BSAF values in terms of species traits. The high BSAFs for *C. volutator* could only be explained by fitting a high ingestion rate 'I' (Figure 3, Table S19), which corresponds to the observed growth of this species of 100 to 150% dry weight. The ingestion rates estimated for *M. balthica* and *N. virens* corresponded well with the range of 0.13-0.62 reported by Thomann et al.[60] (Table S19), whereas the values for *A. marina* were below this range. The low ingestion of *A. marina* correlates with the weight loss observed (Table S9). Elimination rate (k_e) was fitted according to the equation $Logk_e$=-$LogK_{ow}$ + a, and the proportionality parameter 'a' was lowest for *N. virens* and highest for *A. marina* (figure 3B). The k_e for *M. balthica* (between 0 and 0.02) is lower than the earlier reported value of 0.05 for PCBs[301], a difference that remains unexplained.

$Log K_{OM}^{SED}$ was estimated as $Log K_{OM}^{SED}$ =$LogK_{ow}$ – 1.35 with a narrow 90% CI for the intercept of -1.45 to -1.21. This means that K_{OM}^{SED} was about an order of magnitude lower than K_{ow}, which explains the observed base level of the BSAFs of about 10 to 15 (Figure 2). Furthermore, this intercept is lower than the intercept of -0.48 reported by Seth et al.[268] for natural sediment OM, and BSAFs can be <1 for aged field sediments,[331] which implies that bioavailability in the OECD test is higher and overestimates the exposure that might occur under more natural conditions. We conclude that tests with artificial sediment may provide a worst case risk assessment because of the low K_{OM}^{SED} and additional ingestion pathways.

The constant ratio γ between K_{OM}^{SS} and K_{OM}^{SED} had a value of 4.07 (90% CI=3.28 - 4.86), which agrees very well with the average value of 3.73 observed for PCBs in an estuarine field dataset provided by Koelmans et al.[318]. This implies that differences in chemical affinity for sediment and suspended OM play a role in the exposure, and also that it is important to distinguish between ingestion of sediment and of suspended OM fractions (i.e. the parameter β). *A. marina* ingests whole sediment, and had the highest sediment ingestion as expected, although CI intervals were wide (Figure 3, Table S19). *M. balthica* is able to switch between filter feeding and suspension feeding, depending on food availability.[337,338]

The low value of β for *M. balthica* indicates that most OM was taken up by filtering suspended solids from the water column.

Relative importance of uptake pathways

The calibrated model was used to estimate the relative importance of uptake pathways (Eq. 5, Table S22). Chemical uptake from ambient water was estimated to be minor and decreased with increasing $LogK_{ow}$. This means that uptake from OM ingestion dominated for all species, except for *A. marina* (Table S22). *C. volutator* showed high growth rate, which also implies substantial food ingestion, and food contributed up to >95% to the bioaccumulation of the most hydrophobic congeners (Table S22). Sediment uptake was also the major uptake pathway for *M. balthica*, which is in agreement with earlier findings.[10,11] In contrast, *A. marina* did not grow well, which led to reduced or no ingestion, so uptake from the water was calculated to dominate (Table S22). This, however, should be interpreted as a peculiarity of the organism's behaviour in our test systems, and differs from previously published data, in which sediment ingestion dominated.[8,29,42]

In conclusion, we showed that bioaccumulation varied widely among marine benthic invertebrates, with values between 5 and 318. This was explained by food ingestion and implies that EPT is not suitable to assess the exposure of these species. Exposure was higher than expected, due to food ingestion, but also to a particularly low affinity of the OECD test sediment, which implies that OECD tests may provide a worst-case outcome. We have shown how bioaccumulation modelling can be used to link model mechanisms and parameters to species traits. Species-specific traits, such as ingestion and differentiation between sediment and suspended solid particles as food sources in the diet, were important determinants of the extent of bioaccumulation from sediment. The variability in chemical behaviour, species-specific traits and species performance explained the observed high variability of BSAF, and should be taken into account in risk assessment of sediment-bound chemicals. Finally, we showed how a novel test set-up that provides uniform exposure across species tested can be used to increase the sensitivity of tests for detecting the differences in bioaccumulation due to differences in species traits, with obvious implications for toxicity testing.

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Supporting information

Methods and materials

Chemicals and materials

PCBs standards IUPAC numbers 28, 52, 101, 118, 153, 180, chlorpyrifos (CPF) (purity 98.0 %) and chlorphyrifos-D10 (internal standard) were obtained from Dr. Ehrenstorfer. For OECD sediment peat from Klasmann Deilmann Benelux BV, $CaCO_3$ powder from Sigma Aldrich, Germany, quartz sand from Geba 0.06-0.25 mm, Eurogrid, The Netherlands and kaolin from Sigma Aldrich, German was used. An analytical balance (AX204; Mettler Toledo) was used for weighing.

Sediment and water medium

Six batches of sediment with low, medium, or high organic matter content were prepared in prewashed (2 x 0.5 L acetone and 1x 100 mL n-hexane analytical grade) containers of 25 L. Peat dried at 40°C until the weight did not change anymore and afterwards was homogenized by grinding it into 1 mm particles. Peat (1%, 5% or 15%), calcium carbonate powder (1%) and unfiltered natural seawater from the Oosterschelde, the Netherlands were thoroughly mixed six days before spiking to obtain a homogeneous slurry. After spiking the PCB spike solution (see main manuscript) to the slurry, sand (75%) and kaolin clay (20%) were directly mixed through. After 27 d, chlorpyrifos was spiked into the sediment. Salinity (CDC 401, Hach), pH (PHC301, Hach) and temperature (CDC 401, Hach) were measured before spiking the sediments. The sediment had an average (SD) pH of 6.06 (0.14), salinity of 32.6 (0.5)% and a temperature of 12.6 (0.6)°C before spiking.

Acclimatization of test species

All test species were acclimatized under test conditions. *Arenicola marina* was kept in an aquarium with a layer of uncontaminated artificial sand and aerated natural seawater under experimental conditions for 4 d, before the start of the experiment. *Corophium volutator* was kept in a container with sieved natural sediment and aerated natural seawater under experimental conditions for 6 d before the start of the experiment. *Macoma baltica* was kept in an aquarium with a layer of uncontaminated artificial sand and aerated natural seawater under experimental conditions for 5 d before the start of the experiment. *Nereis virens* was kept unfed in an aquarium with aerated natural seawater under experimental conditions for 5 d before the start of the experiment. None of the animals were fed additionally.

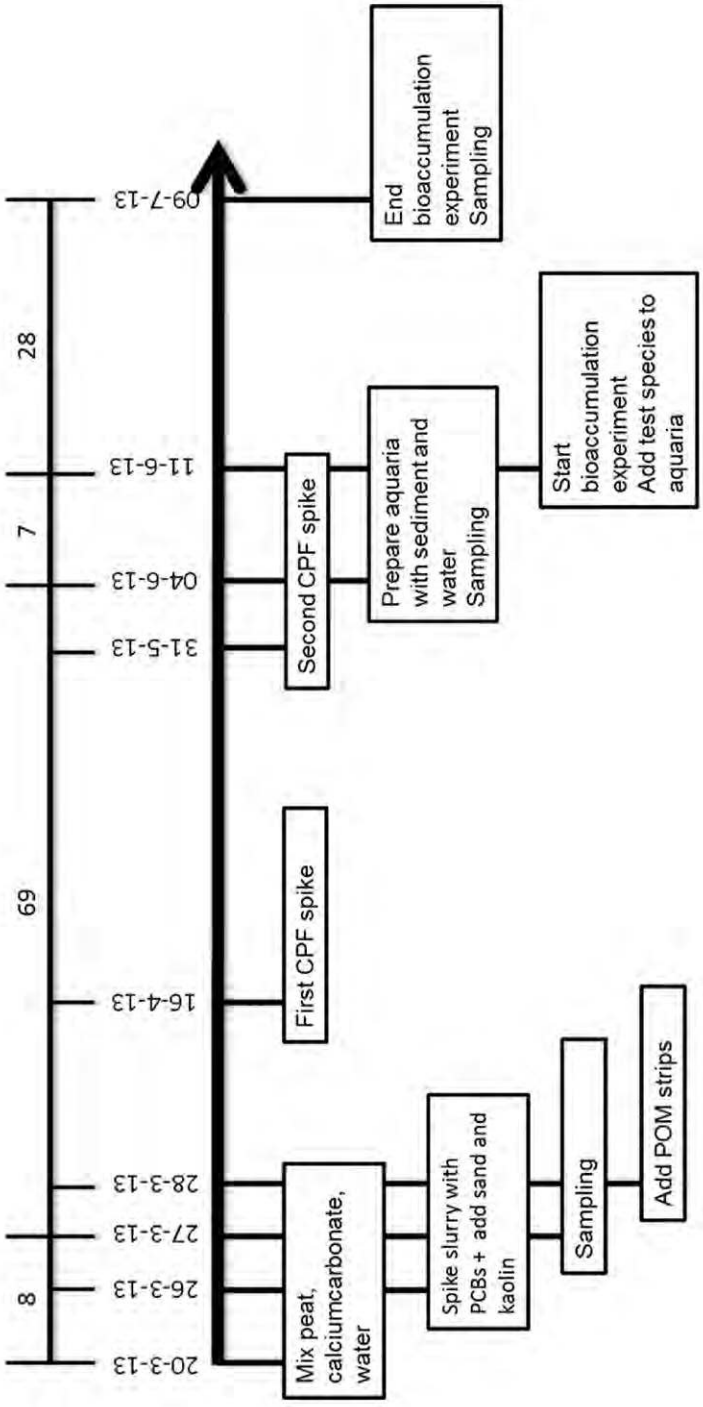

Figure S1. Overview of sediment preparation phase and bioaccumulation experiment for the main test 3. Time scale in days.

Table S1. Traits of *Arenicola marina, Corophium volutator, Macoma balthica* and *Nereis virens.*

Traits[a]	*A. marina*	*C. volutator*	*M. balthica*	*N. virens*
				
Food type	Micro-organisms (bacteria), benthic diatoms, meiofauna and detritus	Particulate organic matter, epipelic (=living on fine sediment) and epipsammic (=living on sand) bacteria and diatoms	Diatoms, deposited plankton, suspended phytoplankton and detritus	Mud, sand, detritus, phytoplankton, plankton and other macrofauna
Size (cm)	11-20	1-2	1-2	11-20
Average lipid content (SD) (%)[b]	1.0 (0.1)	1.5 (0.3)	1.6 (0.3)	1.8 (0.1)
Habit	Burrow dwelling, Infaunal	Burrow dwelling, Infaunal	Burrow dwelling, Infaunal	Burrow dwelling, Infaunal
Respiration type	Gills, body surface	Gills	Gills	Gills
Bioturbation (burrow depth in cm)	U-shaped tubes (15-20)[339]	U-shaped tubes (2-6)[339,340]	Biodiffuser (2-6)[339]	Burrow gallery (6-12)[339,340]
Growth Rate (mm/year)	Insufficient information	8 - 11	3	Insufficient information
Dispersal potential of adult (km)	0.1-1	1-10	0.1-1	1-10
Life span (years)	6-10	<1	6-10	1
Maturity (years)	1-2	<1		<1
Generation time (years)	1-2	<1	1-2	1-2
Reproduction frequency	Annual episodic	Annual episodic	Annual episodic	Semelparous
Reproduction season	Autumn - winter	See additional information	Spring and autumn	Spring - summer
Larval settle period	Not relevant	Not relevant	Insufficient information	Not relevant
Fecundity	316,000 oocytes per female	To ca 50	30000	Insufficient information
Dispersal potential of larvae (km)	1-10	<0.01	>10	Not relevant
Larval settling time	Not relevant	Not researched	1-6 months	Not relevant
Reproduction location	Adult burrow	Adult burrow	Insufficient information	Adult burrow
Development mechanisms	Lecithotrophic, Oviparous	Planktotrophic	Direct development	Oviparous
Depth range	Intertidal	Intertidal		Intertidal
Migratory	Non-migratory / Resident	Non-migratory / Resident	Non-migratory / Resident	Non-migratory / Resident
Growth form	Vermiform segmented, Vermiform annulated, Cylindrical	Articulate	Bivalved	Vermiform segmented
Mobility	Burrower	Swimmer, Crawler, Burrower	Crawler, Burrower	Swimmer, Crawler, Burrower
Reproduction type	Gonochoristic	Gonochoristic	Gonochoristic	Gonochoristic
Salinity (psu)	18- 40	<18-40	<18-40	<18-40
Water flow	Very weak - very strong	Very weak - weak	Weak - moderately strong	Very weak - weak
Wave exposure	Very Sheltered - Moderately Exposed	Extremely Sheltered - Sheltered	Extremely Sheltered - Sheltered	Extremely Sheltered - Sheltered

[a] Information was retrieved from the MarLin database[320] unless stated otherwise [b] Measured in this study in a subsample at the start of the experiment

Table S2. Overview of the three experiments.

	Species included	Test units	Number of animals per test unit at start of the experiment	Average (SD) of total biomass at start of experiment (g)[a]	Average (SD) length (cm)[b]	Sediment (kg WW)	Overlying water (L)	Sediment to water volume ratio (L/L)[c]	Biota tissue to sediment ratio (kg/kg)	Biota tissue to sediment water ratio (kg/kg/kg)	Density (number of individuals/kg WW)	End-points[d]
Test 1	*Arenicola marina*	Round aquarium 5 L	3	8.2 (1.7)	6.7 (1.3)	1.8	3.4	1/3	1/220	1.3E-03	2	S, G
	Corophium volutator	Beaker 600 mL	15	0.13 (0.03)	0.05 - 0.1	0.24	0.5	1/3	1/1846	1.2E-03	63	S, G
	Macoma balthica	Beaker 600 mL	6	2.7 (0.6)	1.11 (0.15)	0.24	0.5	1/3	1/89	2.5E-02	25	S, G
	Nereis virens	Beaker 2 L	5	7.1 (0.7)	6.5 (1.5)	0.8	1.5	1/3	1/113	5.9E-03	6	S, G
Test 2	*Arenicola marina*	Aquaria (35Lx30Wx30H cm) with enclosures	5	11.7 (1.3)	6.7 (1.3)	2.2	8.2	1/6	1/188	6.5E-04	2	S, G
	Corophium volutator		50	0.4 (0.8)	0.05 - 0.1	1.1	3.0	1/4	1/2750	1.2E-04	45	S, G
	Macoma balthica		10	3.2 (0.3)	1.11 (0.15)	1.1	3.0	1/4	1/344	9.6E-04	9	S, G
	Nereis virens		10	13.3 (0.9)	6.5 (1.5)	2.2	8.2	1/6	1/165	7.4E-04	5	S, G
Test 3a	*Arenicola marina*	Aquaria (35Lx30Wx30H cm) with enclosures	5	15.5 (1.5)	9.0 (1.7)	2.5	8.2	1/5	1/161	7.5E-04	2	S, G, B, H
	Corophium volutator		70	0.12 (0.05)	0.05 - 0.1	1.0	3.0	1/5	1/8333	4.0E-05	70	S, G, B, H
	Macoma balthica		25	8.6 (0.6)	1.19 (0.1)	1.0	3.0	1/5	1/116	2.8E-03	25	S, G, B, H
	Nereis virens		10	21.6 (2.1)	8.4 (1.4)	2.5	8.2	1/5	1/116	1.1E-03	4	S, G, B, H
Test 3b	*Arenicola marina*	Aquaria (35Lx30Wx30H cm) without enclosures	5	14.6 (1.5)	9.0 (1.7)	7.0	25	1/5	1/479	8.3E-05	1	S, G, B, H
	Corophium volutator		70	0.16 (0.03)	0.05 - 0.1	7.0	25	1/6	1/43750	9.1E-07	10	S, G, B, H
	Macoma balthica		25	8.7 (0.3)	1.19 (0.1)	7.0	25	1/6	1/805	5.0E-05	4	S, G, B, H
	Nereis virens		10	22.1 (1.8)	8.4 (1.4)	7.0	25	1/6	1/317	1.3E-04	1	S, G, B, H

[a] Biomass was based on wet weight. Weight for *A. marina* was measured before gut clearance, for *C. volutator* weight was measured at the end of the bioaccumulation period and for *M. balthica* weight included flesh and shell. [b] Average and SD length were based on a random subsample at the start of the experiment. Length for *C. volutator* was not measured but based on collection of animals over 500 µm and 1000 µm sieves. Length for *M. balthica* was measured over the widest part of the shell. [c]To calculated sediment to water volume ratio a density of 1.6 L/kg was used to transfer sediment mass to volume. [d]S=survival, G=growth, B=bioaccumulation, H=heaps only for *A. marina*.

Water quality

The water quality variables oxygen, temperature, salinity, conductivity and pH were measured with a Hach (HQ40d) portable multi-meter using the Luminescent Dissolved O_2 probe (LDO101), the conductivity probe (CDC401) and the gel filled pH electrode (PHC 101) or pH meter (SG8-ELK) by Mettler Toledo (bioaccumulation experiment, main test 3). Temperature was measured with the O_2 probe. Ammonium concentrations were measured with the ammonium cell test by Merck with a range of 0.20 - 8.00 mg/l NH_4-N and nitrite concentrations with the colorimetric nitrite test by Merck with a range 0.025-0.5 mg/L NO_2. Phytoplankton concentrations (µg/L) were measured with the Algal Lab Analyzer using a spectrofluorometer (bbe). Turbidity (NTU) was measured with a turbidity meter (TN100; Eutech instruments).

Extraction and analyses

Extraction and analysis followed previously published procedures.[341] Water samples (n=3) of natural seawater were taken to determine background concentrations. Water samples were extracted using C_{18} Empore disks. 200 µL of internal standard solution (PCB112, 80 ng/mL) was added to 200 mL of sample after which the sample was introduced onto the disk and subsequently eluted with 20 mL dichloromethane. The samples were concentrated to 200 µL and transferred to sample vials for analysis.

Invertebrate analysis used mixed samples of surviving individuals per treatment. Biota, sediment and fish food (Tetramin) samples were dried using sodium sulphate (Merck) and extracted by soxhlet extraction using a mixture of pentane/dichloromethane (50:50 v/v). Internal standard solution (1 mL) (PCB112, 80 ng/mL) was added to each sample. For biota samples, half of the extract was dried to gravimetrically determine the fat content. Extracts were then concentrated to 2 ml using a rotavap (Heidolph) and cleaned up on a 25 g florisil column. The extract was run into the column and subsequently eluted using 200 ml of 7% diethyl-ether in pentane. The extract was then concentrated to 1 mL for sediments and 0.5 mL for the biota samples under a gentle flow of nitrogen and transferred to a vial for analysis.

Moisture content was determined gravimetrically after drying for 3 hours at 105°C. Sediment organic matter content was determined gravimetrically after drying at 550°C for 2 hours.

Analyses

Analytical procedures were published before (e.g. Amaraneni[341]). Invertebrate, sediment, water and fish food (1 µL) were injected on a Shimadzu GCMS2010 (GC) coupled to a GC-MS-QP2010 Ultra (MS) detector (Shimadzu,'s Hertogenbosch, the Netherlands). Column used was a 30m x 0.25 mm i.d. HT8 with a film thickness of 0.25 µm. Analysis was performed using Electron Impact (EI) in single ion monitoring (SIM) mode. Injection port and source temperatures were 250 and 200 °C respectively. Oven temperature program started at 90°C, hold for 3 minutes, increased by 20 °C/min to 170°C followed by an

increase by 2.5°C/min to 292. At the end of the program, a column was heated to 320°C for 10 minutes. The following quantifier and qualifier ions were monitored respectively, 256 and 258 for PCB 28, 292 and 290 for PCB 52, 326 and 324 for PCB 101, PCB 112 and PCB118, 360 and 362 for PCB 153, 394 and 396 for PCB 180 and 197 and 314 for chloropyrifos.

Quality assurance

Recovery was between 80-110% for all compounds. Calibration curves consisted of 9 points within a range of 1-650 ng/mL. $R^2 \geq 0.999$ was achieved for each calibration curve for all compounds. Limit of quantification of the PCBs and CPF depended on sample intake, which was typically <1 ng/L for water, <0.1 ng/L for sediment, <0.1ng/g fish food and between <0.03 ng/L and <10 ng/L for biota. Spiked concentrations ranged from 20% to 65% of the nominal concentrations (Table S4). Water background concentrations were below <1 ng/L.

Table S3. Average (SD) measured total sediment concentrations (µg/kg DW) (n=3) compared with the nominal concentration (µg/kg DW) for the three treatments: low, medium and high organic matter content at the start of the experiment.

		Low OM		Medium OM		High OM	
Chemical	**Nominal (µg/kg)**	**Measured (µg/kg)**	**% of nominal**	**Measured (µg/kg)**	**% of nominal**	**Measured (µg/kg)**	**% of nominal**
PCB 28	6	1.2 (0.2)	20	1.9 (1.2)	31	1.4 (0.3)	24
PCB 52	6	1.5 (0.2)	25	2.0 (0.9)	34	1.7 (0.4)	28
PCB 101	6	1.5 (0.2)	24	1.7 (0.6)	28	1.6 (0.3)	27
PCB 118	6	1.8 (0.2)	31	2.1 (0.7)	35	2.7 (0.7)	45
PCB 153	6	1.7 (0.2)	28	1.7 (0.3)	29	1.9 (0.2)	32
PCB 180	6	1.6 (0.3)	26	1.6 (0.3)	27	1.5 (0.1)	25
CPF	3.12	1.7 (0.2)	55	1.8 (0.4)	57	2.0 (0.3)	65

Table S4. Average (SD) measured food concentrations (µg/kg OM) (n=3) compared with the nominal concentration (µg/kg OM) for the three treatments: low, medium and high organic matter content just after spiking.

	Low OM			Medium OM			High OM		
Chemical	**Nominal (µg/kg)**	**Measured (µg/kg)**	**% of nominal**	**Nominal (µg/kg)**	**Measured (µg/kg)**	**% of nominal**	**Nominal (µg/kg)**	**Measured (µg/kg)**	**% of nominal**
PCB 28	142	142 (46)	100	81	93 (34)	115	40	51 (3)	127
PCB 52	142	163 (44)	115	81	90 (50)	111	40	43 (13)	109
PCB 101	142	181 (42)	128	81	92 (54)	113	40	38 (18)	96
PCB 118	142	167 (47)	118	81	95 (46)	117	40	46 (4)	114
PCB 153	142	155 (32)	109	81	97 (52)	119	40	26 (7)	65
PCB 180	142	123 (41)	86	81	58 (59)	71	40	BLD	
CPF	73	BLD		42	BLD		21	BLD	

Model description

Table S5. Parameters for the biota sediment accumulation factor model.

Parameter	Symbol	Unit
Affinity of chemical partitioning to peat in the relation $Log K_{OM}^{SED} = LogK_{OW} + b$	b	-
Biota sediment accumulation factor	$BSAF_t$	-
Chemical assimilation efficiency	α	-
Concentration in invertebrate lipids	C_L	μg×kg^{-1} lipids
Concentration in water	C_w	μg×L^{-1}
Concentration in sediment	C_{OM}^{SED}	μg×kg^{-1} OM
Concentration in suspended solids	C_{OM}^{SS}	μg×kg^{-1} OM
Constant ratio between sorption affinities for suspended matter OM and sediment OM in the relation $K_{OM}^{SS} = \gamma K_{OM}^{SED}$	γ	-
Dermal uptake rate constant	k_w	L×kg^{-1}×d^{-1}
Elimination rate constant	k_e	d^{-1}
Food ingestion rate	I	kg_{OM}×kg_{Lipids}^{-1}× d^{-1}
Fraction of ingested OM originating from the sediment	β (0<β<1)	-
Growth rate constant	k_g	d^{-1}
Lipid water partition coefficient (k_w/k_e)	K_{lip}	L×kg^{-1}
Sediment water partition coefficient	K_{OM}^{SED}	L×kg^{-1}
Species-specific elimination parameter in the relation $LogK_e$=-$LogK_{ow}$ + a	a	-
Suspended solids water partition coefficient	K_{OM}^{SS}	L×kg^{-1}
Time	t	d

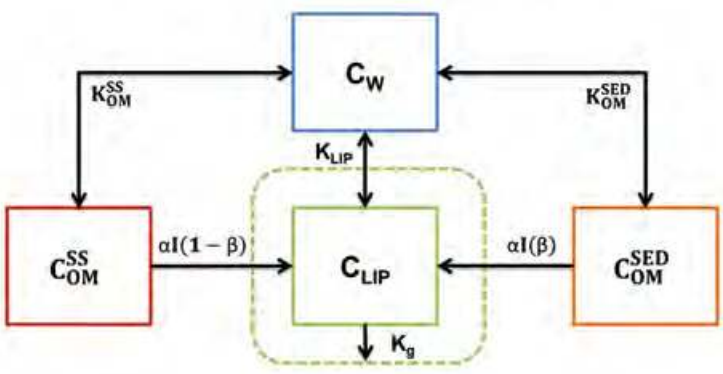

Figure S2. Schematic overview of biota sediment accumulation factor model.

Results

Table S6. Average (SD) water quality values for dissolved oxygen (DO), salinity, conductivity, pH, temperature and ammonium with their range (min-max) for test, 1, 2 and 3.

	Species	DO (mg/L)	Range (mg/L)	Salinity (‰)	Range (‰)	Cond (mS/m)	Range (mS/m)	pH	Range	Temp (°C)	Range (°C)	Ammonium (mg NH_4-N /L)	Range (mg NH_4-N /L)
Test 1	*A. marina*	8.97 (1.41)	5.57-10.16	33.3 (0.6)	31.9-34.4	39.2 (0.9)	33.8-40.7	7.92 (0.23)	7.22-8.29	13.0 (0.4)	12.4-14.3	5.44 (3.57)*	<0.20-11.68
	C. volutator	9.21 (1.09)	2.38-10.26	32.7 (2.3)	28.6-38.4	38.0 (2.3)	33.9-44.1	8.01 (0.17)	7.56-8.37	12.9 (0.3)	12.4-14.4	5.96 (2.32)*	<0.20-8.80
	M. balthica	9.27 (0.74)	5.55-10.11	32.5 (2.7)	26.9-38.8	37.7 (2.7)	32-1-44.2	7.81 (0.15)	7.59-8.17	12.9 (0.3)	12.3-13.6	8.37 (2.58)*	4.60-13.32
	N. virens	8.42 (1.51)	0.14-10.09	34.3 (0.8)	32.2-36.2	40.0 (0.8)	37.9-42.0	7.98 (0.22)	7.41-8.39	12.7 (0.2)	12.2-13.2	14.42 (8.82)*	6.17-31.64
Test 2		8.82 (0.45)	7.38-9.64	34.3 (0.9)	33.5-34.9	40.1 (0.5)	39.0-41.1	8.02 (0.36)	7.59-8.76	12.8 (0.2)	12.2-13.6	4.17 (0.99)*	2.78-5.37
Test 3a		9.00 (0.66)	4.18-9.87	34.4 (0.4)	33.4-35.7	40.4 (0.7)	29.6-41.9	8.09 (0.32)	7.15-9.95	12.8 (0.2)	12.4-14.1	1.85 (2.07)	0.1-10.14
Test 3b		9.39 (0.43)	7.16-9.94	34.4 (0.5)	33.5-35.3	40.7 (0.48)	40.1-42.0	8.07 (0.10)	7.89-8.32	13.2 (0.2)	12.9-13.6	2.60 (2.25)	0.1-6.73

* Values outside detection range were not used for calculation.

Table S7. Average (SD) phytoplankton concentration and turbidity values and range in µg/L, for the bioaccumulation test (Main test 3), for systems with and without enclosure.

	Cyanobacteria (µg/L)	Range (µg/L)	Green algae (µg/L)	Range (µg/L)	Diatoms (µg/L)	Range (µg/L)	Turbidity (NTU)	Range (NTU)
Test 3a	0.25 (0.23)	0-1.39	0.18 (0.30)	0-1.72	0.78 (0.99)	0.27-3.44	7.04 (6.61)	0.86-30.1
Test 3b	0.27 (0.11)	0.05-0.5	0	0-0	0.71(0.31)	0.32-1.33	81.72 (114.65)	1.05-302.00

Table S8. Average survival (SD) (%) during 28 days for test 1, 2 and 3.

Experiment	*OM*	Average survival (SD) %				
	Treatment	***A. marina***	***C. volutator***	***M. balthica***	***N. virens***	***Overall***
Test 1 (n=3)	Low	78 (19)	82 (17)	72 (35)	60 (23)	73
	Medium	100 (0)	82 (8)	100 (0)	53 (42)	84
	High	100 (0)	84 (10)	100 (0)	33 (12)	79
	Natural	100 (0)	98 (4)	94 (10)	46 (12)	85
Test 2 (n=3)	Medium	100 (0)	91 (2)	99 (1)	88 (2)	95
Test 3a (n=4)	Low	85 (30)	66 (26)	98 (2)	83 (15)	83
	Medium	65 (25)	65 (8)	97 (4)	90 (14)	79
	High	85 (19)	47 (15)	99 (2)	90 (18)	80
Test 3b (n=4)	Medium	0 (0)	64 (10)	100 (0)	100 (0)	88*

* A. marina was excluded from the overall value

Table S9. Average relative weight gain/loss per individual (SD) (%) based on wet weight during 28 days for test 1, 2 and 3.

Experiment	*OM*	Average relative growth per individual (SD) (%)			
	Treatment	***A. marina***	***C. volutator***	***M. balthica***[a]	***N. virens***
Test 1 (n=3)	Low	-15.8 (14.1)	32.7 (19.9)	-36.7 (13.4)	-9.6 (16.4)
	Medium	-17.3 (6.3)	27.1 (8.9)	-15.9 (10.0)	-18.1 (26.4)
	High	-17.5 (13.8)	22.2 (19.2)	-7.1 (1.8)	-5.0 (39.4)
	Natural	-21.2 (2.6)	42.1 (29.0)	-6.4 (4.0)	-7.8 (20.3)
Test 2 (n=3)	Medium	-32.7 (7.0)	24.5 (3.9)	- 32.0 (0.8)	-0.9 (9.0)
Test 3a (n=4)	Low	-36.4 (18.3)	115.9 (37.6)	-7.1 (4.3)	-9.6 (11.9)
	Medium	-57.2 (8.8)	101.0 (60.2)	5.1 (11.1)	4.1 (3.3)
	High	-35.2 (7.3)	57.4 (38.4)	12.2 (8.9)	3.2 (3.5)
Test 3b (n=4)	Medium		155.9 (20.3)	19.1 (9.5)	28.0 (2.9)

[a] Flesh only, shell was removed

Table S10. Statistical test for differences in survival (%) of each species between low, medium, high and natural sediment treatments in single species pilot test 1, medium sediment single species (Pilot test 1) and multi species (Pilot test 2), low, medium and high sediment treatments in bioaccumulation test (Main test 3a) and medium sediment treatments for separated and mixed species (Main test 3b). Bold values indicate p<0.05.

Experiment	Compares treatments	Species	F/t/Z/Chi-square	df	p-value
Test 1	*Low, medium, high and natural*	*A. marina*[a]	6.600	3	0.086
		C. volutator[b]	1.462	3,11	0.296
		M. balthica[a]	4.689	3	0.196
		N. virens[a]	1.222	3	0.748
Test 2	*Medium test 1 and test 2*	*A. marina*[c]			
		C. volutator[d]	-1.766	4	0.152
		M. balthica[e]	-1.000		0.700
		N. virens[d]	2.672	4	0.557
Test 3a	*Low, medium and high*	*A. marina*[a]	1.654	2	0.437
		C. volutator[a]	2.423	2	0.298
		M. balthica[a]	0.838	2	0.658
		N. virens[a]	0.789	2	0.674
Test 3b	*Enclosed single species and mixed species with medium OM*	*A. marina*[d]	5.166	6	**0.002**
		C. volutator[d]	0.276	6	0.792
		M. balthica[d]	-1.567	6	0.215
		N. virens[d]	-1.414	6	0.207

[a] Kruskal-Wallis test [b] One-way ANOVA [c] t cannot be computed because the standard deviations of both groups are zero [d] Independent t-test [e] Mann-Whitney U test.

Table S11. Statistical test for differences in relative growth (%) of each species between low, medium, high and natural sediment treatments in single species pilot test 1, medium sediment single species (Pilot test 1) and multi species (Pilot test 2), low, medium and high sediment treatments in bioaccumulation test (Main test 3a) and medium sediment treatments for separated and mixed species (Main test 3b). Bold values indicate p<0.05.

Experiment Compares treatments	Relative weight gain	Species	F/t/Z/Chi-square	df	p-value
Test 1	Wet weight	*A. marina*[a]	0.145	3,11	0.930
		C. volutator[a]	0.518	3,11	0.682
Low, medium, high and natural		*M. balthica*[b]	7.051	3	0.070
		N. virens[a]	0.130	3,11	0.940
	Dry weight	*A. marina*[b]	1.051	3	0.789
		C. volutator[b]	3.512	3	0.319
		M. balthica[a]	3.292	3,11	0.079
		N. virens[a]	0.185	3,11	0.903
	As free dry weight	*A. marina*[b]	0.744	3	0.863
		C. volutator[a]	1.100	3,11	0.404
		M. balthica[a]	3.208	3,11	0.083
		N. virens[a]	0.339	3,11	0.798
Test 2	Wet weight	*A. marina*[c]	2.851	4	**0.046**
		C. volutator[d]	0.458	4	0.671
Medium test 1 and test 2		*M. balthica*[c]	-1.732		0.200
		N. virens[c]	-1.066	4	0.346
	Dry weight	*A. marina*[c]	0.482	4	0.655
		C. volutator[c]	-2.911	4	0.100
		M. balthica[c]	-4.993	4	**0.015**
		N. virens[c]	0.777	4	0.481
	As free dry weight	*A. marina*[c]	-.208	4	0.846
		C. volutator[c]	-2.498	4	0.127
		M. balthica[c]	-4.716	4	**0.018**
		N. virens[c]	0.343	4	0.749
Test 3a	Wet weight	*A. marina*[a]	2.238	2,11	0.163
Low, medium and high		*C. volutator*[b]	3.231	2	0.199
		M. balthica[a]	5.277	2,11	**0.031**
		N. virens[b]	4.885	2	0.087
Test 3b	Wet weight	*A. marina*[c]	-5.552	6	**0.001**
Enclosed single species and mixed species for medium OM		*C. volutator*[c]	-1.729	6	0.134
		M. balthica[c]	-1.924	6	0.103
		N. virens[c]	-10.890	6	**0.000**

[a] One-way ANOVA [b] Kruskal-Wallis test [c] Independent t-test [d] Mann-Whitney U test.

Table S12. Average heaps per individual per day (SD) for *Arenicola marina* during the bioaccumulation test (Main test 3).

Treatment	Average heaps per individual per day (SD)
Low	0.4 (0.1)
Medium	0.5 (0.1)
High	0.4 (0.2)

Table S13. Average (SD) PCB concentrations in the sediments (µg/kg DW) (Main test 3).

	Treatment	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 180	CPF
Sediment µg/kg DW (n=3)	Low OM	1.2 (0.2)	1.5 (0.2)	1.5 (0.2)	1.8 (0.2)	1.7 (0.2)	1.6 (0.3)	1.7 (0.2)
Start	Medium OM	1.9 (1.2)	2.0 (0.9)	1.7 (0.6)	2.1 (0.7)	1.7 (0.3)	1.6 (0.3)	1.8 (0.4)
	High OM	1.4 (0.3)	1.7 (0.4)	1.6 (0.3)	2.7 (0.7)	1.9 (0.2)	1.5 (0.1)	2.0 (0.3)
End	Low OM	1.7 (0.8)	1.9 (1.0)	2.2 (1.1)	1.8 (1.0)	1.8 (0.9)	2.1 (1.6)	BDL
	Medium OM	3.1 (0.2)	3.6 (0.4)	3.5 (0.3)	3.2 (0.4)	3.1 (0.3)	3.7 (0.8)	BDL
	High OM	2.8 (0.3)	3.3 (0.5)	3.5 (0.5)	2.9 (0.2)	3.0 (0.3)	3.4 (0.8)	BDL
	Mixed medium OM	1.4 (0.5)	1.6 (0.5)	1.7 (0.5)	1.3 (0.4)	1.2 (0.4)	1.1 (0.4)	BDL

BDL = below detection limit.

Table S14. Average and SD of PCB concentrations (µg/kg WW) based on wet weight for *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens* for low OM, medium OM, high OM and mixed medium OM treatment (Main test 3).

	Wet tissue concentration (µg/kg ww)													
Treatment	**PCB 28**		**PCB 52**		**PCB 101**		**PCB 118**		**PCB 153**		**PCB 180**		**Lipids**	
	Average	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
Low OM														
A. marina	17.8	4.0	17.3	3.5	14.8	3.6	12.9	3.4	15.3	4.1	5.8	1.6	1.2	0.2
C. volutator	47.3	8.8	61.3	4.3	84.3	4.6	98.3	15.9	91.5	19.2	68.8	42.5	1.2	0.5
M. balthica	24.5	2.4	17.8	3.0	22.3	2.2	20.3	2.2	20.0	2.8	12.2	3.7	2.0	0.1
N. virens	9.1	2.4	10.1	2.0	8.9	1.8	8.4	2.3	9.7	2.4	8.0	2.7	1.6	0.1
Medium OM														
A. marina	10.5	1.7	10.1	1.9	9.7	1.4	8.5	2.2	9.7	2.1	3.9	0.5	1.2	0.1
C. volutator	14.0	4.8	12.8	1.3	12.2	8.2	23.5	6.5	29.0	12.5	BDL		0.6	0.2
M. balthica	10.4	1.2	10.6	2.0	8.2	1.8	13.4	7.1	14.0	9.5	4.0	0.6	1.5	0.4
N. virens	15.5	3.7	11.5	2.4	14.7	2.9	18.8	11.1	20.5	15.6	5.4	2.4	1.6	0.8
High OM														
A. marina	5.0	0.7	5.2	0.6	5.6	0.9	4.7	0.9	5.5	0.9	1.9	0.7	1.2	0.2
C. volutator	BDL		BDL		10.0	-	11.0	-	17.0	-	BDL		1.3	0.2
M. balthica	9.0	1.5	6.9	1.3	9.5	1.3	7.9	1.5	8.2	1.0	3.5	1.7	2.1	0.2
N. virens	3.2	0.4	3.3	0.3	2.8	0.4	2.5	0.5	3.4	0.5	1.8	0.2	1.6	0.1
MS medium OM														
C. volutator	11.5	3.1	13.0	5.4	17.8	7.2	32.3	17.3	33.0	11.4	30.0	19.8	0.5	0.3
M. balthica	12.3	2.2	9.0	1.4	13.3	1.5	11.8	1.7	11.3	1.9	5.8	1.2	1.8	0.2
N. virens	7.2	0.9	7.5	0.7	8.5	0.7	8.2	0.8	9.3	0.8	6.5	0.6	1.6	0.1

- SD could not be calculated because none or only one concentration was above detection limit

Table S15. Average (SD) biota sediment accumulation factor (BSAF) normalized for lipids and organic matter for *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens* for low OM, medium OM, high OM and mixed medium OM treatment (Main test 3).

	BSAF											
Treatment	**PCB 28**		**PCB 52**		**PCB 101**		**PCB 118**		**PCB 153**		**PCB 180**	
	Average	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
Low OM												
A. marina	36.5	8.3	31.7	6.4	23.2	4.9	24.8	5.8	28.4	7.2	9.3*	2.2
C. volutator	110.5**	37.1	131.0**	54.3	156.4**	69.8	217.3**	81.1	195.1**	78.3	129.5*	101.3
M. balthica	30.8	1.6	20.0	3.2	21.5	1.2	24.0	1.6	22.8	2.3	11.9	3.3
N. virens	14.1**	4.2	14.0**	3.3	10.6**	2.7	12.4**	3.9	13.8**	4.1	9.8	3.7
Medium OM												
A. marina	23.1	3.0	19.3	3.2	19.3	2.2	18.1	4.3	21.6	4.2	7.2	0.6
C. volutator	65.2**	24.1	52.1**	13.3	49.2*	30.7	106.1**	35.3	122.3*	66.4	-	-
M. balthica	36.5	37.8	22.8	23.7	30.8	31.5	24.4	22.9	24.8	23.6	9.0	8.1
N. virens	8.8**	1.1	8.4**	0.8	7.6*	0.4	7.4**	0.8	9.1*	0.9	5.3	0.6
High OM												
A. marina	25.1	2.6	22.0**	1.4	22.4*	2.4	22.7*	2.1	25.7*	1.8	8.1	3.5
C. volutator	-	-	-	-	-	-	-	-	-	-	-	-
M. balthica	26.3*	3.7	16.7	3.0	22.1	2.2	22.2	2.2	22.5	2.5	8.1	3.1
N. virens	11.7*	1.8	10.1**	1.1	8.4*	1.5	9.1*	2.0	11.8*	1.9	5.4	0.8
MS medium OM												
C. volutator	99.2*	99.6	93.6*	86.4	137.0**	157.7	259.7*	200.6	318.0*	281.7	154.7	79.3
M. balthica	18.9	3.9	12.3	1.7	16.8	2.5	19.9	3.5	20.9	3.6	10.7	2.3
N. virens	12.2*	2.3	11.3*	1.8	12.0**	1.8	15.3*	2.6	19.2*	3.0	13.4	2.1

- BSAF could not be calculated because concentrations were below detection limit. * Indicates a significant difference between species for one OM treatment with p=0.01-0.05 ** Indicates a significant difference between species for one OM treatment with p=0.001-0.009

Table S16. Effects of organic matter on the biota sediment accumulation factor (BSAF) for each species with Kruskall-Walis test (Test 3a). Bold values indicate p<0.05.

Species		PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 180
Arenicola marina	Chi-Square	6.348	7.477	2.712	2.679	2.053	2.106
	df	2	2	2	2	2	2
	p	**0.042**	**0.024**	0.258	0.262	0.358	0.349
Corophium volutator[a]	Z	-1.732	-2.309	-1.852	-2.309	-1.061	
	p	0.114	**0.029**	0.133	**0.029**	0.400	
Macoma balthica	Chi-Square	3.231	2.634	1.898	2.462	1.862	2.423
	df	2	2	2	2	2	2
	p	0.199	0.268	0.387	0.292	0.394	0.298
Nereis virens	Chi-Square	6.754	7.903	2.325	4.386	4.308	3.500
	df	2	2	2	2	2	2
	p	**0.034**	**0.019**	0.313	0.112	0.116	0.174

[a] High OM content was excluded from the analyses for *Corophium volutator* because the number of insufficient values above detection limit. Test was done with Mann-Whitney U test.

Table S17. Effects of species on the biota sediment accumulation factor (BSAF) for low, medium, high and medium mixed organic matter treatment, with Kruskall-Walis test (Main test 3a).

OM treatment		PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 180
Low ESS	Chi-Square	12.794	13.787	12.706	12.706	12.904	9.154
	df	3	3	3	3	3	3
	p	**0.005**	**0.003**	**0.005**	**0.005**	**0.005**	**0.027**
Medium ESS	Chi-Square	10.263	10.462	8.951	12.129	9.648	2.326
	df	3	3	3	3	3	2
	p	**0.016**	**0.015**	**0.030**	**0.007**	**0.022**	0.313
High ESS[a]	Chi-Square	7.449	9.846	7.420	7.475	8.578	3.500
	df	2	2	2	2	2	2
	p	**0.024**	**0.007**	**0.024**	**0.024**	**0.014**	0.174
Medium MS	Chi-Square	8.769	7.423	9.846	8.769	7.731	5.727
	df	2	2	2	2	2	2
	p	**0.012**	**0.024**	**0.007**	**0.012**	**0.021**	0.057

[a] *Corophium volutator* was excluded from the analyses because the number of insufficient values above detection limit.

Table S18. Comparing biota sediment accumulation factor (BSAF) values between separated single species and mixed species medium OM treatments for each species with Mann-Whitney U test (Main test 3).

Species		PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 180
Corophium volutator	Z	-0.289	-0.289	-0.926	-1.732	-1.061	
	p	0.886	0.886	0.533	0.114	0.400	
Macoma balthica	Z	0.000	-0.289	0.000	-0.866	-1.155	-1.155
	p	1.000	0.886	1.000	0.486	0.343	0.343
Nereis virens	Z	-1.732	-2.021	-2.309	-2.309	-2.309	-2.309
	p	0.114	0.057	**0.029**	**0.029**	**0.029**	**0.029**

Parameter Estimates

Table S19. Parameters and their 90% confidence intervals (Eq. 7) obtained from fitting with data for *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens* using the full model. Ingestion I was fitted separate for low, medium, high and medium mixed organic matter content.

Parameters	CI	*A. marina*	*C. volutator*	*M. balthica*	*N. virens*
	L90	5.1	-	2.8	-
a (-)		5.3	4.3	3.9	2.7
	H90	5.4	5.1	4.3	3.4
	L90	0*	0.5	0*	0.4
β (-)		0.9	0.7	0.2	0.5
	H90	1.0*	0.9	0.4	0.6
I ($kg_{OM} \times kg^{-1} \times d^{-1}$)	L90	0*	2.44	0.16	0.14
Low		0.06	4.60	0.24	0.18
	H90	0.23	6.74	0.32	0.22
	L90	0*	0.68	0.07	0.11
Medium		0.00	1.83	0.12	0.14
	H90	0.14	2.99	0.16	0.17
	L90	0*	0*	0.13	0.13
High		0.01	1.51	0.20	0.16
	H90	0.16	3.49	0.27	0.19
	L90	-	0.92	0.13	0.20
Mixed		0.00	2.33	0.20	0.25
	H90	-	3.74	0.27	0.31
	L90		-1.5		
b (-)			-1.3		
	H90		-1.2		
	L90		3.3		
γ (-)			4.1		
	H90		4.9		
N of experimental data points		66	66	96	96
Parameters	Per species	8	8	8	8
F-ratio value		1.779	1.779	1.741	1.741
SS_{min}		4.3	16.2	12.0	5.9
N of experimental data points			324		
Parameters	Total		26		
F-ratio value			1.394		
SS_{min}			38.5		

'–'= confidence limit not within two orders of magnitude above or below estimated value * parameter set to zero or one because fit was out of constrain boundary, L90= lower boundary of the 90% Confidence Interval, H90= higher boundary of the 90% Confidence Interval.

Model comparison

Table S20. Comparison of reduced model versions with the full model for 324 data points (n) with the F-test (eq 7). The null hypothesis is that the reduced model version is correct, thus when p<0.05 accept full model.

Model	OM food sources	Parameter description	Parameters (#)	SS_{min}	DF	F	p
Full	2	16 I, 4 β	26	38.46	298		
Reduced 1	1	β=1, γ=0	21	39.54	303	1.666	0.143
Reduced 2	2	16 I, 1 β	23	38.46	301	-0.014	*
Reduced 3	2	4 I, 4 β	14	51.82	310	8.622	<0.001
Reduced 4	2	4 I, 1 β	11	51.82	313	6.897	<0.001

* p value could not be calculated because difference in SS_{min} between models is zero.

Table S21. Fraction of Steady State reached* (F_{SS}) (Eq. 6) in 28 d bioaccumulation main tests with *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens* for low, medium, high and medium mixed organic matter content for the full model.

		Fraction of Steady State reached					
Treatment	***Species***	***PCB 28***	***PCB 52***	***PCB 101***	***PCB 118***	***PCB 153***	***PCB 180***
Low	***A. marina***	1.00	0.99	0.77	0.67	0.25	-0.21
	C. corophium	0.90	0.74	0.64	0.62	0.59	0.56
	M. balthica	0.41	0.13	0.01	0.00	-0.04	-0.06
	N. virens	-0.07	-0.09	-0.10	-0.11	-0.11	-0.11
Medium	***A. marina***	1.00	0.99	0.79	0.70	0.31	-0.11
	C. corophium	0.90	0.72	0.61	0.60	0.56	0.53
	M. balthica	0.48	0.23	0.13	0.11	0.08	0.06
	N. virens	0.08	0.05	0.04	0.04	0.04	0.04
High	***A. marina***	1.00	0.99	0.82	0.73	0.39	0.02
	C. corophium	0.87	0.65	0.51	0.49	0.44	0.41
	M. balthica	0.52	0.28	0.18	0.17	0.14	0.12
	N. virens	0.07	0.04	0.04	0.03	0.03	0.03
Mixed	***A. marina***	0.04	0.01	0.01	0.00	0.00	0.00
	C. corophium	0.92	0.78	0.70	0.68	0.65	0.63
	M. balthica	0.54	0.33	0.23	0.22	0.19	0.17
	N. virens	0.25	0.23	0.22	0.22	0.22	0.22

* Fraction of Steady State reached (F_{SS}) with $0< F_{SS}<1$. A value of 0 means that bioaccumulation is zero (t=0) and a value of 1 means that bioaccumulation is at steady state.

Negative values are caused by negative growth, leading to an apparent and artefactual state of 'over-equilibrium' (inversed biodilution).

Table S22. Relative importance of PCB uptake by sediment and suspended particle ingestion in 28 d bioaccumulation main tests with *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens* for low, medium, high and medium mixed organic matter content for the full model.

		% PCB uptake by particle ingestion					
Treatment	***Species***	***PCB 28***	***PCB 52***	***PCB 101***	***PCB 118***	***PCB 153***	***PCB 180***
Low	***A. marina***	2.4	6.4	14.5	17.3	26.5	41.4
	C. corophium	84.7	93.9	97.5	97.9	98.8	99.4
	M. balthica	58.1	79.4	90.6	92.2	95.3	97.6
	N. virens	92.3	97.1	98.8	99.0	99.4	99.7
Medium	***A. marina***	0.0	0.0	0.0	0.0	0.0	0.0
	C. corophium	68.9	86.0	93.9	95.0	97.0	98.5
	M. balthica	39.8	64.7	82.1	84.9	90.7	95.0
	N. virens	90.3	96.3	98.5	98.8	99.3	99.6
High	***A. marina***	0.1	0.4	0.9	1.1	1.9	3.6
	C. corophium	64.6	83.5	92.7	94.0	96.4	98.1
	M. balthica	53.3	76.0	88.7	90.7	94.4	97.0
	N. virens	91.3	96.7	98.7	98.9	99.4	99.7
Mixed	***A. marina***	0.0	0.0	0.0	0.0	0.0	0.0
	C. corophium	73.7	88.6	95.1	96.0	97.6	98.8
	M. balthica	52.7	75.5	88.5	90.5	94.3	97.0
	N. virens	94.3	97.9	99.1	99.3	99.6	99.8

Chapter 5

Trait-based modelling of bioaccumulation by freshwater benthic invertebrates

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Abstract

Understanding the role of species traits in chemical exposure is crucial for bioaccumulation and toxicity assessment of chemicals. We measured and modelled bioaccumulation of PCBs in *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus* and *Sphaerium corneum*. We used a battery test procedure with multiple enclosures in one aquarium, which maximized uniformity of exposure for the different species, such that the remaining variability was due mostly to species traits. The relative importance of uptake from either pore water or sediment ingestion was manipulated by using 28 d aged standard OECD sediment with low (1%) and medium (5%) OM content and 13 months aged sediment with medium OM (5%) content. The range in the magnitude of biota sediment accumulation factors (BSAF) was 3-114 with *C. riparius* < *S. corneum* ≤ *L. variegatus* ≤ *H. azteca*, thus challenging the presumed value of 1-2 typically employed in ecological risk assessment schemes. BSAFs for freshwater taxonomic groups were compared with their marine counterparts showing overlapping values. The dynamic bioaccumulation model with species-specific bioaccumulation parameters fitted well to the experimental data and showed that bioaccumulation parameters were depended on species traits. Enclosure-based battery tests and mechanistic BSAF models are expected to improve the quality of the exposure assessment in whole sediment toxicity tests.

5.1 Introduction

Aquatic ecosystems have been contaminated with xenobiotic organic chemicals for decades. Many of these chemicals are known to concentrate in suspended matter and aquatic sediments[342] and thereby potentially affect benthic invertebrates. Depending on the chemical fugacity gradients, sediments may act as a sink or a source for contaminants.[275,343,344] In aquatic systems, benthic invertebrates provide essential ecological functions such as decomposition and nutrient cycling and are an important food source for higher trophic levels.[87,88] Benthic invertebrates are used in toxicity and bioaccumulation tests to assess the potential impacts of sediment-associated contaminants on aquatic ecosystems both in retrospective and prospective risk assessment.[29,38,56,259]

To date, sediment tests have been developed for a limited number of benthic invertebrates and it is unclear to what extent a read across between species and environments (i.e. freshwater vs. marine) is feasible (Chapter 2[259]). Understanding the variability in bioaccumulation among species would greatly improve the interpretation of test results, the translation of laboratory test results to realistic field settings, and the value of tests in the risk assessment process.[59,345] Bioaccumulation and effects of sediment-bound chemicals can vary among species due to differences in exposure routes and species traits, such as habitat, ingestion rate, metabolic processes and/or diet composition.[49,59,86,259,296,298,300,346,347] In prospective risk assessment, a balanced strategy for sediment toxicity testing of organic compounds requires a motivated selection of species and endpoints. Several recent studies showed that bioaccumulation metrics such as biota sediment accumulation factors (BSAF) show orders of magnitude variation across species for the same chemical.[59,298,343,346] However, the number of studies and the comparability among studies remain limited due to differences in experimental set up. Chapter 4[346] provided bioaccumulation data for four marine invertebrate species using a novel battery test setup that ensures that all species are exposed to exactly the same chemical concentrations in sediment and pore water. This allows for an accurate assessment of exposure pathways such as uptake from water versus uptake via ingestion of food. The observed uptake was generalized using a bioaccumulation model that was capable of explaining the observed differences in uptake per chemical in terms of lipid contents, uptake rates, ingestion rates and elimination rates. Here, we provide the results of a follow-up study where the same methodology is applied to freshwater benthic invertebrates.

The main objective of the current research was to assess the relative importance of exposure pathways on bioaccumulation for four freshwater benthic invertebrates: *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus* and *Sphearium corneum*. Polychlorinated biphenyls (PCBs) were used as test chemicals. A second objective was to interpret the data with a bioaccumulation model as described in Chapter 4[346] that was previously used for describing bioaccumulation in marine invertebrates and to read across the bioaccumulation data of the four freshwater species with their marine counterparts. The range of exposure conditions was extended by varying the organic matter (OM) content of the sediment and by varying the pre-equilibration time of the sediment (i.e. 1 month versus 13 months). PCB concentrations in the sediment pore water were assessed using passive samplers. We hypothesize that by

combining the modeling data to those previously obtained for marine invertebrates, a model-assisted read across for eight species can be achieved.

5.2 Material and methods

5.2.1 Sediment Spiking

Artificial sediment based on OECD[76] was prepared by mixing 20% kaolinite clay, 75% quartz sand and 1% (low) or standard 5% (medium; standard OECD) of grounded peat. First peat was mixed with demineralized water and calcium carbonate and stored in a cooling room (4°C) for 3 days, after which the peat slurry was spiked, thoroughly mixed and the other ingredients were added. Spiking was done according to Chapter 4.[346] The peat slurry was spiked with PCB congeners 28, 52, 101, 118, 153 and 180 dissolved in acetone at a nominal total concentration of 36 µg/kg DW, which is below the toxicity thresholds reported by MacDonald et al.[348]. Acetone was added that each spiking chamber had the same volume of 0.07% (v:v) of acetone in pore water, a volume that has been shown to yield negligible co-solvent effects[82,293,308-310] and is below the recommended level of ISO[122] and OECD.[311] PCBs were used because they are ideal tracers in uptake and accumulation studies with organic chemicals. Six 76 µm polyoxymethylene (POM) Solid Phase Extraction (SPE) passive sampler sheets (0.40 g)[82,349] were added to the sediment. During the pre-equilibration period of 28 days, the sediment was agitated on a roller bank to get (pseudo-)equilibrium between chemicals and sediment prior to the start of exposure. After these 28 days, the POM-SPE passive samplers were retrieved from the slurry, to acquire in situ pore water concentrations at the start of the exposure. One of the sediments with medium OM content was aged for 13 months. The water content of the sediment was between 32% and 34% at the end of the pre-equilibration period.

5.2.2 Test species

Four freshwater benthic invertebrates were used: *Chironomus riparius* (Meigen, 1804) (Arthropoda; suspension/deposit feeder), *Hyalella azteca* (Saussure, 1858) (Crustacea; grazing/deposit feeder), *Lumbriculus variegatus* (Müller) (Annelida; deposit feeder) and *Sphaerium corneum* (Linnaeus, 1758) (Mollusca; facultative suspension feeder). These species were proposed in Chapter 2[259] as a balanced set of freshwater test species, representing different taxonomic groups and species traits. See Table S2 for species-specific traits. Species were acclimatized under experimental conditions with an average (standard deviation (SD)) temperature of 20 (2) °C under average (SD) light of 8.97 (1.32) µmol with a photoperiod of 16 h light: 8 h dark. All the test systems were constantly aerated. Copper-free water was used as overlying water in all stages of the experiment.

L. variegatus were obtained from Alterra, Wageningen, the Netherlands. The worms were cultured in gently aerated aquaria (15L×10W×14H cm) using bleached paper towels as substrate and additionally fed twice a week with two or three sinking fish food pellets (fragments of Trouvit®). Sixty randomly selected healthy adults, with a length ranging from 0.5

to 2.5 cm and showing no signs of fragmentation[350] were added in each assigned enclosure.

Egg ropes of *C. riparius* were obtained from the University of Amsterdam, the Netherlands. The individuals were kept in a gently aerated aquarium (15L×10W×14H cm) with a layer of quartz sand (0.3 kg). The ratio of the depth of the sediment layer and the height of the overlying water was 1:4. During the first 10 days, fine grounded Trouvit®:TetraMin® (20:1) was added (0.25-0.5 mg per larvae per day) as a food source for the young larvae. Slightly more food was added for older larvae (0.5-1 mg per larvae per day) [76]. Five freshly laid *C. riparius* egg ropes were kept in a Petri dish filled with copper free water for 72 h. The hatched first instar larvae, less than 24 h old, were used for the bioaccumulation tests. Seven days after starting the experiment, 22 randomly selected first-instar larvae were carefully introduced in each assigned enclosure. The larvae were allowed to settle for 4 h before aeration of the vessels was restarted.[351]

H. azteca adults (around 7-14 d old) were obtained from Grontmij Nederland B.V., Amsterdam, the Netherlands. Animals were kept in an aerated aquarium (15L×10W×14H cm) with quartz sand under experimental conditions for 1 or 2 d before the start of the experiment. Animals were fed with 1.0 mL of yeast-cerophyll-trout chow (YCT 1.8 g/L) three times a week.[64] At start of the experiment, 40 randomly selected adults of *H. azteca* were added in each assigned enclosure.

S. corneum was collected in clean ditches at the Sinderhoeve, Renkum, the Netherlands. Animals were maintained in an aerated plastic container (32L×21W×18H cm) containing 3 L of copper free water. *S. corneum* were fed with Fishfood TetraMin® (0.6 mg per individual per day) dissolved in deionized water, three times per week. Twenty-five randomly selected individuals of similar size (average shell length of 7.46 mm) were added in in each assigned enclosure.

5.2.3 Experimental design

Three treatments (n=3) were used: artificial sediment containing low OM content with a 28 d ('short') chemical aging time, a medium OM content with a 28 d ('short') chemical aging time and a medium OM content with a 13 month ('long') chemical aging time. The varying OM content and pre-equilibration time provided a wide range of exposures conditions allowing for rigorous model evaluation. A sediment layer (0.20 kg) was added on the bottom of each aquarium, after which 0.72 kg of sediment was added to each enclosure. Then each aquarium was filled with 5.1 L of copper free water.

The test systems were set up seven days before the start of the experiment to enable settling and stabilization of the sediment.[352] Following the methodology described in Chapter 4[346], each species was added to one enclosure inside an aquarium (20L×15W×25H cm) to avoid direct species interactions. Enclosures consisted of stainless steel gauze (RVS 316L) with small mesh (0.5mm) for *C. riparius* and *L. variegatus* and wider mesh (1 mm) for *H. azteca* and *S. corneum*. Aeration caused a gentle water flow through the gauze, which ensured complete mixing of the overlying water. The enclosures with *C. riparius* and *L. variegatus* were complemented with Teflon tape to prevent young animals from escaping to other enclosures.

After 14 days, all enclosures were covered by small mesh gauze to avoid emerged *C. riparius* adults from escaping.

The overlying water was continuously aerated. Dissolved oxygen (DO), temperature, conductivity and pH were measured three times a week in each compartment to check water quality and homogeneity of the overlying water. Ammonium, nitrate, phosphate, chlorophyll (blue algae, green algae and brown algae) and turbidity were measured once a week in a mixed sample containing an equal volume (3 mL) of water from each enclosure. Evaporation was corrected weekly by carefully adding the copper free water. Based upon the nutritional needs of each species standard food was prepared, spiked with PCBs (Table S1) and 1 mL was added to each enclosure three times a week. Chemical concentrations in food were aimed to match the concentrations in the OM in the sediment.

5.2.4 Endpoints

At start, a subsample of the oldest larvae (4th instar, before pupation) of *C. riparius* (100), adults of *H. azteca* (18), *L. variegatus* (21) and *S. corneum* (25) was taken, and their wet weight (dried with filter paper), were measured. Additionally, subsamples of 24 h old *C. riparius* (35 individuals), adults of *H. azteca* (40), *L. variegatus* (60) and *S. corneum* (25) were stored at -20 °C until further chemical and lipid analyses.

Enclosures were checked daily for mortality, burrowing activity, behaviour such as sediment avoidance, faecal pellets of *L. variegatus* and *H. azteca*, and movement of species to other enclosures. Mortality was defined as lack of movement after 30s of gentle stimulation using a fine brush. Additional specific endpoints were assessed for *C. riparius*. The total number of fully emerged, alive male and female midges was recorded over time. Males were identified by their plumose antennae and thin body posture. At the end of the experiment, surviving individuals were counted as adults and offspring to calculate reproduction, wet weight was measured, and samples were stored at -20 °C until further chemical and lipid analyses. For *C. riparius*, almost all larvae developed into adults by the end of the experiment. Therefore, chemical analyses were done in adult flies that had full metamorphosis.

5.2.5 Chemical analysis

Sediment samples and test organisms were extracted and analysed for PCB and lipid content according to previously published procedures (Chapter 4[346] and Amaraneni[341]). Biota, sediment, food samples and POM-SPE passive samplers were soxhlet extracted. Extracts were analysed for the test chemicals using GC-MS, as detailed in the SI. Lipids were quantified gravimetrically.[271] Recovery was between 80-110% for all compounds. For further details on extraction, detection procedures and quality assurance the reader is referred to the Supporting Information (SI) (Table S4).

5.2.6 Data analyses

Biota sediment bioaccumulation factors (BSAF; -) after 28 d were calculated as the concentration in the organism (C_{org}; μg/kg) normalized on the fraction of lipids (f_{lip}; -) divided

by the concentration in the sediment (C_{sed}; μg/kg DW) normalized on the fraction of organic matter (f_{OM}; -) thus BSAF=(C_{org}/f_{lip})/(C_{sed}/f_{OM}). BSAF was also calculated nominalized on organic carbon ($f_{OC}= f_{OM}/1.72$) in order to compare with other literature values. In two cases, lipid concentrations were not available for *H. azteca* and in three cases for *S. corneum*. Therefore, BSAF values were estimated using average concentration of the other replicates of the same treatment or other treatments for the same species. Furthermore, sediment to water partition coefficients (K_P; L/kg) were calculated as C_{sed}/C_{PW} with C_{pw} the chemical concentration in the pore water (µg/L) measured with the POM-SPE passive samplers and organic matter partition coefficients (K_{om}; L/kg) as K_P/f_{OM}.

Factors (OM content, sediment aging and species) affecting BSAF were statistically tested for all cases where sufficient detects were available. Data were checked for normality using Shapiro-Wilk's test and homogeneity of variances using Levene's test. When necessary, data were log transformed, and in case a normal distribution still was not reached, the non-parametric Kruskal-Wallis test was used. Normally distributed data were tested either with an independent t-test or an one-way ANOVA (factor OM treatment) and least significant difference (LSD) was used as a post hoc test with a significance level α=0.05 using IBM SPSS Statistics version 19.

5.2.7 Bioaccumulation modelling

Bioaccumulation modelling was used to link observed bioaccumulation with species-specific traits. Bioaccumulation in invertebrate lipids was modelled according to Chapter 4[346] and described here briefly:

$$BSAF_t = \frac{\frac{k_e K_{lip}}{K_{OM}^{SED}} + \alpha I\left[\beta + (1-\beta)\gamma\right]}{\left(k_e + k_g\right)} \times \left(1 - e^{-(k_e + k_g)t}\right) \quad (1)$$

in which t (d) is time, k_e and k_g (d^{-1}) the rate constants for overall elimination and growth dilution, α (-) the chemical assimilation efficiency (assumed to be independent of food source) and I (≥0, $kg_{OM} \times kg_{LIP}^{-1} \times d^{-1}$) represents the mass of OM ingested per unit of time and organism lipid weight, β (0<β<1) is the fraction of ingested OM originating from the sediment whereas 1-β is the fraction ingested OM originating from the suspended and freshly deposited (sediment top layer) solids and γ is a constant ratio between the sorption affinities for suspended matter OM and sediment OM ($K_{OM}^{SS} = \gamma K_{OM}^{SED}$). The ingested OM thus is assumed to originate partly from suspended solids (SS) from the overlying water and partly from the sediment (SED). Ingestion of multiple food items by benthic invertebrates has been modelled in a similar manner (Chapter 4[346] and Selck et al.[59]). A detailed explanation of Eq. 1 is provided as SI.

The percentages uptake through water is calculated based on Eq. 1 as:

$$\%WaterUptake = \frac{k_e}{k_e + \alpha I\left[\beta + (1-\beta)\gamma\right]\frac{K_{OM}^{SED}}{K_{lip}}} \quad (2)$$

The fraction of steady state reached (F_{SS}, $0<F_{SS}<1$) in the 28 day bioaccumulation test (t=28 d) was calculated as:

$$F_{SS} = 1 - e^{-(k_e + k_g)t} \quad (3)$$

Parameterisation

Parameters in the above model reflect species traits that affect bioaccumulation and the relative importance of these traits and processes can be quantified by parametrization. Eq. 1 was implemented in Microsoft Excel 2010 and the model was fitted to the experimental BSAF data using the Excel Solver tool with scaling of parameters and a relative least-squares criterion. The parameter k_g was obtained from weight measurements and the parameters k_e, I, β and γ were estimated using a two-stage iterative approach (see SI for further details).

Confidence intervals (90% CI) were calculated according to Draper and Smith[319]:

$$SS_{90} = SS_{min}\left(1 + \frac{p}{(n-p)}F(p, n-p, 90\%)\right) \quad (4)$$

with SS_{90} the sum of squares at the 90% confidence contour, SS_{min} the minimum sum of squares, n the number of BSAF measurements (n=139), p the number of estimated parameters (p=21) and F(p,n-p,90%) the F-distribution according to Fisher. Confidence intervals were estimated using n, p and F either for the whole dataset for the general parameters or the species-specific dataset. Negative confidence limits for 'I' and β were set to zero. The full model (Eq. 1) was statistically evaluated against three reduced model versions using the F-test (Eq. S5): 1) only sediment particle ingestion (β=0, γ=0) (p=18), 2) one β for all species (p=18) and 3) one 'I' per species (p=13).

5.3 Results and Discussion

5.3.1 Performance of freshwater benthic invertebrates during the tests

Water quality was constant and similar among enclosures and treatments (Table S5, S6). The minimal concentration of DO was 5 mg/L and the ammonia concentration was less than 1.72 mg N/L in all treatments (Table S6). No cyanobacteria were detected in the overlying water. Green algae were on average less than 0.12 µg/L in all treatments and brown algae ranged from 1.97 to 5.29 µg/L (Table S6).

Survival ranged between 76% and 100% for all species (Table S7, S8). Survival was not adversely affected by the OM treatment (Table S9). For *H. azteca* this agrees with the findings by Suedel and Rodgers[353], who observed no effects on amphipod survival over a 0.12% to 7.8% range of OM content. Reproduction occurred for *H. azteca, S. corneum* and for *L. variegatus* by fragmentation (Table S7). OM treatment had a significant effect on reproduction for *H. azteca* with a higher reproduction in medium long aged sediment compared to low and medium 28d aged sediment (ANOVA, $F(2,6)= 28.555$, $p = 0.001$) (Table S7, S9). The wet weight increased by a factor of 1 to 170 for all species in all

treatments. The highest gain was for *C. riparius*, which developed from 24h old first instar larvae to fourth instar larvae (only calculated for larvae, not for adults). OM treatment had a significant effect on wet weight gain for *H. azteca* (ANOVA $F(2,6)$= 11.209, p = 0.009) and *S. corneum* (ANOVA $F(2,6)$= 5.258, p = 0.048) with a higher wet weight gain in the medium long aged treatment compared to the low and medium 28d aged treatments (Table S7, S9). This indicates that sediment aging had a more important influence than OM content on species performance. Higher growth for *H. azteca* and *S. corneum* and reproduction for *H. azteca* in longer aged sediments may be explained by a better developed microbial community, potentially providing a more variable and stable food source. For *L. variegatus* and *H. azteca*, faecal pellets were observed daily in each treatment, indicating that these animals were feeding which corresponded with the weight gain during the experimental period (Table S7).

C. riparius larvae were found only in the medium short aged OM treatment (2 larvae) and in the 1y medium aged OM treatment (1 larva). Larval development time from the first instar to the fourth instar ranged from 18 days (long aged medium OM content) to 19 days (short aged low and medium OM content). Emergence occurred in all treatments (Table S8). OM treatment had no significant effect on total emergence and sex ratio (Table S9). The mean emergence of *C. riparius* was 73% to 79% (Table S8), which is above the critical level of 70% for controls at the end of the exposure period according to OECD[76].

Despite the efforts to prevent species to move to neighbouring enclosures, *L. variegatus* passed the gauze towards other enclosures. The interaction between *L. variegatus* and the other species might interfere with normal burrowing and feeding behaviour and therefore lead to lower uptake rates and thus lower bioaccumulation for these species.[298] Since *L. variegatus* were found in other enclosures in all treatments, it was assumed that potential species-species interaction was roughly equal among treatments. However, data should be interpreted with this in mind and it is thus recommended to use even smaller mess size to prevent escapes in this test set up. Moreover, occasionally some *L. variegatus* and *S. corneum* individuals showed sediment avoiding behaviour by climbing up the gauze, which could potentially lead to less bioaccumulation via the sediment pathway. *H. azteca* was also observed in the water column however this is common behavior[354] in which they swim up to the surface of the overlying water, quickly move back to the sediment surface and burrow for a few seconds before returning to the overlying water.

5.3.2 Effect of organic matter, aging and species type on chemical partitioning and bioaccumulation

Chemical partitioning

The chemical concentration in the sediment was lower in low OM short aged treatments than for medium OM short and medium OM long aged treatments at the start and at the end of the experiment (Table S10). However, at the start of the experiment, PCB concentrations in pore water were highest for the low OM treatments and lowest for the medium OM short

aged sediment (Table S10). PCB concentration in pore water decreased with increasing hydrophobicity, which is expected based on standard sorption kinetic processes. Aging does not change total sediment concentration but does effect pore water concentrations. It would be expected, however, that short aged sediment would have a higher pore water concentration than long aged sediment.

The partitioning coefficients K_P varied linearly with log K_{ow} ($0.993<r^2<0.998$) (Figure S2). Normalisation to nominal OM content (K_{om}) strongly reduced the difference between the regressions, yielding a regression of $\log K_{om}=1.13\log K_{ow}-1.30$ ($r^2=0.95$; Figure S3), for the combined OECD sediment K_{om} data for all treatments. Using the OM contents measured after 28 d of exposure a regression of $\log K_{om}=1.13\log K_{ow}-1.25$ ($r^2=0.85$) was obtained (Figure S4). This slightly poorer regression might be explained by variability in OM content due to OM feeding and egestion and by some uncertainty in the loss of ignition method to determine OM content. Therefore, the $\log K_{om}$ with nominal OM values was used later as input for the modelling of the BSAF data.

Bioaccumulation

Chemical concentrations in *S. corneum* and *C. riparius* were mainly below the detection limit (Table S11), because of the limited biomass available for analysis. The average magnitudes of the OM normalized BSAFs were: *C. riparius* (3-10) < *S. corneum* (10-17) ≤ *L. variegatus* (7-61) ≤ *H. azteca* (5-114), over a log K_{ow} range of 5.58-7.21 (Figure 1; Table S12). This challenges the presumed value of 1 to 2 typically employed in ecological risk assessment schemes, indicating that the ingestion of particles should not be ignored. These values comply to BSAFs previously reported for marine invertebrates in Chapter 4[346]. The BSAF range found for *L. variegatus* in the present study was higher than the BSAF range 0.2 to 8.8 found for *L. variegatus* in natural sediment.[297,355-358] A range of studies (n=24) gave a median BSAF for PCBs and *H. azteca* of 2.4 (CV 0.9),[359] which is lower than the values in this study. We explain the higher BSAF values in this experiment by the higher bioavailability of PCBs in artificial sediment compared to natural sediment as was explained in Chapter 4[346].

Effect of organic matter content and aging on bioaccumulation

For most PCBs, organic matter and aging treatment had no significant effect on BSAF (Table S13). In general, low OM or medium OM aged had the highest BSAF levels for all species except for *H. azteca* which had the highest BSAF for medium OM for all PCBs. For some individual PCBs, significant OM treatment effects were found. For *C. riparius* (PCB101) (ANOVA, $F(2,4)=28.7$, $p=0.004$) and *S. corneum* (PCB 153) (ANOVA, $F(1,4)=8.57$, $p=0.043$) the low OM treatment had a significant higher BSAF than the medium and medium long aged OM treatments. For *H. azteca* (PCB 52), the medium OM treatment had a significant higher BSAF than the low and medium aged OM treatments (ANOVA, $F(2,5)=6.796$, $p=0.038$; Table S13).

Effect of species traits on bioaccumulation

Of the six PCB congeners, only the BSAFs data for PCB 153 in the low and medium long aged OM treatments were sufficient to compare the impacts on all four species. A statistically significant yet small difference was found for the medium long aged treatment

(Kruskall-Walis, $\chi^2(3)=7.879$, p=0.049). Here *H. azteca* had a significant higher BSAF than *C. riparius* (p=0.019) and *S. corneum* (p=0.036) but did not differ from *L. variegatus*. When BSAF data were available for three or two species, species differed statistically from each other for the medium OM treatment (*C. riparius* < *L. variegatus* < *H. azteca*, p<0.05) and for PCB 52 in the low OM treatment (*H. azteca* < *L. variegatus*; independent t-test, t(3)=-6.807, p=0.006) (Table S12, S14). Exposure to benzo(a)pyrene spiked in natural sediment resulted in an order in BSAF of *H. azteca* < *C. tentans* < *L. variegatus*.[298] Here *H. azteca* had the lowest BSAF while in our findings the BSAF was highest. *H. azteca* spends most of their lifetime in the overlying water and thus responds primarily to contaminants in the overlying water and not to contaminants in the sediment,[354] still they had relative high BSAF values. This suggests that ingestion played a key role in bioaccumulation as observed earlier.[354] *H. azteca* feeds on sediment particles and algal communities on the surfaces of sediments or macrophytes. The more they feed on algae the lower their exposure to sediments is.[360] However, in sediment toxicity tests with artificial sediment, *H. azteca* is more constrained to burrow and feed on sediment than in sediment tests with natural sediments or natural systems.[354] This might lead to worst case effect results in whole sediment toxicity testing. Bivalve exposure may be uncertain because of valve closure and filter-feeders typically accumulate much lower concentrations of contaminants than other organisms,[56] which may explain the high number of non-detects and low BSAF values for *S. corneum*. For *C. riparius*, only adults that had full metamorphosis were analysed. When the larvae develop into adults, the internal concentration might increase slightly due to weight loss of pupae during metamorphosis.[361-363] This indicates that BSAF values for *C. riparius* larvae were lower than the measured BSAFs and thus BSAF differences compared with other species larger. An additional explanation for the low BSAF for *C. riparius* could be the short contact time with the sediment, which was around 17 to 20 days for *C. riparius* whereas the other species were exposed for 28 days.

5.3.3 Modelling bioaccumulation

Model evaluation and parameter accuracy

The full model (Eq. 1) with species and OM-specific parameters provided good fits to the observed BSAF values (Figure 1; Table S12) and provided satisfactory narrow CIs for most of the parameters (Figure 2, Table S16). *S. corneum* (n=8) and *C. riparius* (n=31) had the most non-detects, which affected the precision of the parameter values and CIs (Figure 2, Table S16). Using the F-test criterion, the full model appeared not to be over-parameterised compared to the reduced models (Table S15), which is why the full model is used in the discussion below. This also allows for a direct comparison with parameters that we obtained with the same model for marine species in Chapter 4[346]. Simplifying the proportion of sediment OM ingested (β, i.e. reduced model 1 and 2) in the model did not result in statistically superior fits. Reducing the species-specific ingestion rate 'I' to one per species (reduced model 3) however, resulted in a poorer fit, a differences that was statistically significant (p<0.001, Table S10). We conclude that exposure assessment in sediment toxicity tests requires specific ingestion rates.

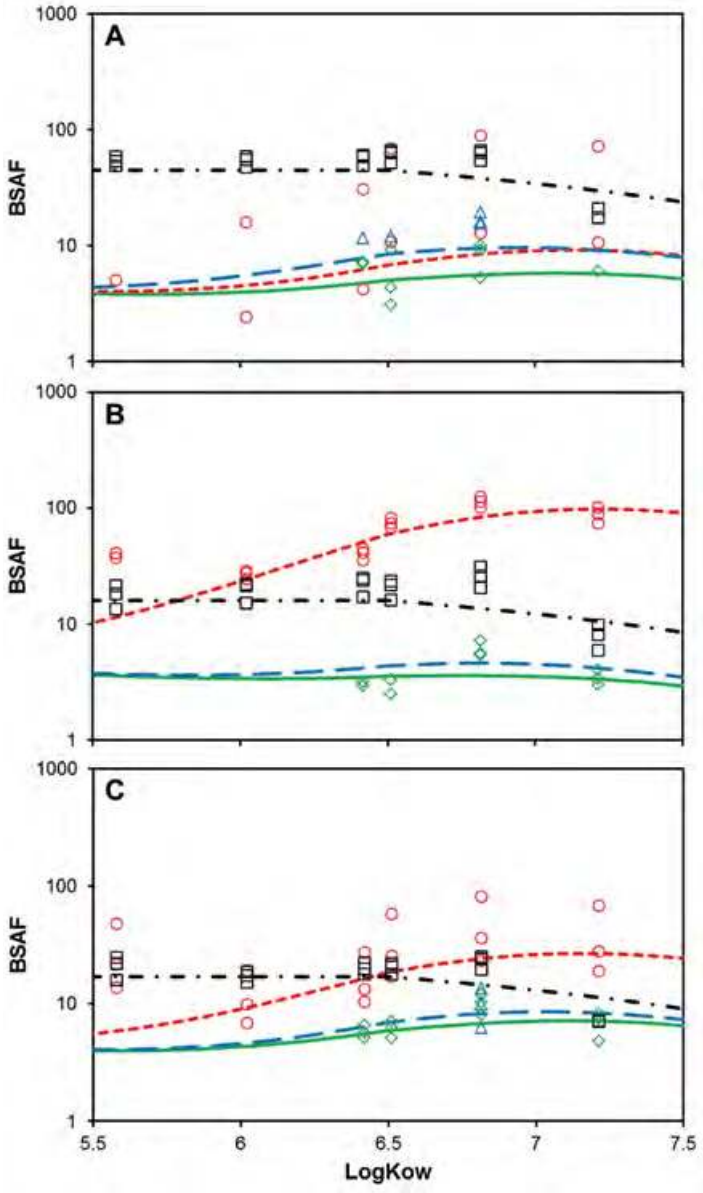

Figure 1. Measured (symbols) and modelled (curves) lipid and organic matter normalized biota sediment accumulation factors (BSAFs) for *Chironomus riparius* (green diamonds ◊, solid line), *Hyalella azteca* (red circles ○, dotted line), *Lumbriculus variegatus* (black squares □, dash dot line) *and Sphaerium corneum* (blue triangle Δ, dashed line), for the treatments: low OM content (**A**), medium OM content as in the OECD test guideline (**B**) and medium aged OM content (**C**).

$LogK_{ow}$ dependence of BSAF

BSAF values for *L. variegatus* were constant or increased slightly until $logK_{ow}$ 6.5 to 7.2, after which BSAF decreased with increasing $logK_{ow}$ of the PCBs (Figure 1). This pattern was observed before and several explanations have been given.[60,329-333,346,359] One explanation is that steady state is not reached. This was however only the case for *L. variegatus* (Table S17). BSAF increased stronger for *H. azteca* in medium and medium aged OM treatments compared to the other species whereas for low OM the increase was similar to *C. riparius* and *S. corneum*. This might reflect uptake by ingestion, which is more important at higher OM content as the PCB concentration is higher.

Discussion of parameters

Ingestion rates 'I' were higher for low OM except for *H. azteca* which has a factor 14 higher ingestion for medium OM then low OM (Table S16, Figure 2A, S5). This might explain the higher BSAF for medium OM for *H. azteca*. Sediment with low OM might contain less nutritional value, which is known to increase ingestion rates.[322] The ingestion rates were just within or above the range of 0.13-0.62 provided by Thomann et al.[60] Elimination rates (k_e) were fitted as $Logk_e$=-$LogK_{ow}$ + a and the parameter 'a' was lowest for *L. variegatus* and highest for *C. riparius* (figure 2B). Elimination rates for *C. riparius* (k_e 0.09-4.08 d^{-1}) were also higher than earlier reported values (0.04-0.28 d^{-1} calculated with $Logk_e$=-0.49$LogK_{ow}$ + 0.08 for sediment with 3% OC)[364] with the main difference between the less hydrophobic PCBs. The parameter γ, i.e. the constant ratio between K_{OM}^{SS} and K_{OM}^{SED}, had a value of 2.3 (90% CI=1.5-3.1), which is close to the average value of 3.2 observed for PCBs in an freshwater field dataset provided by Koelmans et al.[318]. The parameter β distinguishes between ingestion of sediment versus suspended or freshly deposited OM fractions, like for instance the added food. *L. variegatus* ingests whole sediment particles and had the highest β (β=1, 90% CI=0.9-1), as expected. The other species mainly ingested suspended or freshly deposited OM particles, however, upper CIs were high or could not be detected, like for *S. corneum* (Table S16, Figure 2C), making comparison difficult. *S. corneum* can both filter the water column or the top layer of the sediment and *C. riparius* feeds in the first 2 mm of the sediment[365], this specific feeding behaviour can explain the low β.

Importance of uptake routes

The BSAF model was used to estimate the relative importance of uptake pathways. Uptake by particle ingestion increased with increasing $LogK_{ow}$ except for *L. variegatus* where this route dominated for 100% over the whole range of $LogK_{ow}$ (Table S18). For most hydrophobic PCBs, particle ingestion was the dominant uptake route for all species. Particle ingestion as the dominant uptake route was observed earlier for *L. variegatus*,[62,296] indicating the suitability of this species for sediment toxicity testing. For the low PCBs 28 and 52, uptake through water can dominate over particle uptake, especially for *C. riparius* and *S. corneum*.

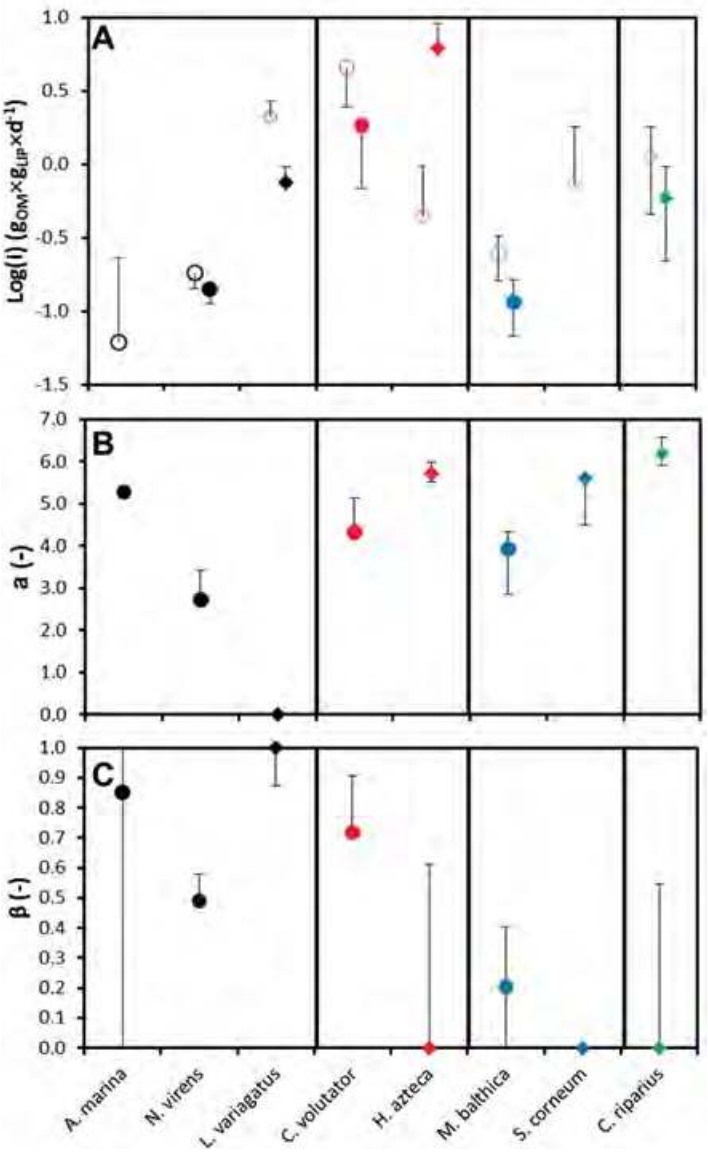

Figure 2. Optimized model parameters and 90% confidence limits for: Ingestion rate (I; g OM x g lipids x d^{-1}) for low (open symbols) and medium (closed symbols) organic matter (**A**), intercept for k_e (a; -) (**B**) and the fraction of ingested OM originating from the sediment (β; -) (**C**) for marine (Chapter 4[346] (circles) and freshwater (diamonds) benthic invertebrate taxonomic groups. For Annalida: *Arenicola marina*, *Nereis virens*, *Lumbriculus variegatus* (black), Crustacean: *Corophium volutator, Hyalella azteca* (red), Mollusca: *Macoma balthica*, *Sphaerium corneum* (blue) and Arthropoda *Chironomus riparius* (green). Parameter values are only included if a 90% confidence limit could be assessed in at least one direction.

5.3.4 Read across between freshwater and marine benthic invertebrates

The present results for freshwater benthic invertebrates can be compared to results obtained in Chapter 4[346] for bioaccumulation in four marine invertebrates. BSAFs for freshwater and marine species within taxon groups show overlapping values for the crustacean *H. azteca* (5-114) <*C. volutator* (49-318), annelida *N. virens* (5-19) <*A. marina* (7-37) < *L. variegatus* (7-61) and molluscs *S. corneum* (10-17) ≤ *M. balthica* (8-36). Both crustaceans had the highest BSAF values, which may be explained by their high ingestion and growth rates compared to other species. The freshwater insect *C. riparius* (BSAF: 3-10), which does not have a marine counterpart, has the lowest BSAF of all species. The comparison of parameters between freshwater and marine species within a taxonomic group was difficult as some data points were not available or CIs were lacking (Figure 2), however a general trend can be given. Ingestion rates for freshwater species appear to be higher than their marine counterparts (Figure 2A). The same holds for the elimination parameter 'a', except for *L. variegatus* which is lower than the marine Annelida (figure 2B). Differences in β are especially high for the crustacean (Figure 2C), indicating differences in feeding habits. These comparisons show that read across among invertebrate species of fresh versus marine ecosystems within a taxonomic group is more similar then a read across among species between different taxonomic groups. Therefore, in a standard suite of benthic test species for prospective sediment risk assessment it is important to include species from different taxonomic groups and with different specific traits such as feeding mode. The tested freshwater (this Chapter) and marine (Chapter 4[346]) species seem good candidates for this standard set of test species, as was suggested in Chapter 2[259]. Moreover, enclosure-based battery tests and mechanistic BSAF models are expected to improve the quality of the exposure assessment in whole sediment toxicity tests in the context of environmental risk assessment.

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Supporting Information

Material and methods

Chemicals and materials

PCBs standards IUPAC numbers 28, 52, 101, 118, 153, 180 were obtained from Dr. Ehrenstorfer.

POM-SPE passive sampler sheets were obtained from CS Hyde Co., Lake Villa, IL.

For OECD sediment peat from Klasmann Deilmann Benelux BV, $CaCO_3$ powder from Sigma Aldrich, Germany, quartz sand from Geba 0.06-0.25 mm, Eurogrid, The Netherlands and kaolin from Sigma Aldrich, German was used.

Balance used for wet weight and dry weight measurements: Sartorius analytic A120S 37040151.

Sediment preparation

Peat was dried at 40°C during 4 days and subsequently ground (particle size ≤ 1mm). After then, three buckets (25L) were used for preparing the peat mix. Before the preparation, they were cleaned using 300mL of technical acetone, 200mL of technical acetone and 100mL of analytical hexane.

POM Preparation

The POM passive samplers were prepared, cut and cleaned using n-hexanol analytical grade (n-hexane Pico grade Promochem® Code SO-1244-BO25) and methanol analytical grade (Methanol HPLC Gradient Grade® Baker J.T.) twice with 30 minutes in between.

Endpoints

- *Weight wet per animal* : weight per individual expressed in mg
- *Body wet weight gained*: expressed as a percentage of the weight gained compare to the initial weight
- *Development time:* larvae of 24 h old to adults
- *Emergence ratio*: expressed as a percentage of the migdes that complete their larval development and became adults
- *Sex ratio*: express by percentage of female of the total emerged adults of *C. riparius*

Water quality

For nutrient ammonium, nitrite-nitrate, phosphate were analyzed with a Continuous flow analyzer (CFA), Skalar Analytical BV Breda, The Netherlands Type SA San plus.

Total P in water was based on NEN6663 including internal extraction UV/Persulfate, ammonium on NEN 6646 without extraction, and nitrate and nitrite on NEN-EN-ISO 13395:1997 nl. Nitrate-nitrite measurements were determined colour metrically on a continuous flow analyser (CFA and FIA). Detection limits were for P total 0.02 mg P/L, phosphate 4 µg P/L, ammonium 0.02 mg N/L, nitrate-nitrite 0.01 mg N/L, and N total: 0.2 mg N/L.

Chlorophyll (blue algae, green algae, and brown algae) concentrations were analysed with PhytoWin v2.13 US with references for Blue: Synechocuccus16.de.REF2/, Green: Dpannonicus16-dec/, and Bronw: Br_US PMDA0172.REF2.

For general water quality parameters the following probes were used: oxygen (DO) with Oxyguard Polaris DO/T-meter, pH with WTW pH310 + sentix 21 pH-electrode, EC with WTW Cond 315i + Tetracon 325 electrode, and turbidity with Eutech TN 100 turbiditymeter.

Figure S1. Aquarium system used in the experiment with their respective enclosures represented by each species tested.

Table S1. Food preparation.

Organic matter content	Total food prepared	Trouvit® : TetraMin® ratio	Solved food
Low	8 mg of finely ground of 20:1 (Trouvit®:Trouvit®)	7.6 : 380 mg	160 mL of yeast-cerophyll-trout chow (YCT)
Medium short and long aged	15 mg of finely ground of 20:1 (Trouvit®:Trouvit®)	14.3 : 715 mg	300 mL of yeast-cerophyll-trout chow (YCT)

Extraction and analyses

Extraction and Clean-up

1 ml of internal standard solution (PCB112, 80 ng/mL) was added to each sample. Samples were dried using sodium sulphate (Merck) and extracted by Soxhlet extraction using a mixture of pentane/dichloromethane (50:50 v/v). For biota samples, extracts were concentrated to approximately 2 mL using a rotavap (Heidolph) and transferred to a test tube. Approximately 200 mg of previously tested clean haring oil was added to the extract as a keeper after which the extracts were dried under a steam of nitrogen and the fat content was determined gravimetrically. The dried fat was subsequently reconstituted in 1 mL iso-octane. One mL sulphuric acid was added to the extracts after which they were

vortexed for 30 s. After the layers had separated, the organic layer was transferred with pentane to a test tube and concentrated to 1 mL and transferred to a vial for determination on GC-MS. For sediment samples the procedure was similar except no fat was added and the extracts were not dried to determine fat content.

Moisture content was determined gravimetrically after drying for 3 hours at 105°C. Organic matter content was determined gravimetrically after drying at 550°C for 2 hours.

Analysis by GC-MS

A sample of 5 µl was injected on a Shimadzu GCMS2010 (GC) coupled to a GCMS-QP2010 Ultra (MS) detector (Shimadzu, 's Hertogenbosch, the Netherlands) using an ATAS Optic3 PTV injector (Shimadzu, 's Hertogenbosch, the Netherlands). Separation was performed using a 30m x 0.25 mm i.d. HT8 column with a film thickness of 0.25 µm. Analysis was performed using Electron Impact (EI) in single ion monitoring (SIM) mode. Injection port temperature started at 75°C and increased with 10°C/sec to 290°C after 90% of the injected sample had evaporated. Source temperatures was 300°C. Oven temperature program started at 75°C, hold for 2 minutes, increase by 23.75°C/min to 170 °C followed by an increase by 2.5°C/min to 300. At the end of the program a column was heated to 320°C for 10 minutes. The following quantifier and qualifier ions were monitored respectively, 256 and 258 for PCB 28, 292 and 290 for PCB 52, 326 and 324 for PCB 101, PCB 112 and PCB118, 360 and 362 for PCB 153, 394 and 396 for PCB 180.

Quality assurance and control

Recovery was typically between 80-110% for all compounds. Calibration curves consisted of 9 points within a range of 1-650 ng/mL. R2≥0.999 was achieved for each calibration curve for all compounds. Limit of quantification of the PCBs depended upon sample intake, typically this was between <0.1 and <30 ng/L. Spiked concentrations ranged from 26% to 75% of the nominal concentrations in the sediment (Table S3) and from 185% to 324% of the nominal concentrations in the food (Table S4).

Table S2. Species specific traits: microhabitat, feeding habit, age tested and lifespan for test species.

Species	Microhabitat	Feeding habit	Age test organisms	Lifespan
Chironomus riparius *(Arthropoda)*	U shaped tube dweller	Suspension/deposit feeder	<24h old larvea	12-23 d from larvae to adult
Hyalella azteca *(Crustacea)*	Burrow, epibenthic	Grazing/deposit feeder	Adults	
Lumbriculus variegatus *(Annelida)*	Burrow, infaunal	Deposit feeder	adults	±14 d for reproduction by fragmentation
Sphaerium corneum *(Mollusca)*	Burrow	Filter feeder/deposit feed (Facultative suspension feeder)	Adults	Mature at 4 mm (sexually mature as early as 3 months old in Europe).

Table S3. Average (SD) measured sediment concentrations (µg/kg DW) (n=3) compared with the nominal concentration (µg/kg DW) for the three organic matter treatments: low OM, medium OM, and medium OM 1 year aged at the start of the experiment.

		Low OM		Medium OM		Medium OM aged	
Chemical	**Nominal (µg/kg)**	**Measured (µg/kg)**	**% from nominal**	**Measured (µg/kg)**	**% from nominal**	**Measured (µg/kg)**	**% from nominal**
PCB 28	6	1.6 (0.1)	27	3.0 (0.1)	50	3.0 (0.2)	51
PCB 52	6	2.3 (0.2)	38	3.7 (0.2)	61	3.8 (0.2)	63
PCB 101	6	2.2 (0.1)	36	3.8 (0.1)	63	3.8 (0.1)	63
PCB 118	6	1.6 (0.3)	26	3.4 (0.7)	56	4.5 (1.2)	75
PCB 153	6	2.1 (0.1)	34	3.8 (0.1)	63	3.7 (0.1)	61
PCB 180	6	2.4 (0.2)	39	3.9 (0.1)	66	3.9 (0.3)	66

Table S4. Average (SD) measured food concentrations (µg/kg OM) (n=3) compared with the nominal concentration (µg/kg OM) for the three organic matter treatments: low OM, medium OM, and medium OM 1 year aged at the start of the experiment.

	Low OM 28d			Medium OM 28d and Medium OM 1year		
Chemical	**Nominal (µg/kg)**	**Measured (µg/kg)**	**% from nominal**	**Nominal (µg/kg)**	**Measured (µg/kg)**	**% from nominal**
PCB 28	172	399 (31)	232	81	150 (10)	185
PCB 52	172	460 (27)	267	81	202 (6)	250
PCB 101	172	426 (16)	247	81	196 (6)	242
PCB 118	172	367 (31)	213	81	160 (4)	198
PCB 153	172	440 (27)	256	81	223 (4)	276
PCB 180	172	438 (32)	254	81	198 (10)	245

Results

Table S5. Water quality parameters (n=156) during 28 day experimental period.

Treatment		**pH**	**Temperature (C°)**	**Dissolved oxygen (mg/L)**	**Cond. (mS/cm)**
Low	Mean	8.38	19.9	8.3	446
	SD	0.10	0.1	0.5	69
	Minimum	8.06	19.6	6.1	288
	Maximum	8.58	20.1	8.8	543
Medium	Mean	8.34	19.9	8.1	534
	SD	0.17	0.1	0.7	74
	Minimum	7.71	19.6	5.5	325
	Maximum	9.39	20.1	8.8	630
Medium aged	Mean	8.31	19.9	8.1	456
	SD	0.18	0.2	0.8	47
	Minimum	7.65	18.7	5.0	313
	Maximum	8.50	20.1	8.7	527

Table S6. Measurements of chlorophyll, turbidity, ammonium, total nitrogen, and phosphate (n=15) during 28 day experimental period.

Treatment		Cyanobacteria (µg/L)	Green (µg/L)	Brown (µg/L)	Turbidity (NTU)	Ammonia (mg N/L)	Nitrate+Nitrite (mg N/L)	Phosphate (µg P/L)
Low	Mean	0.00	0.12	1.97	15.5	0.48	0.78	325.60
	SD	0.00	0.20	1.28	9.5	0.66	0.97	409.37
	Minimum	0.00	0.00	0.40	4.4	0.02	0.00	0.00
	Maximum	0.00	0.60	3.62	35.4	1.72	2.61	1201.91
Medium	Mean	0.00	0.08	5.29	25.1	0.28	0.64	344.59
	SD	0.00	0.17	1.94	19.9	0.52	0.65	346.07
	Minimum	0.00	0.00	2.42	4.4	0.01	0.00	0.00
	Maximum	0.00	0.55	8.22	64.3	1.43	2.26	989.37
Medium aged	Mean	0.00	0.02	3.75	20.7	0.22	0.71	237.96
	SD	0.00	0.05	1.88	16.7	0.29	0.70	286.57
	Minimum	0.00	0.00	0.74	4.6	0.03	0.01	0.00
	Maximum	0.00	0.14	6.35	54.5	1.01	2.26	858.91

Table S7. Average (SD) biological parameters of *Hyalella azteca, Lumbriculus variegatus,* and *Sphaerium corneum* during 28 days exposure in low, medium (short and long aged) organic matter content spiked by PCBs at 20º ± 2º C, light intensity 100-500 lx and 16h photophase.

Species	Treatment	Number of animals	Reproduction (young)[1]	How many times they multiplicated	Survival (%)[a]	Wet weight gain (%)[a]
Hyalella azteca	Low OM content	34	6 a		86 (6) a	75 (37) a
	Medium short OM content	34	9 a		85 (13) a	156 (30) a
	Medium long OM content	39	43 b		99 (1) a	299 (90) b
Lumbriculus variegatus	Low OM content	257	197 a	4.3	100 (0) a	11 (1) a
	Medium short OM content	214	154 a	3.6	100 (0) a	10 (7) a
	Medium long OM content	225	165 a	3.8	100 (0) a	14 (12) a
Sphaerium corneum	Low OM content	22	1.6 a		91 (2) a	16 (14) a
	Medium short OM content	21	1.6 a		84 (11) a	23 (19) a
	Medium long OM content	21	2.0 a		87 (2) a	55 (13) b

[a] Small letters indicate significant differences (α=0.05).

Table S8. Average (SD) biological parameters of *Chironomus riparius* during 28 days exposure in low, medium (short and long aged) organic matter content spiked by PCB's at 20° ± 2° C, light intensity 100-500 lx and 16h photophase.

***Biological parameters of* C. riparius**	Treatments		
	low OM content	**medium short aged OM content**	**medium long aged OM content**
Survival (larvae + adults) (%)	79 (23)	76 (18)	79 (5)
Wet weight gain (%)[a]	16190	15431	16948
Emergence (%)	79 (23)	73 (16)	77 (5)
Female emergence (%)	49 (21.4)	54 (6.4)	53 (8.2)
Sex ratio (F/M)	1.2 (0.8)	1.2 (0.3)	1.2 (0.4)
Development time (d)	19 (1.8)	19 (1.8)	17.5 (1.8)
Development time (F) (d)	19 (1.5)	19.5 (1.3)	17.5 (1.3)
Development time (M) (d)	17 (-)[b]	18 (1.0)	17.5 (1.3)

[a] Wet weight gain was based on one measurement [b]Males only emerged on that day.

Table S9. Effect of organic matter treatment low, medium, and medium aged on biological endpoints.

Species	**Endpoint**	**Test**	**F/ Chi-square**	**df**	**p-value**
Chironomus riparius	Survival	one-way ANOVA	0.039	2,6	0.962
	Total emergence	one-way ANOVA	0.110	2,6	0.897
	Sex ratio (F/M)	one-way ANOVA	0.005	2,6	0.995
	Wet weight gain	one-way ANOVA	n.a.	n.a.	n.a.
Hyalella azteca	Survival	one-way ANOVA	2.874	2,6	0.133
	Reproduction	one-way ANOVA, LSD, data log transformed	28.555	2,6	0.001
	Wet weight gain	one-way ANOVA, LSD	11.209	2,6	0.009
Lumbriculus variegatus	Survival	one-way ANOVA	n.a.	n.a.	n.a.
	Reproduction	one-way ANOVA	1.167	2,6	0.373
	Wet weight gain	one-way ANOVA	0.152	2,6	0.862
Sphaerium corneum	Survival	Kruskal Wallis Test	2.440	2	0.295
	Reproduction	one-way ANOVA	0.034	2,6	0.966
	Wet weight gain	one-way ANOVA, LSD	5.258	2,6	0.048

n.a. data were insufficient or equal for all replicates and treatments.

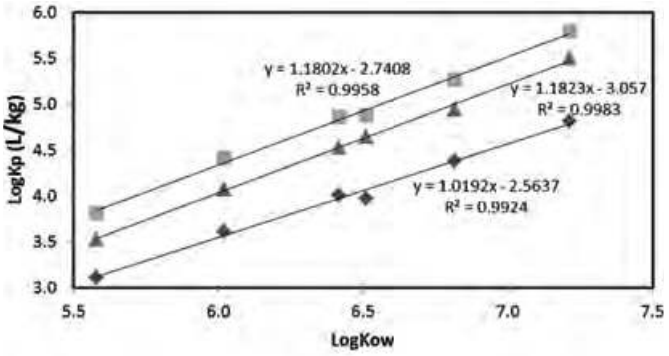

Figure S2. Sediment water partitioning coefficients K_P for low organic matter 28d aged (diamonds), medium organic matter 28d aged (squares), and medium organic matter 13 month aged (triangles).

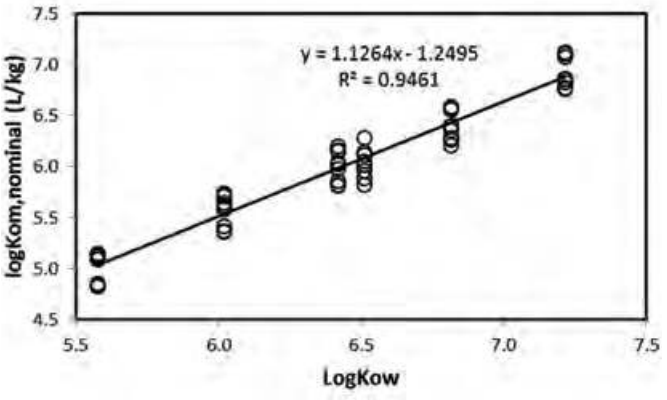

Figure S3. Organic matter water partitioning coefficients K_{om} normalized on nominal organic matter content for low organic matter 28d aged, medium organic matter 28d aged, and medium organic matter 13 month aged.

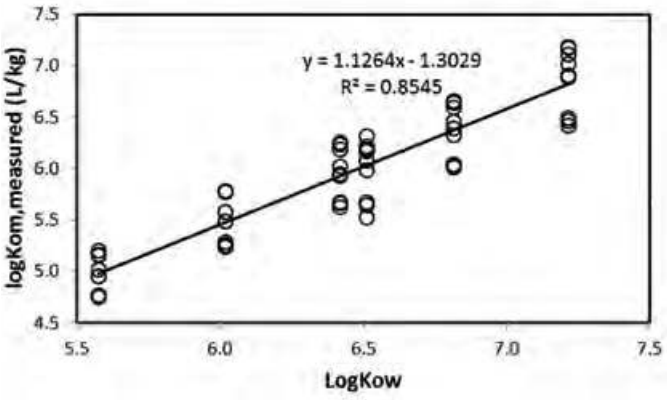

Figure S4. Organic matter water partitioning coefficients K_{om} normalized on measured organic matter content for low organic matter 28d aged, medium organic matter 28d aged, and medium organic matter 13 month aged.

Table S10. Average (SD) sediment concentration (µg/kg DW), pore water concentrations (µg/L), partitioning coefficient sediment pore water (K_p; L/kg), and partitioning coefficient organic matter pore water (K_{om}; L/kg) at start of the experiment.

	Treatment	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 180
Sediment (µg/kg DW) (n=3), t=0	*Low OM*	1.6 (0.1)	2.2 (0.2)	2.2 (0.1)	1.6 (0.3)	2.1 (0.1)	2.4 (0.2)
	Medium OM	3.0 (0.1)	3.7 (0.2)	3.8 (0.1)	3.4 (0.7)	3.8 (0.1)	3.9 (0.1)
	Medium OM aged	3.0 (0.1)	3.8 (0.2)	3.8 (0.1)	4.5 (1.2)	3.7 (0.1)	3.9 (0.3)
Sediment (µg/kg DW) (n=3), t=28	*Low OM*	1.9 (0.2)	2.6 (0.3)	2.5 (0.2)	2.33 (0.5)	2.26 (0.1)	2.76 (0.05)
	Medium OM	3.90 (0.2)	4.56 (0.05)	4.46 (0.05)	4.43 (1.5)	4.23 (0.05)	4.63 (0.05)
	Medium OM aged	3.30 (0.7)	3.96 (0.7)	3.90 (0.7)	3.96 (0.3)	3.80 (0.6)	4.10 (0.6)
Pore water (ng/L) (n=3), t=0	*Low OM*	1.24 (0.09)	0.55 (0.04)	0.21 (0.01)	0.17 (0.01)	0.09 (0.01)	0.04 ($2.89x10^{-3}$)
	Medium OM	0.46 (0.02)	0.14 ($3.46x10^{-3}$)	0.05 ($1.39x10^{-3}$)	0.04 ($1.04x10^{-3}$)	0.02 ($6.76x10^{-4}$)	0.01 ($3.93x10^{-4}$)
	Medium OM aged	0.89 (0.04)	0.32 (0.02)	0.11 (0.01)	0.10 (0.01)	0.04 ($4.50x10^{-3}$)	0.01 ($1.47x10^{-3}$)
K_p (L/kg)	*Low OM*	$1.30x10^3$	$4.11x10^3$	$1.02x10^4$	$9.39x10^3$	$2.42x10^4$	$6.52x10^4$
	Medium OM	$6.57x10^3$	$2.63x10^4$	$7.35x10^4$	$7.65x10^4$	$1.87x10^5$	$6.27x10^5$
	Medium OM aged	$3.40x10^3$	$1.20x10^4$	$3.37x10^4$	$4.49x10^4$	$8.92x10^4$	$3.16x10^5$
K_{om} (L/kg)	*Low OM*	$5.75x10^4$	$1.81x10^5$	$4.52x10^5$	$4.15x10^5$	$1.07x10^6$	$2.87x10^6$
	Medium OM	$1.49x10^5$	$5.98x10^5$	$1.67x10^6$	$1.73x10^6$	$4.25x10^6$	$1.43x10^7$
	Medium OM aged	$9.40x10^4$	$3.33x10^5$	$9.31x10^5$	$1.23x10^6$	$2.47x10^6$	$8.75x10^6$

Table S11. Average concentrations (µg/kg lipids) based on lipids corrected for background concentration matter for *Chironomus riparius, Hyalella azteca, Lumbriculus variegatus*, and *Sphaerium corneum* for low OM 28d, medium OM 28d and medium OM 1year.

	Lipids (%)	Average concentrations (µg/kg lipid)					
Treatment		PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 180
Low OM							
C. riparius	20.7	BDL	BDL	551.60	403.40	584.03	530.76
H. azteca	7.8	300.0	759.1	1346.1	2892.9	3646.1	3646.1
L. variegatus	1.2	3181.4	4441.5	4301.8	4390.2	4357.8	1729.9
S. corneum	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Medium OM							
C. riparius	20.7	BDL	BDL	257.24	248.18	483.55	308.90
H. azteca	0.3	3000.0	2125.0	3000.0	6250.0	9125.0	7875.0
L. variegatus	1.3	1319.0	1689.9	1845.8	1744.0	2065.5	703.1
S. corneum	0.1	BDL	BDL	BDL	BDL	BDL	BDL
Medium OM aged							
C. riparius	8.1	BDL	BDL	497.2	529.7	841.6	608.7
H. azteca	1.2	2023.8	1037.3	1452.8	2894.9	3924.8	3457.5
L. variegatus	1.1	1517.9	1469.2	1712.8	1746.2	1905.1	635.4
S. corneum	0.5	BDL	BDL	BDL	BDL	821.6	BDL

BLD Lipids (%) or low biomass were below detection limit.

5

Table S12. Average biota sediment accumulation factor (BSAF) normalized on lipids and organic matter for *Chironomus riparius, Hyalella azteca, Lumbriculus variegatus*, and *Sphaerium corneum* for low OM 28d, medium OM 28d and medium OM 1year.

	Average (SD) BSAF					
Treatment	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 180
Low OM						
C. riparius	-	-	7.1 (0.2) (n=2)	5.6 (3.2) (n=3)	8.2 (2.5) (n=3)	6.1 (n=1)
H. azteca (n=2)	5.0 (n=1)	9.2 (9.6)	17.4 (18.6)	39.8 (41.0)	51.1 (53.9)	41.6 (43.8)
L. variegatus (n=3)	53.5 (4.5)	53.7 (5.6)	55.6 (6.0)	60.4 (7.6)	61.1 (6.4)	19.7 (1.9)
S. corneum	-	-	11.6 (n=1)	12.1 (n=1)	16.9 (2.1) (n=2)	-
Medium OM						
C. riparius	-	-	3.1 (0.2) (n=2)	2.9 (0.6) (n=2)	6.1 (1.0) (n=3)	3.5 (0.5) (n=3)
H. azteca (n=3)	39.5 (2.0)	27.1 (2.2)	40.5 (4.5)	74.7 (7.4)	114.1 (11.8)	88.4 (14.2)
L. variegatus (n=3)	17.8 (4.1)	19.6 (3.8)	21.9 (4.2)	20.6 (3.9)	26.0 (5.5)	8.0 (2.0)
S. corneum	-	-	-	-	-	-
Medium OM aged						
C. riparius (n=3)	-	-	5.8 (0.7)	6.1 (1.0)	10.1 (2.0)	6.8 (1.8)
H. azteca (n=3)	27.7 (18.0)	12.0 (6.5)	17.0 (9.0)	33.5 (21.5)	47.1 (30.9)	38.5 (26.5)
L. variegatus (n=3)	20.8 (4.5)	17.0 (1.7)	20.0 (2.1)	20.2 (2.2)	22.9 (3.1)	7.08 (0.1) (n=2)
S. corneum (n=3)	-	-	-	-	9.9 (3.6)	-

- BSAF could not be calculated because biota concentration was below detection limit

Table S13. Effects of organic matter and aging on the biota sediment accumulation factor for each species.

Species		**PCB 28**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 180**
Chironomus riparius[a]	F	n.a.	n.a.	28.699	1.463	3.197	5.007
	df			2,4	2,5	2,6	2,4
	p			0.004	0.316	0.113	0.081
Hyalella azteca[b]	Chi-Square/F	2.618	6.796[a]	5.000	3.806	5.000	2.986[a]
	df	2	2,5	2	2	2	2,5
	p	0.270	0.038	0.082	0.149	0.082	0.140
Lumbriculus variegatus[b]	Chi-Square	5.956	5.804	5.600	5.422	5.956	5.139
	df	2	2	2	2	2	2
	p	0.051	0.055	0.061	0.066	0.051	0.077
Spherium corneum[a]	F	n.a.	n.a.	n.a.	n.a.	8.570	n.a.
	df					1,4	
	p					0.043	

n.a. not enough data available for statistics, [a]ANOVA, [b]Kruskall-Walis test.

Table S14. Effects of species on the biota sediment accumulation factor for low, medium, and medium aged organic matter treatment.

OM treatment		**PCB 28**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 180**
Low	Chi-Square/t	n.a.	-6.807[a]	4.500[b]	5.000[b]	7.227[c]	2.143[b]
	df		3	2	2	3	2
	p		0.006	0.105	0.082	0.065	0.343
Medium	Chi-Square/F/t	8.276[a]	2.980[a]	80.845[d]	177.201[d]	175.643[d]	7.200[b]
	df	4	4	2,6	2,6	2,6	2
	p	0.001	0.041	0.000	0.000	0.000	0.027
Medium aged	Chi-Square/t	0.648[a]	-1.303[a]	5.600[b]	5.600[b]	7.879[c]	5.139[b]
	df	4	4	2	2	3	2
	p	0.553	0.262	0.061	0.061	0.049	0.077

n.a. not enough data available for statistics [a]t-test with data of *Hyalella azteca* and *Lumbriculus variegatus*, [b]Kruskall-Walis test with *Chironomus riparius*, *Hyalella azteca*, and *Lumbriculus variegatus*, [c]Kruskall-Walis test with all four species, [d]One-way ANOVA with *Chironomus riparius*, *Hyalella azteca*, and *Lumbriculus variegatus.*

Model

Model comparison

Table S15. Comparison of reduced model versions with the full model for 324 data points (n) with the F-test. The null hypothesis is that the reduced model version is correct, thus when p<0.05 accept full model.

Model	**OM food sources**	**Parameter description**	**Parameters (#)**	**SS_{min}**	**DF**	**F**	**p**
Full (regress K_{om} nom)	2		21	19.32	118		
Reduced 1	1	β=1, γ=0	18	19.32	121	$-4.38*10^{-8}$	*
Reduced 2	2	1 β for all species	18	19.32	121	$-4.38*10^{-8}$	*
Reduced 3	2	1 'I' per species	13	42.22	126	17.487	<0.001

* p value could not be calculated because difference in SS_{min} between models is negative.

Parameter estimates

Table S16. Parameters and their 90% confidence intervals obtained from fitting with data for *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus*, and *Sphaerium corneum* using the full model. Ingestion 'I' was fitted separate for low, medium, and medium aged organic matter content.

Parameters	**CI**	***C. riparius***	***H. azteca***	***L. variegatus***	***S. corneum***
a (-)	L90	5.9	5.5	0.0*	4.5
		6.2	5.7	0.0	5.6
	H90	6.6	6.0	-	-
β (-)	L90	0.0*	0.0*	0.9	0.0*
		0.0	0.0	1.0	0.0
	H90	0.5	0.6	1.0*	-
I ($kg_{OM} \times kg_{LIP}^{-1} \times d^{-1}$) Low	L90	0.46	-	1.52	-
		1.12	0.44	2.11	0.73
	H90	1.79	0.99	2.70	1.79
Medium	L90	0.22	3.23	0.54	-
		0.59	6.15	0.75	0.14
	H90	0.96	9.07	0.97	-
Medium aged	L90	0.80	0.84	0.58	-
		1.44	1.90	0.81	0.36
	H90	2.09	2.96	1.04	1.11
γ (-)	L90			1.5	
				2.3	
	H90			3.1	
N of experimental data points	Per species	31	47	53	8
Parameters		6	6	6	6
F-ratio value		2.024	1.923	1.903	9.326
SS_{min}		2.6	11.2	5.2	0.3
N of experimental data points	Total			139	
Parameters				21	
F-ratio value				1.473	
SS_{min}				19.3	

'–'= confidence limit not within two orders of magnitude above or below estimated value * parameter set to zero or one because fit was off constrain boundary, L90= lower boundary of the 90% Confidence Interval, H90= higher boundary of the 90% Confidence Interval.

Table S17. Fraction of Steady State reached* (F_{SS}) (Eq. 6) in 28 d bioaccumulation tests with *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus*, and *Sphaerium corneum* for low, medium, and medium aged organic matter content for the full model.

		Fraction of Steady State reached					
Treatment	***Species***	***PCB 28***	***PCB 52***	***PCB 101***	***PCB 118***	***PCB 153***	***PCB 180***
Low	*C. riparius*	1.00	1.00	1.00	1.00	1.00	1.00
	H. azteca	1.00	1.00	1.00	0.99	0.94	0.76
	L. variegatus	0.10	0.10	0.10	0.10	0.10	0.10
	S. corneum	1.00	1.00	0.99	0.97	0.84	0.56
Medium	*C. riparius*	1.00	1.00	1.00	1.00	1.00	1.00
	H. azteca	1.00	1.00	1.00	1.00	0.96	0.84
	L. variegatus	0.09	0.09	0.09	0.09	0.09	0.09
	S. corneum	1.00	1.00	0.99	0.97	0.85	0.58
Medium aged	*C. riparius*	1.00	1.00	1.00	1.00	1.00	1.00
	H. azteca	1.00	1.00	1.00	1.00	0.97	0.89
	L. variegatus	0.11	0.11	0.11	0.11	0.11	0.11
	S. corneum	1.00	1.00	0.99	0.98	0.88	0.67

* Fraction of Steady State reached (F_{SS}) with $0< F_{SS}<1$. A value of 0 means that bioaccumulation is zero (t=0) and a value of 1 means that bioaccumulation is at steady state.

Table S18. Relative importance of PCB uptake by sediment and suspended particle ingestion in 28 d bioaccumulation tests with *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus*, and *Sphaerium corneum* for low, medium, and medium aged organic matter content for the full model.

		% PCB uptake by particle ingestion					
Treatment	***Species***	***PCB 28***	***PCB 52***	***PCB 101***	***PCB 118***	***PCB 153***	***PCB 180***
Low	*C. riparius*	12.7	31.4	56.2	61.9	75.4	87.0
	H. azteca	14.6	35.0	60.1	65.6	78.3	88.8
	L. variegatus	100.0	100.0	100.0	100.0	100.0	100.0
	S. corneum	26.8	53.5	76.4	80.3	88.5	94.4
Medium	*C. riparius*	7.1	19.3	40.2	45.9	61.6	77.9
	H. azteca	70.3	88.2	95.4	96.4	98.0	99.1
	L. variegatus	100.0	100.0	100.0	100.0	100.0	100.0
	S. corneum	6.6	18.2	38.4	44.1	59.9	76.6
Medium aged	*C. riparius*	15.7	37.0	62.2	67.6	79.8	89.6
	H. azteca	42.2	69.7	86.6	89.1	93.9	97.1
	L. variegatus	100.0	100.0	100.0	100.0	100.0	100.0
	S. corneum	15.2	36.2	61.4	66.8	79.2	89.3

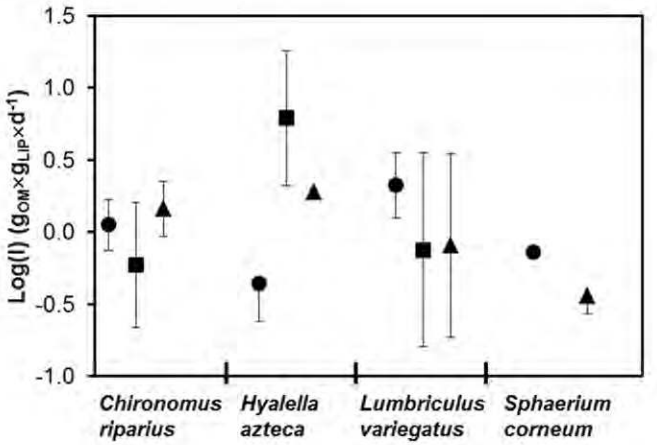

Figure S5. Optimized model parameters and 90% confidence limits for: Ingestion rate (I; g OM x g lipids x d^{-1}) for low (circles), medium (squares), and medium aged (triangles) organic matter for *Chironomus riparius, Hyalella azteca, Lumbriculus variegatus*, and *Sphaerium corneum*. Parameter values are only included if a 90% confidence limit could be assessed in at least one direction.

Chapter 6

Molecular assessment of bacterial community dynamics and functional endpoints during sediment bioaccumulation tests

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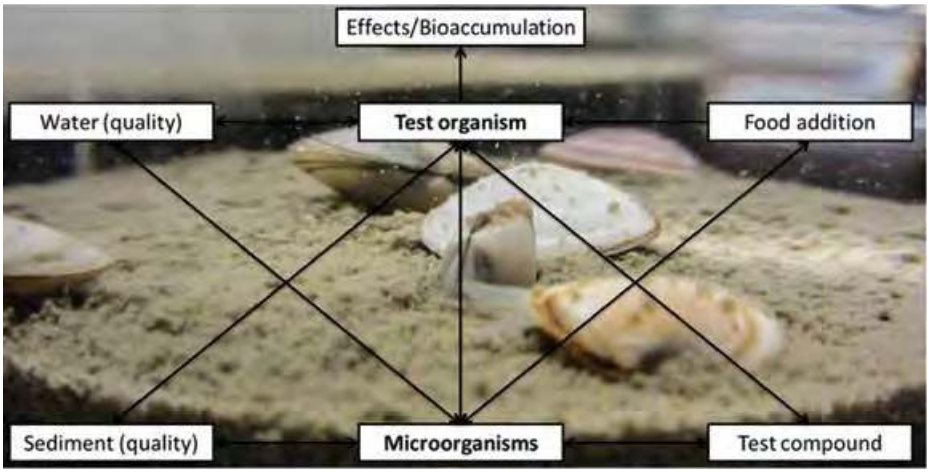
Abstract

Whole sediment toxicity tests play an important role in environmental risk assessment of organic chemicals. It is not clear, however, to what extent changing microbial community composition and associated functions affect sediment test results. We assessed the development of bacterial communities in artificial sediment during a 28 day bioaccumulation test with polychlorinated biphenyls, chlorpyrifos and four marine benthic invertebrates. DGGE and 454-pyrosequencing of PCR-amplified 16S rRNA genes were used to characterise bacterial community composition. Abundance of total bacteria and selected genes encoding enzymes involved in important microbially-mediated ecosystem functions were measured by qPCR. Community composition and diversity responded most to the time course of the experiment, whereas organic matter (OM) content showed a low but significant effect on community composition, biodiversity and two functional genes tested. Moreover, OM content had a higher influence on bacterial community composition than invertebrate species. Medium OM content led to the highest gene abundance and is preferred for standard testing. Our results also indicated that a pre-equilibration period is essential for growth and stabilization of the bacterial community. The observed changes in microbial community composition and functional gene abundance may imply actual changes in such functions during tests, with consequences for exposure and toxicity assessment.



6.1 Introduction

Sediment microbial communities play an important role in ecosystem functions like nutrient cycling, primary production and decomposition.[162] Microbial communities have a large influence on abundance and diversity of benthic invertebrates by controlling carbon dynamics[162] and providing a food source.[163,166,366,367] On the other hand, benthic invertebrates can affect microorganisms for instance by bioturbation.[273,368-370]

Microorganisms influence the degradation and bioavailability of contaminants that accumulate in aquatic sediments[371-373] by adsorption,[374] bioaccumulation[328] and biodegradation.[375,376] In turn, chemicals that enter the environment might affect microbial community structure and function[40,328,377] and therewith cause effects at higher trophic levels.[378-380] Hence, microbial communities constitute an important endpoint in sediment quality assessment,[167,259] since they are ecologically relevant,[381] might affect environmental transformation of chemicals[167] and are sensitive to chemicals.[40]

Effects of contaminants in aquatic sediments can be assessed by sediment toxicity testing.[259] Natural sediments are highly complex and heterogeneous in time and space. Therefore, artificial sediments are often used to standardize toxicity test procedures and to allow for more comparable outcomes. Microbial communities, however, are poorly developed in artificial sediments compared to natural sediments.[352,382] Nevertheless, the presence of microbial communities in artificial sediment, even when poorly developed, still might directly or indirectly influence the quality of sediment and water,[352] chemical behaviour, food availability, symbioses and other processes (Figure S1). Such processes may already start during the sediment equilibration period, which is a common stage of sediment preparation, following spiking. Eventually, microbes may affect the outcome of standard tests with higher organisms.[382] For instance, the bioavailability of chlorpyrifos for *Chironomus riparius* increased with the presence of microbes and biofilms.[328]

Ideally, benthic invertebrate toxicity tests should be performed with single species, in order to avoid interactions that might influence test outcomes. However, it is difficult to exclude microorganisms during an invertebrate test or during any sediment test. Absence of microorganisms would also make such tests less ecologically relevant, since sediment microbial communities play an important role in ecosystem functions.[162] Here we argue that because unavoidable microorganisms might influence test results, there is a need to understand microbial community development in artificial sediments during sediment tests.[382] Moreover, toxicity tests using sediment microorganisms often focus on evaluating effects on single species[383] or on global microbial endpoints, such as microbial community density.[384] Such approaches may fail to detect effects on microbial community composition, structure and/or function. Therefore, measurements of ecologically relevant endpoints associated with benthic microbial communities in standardised sediment toxicity tests are also needed (Chapter 2[259]).

The aim of the present study was to assess the development of bacterial communities and selected genes involved in important microbially mediated ecosystem functions,

during pre-equilibration and exposure stages of a whole-sediment test. A 28-day bioaccumulation experiment was conducted with four marine benthic invertebrates on artificial sediment spiked with six polychlorinated biphenyls (PCBs) and chlorpyrifos (CPF) at concentrations non-toxic for invertebrates as described in Chapter 4.[346] Denaturing gradient gel electrophoresis (DGGE) and 454-pyrosequencing of PCR-amplified bacterial 16S ribosomal RNA (rRNA) gene fragments were used to investigate bacterial community structure and composition in the artificial sediment. Bacterial abundance was measured by quantitative polymerase chain reaction (qPCR), as well as abundance of selected genes encoding enzymes involved in important microbially mediated ecosystem functions, such as nitrogen-fixation, ammonia-oxidation, denitrification, sulphate-reduction and degradation of organophosphate compounds like CPF. To our knowledge, this is the first study to describe bacterial community dynamics during a bioaccumulation test using a complementary set of state of the art molecular tools.

6.2 Materials and Methods

6.2.1 Sediment bioaccumulation experiment

The bioaccumulation experiment is described in Chapter 4.[346] Here we provide a brief summary. Visual representation of the experiment (Figure S2) and details on methods and chemicals used are provided as supplementary information (SI). A 28-day sediment bioaccumulation test was performed in a temperature-controlled room of 14°C under average (standard deviation (SD)) light conditions of 21 (2) lux with a photoperiod of 16h light: 8h dark. Four marine benthic invertebrate species were used: *Arenicola marina* (Linnaeus, 1758) (annelid; sub-surface deposit feeder), *Corophium volutator* (Pallas, 1766) (crustacean: detritus feeder), *Macoma balthica* (Linnaeus, 1758) (mollusc; facultative suspension feeder) and *Nereis virens* (Sars, 1835) (polychaete; omnivore). These species live burrowed in the sediment. Four treatments (n=4) were used: enclosed single species at nominal low (1%), medium (5%) and high (15%) organic matter (OM) content and 'mixed species' at medium OM content. In the 'mixed species' treatment, all four species were tested together in the same aquarium (35L×30W×30H cm). For the enclosed single species treatments, direct species interaction was avoided by introducing four enclosures per aquarium, using fine mesh gauze.[346]

Standard sediment was prepared according OECD guideline 218[76] with small modifications. This included varying the quantity of peat, to obtain the aforementioned low, medium and high OM content treatments.[346] Peat was dried (40°C) and grinded before being used for sediment preparation. After grinding, three random samples were taken and kept at -20ºC until further analyses.

Sediment was spiked with six PCB congeners, i.e. 28, 52, 101, 118, 153, 180 and CPF. PCBs were chosen as a representative of legacy compounds (POPs) and as relatively inert chemicals with a dose below toxicity thresholds for invertebrates and therefore an ideal tracer chemical for bioaccumulation. CPF was chosen as a representative of insecticides,

which are a contrasting chemical group (e.g. regarding their degradability and usage patterns) as compared to PCBs.[346] The nominal concentration for sum PCBs was 36 µg/kg dry weight and for CPF it was 3.12 µg/kg dry weight. The total chemical concentration was the same for all treatments, however, pore water concentrations differed because of the differences in OM content. To allow for (pseudo-) equilibrium between chemicals and sediment prior to the start of exposure, sediment was agitated for 69 days on a roller bank in the dark at room temperature. Control sediment received the same amount of solvent, i.e. acetone, as the treated sediment.[346]

Unfiltered natural seawater from the Eastern Scheldt, the Netherlands, was used as pore water and overlying water. The volume of overlying water was approximately 25 L and the wet sediment to overlying water volume ratio in the aquaria was kept at 1 to 5 for the enclosed single species test and 1 to 6 for the mixed species test. Water flow was possible through the gauze and was enhanced by aeration to ensure complete mixing of overlying water. Invertebrates were added 7 days after the sediment water system was prepared to allow for better physical-chemical stability as has been recommended by Verrhiest et al.[352] Invertebrates were fed with spiked ground fish food (TetraMin) suspended in deionised water, three times per week after the first week of the experiment.[346] The water quality variables oxygen, temperature, salinity, conductivity and pH were measured three times a week.

6.2.2 Sediment collection for microbial analysis

Sediment samples for microbial analyses were taken at the start of the pre-equilibration of the sediment (t=-69 days), at start (t=0 d) and at the end (t=35 d) of the bioaccumulation test. Note that the duration of the bioaccumulation experiment was 28 days, starting after a stabilization period of 7 days. Therefore, the end of the bioaccumulation experiment is referred to as t=35 d. Pre-equilibration samples were taken after adding the sediment compounds and mixing them thoroughly on a roller bank for 1 day (t=-69 d) (Figure S2). If more than one container was used for sediment preparation, subsamples from each container were mixed and three random samples were taken. At the end of the pre-equilibration period (t=0 d), which was the start of the experiment, containers with the same sediment were thoroughly mixed and three random samples were taken. At the end of the experiment (t=35 d) invertebrate test species were removed, sediment from each enclosure was mixed and a sample was taken. For the treatments without enclosure, the whole sediment was mixed and a sample taken, after removal of the test species. Samples were stored at -20°C until further analyses. In addition, samples of control and spiked medium OM sediment were taken during the sediment preparation phase at t=-69 d, t=-62 d, t=-55 d and t=-41 d in a similar way as described above.

6.2.3 Total abundance of bacteria and selected functional genes

Total DNA was isolated from all sediment and peat samples using the FastDNA Spin kit for soil (MP Biomedicals) according to manufacturer's protocol. Sediment samples of all OM contents collected during the pre-equilibration period (t=-69 d) and the start of the

bioaccumulation experiment (t=0 d) were used for DNA isolation, yielding in total 18 samples. However, for the sediment samples at the end of the bioaccumulation period (t=35 d), only low and medium OM content samples were extracted, giving rise to in total 36 samples. Analysis of all samples was not feasible; therefore high OM content samples were left out as less chemical effect on the bacterial community was expected because of lower bioavailability. qPCR was used to determine the abundance of total bacteria (16S rRNA gene), nitrogen-fixing bacteria (*nifH* gene), ammonia-oxidizing bacteria (*amoA* gene), denitrifying bacteria (*nosZ* gene), sulphate-reducing bacteria (*dsrA* gene) and bacteria capable of hydrolyzing organophosphate compounds (*opd* gene). For peat samples, only total bacterial abundance was quantified. qPCR reactions were performed in a 384-well plate (Bio-Rad, Veendaal, the Netherlands) using a CFX384 Real-Time PCR Detection system (Bio-Rad, Veendaal, the Netherlands). All samples were analysed in triplicate and reactions were carried out in a total volume of 10 µL. qPCR reactions targeting total bacteria, nitrogen-fixing and ammonia-oxidizing bacteria were performed according to Rico et al.[385]. Abundance of the denitrification gene *nosZ* was quantified according to Veraart et al.[386]. Abundance of the *dsrA* gene was quantified according to Foti et al.[387]. Abundance of the *opd* gene was quantified using primers 3F and 3R described by Singh et al.[388]. For each qPCR reaction, a standard curve comprising 10-fold serial dilutions of the target gene was included. Standards were obtained by amplifying the target genes from bacterial sources known to harbour one or more genes of interest. Specificity of target gene fragment amplification was checked by melting curve analysis for each qPCR reaction. Primer combinations and cycle conditions are described in Table S1.

6.2.4 Bacterial community structure and composition

In the same sediment samples used for qPCR, bacterial community composition was investigated by 454-pyrosequencing (Roche Diagnostics, Germany) of the 16S rRNA gene. Amplicons were generated by PCR amplification of the V1 and V2 regions of the 16S rRNA gene (Table S2) and sequenced using an FLX genome sequencer in combination with titanium chemistry (GATC-Biotech, Constance, Germany). Preparation of sediment samples for sequencing was done according to Dimitrov et al.[389]. Bacterial community structure of medium OM sediment samples taken during pre-equilibration period of the control and spiked sediments, were furthermore analysed by DGGE fingerprinting of PCR amplicons. Total DNA extraction, PCR reactions and DGGE were performed according to Lin et al.[390].

6.2.5 Data analyses

Raw 454-pyrosequencing data were processed and sorted using default parameters in the Quantitative Insights Into Microbial Ecology pipeline (QIIME) version 1.7.0[391]. Principal Coordinates Analyses (PCoA) were performed using un-weighted and weighted UniFrac distances. Unifrac is a method of calculating distance between microbial communities taking into consideration phylogenetic information, where only presence/absence (un-weighted) or relative abundance (weighted) of operational taxonomic units (OTUs) can be taken into account. PCoA plots were used to visualize similarities or dissimilarities among samples taken at start (t=-69 d) and end (t=0 d) of the pre-equilibration period as well as at the end of the actual bioaccumulation test (t=35 d). Statistical differences between samples taken at

different sampling times were tested using analysis of similarity (ANOSIM) by permutation with 999 replicates, as implemented in QIIME. OTUs were defined at a 97% sequence identity threshold. In order to avoid bias introduced by sequencing depth, all samples were rarefied to an equal number of sequences.

DGGE band detection and quantification of band intensity were performed using Bionumerics software version 4.61 (Applied Maths, Sint-Martens-Latem, Belgium). Multidimensional Scaling (MDS) was performed in order to compare bacterial communities present in sediment samples taken from the pre-equilibration phase, which had been analysed by DGGE. MDS analysis was performed using Bionumerics software version 4.61.

Bacterial 16S rRNA, *nifH*, *amoA*, *nosZ*, *dsrA* and *opd* gene abundance data and Shannon diversity index (16S rRNA gene) were checked for normality with Q-Q plots and Shapiro-Wilk tests and for equality of variances with Levene's test. Log transformation was used for data that were not normally distributed, however, in case data were still not normally distributed the non-parametric Kruskal-Wallis test with pairwise comparison was used. Data for which assumptions were met were tested either with a t-test or with a two-way ANOVA (factors: OM or time or species) with a significance level $\alpha=0.05$ using SPSS version 19. The least significant difference (LSD) was used as a post hoc test for main effects. When an interaction effect was detected with two-way ANOVA, an LSD test adjusted for multiple pairwise comparisons was used to detect differences.

6.3 Results and discussion

6.3.1 Chemical exposure, survival of benthic invertebrate species and water quality

Results of the bioaccumulation experiment are described in Chapter 4.[346] In brief, because of the experimental design, concentrations in the sediment were similar for treatments and stayed relatively constant during the experiment for PCBs. PCBs are chemically and biologically stable and can persist in sediments and soils for years.[312,392] In contrast, at the end of the experiment, the concentration of CPF was below the detection limit in all treatments, which might be explained by volatilization, biologically-mediated and surface-catalysed hydrolysis, oxidation and photolysis.[388,393,394] A previously reported halve-life time (DT_{50}) for CPF in water-sediment systems was 36.5 days.[267] Survival of invertebrates ranged from 47% for *C. volutator* to 60% and higher for *A. marina*, *M. balthica* and *N. virens* in all treatments. Survival for *A. marina* in the mixed species was 0% probably due to predation by *N. virens*.[346] A good water quality was maintained during the test, and variation of temperature, pH, DO and conductivity among enclosures was low (Chapter 4).

6.3.2 Gene abundance during pre-equilibration phase and bioaccumulation test

A selection of genes was used to quantify overall bacterial abundance as well as to target important ecosystem functions mediated by microorganisms in sediments. This enabled us to address to what extent presence and abundance of such genes are affected by the various steps during artificial sediment pre-equilibration and bioaccumulation testing, by varying OM content and by presence of benthic invertebrates.

General patterns

Overall, abundance of all genes targeted here was low or below detection limit (highest 10-fold serial dilution of the qPCR control where amplification was observed) at the start (t=-69 d) and end (t=0 d) of the pre-equilibration period and increased during the pre-equilibration and bioaccumulation period of the experiment, especially for medium OM (Figure 1, S3, S4, Table S3). The total bacterial abundance, as measured by 16S rRNA gene-targeted qPCR, ranged between 7.8×10^6 to 6.6×10^8 copies/g wet sediment for all treatments and time points (Table S3), which lies in the lower range found for natural marine sediment ($2x10^7$ to $3x10^9$ copies/g wet sediment, calculated assuming 3.6 16S rRNA gene copies per cell and an average marine sediment density[395] of 1.7 g/cm^3).[44,45396,397] Abundances of functional genes in the artificial sediment were up to an order of magnitude of 7 lower than those found in natural marine sediment (Table S4).[396-401] These findings correspond with the conclusion of Goedkoop et al.[382] and Verrhiest et al.[352] that artificial sediment is a poor replacement for natural sediment. However, if impacts of microbes on test results were to be minimized, then artificial sediments would be a better choice, even though the ecological relevance decreases. Bacterial communities in artificial sediment originate mainly from the sediment components and any other bacterial source during preparation (e.g. bacteria present in the air) and therefore might differ from a natural sediment bacterial community.[382] After grinding, the total bacterial abundance in peat was higher than the bacterial abundance in the sediment at start of the pre-equilibration period (t=-69 d) (Table S3). Consequently, it can be assumed that peat was the main bacterial source. The seawater that was used to prepare the sediment might have been another main source, however, bacterial abundance in the seawater was not measured.

At the start and end of the pre-equilibration period (t=-69 d and t=0 d), *nosZ* was only detected in some cases (Figure S3D, S4D), whereas *dsrA* and *opd* abundances were all below the detection limit. At the end of the bioaccumulation period (t=35 d), however, these genes were detected, with highest values found for *nosZ* and *dsrA* (Table S3). This suggests that during the testing phase bacterial growth might be stimulated by changing conditions during the experimental period, such as increased concentrations of nutrients in general, as well as specialized feeding of bacteria on the spiked chemical e.g. bacteria capable of hydrolyzing organophosphate compounds (*opd* gene). Studies conducted in soils have demonstrated the importance of microbial activity for the degradation of CPF, where degradation half-lives were significantly longer in sterile soil (abiotic degradation) compared to natural soils (abiotic and biotic degradation).[388,393,402,403] Moreover, DT_{50} for aquatic photolysis (29.6 days) and hydrolysis (25.5 days) are much longer than the total DT_{50} in the aquatic phase (5 days),[267] indicating that biodegradation dominates degradation in sediments.[393] Consequently, it is plausible that the disappearance of the organophosphate CPF during the bioaccumulation test can be explained by an increased abundance of bacteria capable of hydrolyzing organophosphate compounds as quantified by *opd* gene-targeted qPCR. Previously, a similar relationship between functional gene abundances and chemical degradation has been shown e.g. for chloroethenes and hexachlorobenzene,[404-406] which further supports the plausibility of this explanation.

Additionally, bacteria can be introduced either with the added invertebrate test species[55,56] and/or by experimental procedures and environmental surrounding (e.g. air). Moreover, bioturbation by invertebrates may positively influence bacterial abundance and diversity.[368-370] For example, Dollhopf et al.[401] showed that bioturbation delivered oxygen to sediment microorganisms, enhancing coupled nitrification-denitrification in salt marsh sediment, consequently increasing the abundance of genes related to such processes.

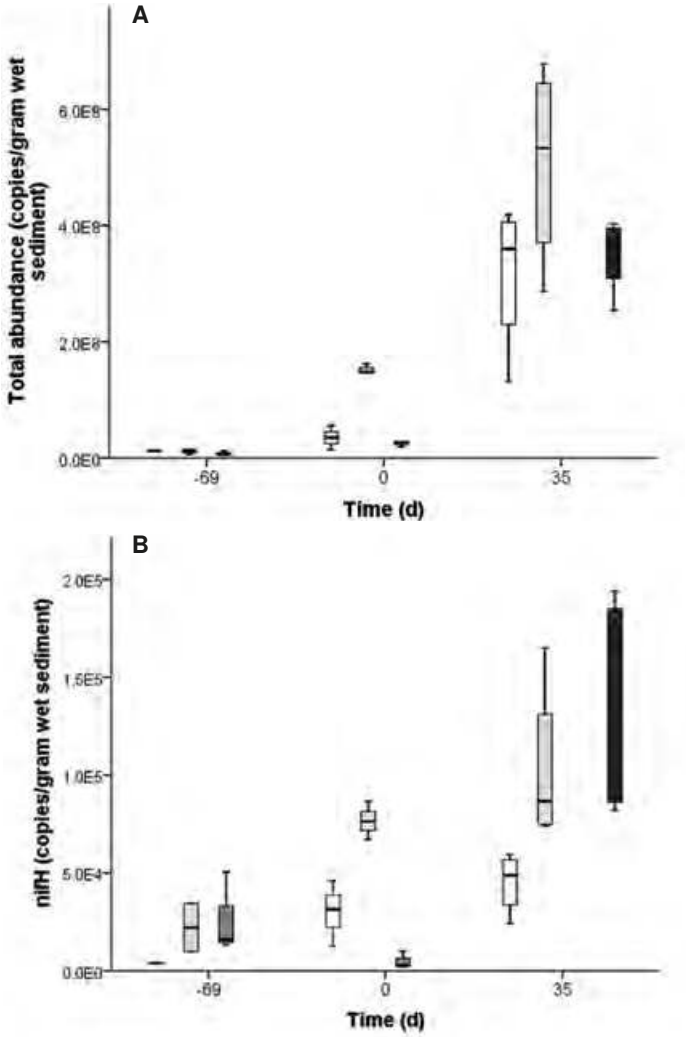


Figure 1. Gene abundances (copies/gram wet sediment) for total bacterial 16S rRNA gene (**A**) and *nifH* (**B**) at the start of the pre-equilibration period (t=-69 d, n=3), at the end of the pre-equilibration period/start of experiment (t=0 d, n=3) and at the end of the bioaccumulation experiment (t=35 d, n=4) for low (white), medium (light grey), high (medium grey) and medium mixed species (dark grey) organic matter content. Note different scales on y-axes. For an overview of the pre-equilibration period only, check Figure S4.

Effect of time, OM and species

At the start of the pre-equilibration period (t=-69 d), no difference was found for total bacteria, *nifH* and *amoA* abundance between the OM treatments except for *nifH* at low OM. At the end of the pre-equilibration period (t=0 d) however, the abundance of all detected genes was higher in the medium OM than in the low and high OM treatment (Figure S3, S4, Table S3). Based on the lower pore water concentrations of PCBs and CPF and the higher nutrient availability at high OM, the highest bacterial abundance would be expected at high OM instead of medium OM.

Total bacterial abundance differed significantly between start (t=0 d) and end (t=35 d) of the bioaccumulation test for both low and medium OM, whereas *nosZ* and *dsrA* abundances were different between start and end for medium OM only (independent t-test; two-tailed $p<0.05$, Table S3, S5, Figure 1A, S3D, S3E). For *amoA* no significant differences could be found in neither of the OM treatments, despite the high numerical increase in abundance (Figure S3C, Table S3, S5). For *nosZ* and *dsrA* for low OM and *opd* for low and medium OM treatments, no statistical tests were performed as values at t=0 d were below detection limit. However, a similar numerical increase in abundance occurred as observed also for *amoA* (Figure S3D, S3E, S3F, Table S3). The gene *nifH* did neither showed significant differences nor a high numerical increase in abundance between start and end of the bioaccumulation test (Figure 1B, Table S5).

At the end of the bioaccumulation period (t=35 d), abundance for almost all targeted genes was lower for *A. marina* and *C. volutator* compared to treatments with *N. virens* and *M. balthica* (Figure 2, S5). No significant interaction was detected between the OM content and invertebrate species on total bacterial abundance, neither on any of the targeted functional genes in the sediment (2-way ANOVA, $p>0.05$, Table S6). There was, however, a significant main effect of OM content on the total bacterial, *nifH* and *dsrA* abundance in the sediment at t=35 d (Table S6, $p<0.05$), where low OM content had lower abundance than medium OM.

Moreover, a significant main effect ($p<0.05$) of benthic invertebrate species on *amoA* (Figure S5C) and *nosZ* (Figure S5D) in the sediment was detected. Gene abundances in sediments with *A. marina* and *C. volutator* were more similar to each other than those observed in sediments with *M. balthica* and *N. virens*. The highest difference was observed between *A. marina* with low abundance and *M. balthica* with high abundance. As mentioned before, bioturbation can stimulate bacterial growth, thus leading to increased bacterial abundance. *A. marina* and *C. volutator* share the same bioturbation mechanism: creating and irrigating U-shaped tubes in the whole sediment or in the top 2 cm of the sediment.[340] In contrast, *N. virens* creates and irrigates burrow galleries in the whole sediment,[340] whereas *M. balthica* burrows itself in the first 2-6 cm of the sediment and is a biodiffuser.[339] The type of bioturbation determines the magnitude of the effect[339,340] and explains that species with more similar bioturbation strategies show a greater similarity in bacterial abundance. However, for specific functional processes this might be different. For example, *C. volutator* and *M. balthica* increase the flux of nitrate from sediment to the overlying water, whereas

A. marina and *N. virens* increase the nitrate flux from overlying water to sediment.[339,370] All species have been reported to increase the flux of ammonium from the sediment to overlying water.[339] Differences in fluxes were explained with the depth distribution of nutrients in pore water, irrigation activity and microbial activity in faecal pellets.[339,370]

At the end of the rolling period (t=0), the medium OM treatment showed no significant difference in abundance between the enclosed single species and mixed species treatment, for any of the genes (independent t-test, $p>0.05$, Table S7). In mixed species systems, it can be expected that the bioturbation activities of the species with the highest impact will dominate the effects of the other bioturbating species, rendering them less visible.[340]

In summary, our results show that variables during a sediment test, such as OM content, time and added invertebrate species, affected functional endpoints, such as the abundance of nitrogen-fixing bacteria, ammonia-oxidizing bacteria, denitrifying bacteria, sulphate-reducing bacteria and bacteria capable of hydrolyzing organophosphate compounds. Additional tests will be needed to determine whether the effects found here with respect to effects on microbial composition and general and pollutant-specific functions can be generalized to other chemicals.

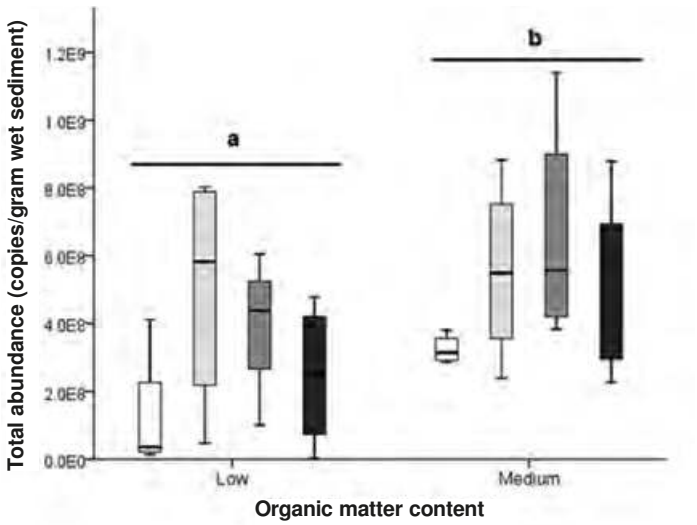


Figure 2. Total bacterial abundance (copies/gram wet sediment) at the end of the bioaccumulation experiment (t=35 d, n=4) at low and medium organic matter content for *Arenicola marina* (white), *Nereis virens* (light grey), *Macoma balthica* (medium grey) and *Corophium volutator* (dark grey). Lines indicate no significant difference in abundance between species within a treatment. Small letters indicate significant differences in abundance between treatments ($\alpha=0.05$).

6.3.3 Bacterial community composition during pre-equilibration and bioaccumulation stages of the test

During the pre-equilibration period, control and spiked sediment with medium OM content showed a similar bacterial community structure, based on DGGE profiles (Figure S6). Control and spiked sediment differed most at the beginning of the pre-equilibration period (t=-69 d), becoming more similar at the end of the pre-equilibration period (Figure S6). However, it seems unlikely that PCBs spiked into the sediment could alter the sediment bacterial community so quickly, that is, in such a way that the bacterial communities in the control and spiked sediment would differ already after a single day of mixing. Previous work showed effects of PCBs on structure, composition and function of microbial communities in sediment and soil, however, after a much longer time (1-8 months).[407,408] Therefore, differences between control and spiked sediment at the start of the pre-equilibration period might reflect insufficient mixing of the sediment after all components had been mixed for one day. Bacterial community appeared to develop in a similar way over time in spiked and control sediment, with community structure of both treatments being very similar at the last two sampling dates. No major difference was observed between control and spiked sediment during the pre-equilibration phase (Figure S6).

Samples of PCBs and CPF spiked sediment from the pre-equilibration period and the bioaccumulation experiment, containing low, medium and high concentrations of OM, were subsequently analysed by 454-pyrosequencing to obtain a more detailed view on potential changes in microbial community structure than is possible by DGGE analysis. A total of 444304 16S rRNA gene sequences with an acceptable quality were obtained with an average of 8228 reads per sample, being 4557 reads the lowest and 13935 reads the highest number (average read length = 300 base pairs). Based on 97% sequence similarity as threshold, a total of 1632 OTUs was found.

Sequencing analysis revealed that *Proteobacteria* was the major bacterial phylum present in the sediment samples (Figure 3, Table S8, S9). At the start of the pre-equilibration period (t=-69 d), sediment containing low and medium OM content showed a similar relative abundance of *Proteobacteria*, which was higher than that in high OM sediment. Similar relative abundance was also observed for the phyla *Acidobacteria* and *Actinobacteria*, however, sediment with high OM content presented a higher relative abundance of these groups. The phylum *Bacteroidetes* was present at higher relative abundance in low OM content sediment, whereas *Firmicutes* were observed only in the high OM content sediment (Figure 3, Table S8). Despite the fact that peat samples were not included in the sequence-based analysis, the bacterial profiles obtained from sediment samples at the beginning of the pre-equilibration period (t=-69 d) give an indication of the relative abundance of different bacterial phyla in peat. For example, the fact that *Firmicutes* were observed only in sediment with high OM content suggests that this bacterial phylum represents only a minor component in the peat-associated microbial community. Moreover, varying the OM content was enough to produce artificial sediment with significantly different bacterial community compositions, as was demonstrated by ANOSIM (un-weighted UniFrac R=0.85,

p=0.001; weighted UniFrac R=0.83, *p*=0.001). At the end of the pre-equilibration period (t=0 d) the relative abundance of *Proteobacteria* was similar to the initial level observed for sediment samples with low and medium OM content, whereas the sediment with high OM content showed a higher relative abundance compared to its initial value. *Acidobacteria*, *Actinobacteria*, WPS-2, *Planctomycetes* and *Firmicutes* decreased in relative abundance at the end of the pre-equilibration period, whereas *Bacteroidetes* increased considerably in all sediment samples (Figure 3, Table S8). At the end of the pre-equilibration period (t=0 d) bacterial communities in all sediment samples were more similar than at the beginning of the pre-equilibration period, as indicated by ANOSIM (un-weighted UniFrac R=0.30, *p*=0.005; weighted UniFrac R=0.16, *p*>0.05), which confirms the results of the DGGE analysis. Observed richness (i.e. number of OTUs) as well as diversity, as indicated by the Shannon index (Figure S7), were consistently higher for sediment samples at the beginning of the pre-equilibration period (t=-69 d) compared to those at the end of pre-equilibration (t=0 d). For low OM content, however, the Shannon index increased significantly during the bioaccumulation test whereas for medium OM the diversity was similar between t=0 d and t=35 d (Figure S7, Table S5). At the end of the bioaccumulation test (t=35 d), there were no differences in bacterial diversity between the test species but there was a significant difference between low and medium OM content (Figure S7, Table S6).

At the end of the bioaccumulation test (t=35 d), *Proteobacteria* was still the most abundant phylum present in the sediment samples (Figure 3, Table S9). *Bacteroidetes'* relative abundance increased in all sediment samples collected at the end of the bioaccumulation test (t=35 d), compared to relative abundance values at the beginning (t=-69 d) and end of the pre-equilibration period (t=0 d). Values were consistently higher in sediment samples containing medium OM content, compared to low OM content (Figure 3, Table S8, S9). The relative abundance of *Firmicutes* had also increased by the end of the bioaccumulation test (t=35 d). *Acidobacteria* and *Actinobacteria* relative abundances at the end of the bioccumulation test (t=35 d) were similar to values observed at the end of pre-equilibration period (t=0 d) (low and medium OM content) (Figure 3, Table S8, S9). Bacterial community composition of all sampling points was compared using PCoA analysis and un-weighted and weighted UniFrac distances (Figure 4), which showed grouping of samples according to time rather than to OM content, especially for un-weighted UniFrac (ANOSIM, un-weighted UniFrac R=0.81, *p*=0.001; weighted UniFrac R=0.74, *p*=0.001). However, when only comparing samples taken at the end of the bioaccumulation test (t=35 d) a clear separation between sediment containing low and medium OM content was observed, indicating that OM content had a direct influence on bacterial community composition or indirectly via chemical concentrations in the pore water, which in turn depend on OM content (Figure 4) (ANOSIM, un-weighted UniFrac R=0.30, *p*=0.036; weighted UniFrac R=0.53, *p*=0.007). PCoA analysis also showed that OM content had a higher influence on bacterial community composition than invertebrate species, especially for weighted UniFrac (Figure 4). Diversity decreased during the pre-equilibration period and increased during the bioaccumulation test, reaching similar diversity values observed at the beginning of the pre-equilibration period (t=-69 d). The observed bacterial richness showed the same pattern (Figure S7).

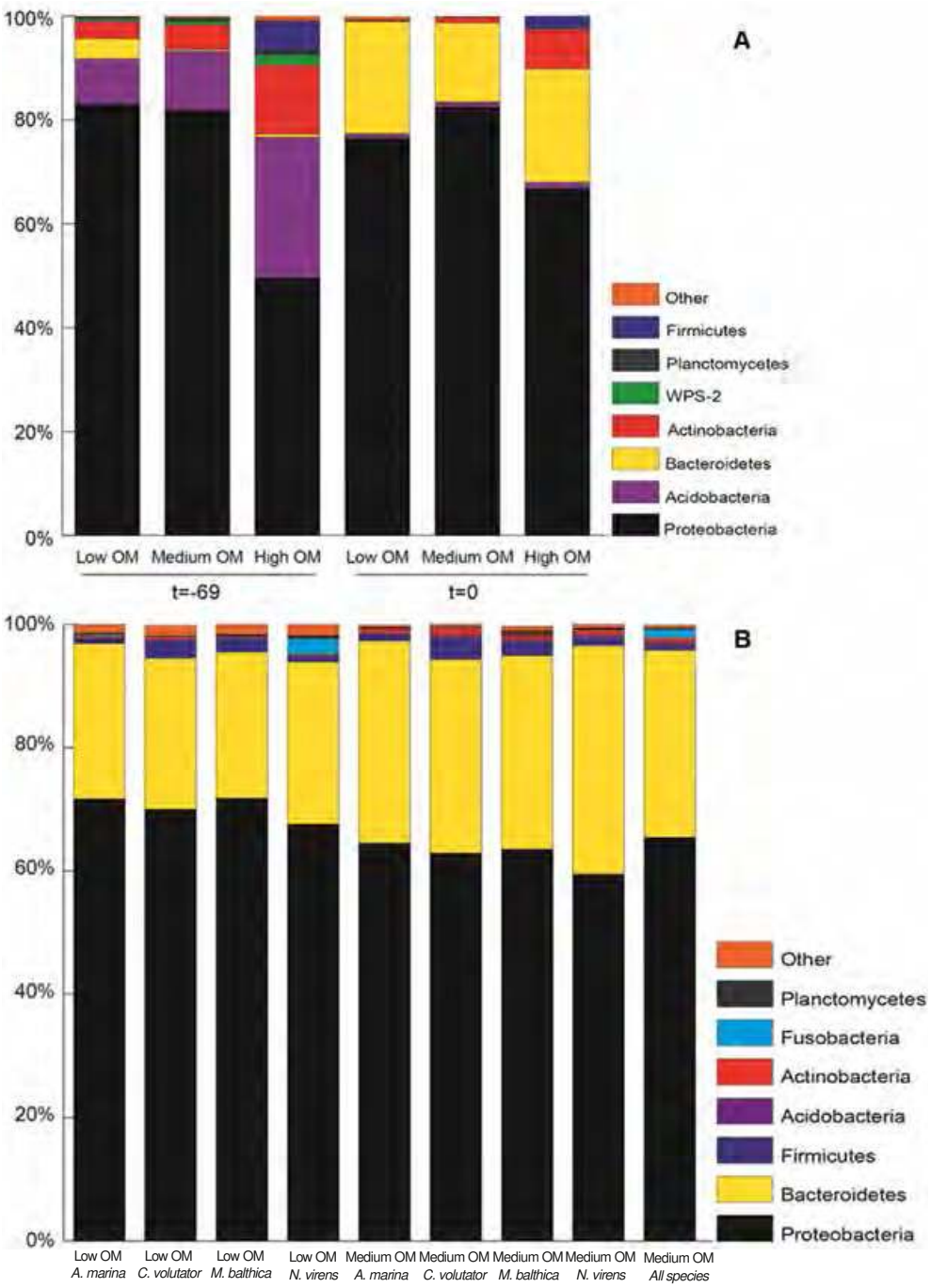


Figure 3. Relative abundance of bacterial phyla detected in sediment samples based on 454 pyrosequencing of 16S rRNA gene fragments, at the beginning (t=-69 d) and end of the pre-equilibration period (t=0 d) (**A**) and at the end of the bioaccumulation test (t=35 d) (**B**). All phyla contributing to less than 1% of the total bacteria were grouped as 'Other'.

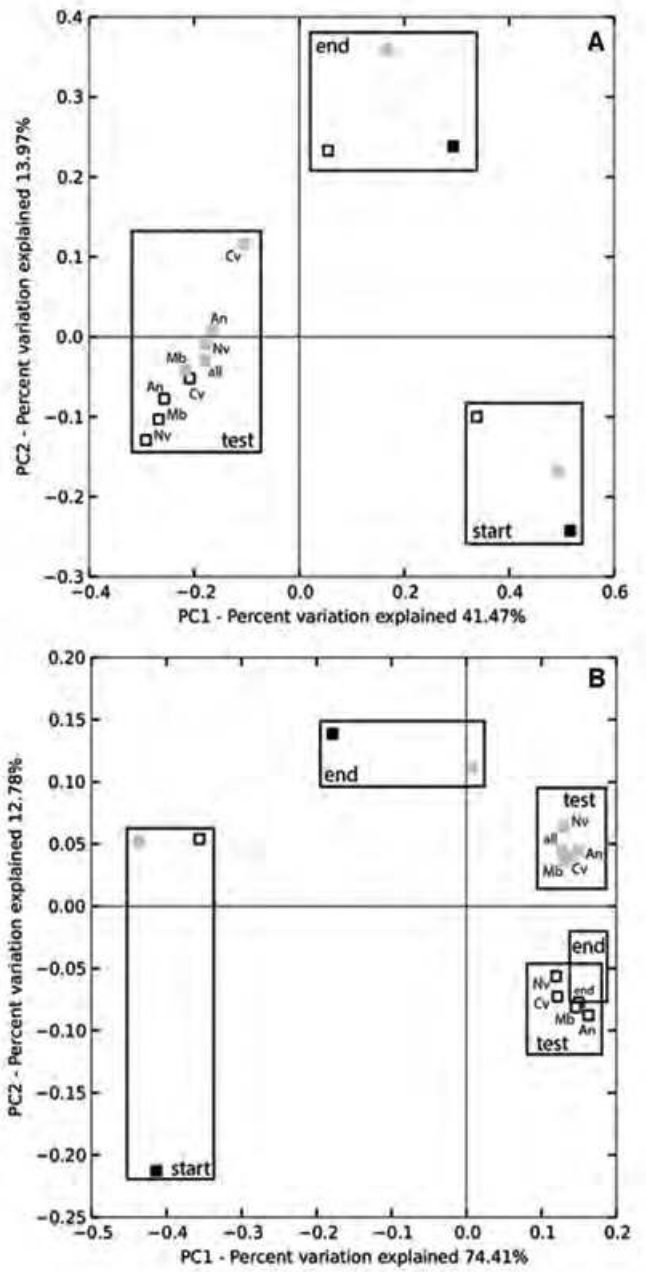


Figure 4. PCoA plots of unweighted (A) and weighted (B) UniFrac distances of sediment samples collected during pre-equilibration phase and bioaccumulation test. Sampling dates are shown as start (t=-69 d), end (t=0 d) and test (t=35 d). OM content is depicted as white (low OM), light grey (medium OM) and black (high OM) squares. Am = *Arenicola marina*, Nv = *Nereis virens*, Mb = *Macoma balthica*, Cv = *Corophium volutator* and all = all invertebrate species together. ANOSIM, un-weighted UniFrac R=0.81, *p*=0.001; weighted UniFrac R=0.74, *p*=0.001.

Establishing a direct link between bacterial community composition observed in the sediment samples and results of the qPCRs assays is difficult. Important microbially mediated ecosystem functions, including those targeted here, are often performed by a wide range of microorganisms. Such functional redundancy may also be reflected at the DNA level, meaning that functional genes frequently do not present a completely conserved DNA sequence across different organisms. Furthermore, next-generation sequencing results often do not provide the necessary taxonomical depth for a detailed classification of observed OTUs. An attempt to predict functional composition based on the 16S rRNA gene information obtained by sequencing was done using the software PICRUSt.[409] However, quality control of PICRUSt predictions indicated that results were not trustable for the dataset described here due to insufficient coverage of annotated genomes related to organisms found in this study in the underlying database. Therefore, in order to acquire detailed molecular information about ecosystem functions associated with a certain sediment sample, either a metagenomics or metatranscriptomics study would be required, as these provide direct sequence information with respect to a microbial community's functional capacity and actual activity as reflected in actively expressed genes.[410-413]

6.4 Implications

This study showed that microbial communities changed as a function of time and as a function of organic matter content. Effects of invertebrate species, however, were only detected for two genes (*amoA* and *nosZ*). OM content more strongly affected bacterial dynamics than invertebrate species. The treatment with medium OM content had the highest gene abundance, and in the light of ecological relevance thus is to be preferred in standard sediment tests, which matches the recommendation by the OECD to use 5% OM by default. Our results also indicated that besides the equilibration of spiked chemicals, a pre-equilibration period is also essential for growth and stabilization of the bacterial community. Therefore, the seven-day pre-equilibration period recommended by the OECD might need to become obligatory, with an extended pre-equilibration period for persistent hydrophobic chemicals with slow sorption kinetics. With the introduction of invertebrate species in the test system, bacterial biodiversity increases, which might change the dynamics of the microbial community already present. Invertebrate species might as well directly contribute to microbial community dynamics by reworking of the sediment via e.g. bioturbation and feeding on bacteria.[368]

We showed that during a bioaccumulation experiment in an OECD set up, the bacterial diversity and community composition as well as functional endpoints such as: the abundance of nitrogen-fixing bacteria, ammonia-oxidizing bacteria, denitrifying bacteria, sulphate-reducing bacteria and bacteria capable of hydrolyzing organophosphate compounds were significantly affected by the test conditions. This is especially important (a) for functions that affect chemical exposure, like in the present case the ability to hydrolyze organophosphate compounds and (b) for functions that affect the water quality

variables driving the performance of the test species. After all, such changes can affect the outcomes of the tests for the target species in an unpredictable manner and limit the reliability of the subsequent steps in the risk assessment. A similar test set up without invertebrates could be used to assess microbial endpoints from which community level dose response relationships could be derived. For instance, a standard inoculum could be applied to standard sediment, after which community composition and gene abundance patterns are assessed as a function of chemical dose. In terms of ecological relevance, however, having a mixed species system that includes microbes as well as invertebrates remains closer to reality.

Acknowledgements

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Supporting Information

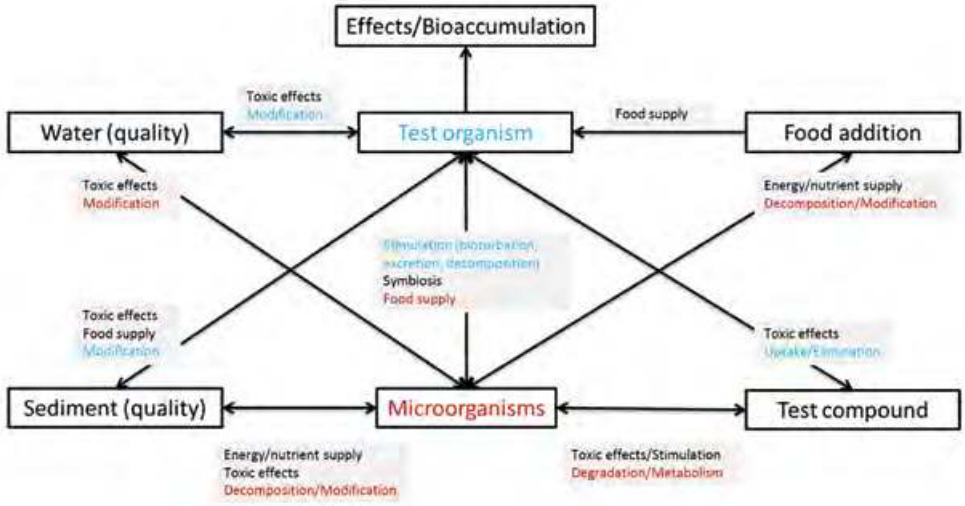

Figure S1. Illustration depicting possible influences that microorganisms might have on toxicity and bioaccumulation test results (adapted from Goedkoop et al.[382]).

Materials and methods

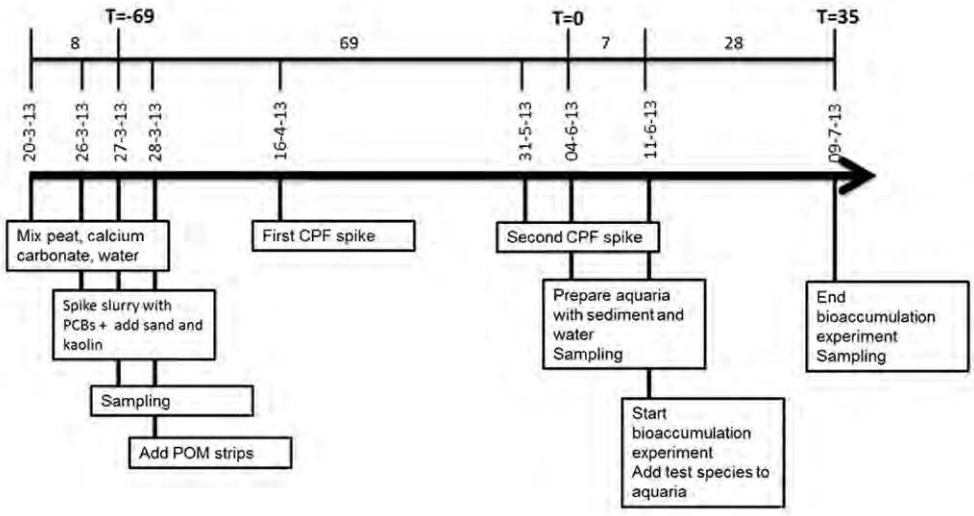

Figure S2. Experimental time (days) scheme of steps during pre-equilibration (from t=-69 d until t=0 d), stabilizing period (from t=0 d until t=7 d) and bioaccumulation test (from t=7 d until t=35 d).

Table S1. Primers and cycle conditions used in the quantitative PCR reactions.

Target gene	Primers	Cycle conditions	qPCR Standards	References
16S rRNA	BACT1369F PROK1492R	95 °C – 3min; 40 cycles of 95 °C – 30 sec, 56 °C – 45 sec, 72 °C 60 sec	*Escherichia coli* (genomic DNA)	414
nifH	nifHF nifHR	95 °C – 3min; 40 cycles of 95 °C – 30 sec, 63 °C – 45 sec, 72 °C 60 sec	*Pseudomonas stutzeri* DSM 4166 (genomic DNA)	415
amoA	amoA-1F amoA-2R	95 °C – 3min; 40 cycles of 95 °C – 30 sec, 55 °C – 45 sec, 72 °C 60 sec	*Nitrosospira multiformis* ATCC25196 (cloned gene fragment)	416
nosZ	nosZ2F nosZ2R	95 °C – 3min; 40 cycles of 95 °C – 15 sec, 65 °C – 30 sec, 72 °C 30 sec	*Pseudomonas nitroreducens* DSM 1650 (genomic DNA)	417
dsrA	DSRp2060F DSR4R	95 °C – 3min; 40 cycles of 95 °C – 40 sec, 55 °C – 40 sec, 72 °C 60 sec	*Desulfitobacterium sp.* (cloned gene fragment)	387
opd	3F 3R	95 °C – 3min; 40 cycles of 95 °C – 30 sec, 57 °C – 3 sec, 72 °C 60 sec	*Sphingomonas sp.* DSM 16637 (genomic DNA)	388

Table S2. Primers and cycling conditions used for targeting bacterial community present in sediment samples.

Primers	Sequence 5'– 3'	Cycle condition	References
27F-DegS	GTTYGATYMTGGCTCAG	95 °C – 2min; 30 cycles of 95 °C – 30 sec, 56 °C – 45 sec, 72 °C – 60 sec	418
338R-I	GCWGCCTCCCGTAGGAGT		419
338R-II	GCWGCCACCCGTAGGTGT		419

Table S3. Average and standard deviation (SD) of total bacterial, *nifH*, *amoA*, *nosZ*, *dsrA* and *opd* abundance in copies/gram wet sediment and percentages of the specific genes compared to the total bacteria at start of the pre-equilibration time (t=-69, n=3), at the end of the pre-equilibration time/start of experiment (t=0, n=3), pure peat after grinding, and at the end of the bioaccumulation test (t=35, n=4) at low, medium, and high organic matter content for *Arenicola marina*, *Nereis virens*, *Macoma balthica*, *Corophium volutator and mixed species*.

			Total bacteria		*nifH*			*amoA*		
Time	**OM content**	**Species**	**Average (copies/g wet sediment)**	**SD**	**Average (copies/g wet sediment)**	**SD**	**% of total abundance**	**Average (copies/g wet sediment)**	**SD**	**% of total abundance**
-69	Low		1.24E+07		3.85E+03		0.03	BDL[a]		
-69	Medium		1.09E+07	4.80E+06	2.21E+04	1.73E+04	0.20	2.51E+02	1.53E+02	0.0023
-69	High		7.75E+06	3.71E+06	2.66E+04	2.08E+04	0.34	2.71E+02	1.52E+02	0.0035
0	Low		3.49E+07	2.06E+07	3.00E+04	1.66E+04	0.09	3.37E+01		0.0001
0	Medium		1.52E+08	8.53E+06	7.67E+04	9.78E+03	0.05	7.47E+02	2.06E+02	0.0005
0	High		2.48E+07	4.66E+06	4.89E+03	4.44E+03	0.02	5.46E+01	1.31E+01	0.0002
0	Pure peat		2.54E+07	2.23E+07						
35	Low	*Arenicola marina*	1.24E+08	1.92E+08	1.99E+04	2.84E+04	0.02	1.33E+03	1.90E+03	0.0011
35	Low	*Nereis virens*	5.03E+08	3.58E+08	5.91E+04	4.46E+04	0.01	1.47E+04	1.03E+04	0.0029
35	Low	*Macoma balthica*	3.96E+08	2.12E+08	6.74E+04	4.56E+04	0.02	1.72E+04	1.27E+04	0.0043
35	Low	*Corophium volutator*	2.47E+08	2.14E+08	2.87E+04	2.16E+04	0.01	8.83E+03	5.14E+03	0.0036
35	Medium	*Arenicola marina*	3.24E+08	4.29E+07	9.22E+04	5.40E+04	0.03	2.46E+03	1.21E+03	0.0008
35	Medium	*Nereis virens*	5.54E+08	2.70E+08	9.27E+04	2.94E+04	0.02	5.81E+03	2.50E+03	0.0010
35	Medium	*Macoma balthica*	6.59E+08	3.39E+08	8.48E+04	2.08E+04	0.01	1.46E+04	2.10E+04	0.0022
35	Medium	*Corophium volutator*	4.94E+08	2.81E+08	1.43E+05	1.78E+05	0.03	6.49E+03	5.48E+03	0.0013
35	Medium	Mixed species	3.52E+08	6.73E+07	1.35E+05	5.75E+04	0.04	7.42E+03	3.35E+03	0.0021

[a] BDL=Below Detection Limit

Table S3 continued.

			nosZ			*dsrA*			*opd*		
Time	**OM content**	**Species**	**Average (copies/g wet sediment)**	**SD**	**% of total abun-dance**	**Average (copies/g wet sediment)**	**SD**	**% of total abun-dance**	**Average (copies/g wet sediment)**	**SD**	**% of total abun-dance**
-69	Low		BDL[a]			BDL			BDL		
-69	Medium		BDL			BDL			BDL		
-69	High		1.01E+04	6.01E+03	0.13	BDL			BDL		
0	Low		BDL			BDL			BDL		
0	Medium		1.37E+06	1.31E+05	0.90	2.33E+03	7.68E+02	0.002	BDL		
0	High		3.77E+03		0.02	BDL			BDL		
35	Low	*Arenicola marina*	7.20E+05	1.31E+06		1.56E+04	2.45E+04		3.96E+04		
35	Low	*Nereis virens*	5.43E+06	3.89E+06	0.58	2.28E+05	3.49E+05	0.01	6.82E+04	3.52E+04	0.032
35	Low	*Macoma balthica*	7.84E+06	4.95E+06	1.08	2.09E+05	1.44E+05	0.05	1.22E+05	9.16E+04	0.014
35	Low	*Corophium volutator*	4.38E+06	3.37E+06	1.98	2.56E+04	2.00E+04	0.05	3.00E+04	1.58E+04	0.031
35	Medium	*Arenicola marina*	4.26E+06	2.33E+06	1.77	7.63E+05	2.54E+05	0.01	3.76E+04	2.56E+04	0.012
35	Medium	*Nereis virens*	5.60E+06	3.15E+06	1.31	6.68E+05	2.57E+05	0.24	2.71E+04	1.56E+04	0.012
35	Medium	*Macoma balthica*	9.59E+06	2.09E+06	1.01	4.72E+05	3.07E+05	0.12	1.13E+05	1.45E+05	0.005
35	Medium	*Corophium volutator*	4.91E+06	1.99E+06	1.46	9.21E+05	1.27E+06	0.07	4.60E+04	3.06E+04	0.017
35	Medium	Mixed species	3.38E+06	1.61E+06	0.99	2.98E+06	4.86E+05	0.19	2.29E+04	8.99E+03	0.009

[a] BDL=Below Detection Limit

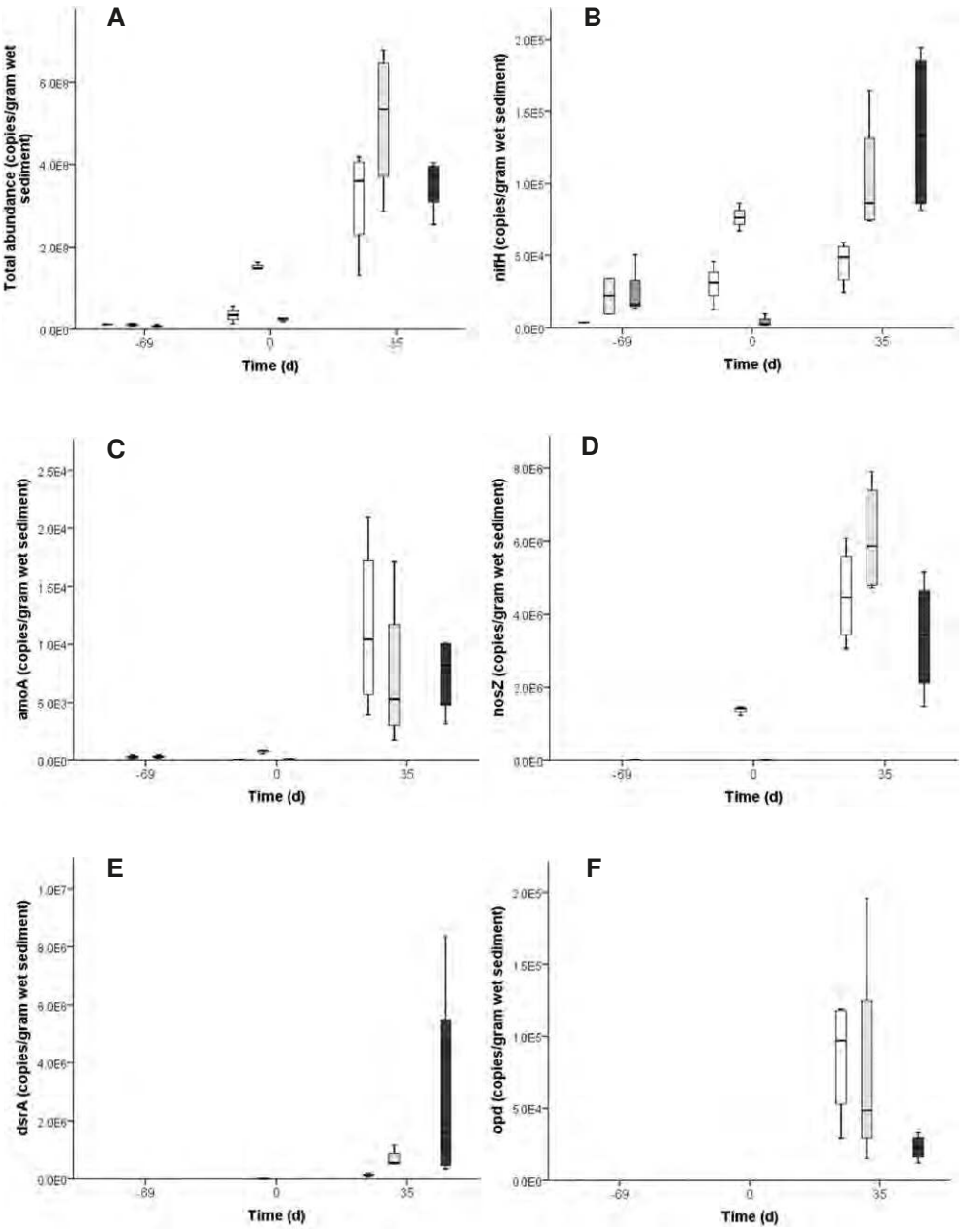

Figure S3. Gene abundances (copies/gram wet sediment for (**A**) total bacterial 16S rRNA gene (**B**) *nifH*, (**C**) *amoA,* (**D**) *nosZ*, (**E**) *dsrA* and (**F**) *opd,* at start of the pre-equilibration period (t=-69 d, n=3), at the end of the pre-equilibration period/start of experiment (t=0 d, n=3) and at the end of the bioaccumulation experiment (t=35 d, n=4) for low (white), medium (light grey), high (medium grey) and medium mixed species (dark grey) organic matter content. Note different scales on y-axes. For an overview of the pre-equilibration period only, check Figure S4.

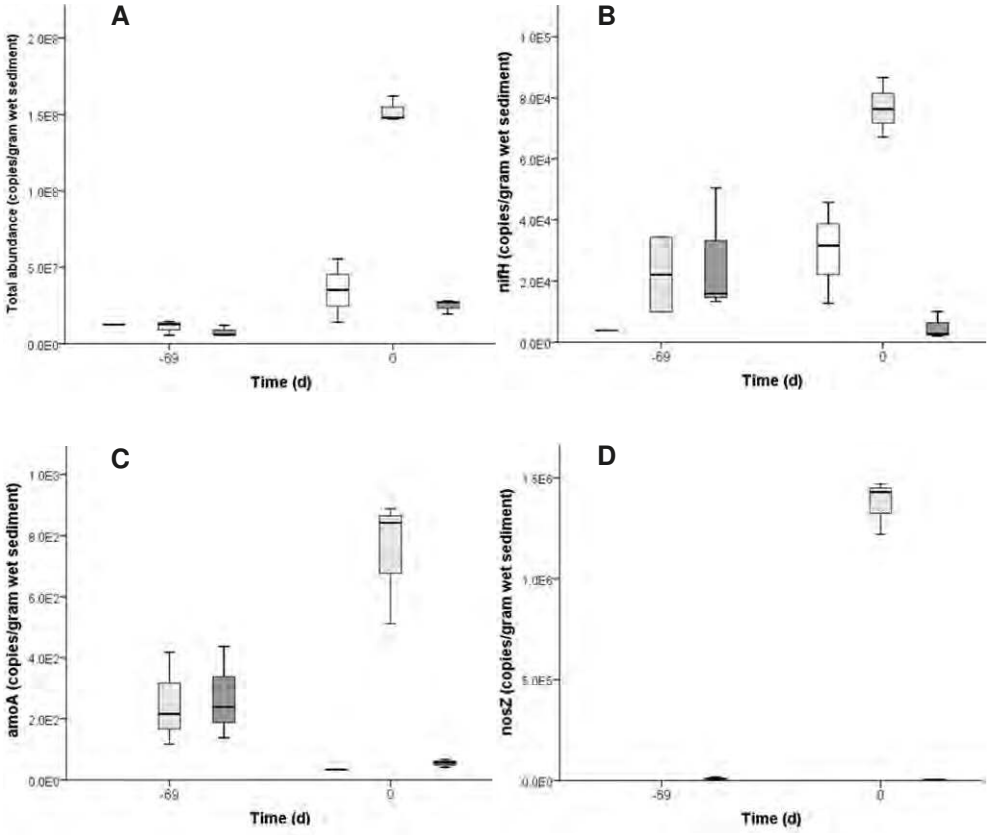

Figure S4. Total bacterial abundance (copies/gram wet sediment) (**A**), *nifH* abundance (copies/gram wet sediment) (**B**) *amoA* abundance (copies/gram wet sediment) (**C**) and *nosZ* abundance (copies/gram wet sediment) (**D**) at start of the pre-equilibration time (t=-69, n=3), at the end of the pre-equilibration time/ start of experiment (t=0, n=3) for low (white), medium (light grey) and high (medium grey) organic matter content.

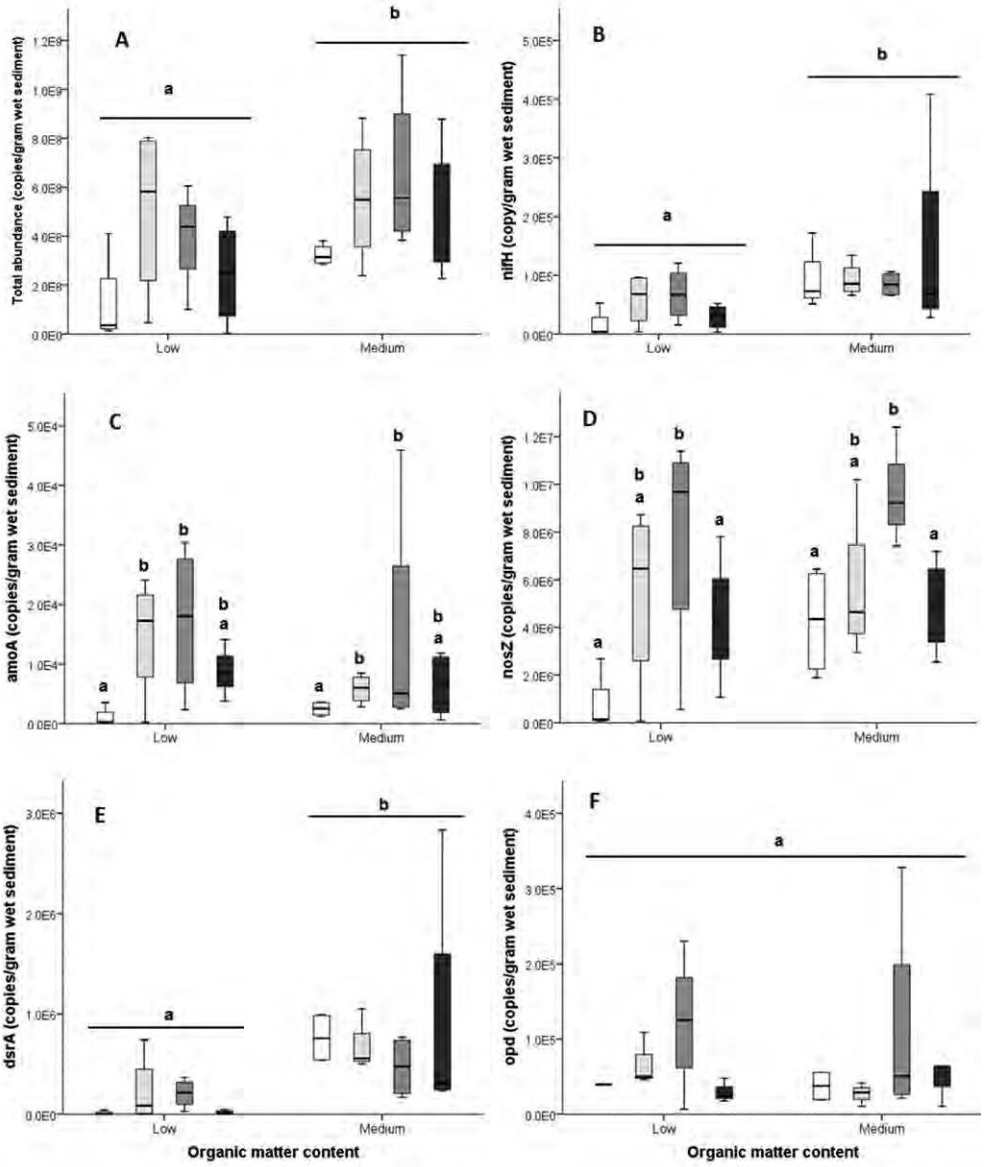

Figure S5. Total bacterial abundance (copies/gram wet sediment) (**A**), *nifH* abundance (copies/gram wet sediment) (**B**), *amoA* abundance (copies/gram wet sediment) (**C**), *nosZ* abundance (copies/gram wet sediment) (**D**), *dsrA* abundance (copies/gram wet sediment) (**E**) and *opd* abundance (copies/gram wet sediment (**F**) at the end of the bioaccumulation experiment (t=35 d, n=4) at low and medium organic matter content for *Arenicola marina* (white), *Nereis virens* (light grey), *Macoma balthica* (medium grey) and *Corophium volutator* (dark grey). Lines indicate no significant difference in abundance between species within a treatment. Small letters indicate significant differences in abundance between treatments (α=0.05).

Table S4. Overview of gene abundance in natural marine sediment.

Gene	Sediment	Abundance (min - max)	Unit	Remarks	Refs	This study (min-max)
16S	Marine	$2x10^7$ - $3x10^9$	copies/g wet sediment	Assumptions: 3.6 copies per cell. Density of sed 1.7 g/cm^3 [S395]. No EMA treatment[a]	[S396]	$7.8x10^6$ – $6.6x10^8$
16S	Marine	$4.7x10^7$ - $2.6x10^9$	copies/g wet sediment	Assumptions: 3.6 copies per cell	[S397]	
amoA	Salt marsh	$5.6x10^4$ - $1.3x10^6$	copies/g wet sediment		[S401]	$3.4x10^1$ – $1.7x10^4$
amoA *(AOB)*	Marine	$6.55×10^4$ - $3.26×10^7$	copies/g sediment		[S398]	
nifH (group NB3)	Marine	$1.5x10^6$ - $1.5x10^8$	copies/g sediment		[S399]	$3.9x10^3$ – $1.4x10^5$
nifH (group NB7)	Marine	$1X10^6$ - $1.5x10^8$	copies/g sediment		[S399]	
dsrA (distribution of SRB)	Marine	$1.7x10^6$ - $2.8x10^8$	copies/g wet sediment	Assumptions: 1 copies per cell. Density of sediment 1.7 g/cm^3 [S395]. No EMA treatment[a]	[S396]	$2.3x10^3$ – $3.0x10^6$
dsrA (distribution of SRM)	Marine	$8x10^5$ (min) $5.1x10^7$ (mean)	copies/g wet sediment	Assumptions: 1 copies per cell	[S397]	
nosZ	Estuarine wetland	$1.9x10^6$ - $2.9x10^7$	copies/g dry soil	Assumption: fraction of water 0.9 (in first 1 cm)	[S400]	$3.8x10^3$ – $9.6x10^6$

[a] Ethidium monoazide (EMA) is a specific treatment to avoid the qPCR quantification of dead cells or free DNA.

Table S5. Difference of total bacterial, *nifH*, *amoA*, *nosZ* and *dsrA* abundance and Shannon diversity index between start (t=0) and end (t=35) of bioaccumulation test was tested with an independent t-test. *opd*, *nosZ* and *dsrA* for low OM could not be tested as values at t=0 were below detection limit. Values between brackets show degrees of freedom. Reported p values are two-tailed and significant values are shown in bold.

	Total abundance		*nifH*		*amoA*		*nosZ*		*dsrA*		Shannon	
	t	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Low OM	(5) 3.642	**0.015**	(5) 1.245	0.268	(3) 1.363	0.266					-8.343	**0.000**
Medium OM	(3.018) 4.063	**0.027**	(5) 1.028	0.351	(5) 1.652	0.159	(3.060) 6.043	**0.009**	(5) -3.999	**0.01**	-1.089	0.352

Table S6. Effect of organic matter and species on total bacterial, *nifH*, *amoA*, *nosZ, dsrA* and *opd* abundance at the end of the bioaccumulation experiment (t=35 d) with a two way-ANOVA. Values between brackets show degrees of freedom. p values in bold are significant.

	Total abundance		*nifH*[1]		*amoA*[5]		*nosZ*		*dsrA*[1]		*opd*[5]		Shannon	
	F	**p**	**X²**	**p**	**F**	**p**	**F**	**p**	**X²**	**p**	**F**	**p**	**F**	**p**
OM	(1, 24) 4.424	**0.046**	(1) 7.225[2]	**0.007**	(1, 22) 0.000	0.995	(1, 22) 1.546	0.227	(1) 14.946[2]	**0.000**	(1, 15) 0.292	0.597	(1, 24) 7.607	**0.011**
Species	(3, 24) 2.605	0.075	(3) 3.588[3]	0.31	(3,22) 3.525	**0.032**	(3, 22) 4.876	**0.010**	(3) 4.367[3]	0.224	(3, 15) 0.68	0.578	(3, 24) 1.119	0.361
OM x Species	(3, 24) 0.286	0.835	(3) 0.728[4]	0.867	(3, 22) 0.894	0.460	(3, 22) 0.361	0.782	(3) 2.184[4]	0.535	(3,15) 0.368	0.777	(3, 24) 1.123	0.360

[1]*nifH* and *dsrA* were analysed with the Kruskal-Wallis test, tests were done for difference in OM[2], differences in species at low OM[3] and medium OM[4]
[5]*amoA* and *opd* were log transformed to meet the normality assumption.

Table S7. Effect of single species versus mixed species at medium organic matter at the end of the bioaccumulation experiment (t=35 d) on total bacterial, *nifH*, *amoA*, *nosZ, dsrA* and *opd* abundance tested with an independent t-test. Values between brackets show degrees of freedom. Reported p values are two-tailed.

Total abundance		nifH[1]		amoA		nosZ		*dsrA*[2]		*opd*[1]	
t	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**X²**	**p**	**t**	**p**
(6) 1.661	0.148	(6) -0.887	0.409	(6) -0.013	0.990	(6) 2.421	0.052	(1) 0.333	0.564	(6) 1.552	0.172

[1]*nifH* was log transformed to meet the normality assumption
[2]*dsrA* was analysed with the Kruskal-Wallis test

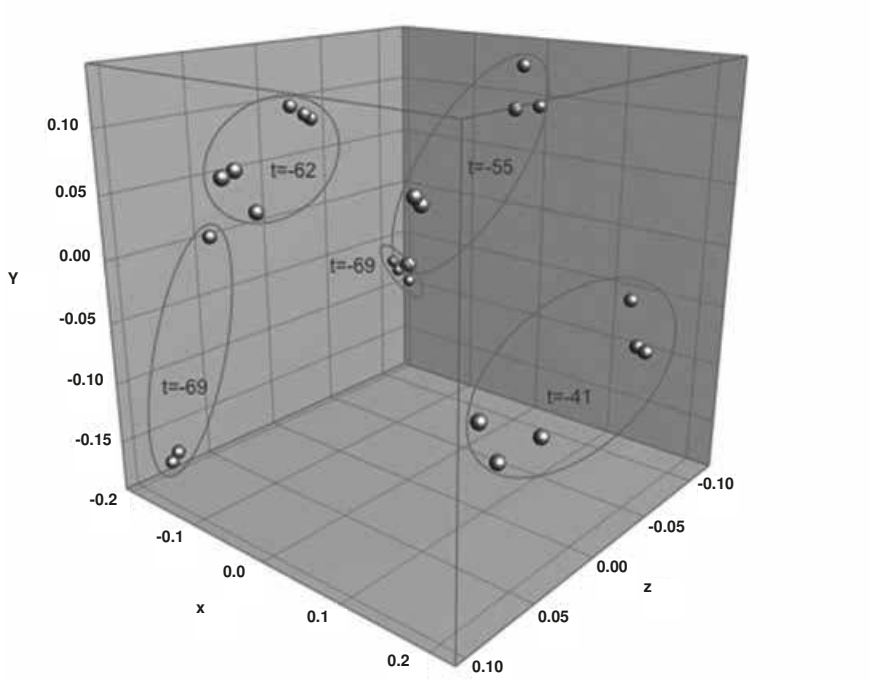

Figure S6. MDS plot of the DGGE profiles obtained from control and spiked artificial sediments (medium OM content) during the pre-equilibration period. Samples were analysed in triplicate and all replicates are represented.

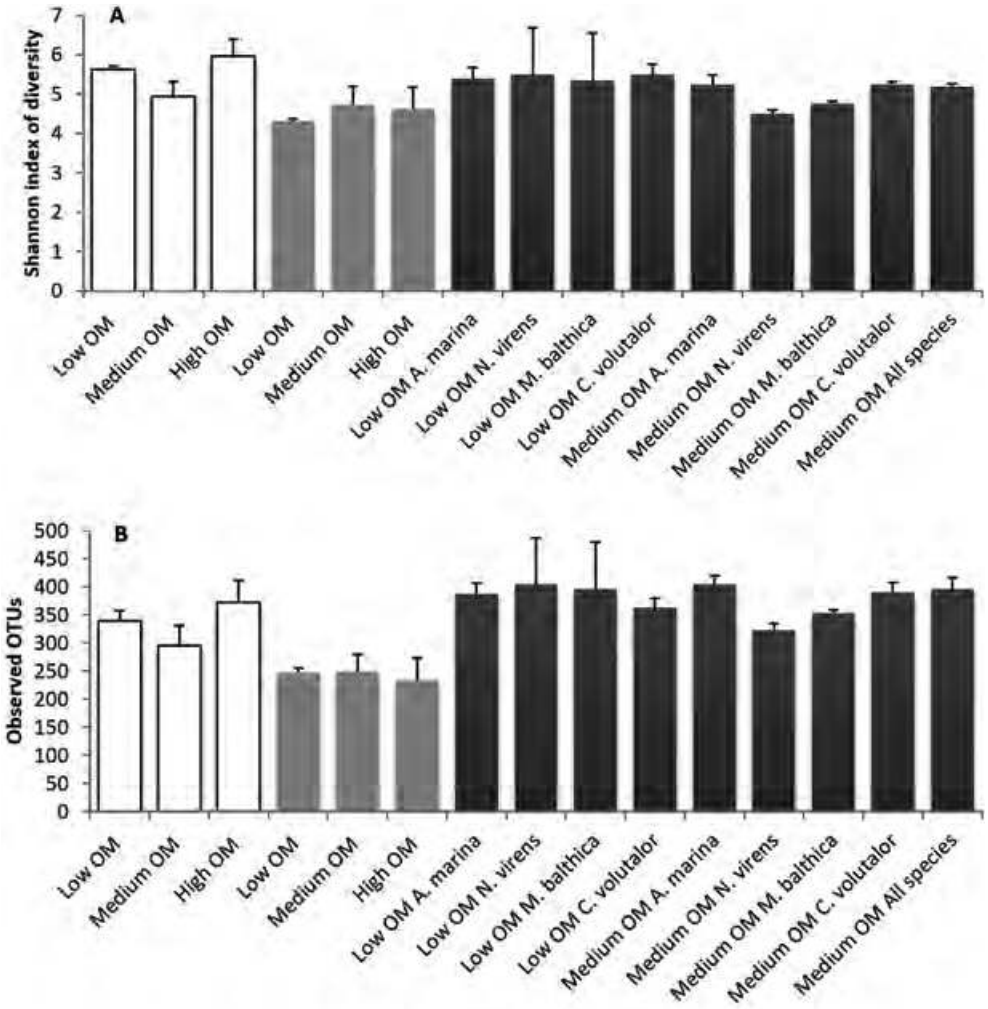

Figure S7. Shannon index of diversity (**A**) and observed OTUs (**B**) of the sediment samples collected during the experiment. White bars represent beginning of pre-equilibration period (t=-69 d), whereas light grey bars represent end of the pre-equilibration period (t=0 d). Dark grey bars represent end of the bioaccumulation test (t=35).

Table S8. Relative abundance of bacterial phyla detected in sediment samples based on 454 pyrosequencing of 16S rRNA gene fragments, during the pre-equilibration period. All phyla contributing to less than 1% of the total bacteria were grouped as 'Other'.

Bacterial phyla	Low OM t=-69	Medium OM t=-69	High OM t=-69	Low OM t=0	Medium OM t=0	High OM t=0
Proteobacteria	83.1	81.9	49.6	76.5	82.5	66.8
Acidobacteria	8.7	11.4	27.1	0.9	1	1.2
Bacteroidetes	3.9	0.3	0.6	21.5	15.2	21.8
Actinobacteria	3.3	4.8	13.3	0.3	1.1	7.7
WPS-2	0.4	0.7	1.9	0	0.1	0.2
Planctomycetes	0.3	0.3	1	0	0	0
Firmicutes	0.1	0.3	5.6	0.1	0	2.3
Other	0.2	0.3	0.9	0.6	0	0

Table S9. Relative abundance of bacterial phyla detected in sediment samples based on 454 pyrosequencing of 16S rRNA gene fragments at the end of bioaccumulation test (t=35 d). All phyla contributing to less than 1% of the total bacteria were grouped as 'Other'.

Bacterial phyla	Low OM Aronicola	Low OM Corophium	Low OM Macoma	Low OM Nereis	Medium OM Aronicola	Medium OM Corophium	Medium OM Macoma	Medium OM Nereis	Medium OM All animals
Proteobacteria	71.7	70	71.8	67.6	64.5	62.9	63.5	59.5	65.4
Bacteroidetes	25.2	24.5	23.7	26.3	32.8	31.4	31.4	37	30.4
Firmicutes	0.6	2.7	2.3	0.8	0.9	3.4	2.3	1.3	0.8
Acidobacteria	0.3	0.4	0.2	0.3	0.4	0.5	0.3	0.5	0.5
Actinobacteria	0.3	0.4	0.2	0.2	0.7	1.2	0.7	0.8	0.7
Fusobacteria	0.3	0	0	2.5	0.1	0	0	0.1	1.4
Planctomycetes	0.2	0.1	0.2	0.5	0.1	0.2	0.7	0.2	0.2
Other	1.4	1.7	1.6	1.8	0.4	0.4	0.8	0.6	0.5

Chapter 7

Dynamics and recovery of a sediment exposed *Chironomus riparius* population: A modelling approach

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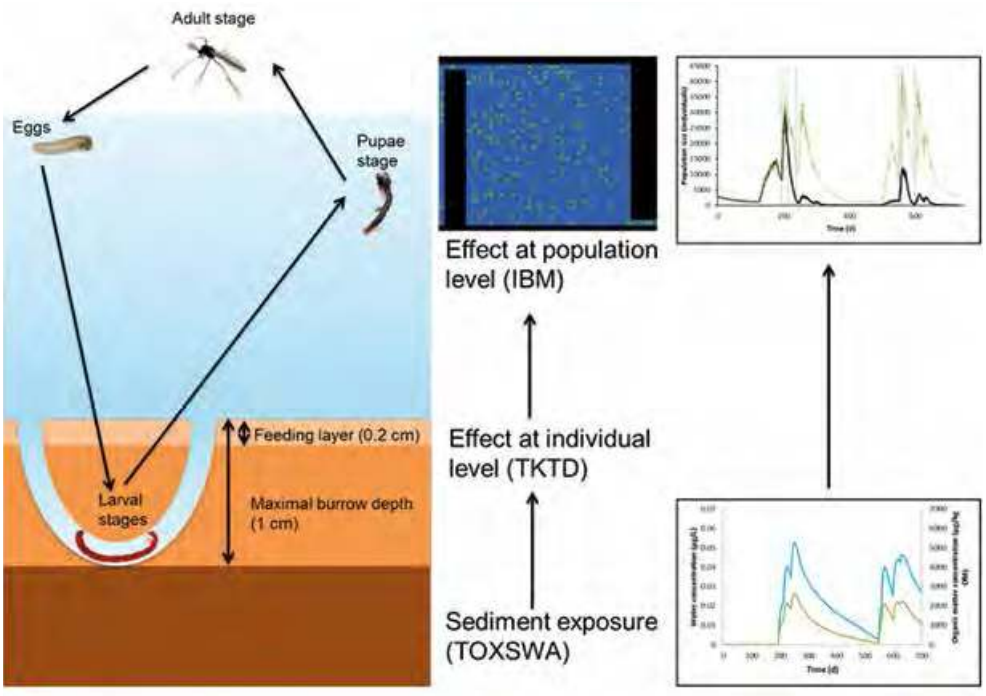
Abstract

To assess risks of sediment-bound contaminants, larger temporal and spatial scales have to be addressed than can be covered in laboratory tests. Although models can address these scales, they usually lack the coupling between chemical fate in the sediment, toxicokinetic-toxicodynamic processes in individuals and the propagation of individual-level effects to the population. We developed a population model that includes all these processes and assesses the importance of chemical uptake routes on damage and recovery of a *Chironomus riparius* population after pulsed chlorpyrifos exposure. We show that particle ingestion is an important additional exposure pathway affecting *C. riparius* population dynamics and recovery. Neglect of particle ingestion underestimates damage and recovery times, which implies that risks of sediment-bound chemicals are underestimated. Additional scenario studies showed the importance of selecting the biologically relevant sediment layer and the use of long term data output.



7.1 Introduction

Hydrophobic organic chemicals accumulate in sediments[342] and may pose high risks to benthic organisms. In the context of prospective environmental risk assessment (ERA), single species laboratory tests are widely used to assess effects of chemicals on aquatic and benthic species.[259] Such tests, however, do not evaluate effects at the population and/or community level as only a relatively small number of individuals of the same species are exposed in isolation.[420] Community level microcosm and mesocosm tests overcome limitations of single species laboratory tests and add ecological realism and complexity by including different levels of biological organization. However, there is insufficient experience on how results of these tests relate to long-term field effects.[201] Moreover, microcosm and mesocosm are limited in size and functional connectivity compared to real ecosystems and often do not include top-predators or allow for recolonization. These limitations may influence assessment of recovery times of affected species.[23] Mathematical population models can account for such processes. Several single-species models, including individual based models (IBM), have been used to predict species responses at the population and landscape/watershed level.[23,221,421-425] These models are available for species like the freshwater species *Asellus aquaticus,*[421,423] *Chironomus riparius,*[421,424,425] *Gammarus pulex,*[421,422] C*loeon dipterum*[421] and the marine species *Corophium volutator*.[221]

Assessment of sediment-bound chemicals usually only accounts for exposure from the water phase based on equilibrium partitioning theory (EPT) and therewith neglecting exposure via ingested sediment and other species traits driving bioaccumulation. For several species, including the standard test species *C. riparius*, it has been shown that exposure via the sediment compartment cannot be ignored.[62,98,296,300,301,346,426] There are several bioaccumulation models available that do describe accumulation of sediment bound chemicals into aquatic food webs, also accounting for ingestion of prey and/or sediment.[69,81,83,279,301,304,315,316,427-429] It is unclear, however, to what extent effects of sediment-bound chemicals at the individual level translate to effects on the population level. Currently, population models for prospective ERA of sediment-bound chemicals are still insufficiently developed, because they do not account for species-specific differences in bioaccumulation and lack exposure via the sediment compartment. There is thus a need to develop models that cover large temporal and spatial scales and couple chemical fate in the sediment, toxicokinetics and toxicodynamics of the chemical within individuals and propagate individual-level effects to the population level, to assess risks of sediment-bound chemicals in the context of prospective ERA.

The aim of this paper was to assess the importance of the sediment uptake route on the effects of sediment-bound organic chemicals on the density and recovery of a *C. riparius* population after a pulsed exposure to the pesticide chlorpyrifos (CPF). This was done by obtaining CPF exposure profiles for pore water and organic matter in the sediment for a standard European pond scenario. The exposure profiles were used as input to an IBM. The individuals in the IBM were equipped with a toxicokinetic toxicodynamic (TKTD) sub-model for effect at the individual level. The TKTD model for the first time integrated

both the water exposure and sediment uptake route, which allowed for the modeling of CPF ecotoxicologically relevant concentrations and bioaccumulation in a species-specific way. *C. riparius* was used as a model species as it is one of the few benthic standard test organisms. CPF is an often studied insecticide and was used as a model compound. Scenario studies were performed to investigate the relative importance of variation in food intake, exposure layer depth, chemical sorption affinity (K_{oc}) and organic matter content in the sediment on the population dynamics and recovery. This was done in order to assess possible implications for toxicity testing methodologies and model approaches in the context of prospective ERA for sediment-bound chemicals.

7.2 Materials and Methods

7.2.1 Model species and compound

C. riparius (Meigen, 1804; Diptera, Chironomidea) has four main life stages in three different environmental compartments (Figure S1) and is used as a standard test species e.g. in OECD 218[76], 219[75] and 233[78]. In the first life stage the eggs are deposited on the water surface and the second stage consists of four larvae instar stages. In the third stage pupae float below the water surface and in the fourth stage adults emerge into the aerial compartment. In the larval stage, the first instar is mainly planktonic whereas the other three live in sediment.[430] The larvae construct stable U-shaped burrows in the sediment down to 1 cm depth (Figure S1). In the third and fourth instar, larvae have a body length between 5 to 12 mm.[431] Larvae feed mainly on silt particles and microdetritus fragments[432] in the first 2 mm of the sediment.[365]

CPF is a widely used neurotoxin organophosphate insecticide that acts by inhibiting the cholinesterase enzymes: acetylcholinesterase and butyrylcholinesterase in the nervous system. CPF has a $\log K_{ow}$ of 4.66[267], a reported K_{oc} of 8151 L/Kg[267], a DT_{50} of 25.5 d for hydrolysis at pH of 7 in water[267] and a DT_{50} of 36.5 d in a water-sediment study[267]. DT_{50} values were used to parameterize transformation in water and in sediment. CPF has a reported acute 96 h LC_{50} geometric mean of 0.17 µg/L[433] and a chronic 28 day no observed effect concentration (NOEC) of 0.1 µg/L[267] for *C. riparius* for exposure via water only. For sediment exposure this is 1.58 µg/g organic carbon (OC) for acute 96 h LC_{50} and 0.32 µg/g OC for chronic 28 day NOEC.[433] For microcosm and mesocosm studies a consistent NOEC of 0.1 µg/L has been observed.[434]

7.2.2 Model description

Modelling chemical exposure

The chemical fate model Toxic Substances in Surface Waters (TOXSWA)[435] was used to calculate CPF exposure concentrations in sediment for a standard FOCUS[436] pond scenario (30 x 30 x 1 m) using the meteorological data from station Weierbach (Germany). The pond was modelled with an inflow and outflow. CPF entered the pond through spray drift, run off and erosion. In the standard exposure scenario, the sediment was assigned an OM content of 9%, a porosity of 60% and the pond had 15 mg/L of suspended solids with 9% organic matter (OM) in the water layer.[436] Exposure profiles in sediment were calculated using the concentration simulated as a function of depth in the sediment. The calculation of the depth profiles used the assumption of sorption equilibrium, which was based on the nonlinear Freundlich isotherm. The Freundlich exponent was set at the default value of 0.9.[437]

For the CPF concentration in pore water (C_W; µg L^{-1}) an average concentration in the first cm of the sediment was used, which equals the maximum burrow depth of *C. riparius*.[431] For the CPF concentration in OM (C_{OM}; µg kg^{-1}) an average concentration in the OM in the top 0.2 cm of the sediment was used, which equals the maximal feeding depth.[365] Details on scenario, substance loadings and substance properties are provided as Supporting Information (Table S1).

An application scheme of three CPF applications of 0.8 kg/ha active ingredient per year was used, which was based on the application scheme that is registered for apples in the United Kingdom. CPF is applied with an interval of minimally 10 days in the period from the first of July till the end of September (late application). A temperature data set of 20 years from the Weierbach meteorological station in Germany was used to obtain monthly water temperatures. In the 20 year period, the average temperature was 11 °C and a ranged from 4 °C to 23 °C (Figure S2).

Three additional main scenarios were defined (Table 1): 1) To determine the effect of exposure layer thickness and thus the biologically relevant sediment layer thickness, the standard scenario was calculated for average exposure for OM over the first 0.2 cm (shallow) and 1 cm (deep). 2) To determine the effect of OM two additional OM scenarios were defined besides the standard scenario: a) a low OM (1% OM) scenario and b) a medium OM (5% OM) scenario. The medium OM scenario resembles the OM content of standard artificial sediment as used in sediment toxicity tests (e.g. OECD 218,[76] 225[77] and 233[78]). 3) The strength of the sorption of organic chemicals to sediment OM as quantified by the K_{oc} value can vary widely depending on the sediment and method used.[438] For natural sediments, a high K_{oc} value indicate a sediment with a high sorption capacity e.g. by presence of high surface area carbonaceous materials, such as black carbon.[439,440] To account for the high variability in K_{oc} values available for field sediments ranging from 973 to 31000 L/kg,[393,441] two K_{oc} scenarios were defined additional to the standard K_{oc} of 8151 L/Kg used: a) low K_{oc} (3x lower) scenario and b) high K_{oc} (3x higher) scenario. TOXSWA uses K_{om} as input value, which was calculated as K_{om}=K_{oc}*0.58 (Table 1).

Table 1. Overview of exposure and ecological scenarios. X indicates the scenario combinations used.

	Model period	CPF	OM	Exposure depth (cm)[a]		K_{oc}	Ingestion factor of normal				
Scenario	**(years)**	**exposure**	**(%)**	**PW**	**OM**	**Factor**	**0**	**0.5**	**1**	**1.5**	**2**
Control	2, 20	No	9						x		
Standard shallow	2, 20	Yes	9	1	0.2	1	x	x	x	x	x
Standard deep	2	Yes	9	1	1	1			x		
Low OM	2	Yes	1	1	0.2	1			x	x	
Medium OM	2	Yes	5	1	0.2	1			x		
Low K_{oc}	2	Yes	9	1	0.2	0.3	x		x		
High K_{oc}	2	Yes	9	1	0.2	3	x		x		

[a] The average exposure over that depth was used as model input. OM=organic matter and PW=pore water

Modelling effects at the individual level

To link exposure to individual effect the TKTD threshold damage model (TDM)[442] was extended to include ingestion of organic matter particles. The TDM model consists of a toxicokinetic part (Equation 1 and 2) accounting for chemical uptake, biotransformation and elimination processes and a toxicodynamics part (Equation 3). The TDM model includes stochastic death, results in a mortality probability and is part of the more comprehensive General Unified Threshold model for Survival (GUTS).

Following earlier bioaccumulation models[81,301,315,316], the dynamics of the internal concentration C_{int} (µg mg^{-1} WW) for benthic invertebrates, was modeled as (described in Chapter 4[346]):

$$\frac{dC_{int}(t)}{dt} = k_{in}C_W + \alpha I\left[\beta C_{OM}^{SED} + (1-\beta)C_{OM}^{SS}\right] - k_e C - k_g C \quad (1)$$

with k_{in} (mL mg^{-1} WW d^{-1}) being the first-order rate constant for dermal uptake, k_e (d^{-1}) the rate constants for overall elimination, k_g (d^{-1}) growth dilution, α (-) the chemical assimilation efficiency (assumed to be independent of food source) and I (mg OM mg^{-1} WW day^{-1}) the mass of OM ingested per unit of time and organism WW. C_{OM}^{SED} represents the chemical concentration (µg kg^{-1}) in organic matter in sediment and C_{OM}^{SS} in suspended or freshly settled solids and β (0<β<1) is the fraction of ingested OM originating from the sediment. For *C. riparius*, β was estimated to be zero (Chapter 5[426]), in which case equation 1 can be simplified to:

$$\frac{dC_{int}(t)}{dt} = k_{in}C_W + k_{ing}C_{OM} - k_{out}C \quad (2)$$

in which $k_{ing} = \alpha I$ is the effective ingestion rate constant (mg OM mg^{-1} WW day^{-1}), $k_{out} = k_e + k_g$ the overall elimination rate constant (day^{-1}), and $C_{OM}^{SS} = C_{OM}$ the chemical concentration in OM in the first 0.2 cm of the sediment. Note that for the model input, C_w is recalculated to nanomol mL^{-1} and C_{OM} to nanomol mg^{-1}.

The toxicodynamics part (Equation 3) accounts for damage accrual and recovery:

$$\frac{dD(t)}{dt} = k_k C_{int}(t) - k_r D(t) \quad (3)$$

$$\frac{dH(t)}{dt} = \max\left[D(t) - threshold, 0\right] \quad (4)$$

The first term in equation 3, simulates the accrual of damage in time as a function of the internal concentrations and the second term the recovery/repair of damage. In which k_k is a killing rate constant (mg nanomole^{-1} day^{-1}), k_r the damage recovery/repair rate constant (day^{-1}) and D(t) is damage (-). In equation 4, dH(t) is the hazard rate which describes the probability of a individual to die at a given time t. When the threshold for damage is exceeded the hazard rate is positive.

As was motivated previously by Baveco et al.[421], coefficients for *Chaoborus obscuripes*[443,444] (Table S2) were used, belonging to the order Diptera like *C. riparius*. Static individuals were assumed by ignoring growth dilution, which is common in TKTD models.[445] To set the effective ingestion rate k_{ing}, a chemical assimilation efficiency α of 0.8,[60] an ingestion rate of 0.325 mg food per mg larval DW per day for natural sediment[446] and a DW:WW ratio of 0.142[447] were assumed, resulting in k_{ing} = 0.8 * 0.325 * 0.142 = 0.0369 mg OM mg^{-1} WW day^{-1}.

Modelling effects at the population level

To link individual level effect to effect on population dynamics, a previously published IBM for *C. riparius*[424] was used. This spatiotemporally explicit model incorporated the basic life-history of *C. riparius*, with individuals developing through egg, larval, pupal and adult stages, and included the basic processes of temperature-dependent growth and development, reproduction, background and density-dependent mortality and aerial dispersal of adults. Coefficient values were identical to the ones used in the original model, except for the rate of density-dependent mortality. Following Baveco et al.[421], this rate was set to 0.003 ind^{-1} m^2 d^{-1} instead of 0.001 ind^{-1} m^2 d^{-1}, in order to simulate population dynamics at a lower density level. Dynamics were simulated on a grid with 1 m^2 cell size. Adult (swarming) movement was simulated as a random-walk (consisting of 10 steps to a randomly-chosen neighbor cell), with adults ending up outside the waterbody returning to the nearest location with water. The IBM was implemented in NetLogo version 5.0.3.[448]

The same temperature dataset from the Weierbach meteorological station was used as in the exposure modelling. Temperatures below 8°C limit growth in winter (Figure S2). The spatial setup for the test simulation was similar to the exposure scenario, i.e. a pond of 30 by 30 meter. Conditions and concentrations were assumed to be uniform over the pond.

Life stages can differ in their sensitivity towards toxicants, with earlier instars larvae being more sensitive than later instars.[425,449,450] For ERA it is important to at least consider the most sensitive life stages, which is why only larvae were exposed to the chemicals in sediment and (pore) water.

Five scenarios for OM ingestion were simulated with a range of values for k_{ing}: zero ingestion (f0), a factor 0.5 of normal ingestion (f0.5), normal ingestion (f1), a factor 1.5 of normal ingestion (f1.5) and a factor 2 of normal ingestion (f2) (Table 1). Additionally, a control scenario without exposure but with normal ingestion (f1) was run to have the baseline population dynamics. The scenarios were run for 2 or 20 years and because of the stochastic nature of the model the average of 20 runs were taken. Variation between runs was relatively small, due to the combination of the extent of the system and spatially uniform exposure, which resulted in a relatively 'well-mixed' situation. All exposure and effect simulations started on January first. Each year CPF was applied, as defined in the FOCUS scenario.

The fraction of recovery of the exposed population was calculated by dividing daily abundance of the exposed population by the abundance of the control population. The exposed population was considered recovered if the abundance reached 95% of the abundance of the control.

7.3 Results and discussion

7.3.1 General exposure patterns

CPF concentrations in overlying water, pore water, total sediment and organic matter varied between years due to varying weather conditions (Figure S3). Rain events influenced run off and the time of application CPF. After the last application of the year, concentrations returned to negligible levels preceding the first application in the following year. Concentrations in sediment decreased with increasing sediment depth (Figure S4). For the standard scenario (9% OM), maximal CPF concentrations in overlying water were 1.85 µg L^{-1}, in pore water 0.08 µg L^{-1} (0-1 cm), in total sediment 76 µg kg^{-1} (0-1 cm) and in OM 3708 µg kg^{-1} (0-0.2 cm). The CPF concentration in overlying water and in OM exceeded the 96h LC_{50} for *C. riparius* for water and sediment[433], indicating potential acute effects.

7.3.2 General population dynamics

Non-exposed and exposed populations showed similar population dynamics but population size differed with lower densities for exposed populations (Figure 1, 2, 3). The population had a multivoltine life cycle with three to four generations per year, the first starting in the beginning of May and the last starting in September. The number of generations was controlled by temperature.[451] Maximal growth occurs at around 25 °C and under laboratory conditions one generation takes around 35 days at 21 °C.[452] The highest population size was reached during the second and third generation. During winter, low temperatures and shorter day length, not accounted for in the model, induced a diapause in third and fourth instar *C. riparius* larvae[453] and population size decreased to a minimum until the first generation in the following year. Similar population dynamics have been observed previously for a natural population in an uncontaminated temperate lowland stream[454], which supports the validity of the present simulations.

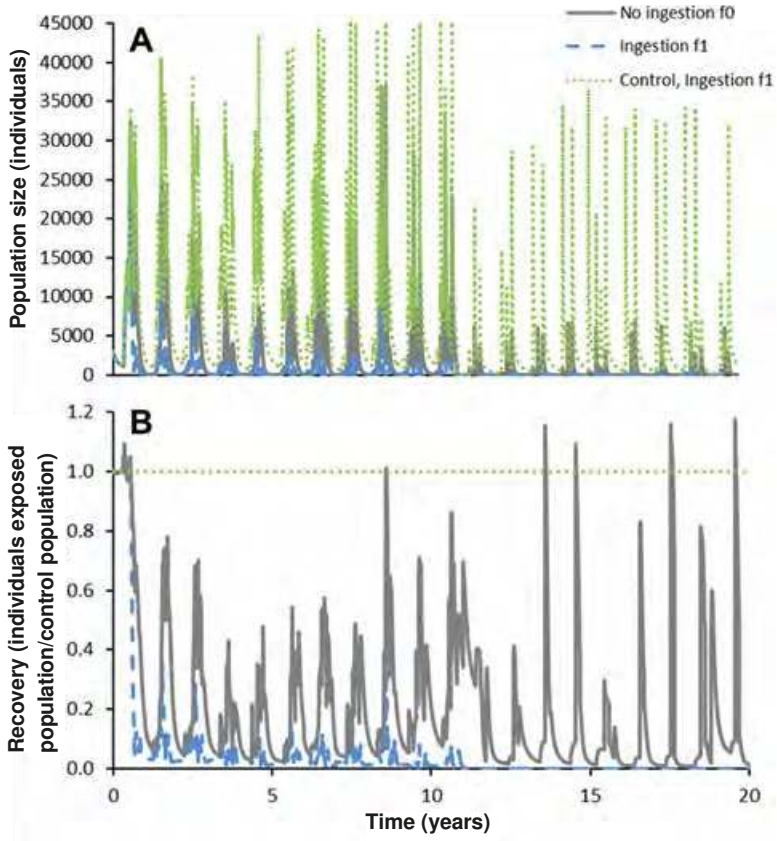


Figure 1. Long term effects of pulsed chlorpyrifos exposure on *Chironomus riparius* population dynamics (number of individuals; Panel **A**) and recovery (Panel **B**). Recovery is calculated as number of individuals in the exposed population divided by that number for the control population. The simulations were done for sediment with a high organic matter content (9%) over a period of 20 years. The grey solid line (——) indicate the 'no ingestion' (f0) scenario and the blue dashed line (— — —) indicates the 'normal ingestion' scenario. The green dotted line (•••••) indicates the dynamics of a non-contaminated control population of chlorpyrifos.

Long term effects

The 20-year simulations indicated that there was natural inter-annual variation in peak density (Figure 1). In year 12 there was an apparent, possibly temperature-related, decline in peak numbers compared to the previous years both for the non-spiked and spiked populations. However, after 12 years of annual CPF applications, the population with normal ingestion (f1) got extinct whereas the population without ingestion (f0) survived (Figure 1A). The extinction of the population with the normal ingestion scenario can be explained by the population density from the previous year being too low to survive the new CPF application. Additionally, combined stressors might be an explanation e.g. climate and chemical stress. In the model, temperature is assumed to influence the development rate of the larvae stages and larvae stopped growing below 8 °C. Therefore, if the development

rate is low, average exposure to individuals lasts longer and thus higher effects and lower recovery are expected. Indeed, a previous study showed that low temperatures (<8 °C) are an additional stress factor for *C. riparius* next to the stress imposed by contaminants.[455]

Recovery was low for the scenario with normal ingestion whereas full recovery occurred for the population without ingestion (Figure 1B). Recovery potential was found to increase with an increasing size of the modelling area[421] and the presence of uncontaminated areas which increase the recolonization potential and the possibility to avoid contaminated areas due to movement.[124,456,457] Therefore, recovery in a pond which is limited in size and has no uncontaminated areas may be lower than in most natural systems. Moreover, the time at which the application takes place, e.g. spring, is important for the effect on population size and subsequent recovery, due to differences in life stage sensitivity and population dynamics at the time of application during e.g. the reproductive period.[423]

For prospective ERA, it is important to run models for a long time period (e.g. 20 instead of 2 years) to grasp the full impact of long term pesticide exposure on benthic populations. However, we aimed at identifying the major differences on short term effects first and therefore our scenarios used a two year period.

Influence of particle ingestion

For the first time, exposure via sediment was incorporated in the TKTD model and it was shown that adding realism by including ingestion as an exposure pathway resulted in higher exposure, higher mortality, lower population densities and a slower recovery rate compared to scenarios with exposure from pore water only (Figure 1, 2, 3). After the first application of CPF on day 193, the simulated number of individuals, for both populations with and without ingestion, kept increasing but did not reach the maximum number of the control population as observed for the second generation (Figure 2A). After the second and third application of CPF in the first year, the population with ingestion showed larger effects than the population without ingestion. Recovery during winter was slow, which resulted in a small first generation population size in the following year before application (Figure 2B). The maximum population size and recovery of the second generation in the second year depended on the ingestion rate. For none of the scenarios 95% recovery was reached, with the highest recovery of 92% being observed for the population without ingestion. Thus accounting for realistic simultaneous exposure via pore water and ingestion of contaminated particles resulted in a larger and earlier impact and a delayed recovery compared to exposure via pore water only (Figure 2). The higher the ingestion rate, the more profound these effects were. Despite the fact that bioaccumulation models for invertebrates have included ingestion since the 80's of the past century,[304] many risk assessment schemes still rely on aqueous phase exposure based on EPT. This is not new as such, but here we show for the first time how neglecting of chemical exposure via food ingestion can lead to an underestimation of risk of sediment-bound chemicals on the population level. We conclude that exposure via the sediment compartment should be taken into account in ERA.

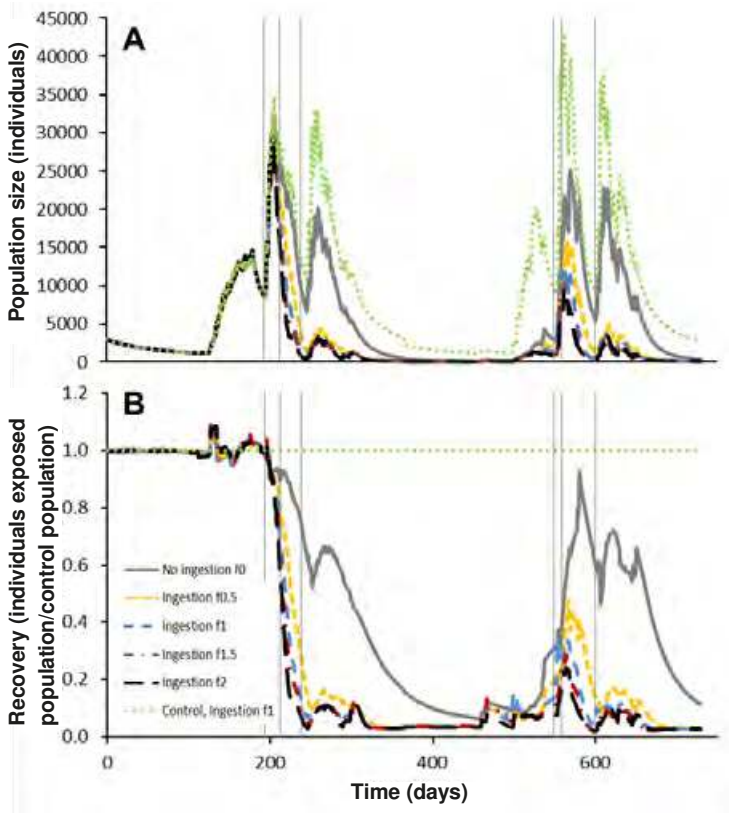


Figure 2. Shorter term effect of pulsed chlorpyrifos exposure on *Chironomus riparius* population dynamics (number of individuals; Panel **A**) and recovery (Panel **B**) for different ingestion scenarios. Recovery is calculated as number of individuals in the exposed population divided by that number for the control population. The simulations were done for sediment with a high organic matter content (9%) over a period of 2 years. Ingestion scenarios include: 'no ingestion' i.e. aqueous exposure only (f0; grey solid line; ——), '50% of normal ingestion' (f0.5; orange squared dotted line; – – –), 'normal ingestion' (f1; blue dashed line; — — —), '150% of normal ingestion' (f1.5; red dash dotted line; – • –), and '200% of normal ingestion' (f2; black long dash line; — —). The green dotted line (•••••) indicates the dynamics of a non-contaminated control population. Vertical lines indicate applications times of chlorpyrifos.

7.3.3 Exposure scenarios

Influence of exposure layer thickness

Concentration in sediment decreased with increasing depth for pore water, total sediment and organic matter (Figures S3, S4). In earlier studies, more than 90% of the total mass of CPF was found in the top 1.5 cm of the sediment in the first week after application[434] and 96% of the total mass was found in the top cm of the sediment with an organic carbon content of 12% (= 21% OM) after one day of exposure[393,458]. These studies reported that CPF reached deeper sediment layers with increasing time, a pattern that also was observed for carbendazim.[459] The vertical distribution of a pesticide in the sediment is driven by processes like diffusion in

pore water, adsorption, degradation and bioturbation.[459] Bioturbation can affect the physical, chemical and biological parameters[460] and the distribution of chemicals[461] in the sediment and water compartment. Bioturbation was not explicitly included in the TOXSWA model. However, based on observations done on other *Chironomus* sp.[462] it is plausible that *C. riparius* mixes the first cm of the sediment by construction and ventilation of the U-shaped burrows.

The thickness of the exposure layer had an effect on the dynamics and recovery of the population (Figure S5). An exposure layer thickness of 0.2 cm for feeding on OM led to a lower population size and slower recovery compared to an exposure layer thickness of 1 cm. Thus the 0.2 cm scenario (shallow), which was tuned to species specific feeding habits led to a worst-case effect assessment and would therefore be the preferred scenario for ERA. Which sediment layer thickness should be considered biologically relevant depends on species specific traits and should therefore be specified per species. Moreover, the vertical distribution of chemicals and thus exposure can be influenced by mixing of sediment due to bioturbation, as discussed above. This may influence the thickness of the biologically relevant sediment layer or may affect concentration profiles within that layer. Assessment of the effects of mixing on exposure however, is not straightforward. Due to the trade-off between opposing processes such as *attenuation* of exposure due to particle mixing and *increased* exposure due to turbulent dispersion driven enhancement of the chemical influx at the sediment-water interface, the net effect of bioturbation on exposure is difficult to predict.[459,461,463,464] Exposure might lead to decreased bioturbation, providing an intrinsic feedback between toxicity and fate.[459,465]

Consequently, in order to select the appropriate biologically relevant sediment layer thickness in prospective ERA it is important to carefully consider vertical heterogeneity in combination with species specific traits and possible bioturbation activity. The future incorporation of mixing processes and feedback mechanisms in the TOXSWA and TKTD models would support the development of more ecologically relevant scenarios to assist the prediction the biologically relevant sediment layer and effects of bioturbation in contaminated sediments on benthic populations.

Influence of organic matter content

CPF concentration in the total sediment decreased with decreasing OM content, whereas the CPF concentration in pore water and OM increased with decreasing OM content (Figure S6). Lowering the sediment OM content led to higher concentration of CPF in the OM, which also explains the increase of the CPF equilibrium concentration in the pore water. At lower OM content CPF diffused deeper into the sediment because the diffusion was less retarded due to sorption (Figure S4), leading to more CPF in the total sediment. With an equal amount of OM ingested in all OM treatments larger effects of CPF on population dynamics and recovery were expected for low OM because CPF concentration was higher in low OM than in high OM. This was, however, not observed in our simulations (Figures S7A, S7B), which we explain from the existence of a threshold in the modeled damage (Eq. 4). We conclude that once the threshold of 3.3×10^{-8} is exceeded by the damage in the TD model part (Eq. 4), the output is insensitive to higher CPF exposure (Figure S7). This was checked by manipulating the input

of the TDM (simulated for a single, imaginary, long-lived individual) by: 1) changing the CPF concentration simultaneously in pore water and OM (factors 0, 10^{-2}, 10^{-4} or 10^{-6}), 2) the fraction of ingestion (no ingestion (f0) or normal ingestion (f1)) or 3) a combination of both (factor 10^{-2} and no ingestion). When accounting for normal ingestion (f1) and lowering the concentration in pore water and OM with a factor of 10^{-2}, the damage decreases while survival increased rapidly (Figure S7). Only a decrease of CPF concentration with a factor of 10^{6} led partly to internal damage levels below the threshold after the first CPF exposure whereas nearly full survival was reached when CPF concentration was decreased with a factor 10^{4} and higher. This indicates that survival responded faster to decreasing concentrations in pore water and OM than damage. Survival reached nearly 100%, whereas damage did not decreased below the threshold yet (Figure S7). Setting ingestion to zero (f0) and lowering concentrations in pore water and OM led to similar patterns. Changes in the high OM scenario were slightly more pronounced than in the low OM scenario, possibly due to lower starting concentrations in pore water and OM in high OM compared to low OM scenario (Figures S7B, S7C).

Cammen[322] found that ingestion of particles is negatively correlated with OM content in the sediment. Thus it would be expected that *C. riparius* in the low OM scenario has a higher ingestion, potentially leading to higher effects. We simulated this by comparing the low OM and a higher ingestion factor (1.5) scenario with a scenario with a high OM and a lower ingestion factor (0.5). As expected, the populations in the low OM scenario were more affected than in the high OM scenario, especially in the second year (Figure S8). Moreover, OM content and thus the amount of food available can affect sublethal and lethal endpoints at the individual level, like emergence success, that may extrapolate to effects at population level.[451,452,466-469] It is thus important to consider food quality and quantity when including ingestion as an additional exposure pathway in ERA.

Effect of sorption strength

Varying the K_{oc} value influenced exposure (Figure 3). A three times lower K_{oc} value resulted in a maximal 3.4 times higher CPF concentration in pore water and a 3 times lower CPF concentration in OM. A three times higher K_{oc} value resulted in a maximal 1.4 times lower concentration in pore water and a 3 times higher concentrations in OM compared with the scenario that used the default K_{oc} (Figure 3C, 3D).

This variability in sediment sorption properties (varying K_{oc}) led to clear differences in population size and recovery times for a population without ingestion, whereas there was no difference between the K_{oc} scenarios when ingestion was taken into account (Figure 3A, 3B). We explain this with the threshold for damage, as described above. Damage probably already exceeds the threshold for the low K_{oc} scenario for a population with ingestion. Consequently, scenarios with higher exposure are less sensitive. Therefore, the present model parameterization is only sensitive to differences in K_{oc} when ingestion is excluded. However, for species with different TKTD parameter values, sensitivity to varying K_{oc} values may differ. Therefore, for an appropriate effect assessment it is important to carefully measure and select the appropriate K_{oc} value for the chemical under evaluation.

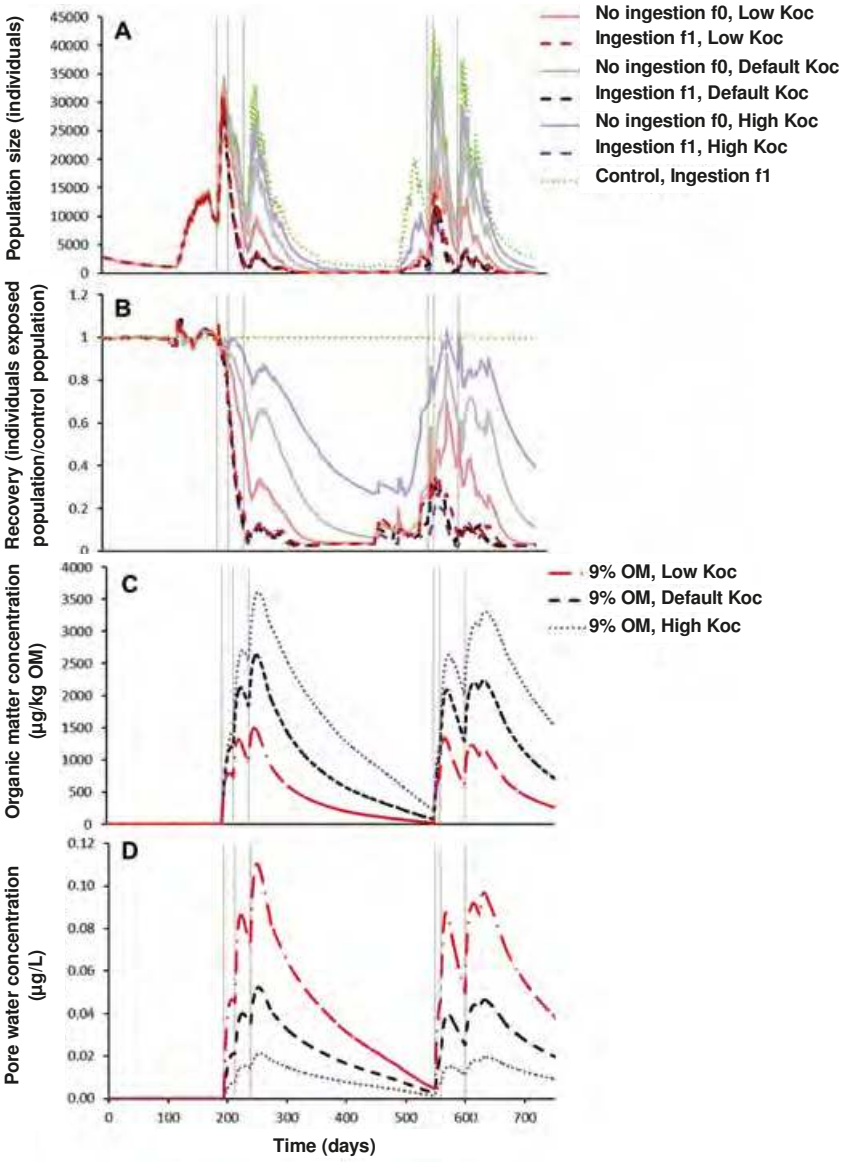


Figure 3. Short term effect of pulsed chlorpyrifos exposure on *Chironomus riparius* population dynamics (number of individuals; Panel **A**) and recovery (Panel **B**) for different sorption strength (K_{oc}) and ingestion scenarios. Recovery is calculated as number of individuals in the exposed population divided by that number for the control population. The simulations were done for sediment with a high organic matter content (9%) over a period of 2 years. K_{oc} scenarios include: default sorption strength (black; Default K_{oc}), 3x lower K_{oc} (red; Low K_{oc}) and 3x higher K_{oc} (purple; High K_{oc}). In panel **A** and **B,** the light grey (——), red (——) and purple (——) solid lines indicate the effect and recovery when ignoring ingestion, which shows that changing K_{oc} has considerable effect. However, when ingestion is taken into account (— — — dashed lines), a change in K_{oc} does not show a change in the simulation results. Panel **C** describes the chlorpyrifos concentrations in sediment organic matter over 0.2 cm (µg kg^{-1}) and panel **D** the concentration in pore water over 1 cm (µg L^{-1}). The green dotted line (•••••) in panel **A** and **B** indicates the dynamics of a non-contaminated control population. Black vertical lines indicate applications times of chlorpyrifos.

7.4 Conclusions and outlook

In the present study, the TKTD model for the first time integrated both the chemical exposure from pore water and sediment and showed the importance of the latter exposure pathway for *C. riparius* population dynamics and recovery. Accounting for chemical exposure from the pore water only, as advocated by EPT, could underestimate the risks of sediment-bound chemicals in ERA. This has been shown earlier for bioaccumulation, and is here translated to internal exposure, damage, toxicity and population effects and recovery.

Additional scenario studies showed the importance of selecting the appropriate K_{oc} value, the biologically relevant sediment layer and long term data output for population modelling. This further illustrates the usefulness of TDTK based population modeling as a tool in prospective risk assessment.

The presented model framework is general and can easily be used for other species with similar life cycles, by defining the species' specific TKTD parameters and ingestion rates. New developments in the field of TKTD modelling show that simplified models can be parameterized using standard toxicity data when the sole purpose of the parameterized model is to predict the effects of time varying exposure or of prolonged exposure.[470]

Ideally, population models include effects at sublethal endpoints at the landscape level, landscape management e.g. dredging and buffer zones[471] and ecological relevant processes such as recolonization by flying or drifting animals.[424] These models are useful tools complementary to higher experimental tier tests in prospective ERA. A further outlook would be to connect aquatic and terrestrial ecosystem compartments in a landscape food web approach as *C. riparius* can transfer chemicals from one compartment to the other.[363]

Acknowledgements

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Supporting information

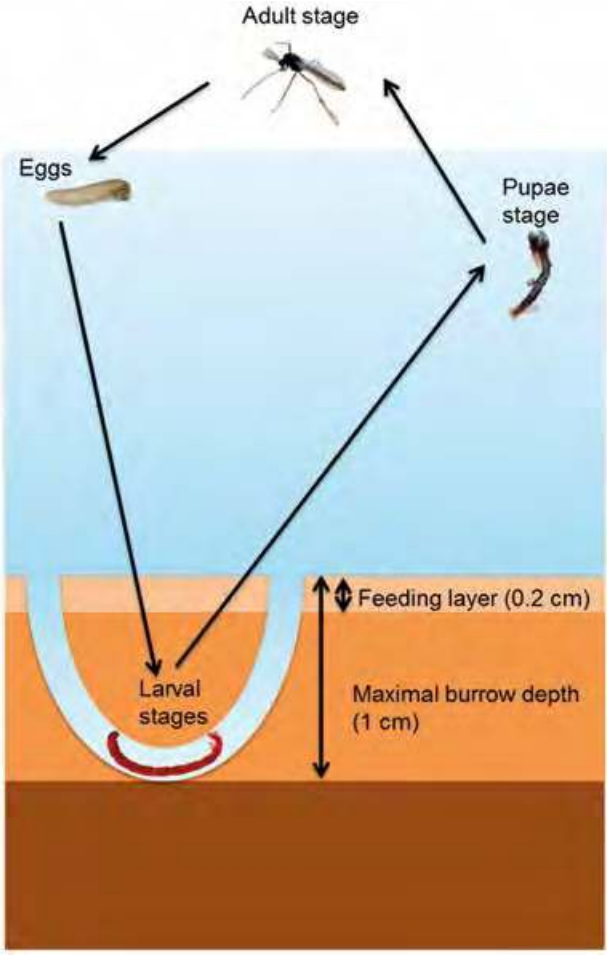

Figure S1. Life cycle of *Chironomus riparius* and details on U-shaped burrow construction and feeding layer thickness.

Table S1. Values of scenario, loadings and substance properties used for calculation of concentration in water and sediment with TOXSWA.

Property	Value
Pond (FOCUS, Weiherbach meteo)[1]	
Dimensions	30 x 30 m
Water depth	1.01 – 1.02 m
Flow velocity	0.2 – 4.9 m/d
Concentration suspended solids	15 mg/L
Mass ratio of organic matter in suspended solids and in sediment	0.09
Bulk density sediment	800 kg/m^3
Porosity sediment	0.6
Loadings	
Application rate substance to field[2]	3 x 0.8 kg/ha in July-August
Spray drift deposition on pond	1.2 %
Annual runoff of water from field	3 - 67 mm/m^2
Annual runoff of substance into pond	9 – 792 mg
Annual erosion of substance into pond	0 – 54 mg
Chorpyrifos[3]	
Molar mass	350.89 g/mol
Saturated vapour pressure	1.43 mPa
Solubility	1.05 mg/L
Transformation half-life in water[4]	25.5 d
Transformation half-life in sediment[5]	36.5 d
Molar activation energy for transformation[6]	65.4 kJ/mol
Sorption coefficient for organic matter[7], K_{om}	4728 L/kg
Freundlich exponent[6], N	0.9
Reference concentration Freundlich exponent[6]	1 mg/L
Diffusion coefficient[6]	0.43 mm^2/d

[1] FOCUS, 2001

[2] Use in apples post blossom in UK, http://uk.dowagro.com/wp-content/uploads/2014/02/Dursban-WG-label-text-160657-05.pdf

[3] Footprint[267]

[4] From hydrolysis rate in water at pH 7

[5] From transformation rate in water-sediment study

[6] FOCUS default value

[7] K_{om} = 0.58 x K_{oc}

Table S2. Coefficients for the *Chironomus riparius* TKTD model

Description	Coefficient	*Value*	*Unit*	*Reference*
Effective ingestion rate constant	k_{ing}	0.0369	[mg OM mg^{-1} WW day^{-1}]	[446]
Intake rate constant	k_{in}*	0.318	[mL mg^{-1} day^{-1}]	[443]
Elimination rate constant	k_{out}*	0.131	[day^{-1}]	[443]
Killing rate constant	k_k*	88.0	[mg nanomole^{-1} day^{-1}]	[444]
Rate constant damage recovery	k_r*	0.518	[day^{-1}]	[444]
Damage threshold	threshold*	3.3x10^{-8}	[-]	[444]

* Values are based on values for *Chaoborus obscuripus*

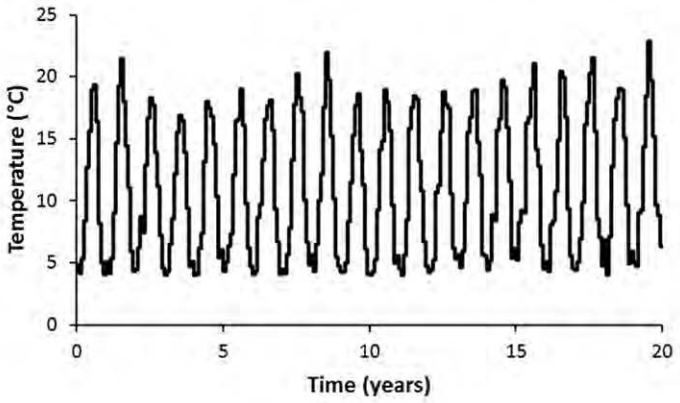

Figure S2. Air temperature during 20 years measured at the Weierbach meteo station (Germany).

Result

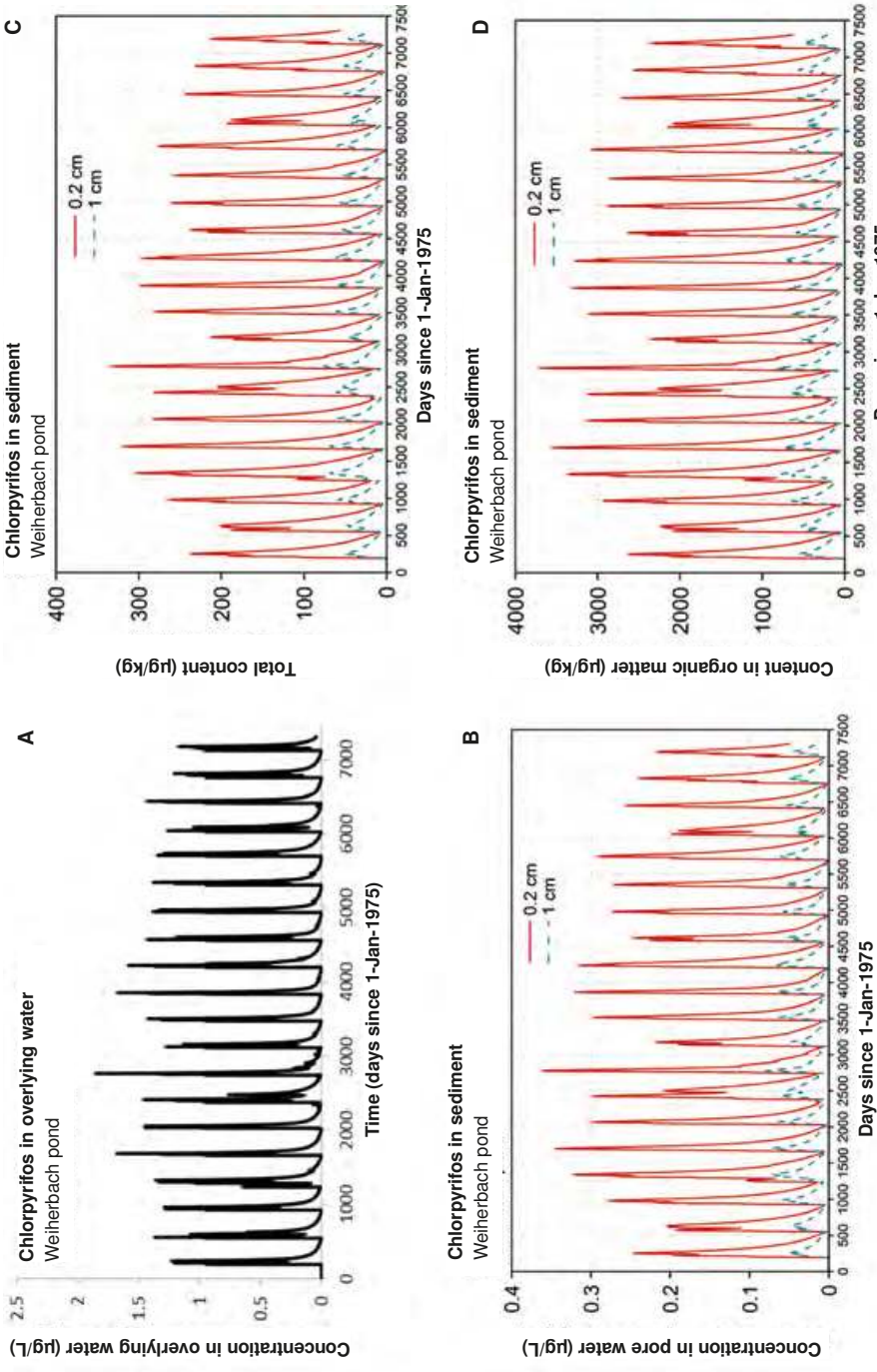

Figure S3. Average chlorpyrifos concentrations in overlying water (µg/L) **(A)**, pore water (µg/L) **(B),** total sediment (µg/kg) **(C)** and organic matter (µg/kg) **(D)** for the standard scenario high organic matter (9%) in the Weiherbach pond. Concentrations in sediment and pore water are expressed in the first cm (blue dotted line; — — —) and first 0.2 cm (red solid line; ——) of the sediment. Note the different scale on the Y-axes.

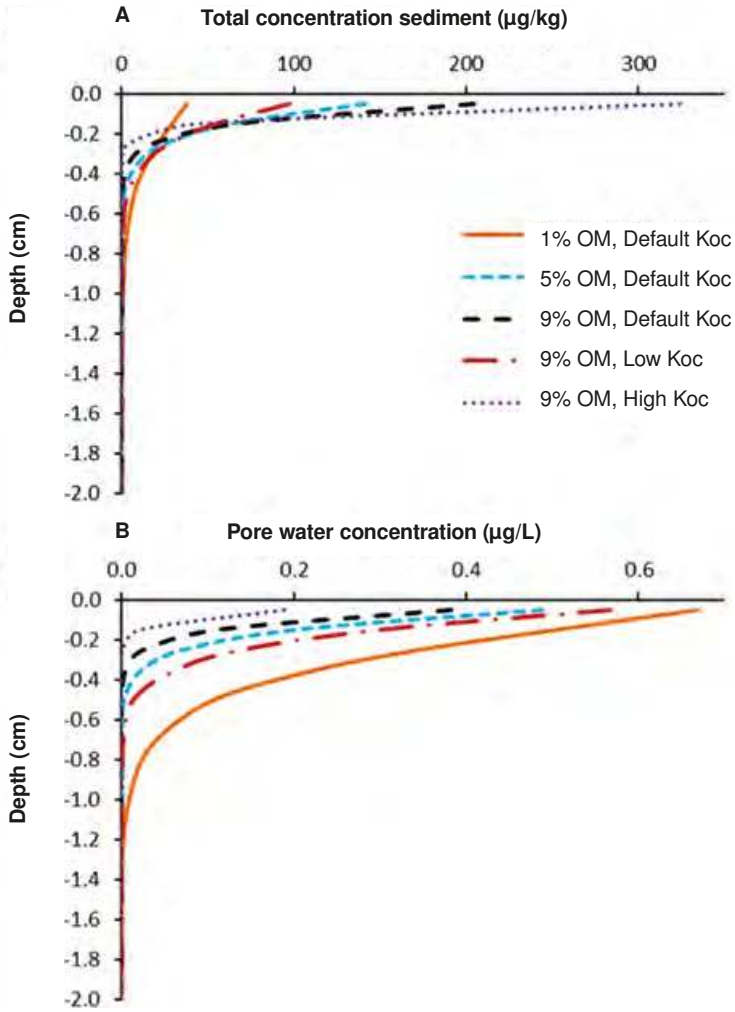

Figure S4. Vertical exposure profiles of CPF concentration in total sediment (**A**) and pore water (**B**) over the first 2 cm of the sediment layer in the Weiherbach pond at 8 September 1975 in the first year of exposure when sediment concentrations are highest. For the default sorption strength (Default K_{oc}) organic matter was varied: low organic matter content (1%; Orange solid line; ——), medium organic matter content (5%; blue squared dotted line; – – –) and high organic matter (9%; black dashed line; — — —). For high organic matter sorption strength was varied: 3 times lower K_{oc} (Low K_{oc}; red long dash dot line; — —), default sorption strength (Default K_{oc}; black dashed line; — — —) and a 3 times higher K_{oc} (Low K_{oc}; purple round dot line; •••••).

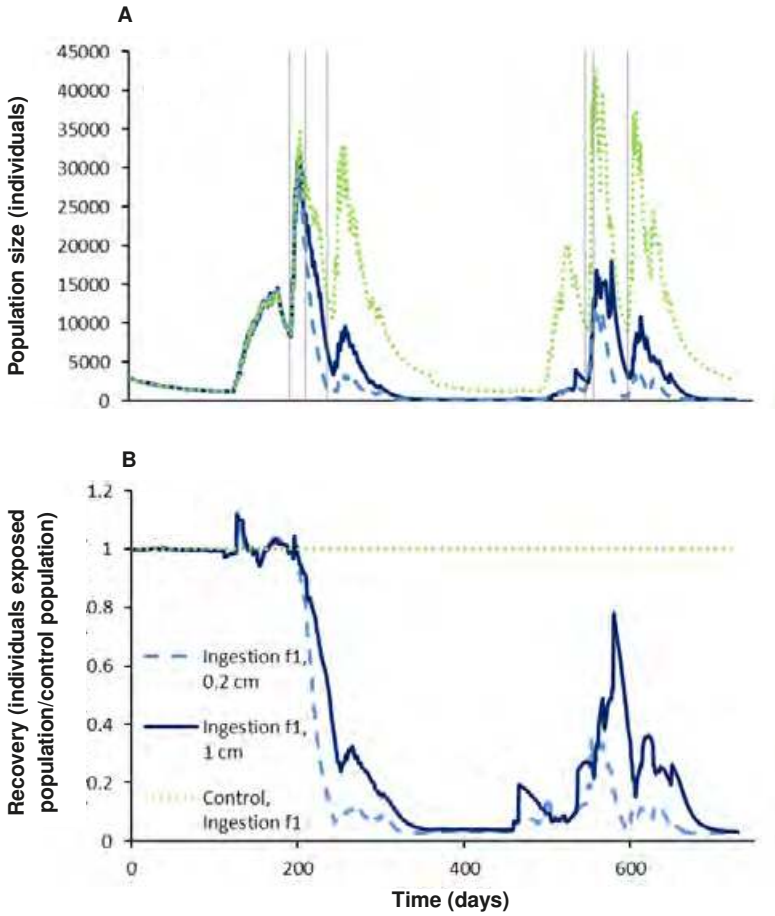

Figure S5. Shorter term effect of pulsed chlorpyrifos exposure on *Chironomus riparius* population dynamics (number of individuals; Panel **A**) and recovery (Panel **B**) with a normal ingestion rate for two exposure layer thicknesses. Recovery is calculated as number of individuals in the exposed population divided by that number for the control population. The simulations were done for sediment with a high organic matter content (9%) over a period of 2 years. Exposure layer thickness include: a) average concentrations for pore water over the first 1 cm in combination with organic matter over the first 0.2 cm (light blue dashed line; — — —) and b) average concentrations for pore water and organic matter over the first cm (dark blue solid line; ——). The green dotted line (•••••) indicates the dynamics of a non-contaminated control population. Vertical lines indicate applications times of chlorpyrifos.

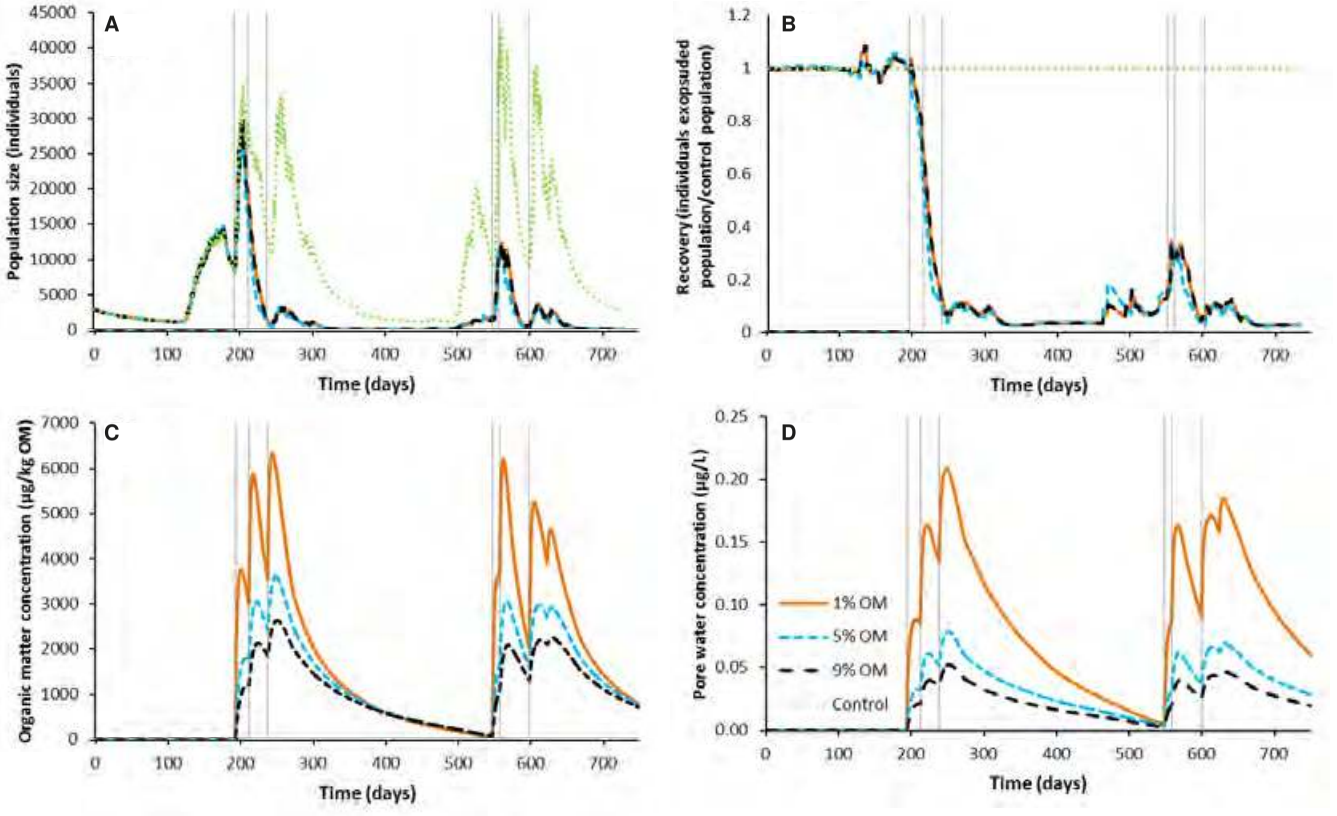

Figure S6. Shorter term effect of pulsed chlorpyrifos exposure on *Chironomus riparius* population dynamics (number of individuals; Panel **A**) and recovery (Panel **B**) with a normal ingestion rate for different organic matter scenarios. Recovery is calculated as number of individuals in the exposed population divided by that number for the control population. The simulations were done for sediment with a high organic matter content (9%) over a period of 2 years. Organic matter (OM) scenarios include: low OM content (1%; orange solid line; ——), medium OM content (5%; blue squared dotted line; – – –) and high OM (9%; black dashed line; — — —). Panel **C** describes the chlorpyrifos concentrations in sediment organic matter over 0.2 cm (µg kg^{-1}) and panel **D** the concentration in pore water over 1 cm (µg L^{-1}). The green dotted line (•••••) in panel **A** and **B** indicates the dynamics of a non-contaminated control population. Black vertical lines indicate applications times of chlorpyrifos.

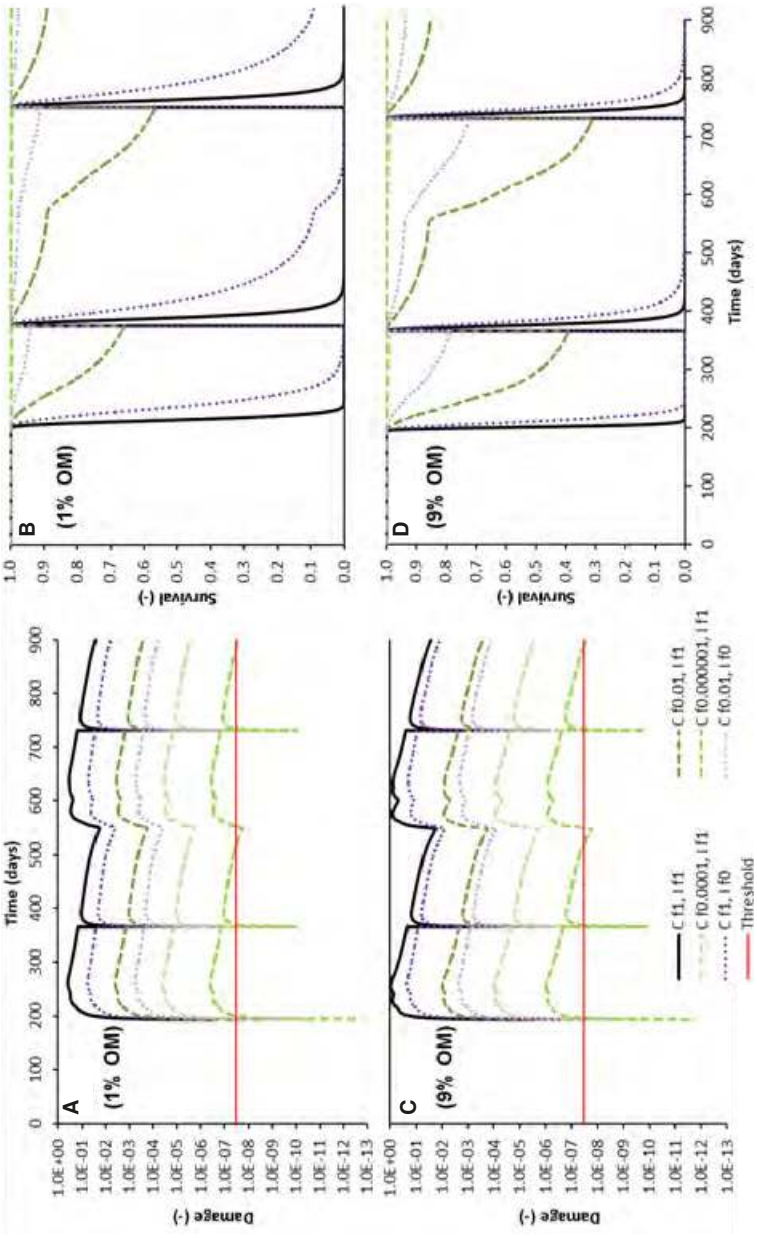

Figure S7. Damage (-) **(A, C)** and survival (-) **(B, D)** for a single, imaginary, long-lived individual *Chironomus riparius* individual to variation in chlorpyrifos concentration in pore water and organic matter and ingestion for normal ingestion f1 (solid or dashed lines) and no ingestion f0 (round dotted lines) for low (1% OM) **(A, B)** and high (9% OM) **(C, D)** scenarios. Normal scenario (black solid line; ——), 10^2 times lower concentrations (dark green dashed line; — — —), 10^4 times lower concentrations (light green dashed line; — — —), 10^6 times lower concentrations (bright green dashed line; — — —), normal scenario without ingestion (purple round dotted line; ••••), no ingestion and 10^2 times lower concentrations (light purple round dot line ••••). Note that background mortality was not taken into account and there were new individuals at the start of the year. Horizontal red line (——) in panel A and C indicates the damage threshold of 3.3×10^{-8}.

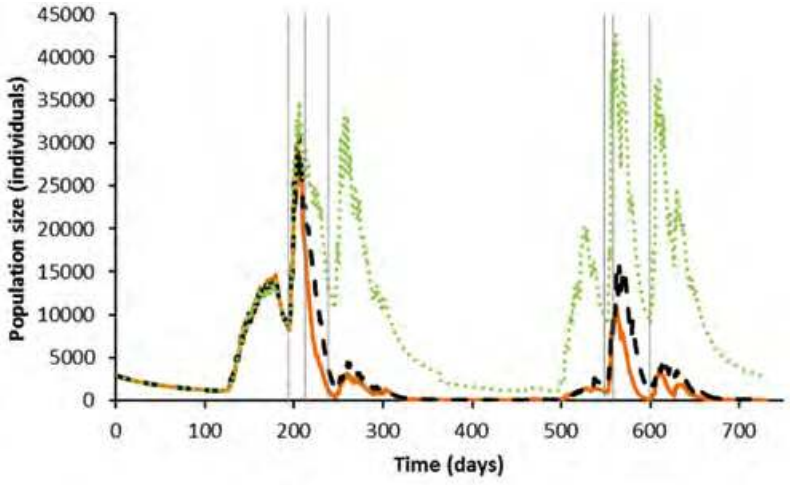

Figure S8. Shorter term effects of chlorpyrifos concentrations on *Chironomus riparius* population size dynamics (individuals) over a period of 2 years for low organic matter content (1%; Orange solid line; ——), with high ingestion (f1.5) and high organic matter (9%; black dashed line; — — —) with low ingestion (f0.5). The green dotted line (•••••) indicates the dynamics of a non-contaminated control population. Vertical lines indicate applications times of chlorpyrifos.

Chapter 8

Prospective environmental risk assessment for sediment-bound organic chemicals: A proposal for tiered effect assessment

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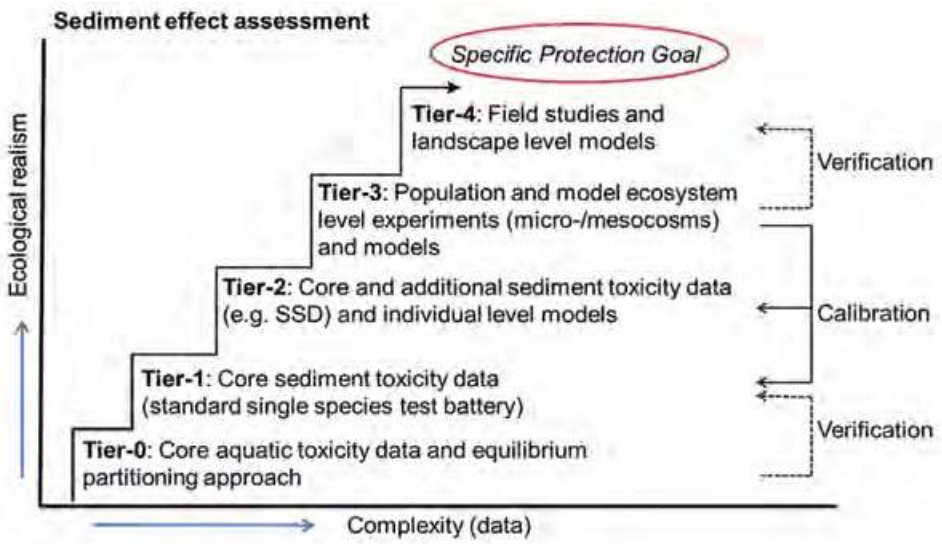
Abstract

A broadly accepted framework for prospective environmental risk assessment (ERA) of sediment-bound organic chemicals is currently lacking. Such a framework requires clear protection goals, evidence-based concepts that link exposure to effects and a transparent tiered-effect assessment. In this paper, we provide a tiered prospective sediment ERA procedure for organic chemicals in sediment, with a focus on the applicable European regulations and the underlying data requirements. Using the ecosystem services concept, we derived specific protection goals for ecosystem service providing units: microorganisms, benthic algae, sediment-rooted macrophytes, benthic invertebrates and benthic vertebrates. Triggers for sediment toxicity testing are discussed.

We recommend a tiered approach (Tier 0 through Tier 3). The Tier-0 approach is a cost-effective screening based on chronic water-exposure toxicity data for pelagic species and equilibrium partitioning. A Tier-1 approach can be based on spiked sediment laboratory toxicity tests with standard benthic test species and protocol test methods. If chronic toxicity data for both standard and additional benthic test species are available, the Species Sensitivity Distribution (SSD) approach is a more viable Tier-2 option than the geometric mean approach. Criteria for the application of the SSD approach in sediment risk assessment are discussed. We propose microcosm and mesocosm experiments with spiked sediment as a Tier-3 approach. Ecological effect models can be used to supplement the experimental tiers. A strategy for unifying information from various tiers by experimental work and exposure and effect modelling is provided.



8.1 Introduction

Aquatic sediments are an important part of the aquatic ecosystem, providing critical ecosystem services and functions.[3] The sediment compartment acts as a sink for hydrophobic organic chemicals, which can affect the services and functions provided. Therefore, sediment should be considered in environmental risk assessment (ERA) whereas it is currently underrepresented. A conceptual prospective sediment ERA framework for organic chemicals is lacking.[21,259] Such a framework requires clear protection goals, evidence-based concepts for linking exposure and effects, and a transparent tiered-effect assessment procedure for sediment organisms and processes. Furthermore, harmonization of data requirements, test protocols and ERA frameworks between existing regulations/directives would be beneficial.[20,259]

The aim of this paper was to provide guidance to establish a prospective ERA framework for organic chemicals in sediments of freshwater, estuarine and marine ecosystems. In this paper we focus on European regulations and underlying data requirements for prospective sediment ERA, but also address useful concepts developed in North America within the context of sediment ERA. A synthesis of existing approaches and new scientific insights and data is provided, showing how a rational prospective assessment can be performed cost-effectively. Our analysis starts by defining specific protection goals based on the ecosystem services concept, which in turn is based on the ecological role and functions provided by benthic organisms. We then present and discuss trigger values for sediment testing and data requirements within current European risk assessment frameworks. Current procedures for exposure and effect assessment, including the use of models, are presented and recommendations are given. Finally, several case studies are provided as 'proof of concept' and to illustrate the general features of the framework. The concepts underlying this paper were discussed with representatives of government, industry and academia during a workshop in Wageningen, the Netherlands, in February 2014 (for list of participants see Table S1). Discussions, remarks and recommendations from the workshop were used to improve this paper.

8.2 Benthic organisms

Aquatic sediment is a complex heterogeneous matrix that covers a large part of earth's surface (freshwater 0.5%, marine 74%).[2] In this paper, sediment is defined as all unconsolidated material of fine, medium and coarse grain minerals and organic particles that make up the bottom of aquatic ecosystems.[162,472] The numerous benthic organisms that inhabit the sediment compartment fulfil a wide variety of crucial ecosystem functions. The benthic food chain and processes in the sediment compartment are not only connected with pelagic organisms and processes, but also with terrestrial soils. Soils, freshwater and marine sediments are closely interlinked as well, e.g. via groundwater systems, and have many functions in common.[3] Contamination and other anthropogenic pressures can negatively influence critical functions provided by benthic organisms. Protection of benthic

organisms is essential for ecosystem functioning and the sustainable use of services provided by nature.

Landscape and local factors such as geology, hydrology and water chemistry influence the sediment habitat and therewith the diversity and structure of benthic communities.[3,473] In general, sediment can be divided into two types: soft bottom sediments and hard substrates, each containing different benthic organism groups.[474] Low flow velocities and fine sediment particles characterize soft bottom sediments. Hard substrates are often found in high-energy areas, such as areas with high flow velocity and wave impact. Chemicals with high hydrophobicity that end up into surface waters tend to accumulate in soft bottom sediments due to sorption to sediment particles. In toxicity tests for prospective sediment risk assessment of organic chemicals, soft sediment is mostly used as a test matrix in the form of artificial sediment. In this paper, we focus on the soft bottom sediment benthic community.

In this paper we define benthic organisms as follows: organisms that spend their full life cycle, or an important part thereof, living on sediment (epibenthos) or in sediment (endobenthos). For these species, exposure via the sediment compartment may contribute to contaminant-mediated effects. This is not adequately covered by ERA that are based on exposure in other environmental compartments.

Ecosystem processes performed by benthic organisms cover a wide range of temporal and spatial scales. On the micro scale, populations of microbenthos, which usually have a life cycle of hours to days (including bacteria, fungi, ciliate protozoans, flagellates, and diatoms), perform processes such as nitrogen and phosphate transformation, carbon mineralization and photosynthesis. Meiobenthos populations, which may have a life cycle of days to weeks (including nematodes, harpacticoid copepods, turbellarians, and Gastrotricha), regulate microbenthos populations and are characterized by a variety of feeding strategies.[3,475] Macrobenthos populations, which have a life cycle of months to years (including rooted macrophytes and larger invertebrates such as crustaceans, larvae of dipterans, bivalves, and annelid worms), may act as ecosystem engineers by either mixing or stabilizing sediments. In addition, they produce organic matter (macrophytes in particular) and consume dead organic matter and associated microbenthos (detritivores) or serve as food for other benthic organisms (carnivores). For vertebrates such as fish, amphibians, birds and mammals, macrobenthos may be an important food source and consequently may be subject to exposure via food web transfer. Vertebrates may have a relatively large habitat range, and their life span may cover several years. Classification of benthic organisms based on size is not strictly coupled to taxonomic groups. This is because different species within a taxonomic group, and even different life stages of the same species, may belong to different size classes. For example, Gerino et al.[474] classified macroinvertebrates in functional groups based on mechanical activities they perform, e.g. bioturbation or feeding strategies. More detailed information on the ecology of benthic organisms is provided in review papers dealing with benthic bacteria,[381] marine fungi,[476] marine meiobenthos,[477] micro- and meiobenthos[478] and freshwater benthic invertebrate species.[87]

8.3 Ecosystem functions and services provided by benthic organisms

An overview of protection goals in EU directives is given by Hommen et al.[479]. Until now, protection goals for benthic organisms have only been defined in general terms.[259] Defining specific protection goals is a crucial starting point in ERA. To operationalize the general protection goals mentioned in legislation, the ecosystem service concept has been proposed [121,480]. Ecosystem services are the stocks of natural capital from which humans benefit.[481] The concept has been developed primarily as a communication tool to explain societal dependence on nature and as a framework to help decision makers implement policies and measures that support human wellbeing, including sustainable management of the environment. Specific protection goals for water organisms in edge-of-field surface waters subject to pesticide exposure were derived with this method by the European Food Safety Authority (EFSA)[220,482]. In a recent European Chemicals Agency (ECHA) workshop (Helsinki, 2013), it was recognized that this concept could also be applied to derive specific protection goals for benthic ecosystems.[20] Wall et al.[3] provided an extensive overview of ecosystem functions and services in soils and sediments, whereas Levin et al.[483] reviewed ecosystem functions provided by benthic communities in estuaries and coastal wetlands. Covich et al.[473] reviewed the role of biodiversity in the functioning of freshwater and marine benthic ecosystems.

Based on these reviews, and following the approach originally developed by EFSA[220], we classified the ecosystem services provided by benthic organisms and ecosystems in freshwater and marine sediment into four groups according to the Millennium Ecosystem Assessment[484] (Table 1): 1) provisioning ecosystem services i.e. products obtained by humans, 2) regulating ecosystem services i.e. regulating processes beneficial for humans, 3) cultural ecosystem services, i.e. important conditions for humans related to aesthetic, spiritual, educational and recreational values and benefits, and (4) supporting ecosystem functions, i.e. ecosystem functions that support ecosystem sustainability and therewith the provisioning, regulating and cultural services. For each service provided by benthic organisms, we assessed the relative importance on this service on a subjective scale from low 1) to high 3). Moreover, we identified the ecosystem service providing units (SPUs), also referred to as key drivers by EFSA[220] and Nienstedt et al.[121]. SPUs are the main taxonomic groups of organisms providing each service (Table 1).

Freshwater and marine benthic ecosystems may provide similar ecosystem services (Table 1) and overall, similar taxonomic and/or functional groups of benthic organisms provide these services. However, certain taxonomic groups are largely restricted to either freshwater sediments (e.g. insects) or marine sediments (e.g. Echinodermata). Important SPUs include microorganisms, benthic algae, benthic invertebrates, sediment rooted macrophytes and benthic vertebrates.

8.3.1 Dealing with vulnerable key species

Current approaches in prospective risk assessment aim to provide sufficient protection to a wide array of non-target species. Vulnerable key species are of particular importance. When selecting indicator species for testing, it should be considered whether the lower-tier approaches (those based on standard test species and the application of an assessment factor) sufficiently protect these vulnerable benthic taxa. Vulnerable key species are species that fulfil a highly important role in the ecosystem, have a high risk of exposure (e.g. low avoidance potential), are very sensitive to chemical stress due to specific traits (e.g. poor detoxification mechanism, feeding habit, low elimination rate) and have a low recovery potential (e.g. low recolonisation potential, long generation times). These characteristics make it difficult to culture and test these species in the laboratory. Moreover, it is difficult to identify the most vulnerable key species of each SPU group and type of ecosystem, as many species have a high plasticity, fulfil a variety of functions and might change function depending on their life stage and/or type of ecosystem where they dwell. Furthermore, the vulnerability concept of benthic species and the impact of organic contaminants have not received much attention in the scientific literature. Two approaches are possible. First, traits might be used to identify these species. For instance, nitrifying bacteria that oxidize nitrite to nitrate are slow growing specialists [381] and might therefore be good indicators of a vulnerable key group for benthic microbes. Second, the mode of action of the chemical might determine which main group of species is more sensitive. For instance, herbicides are designed to kill plants and would therefore be expected to mainly impact non-target benthic algae and macrophytes. However, even after identifying the most sensitive group to one herbicide, no single species is most sensitive to all herbicides [485]. An important research need is therefore to find a good method to identify vulnerable key benthic species.

Table 1. Estimated importance of ecosystem services provided by freshwater and marine benthic organisms based on a subjective scale from low (1) to high (3), potentially influenced by organic contaminants. For each service, the most important benthic organism groups are identified.

Millennium Ecosystem category	Ecosystem services	Freshwater benthic ecosystems	Marine benthic ecosystems	Related soft bottom benthic organisms
Provisioning services (Products obtained by humans)	Food	2	3	Consumable benthic fish, shellfish, and macrophytes
	Fibre, construction materials	2	1	Emergent macrophytes (e.g. thatched roofs)
	Genetic resources	3	3	All species harvested by man
	Natural medicines and biochemical substances	1	2	Potentially all species
	Ornamental resources	2	2	Aquaria and garden pond macrophytes, invertebrates and vertebrates
	Fuels and energy	3	2	Peat, mangrove wood
	Biological products	2	2	Invertebrates for fish bait (e.g. *Nereis* sp., *Arenicola marina*, *Lumbriculus variegatus*)
Regulating services (Beneficial regulation)	Pest and disease regulation	1	1	Benthic fish and invertebrates (e.g. that control aquatic species that act as host for parasites and diseases)
	Sediment bioremediation	3	3	Bacteria, fungi, microfauna, macrophytes, bioturbating invertebrates and vertebrates
	Water purification	3	3	Bacteria, fungi, microfauna, macrophytes, bioturbating invertebrates and vertebrates
	Climate regulation	2	2	Bacteria (e.g. methane production)
	Shore, bank, and sediment stabilization	3	3	Macrophytes, biofilms (microbes and algae), sediment-stabilizing invertebrates
	Hydrological regulation	2	2	Macrophytes
	Pollination	2	1	Aquatic and semi-aquatic insects that pollinate vascular plants and that have benthic larval stages (e.g. Ephydridae)
	Air quality regulation	2	2	Rooted macrophytes and benthic algae
	Invasion resistance	2	2	All native benthic organisms having similar niche as invasive species

Table 1 (Continued).

Millennium Ecosystem category	Ecosystem services	Freshwater benthic ecosystems	Marine benthic ecosystems	Related soft bottom benthic organisms
Cultural services	Education and inspiration	3	3	All benthic organisms
	Aesthetic values	2	2	Benthic red list species
	Recreation and ecotourism	3	3	Rooted macrophytes, benthic fish,
	Spiritual and religious value	2	2	Potentially all species (including benthos)
	Cultural heritage	2	1	All characteristic benthic organisms of man-made aquatic ecosystems (e.g. channels, ditches, peat excavations)
Supporting services (to facilitate other ES)	Sediment formation and structuring	3	3	Macrophytes, bioturbating invertebrates and vertebrates, bacteria and fungi
	Photosynthesis	3	3	Rooted macrophytes, benthic algae, photosynthesising bacteria in biofilms
	Primary and secondary food production	3	3	All benthic organisms
	Nutrient cycling	3	3	All benthic organisms
	Decomposition and mineralization	3	3	All benthic detritivores, microbes
	Food web control mechanisms	3	3	Benthic vertebrates, invertebrates and microbes (including pathogens)
	Provision of habitat and shelter	3	3	Rooted macrophytes, biofilms

8.4 Specific protection goals for sediment risk assessment

Specific protection goals for SPUs are presented in Table 2. These goals are defined in terms of the ecological entities and attributes to be protected. Ecological entities concern the relevant level of biological organization to consider and attributes determine which endpoint to assess.[121] Each specific protection goal must be addressed by a different environmental RA scheme. This is particularly the case when addressing spatial differentiation in specific protection goals with various options, such as a threshold option (accepting negligible impacts on sensitive endpoints only) or a recovery option (accepting temporal impacts followed by a return to the base line).

Table 2. Proposed protection goals for benthic organisms with their ecological entity and attribute based on the ecosystem services concept.

Organism group	Ecological entity	Attribute
Microorganisms	Functional group	Processes
Benthic algae	Population	Abundance, Biomass
Sediment rooted macrophytes	Population	Abundance, Biomass, Cover
Benthic invertebrates	Population	Abundance, Biomass
Benthic vertebrates	Individual to population	Survival, Growth, Abundance, Biomass

Microorganisms are of major importance for many functions such as nutrient cycling, decomposition and water purification.[162] The functional redundancy and recovery potential of microorganisms is high.[163] We therefore followed the proposal of Nienstedt et al.[121] to protect microorganisms on the level of functional group and focused on functional measurement endpoints in ERA. However, generating quantitative data on microbial diversity in polluted sediments is still important, since this type of information likely provides insight into causal relationships between microbial composition and shifts in processes mediated by microbes as discussed in Chapter 2 and 6[259,323] For benthic algae, macrophytes and invertebrates, we propose the population as the ecological entity to be protected, since the functional redundancy concept is more difficult to apply to several provisioning and cultural ecosystem services provided by these organisms. In particular, rooted aquatic macrophytes and benthic invertebrates might include vulnerable key species that require protection at the population level to guarantee the protection of structural and functional biodiversity of benthic communities. Again following the line of reasoning of Nienstedt et al.[121] for benthic vertebrates, we selected the individual-to-population level as an ecological entity to avoid mortality due to acute toxicity and prevent suffering of individual animals due to sediment exposure. The SPUs that we have proposed for benthic organisms, as well as their ecological entities and attributes to be considered in the ERA of organic contaminants in sediments (Tables 1, 2), are similar to those identified by EFSA [482] in their derivation of specific protection goals for water organisms for prospective ERA of pesticides. An important question in this context is the extent to which the specific protection goals that have been derived for water-column organisms already cover/protect benthic organisms.

The acceptability of an effect can be specified for each SPU by quantifying the acceptable magnitude of an effect and the associated temporal and spatial scale. Figure 1 shows possible options for a spatial-temporal differentiation of acceptable effects. Defining the spatial scale for an appropriate sediment ERA, particularly the spatial scale of possible acceptable effects, can be challenging. In most cases, sediments and sediment organisms are not the target of chemical applications, but sediments can act as a sink for chemicals from elsewhere. For example, with the exception of rice paddy fields, agrochemicals such as pesticides are not directly applied in aquatic ecosystems, but edge-of-field surface waters (e.g. ditches) might be considered a transition zone between agricultural fields (target site) and larger surface waters such as lakes and rivers (non-target site). Moreover, exposure might be very heterogeneous, both horizontally (sediment surface) and vertically (depth of the sediment profile). For example, antibiotics and biocides are used in aquaculture cages, and these chemicals eventually reach the sediment.[486,487] In this case, it would be useful to consider the situation in a 3D profile and define the area under and around the cages as an indirect target area. A more complicated example concerns antifouling paints on ships, as they travel large distances. Consequently, contamination from antifouling substances has been found worldwide in sediments.[488] Harbours often are sedimentation areas for contaminated particles[275,344] and might therefore be considered as a main accumulation site – or 'hot spot' – for exposure to antifouling agents. Suspended solids also should be considered, which might carry contaminants away from the target or hot spot area. An important question is whether exposure via suspended solids should be addressed in aquatic or in sediment risk assessment schemes. A pragmatic approach could be to consider only settled particles in sediment ERA.

Thus, to sustain ecosystem structure and functioning, the effects of sediment-bound contaminants should be either preventable or reversible, even at target and/or hot spot sites. However, recovery of the selected attributes of the relevant ecological entities might be variable depending on the persistence of the chemical, its bioavailability and the ability of the affected benthic organisms to recover. Note that it is the responsibility of risk managers and policy makers to define the acceptable spatial and temporal effects.

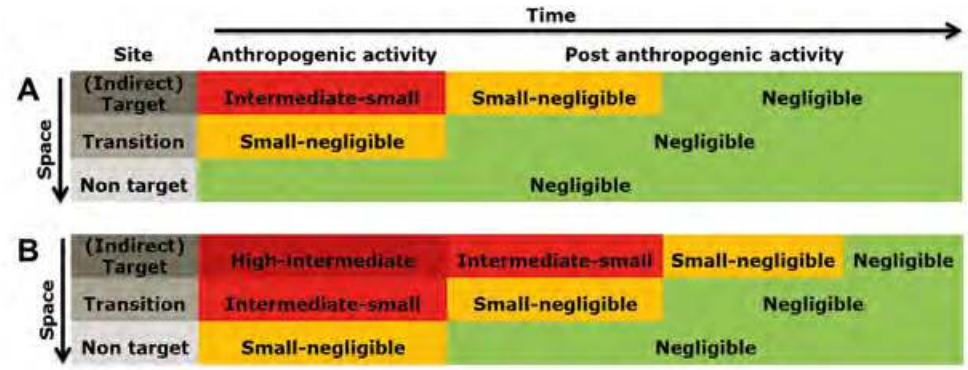


Figure 1. Example for a strict (A) and a less strict (B) option to define the magnitude of acceptable effects on a temporal and spatial scale. Note that sediments not often are target sites for application of regulated organic chemicals, but often hot spot sites of sediment exposure can be identified.

8.5 Triggers for prospective sediment risk assessment in European regulatory frameworks

Ideally, triggers for conducting a sediment ERA should be based on the physicochemical properties of the test compound that affect its adsorption and persistence in the sediment and on its toxicity potential for benthic organisms (Fig 2). Maund et al.[489] proposed the following triggers for sediment testing of pesticides: 1) an *adsorption trigger* consisting of an organic carbon-water partitioning coefficient (K_{oc}) greater than or equal to 1000 (or log K_{oc}>3), 2) a *persistence trigger* consisting of a laboratory aerobic soil half-life time greater than or equal to 30d, and 3) a *toxicity trigger* consisting of a 48h median effect concentration (EC_{50}) to *Daphnia* of less than 1 mg/L or a 21d no observed effect concentration (NOEC) of less than 0.1 mg/L in water-only toxicity tests.

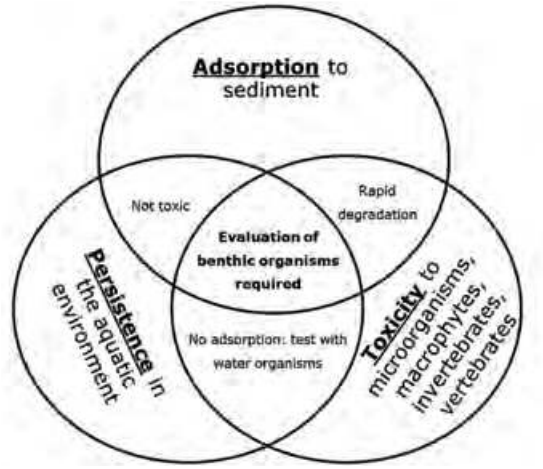


Figure 2. Theoretical basis for defining triggers for sediment toxicity studies based on Maund et al. [489]. The circles describe the three chemical characteristics that should be evaluated, and the overlap between the circles indicates the decision-making process for combinations of those characteristics.

Criteria that are currently required to trigger sediment toxicity testing differ between existing European regulations and directives dealing with prospective ERAs (Table 3). The persistence trigger (more than 10% of the applied radioactivity of the parent in sediment after day 14) is used for pesticides and medicinal products for humans, while the adsorption trigger (log K_{oc} or log K_{ow} >3) is used for chemicals under REACH, biocides and veterinary medicinal products.

In most regulatory documents, except those for pesticides, the toxicity trigger for sediment ERA is initially based on equilibrium partitioning (EP) and toxicity data for pelagic organisms (Table 3). EP theory states that partitioning of a chemical between two phases is governed by the chemical affinity of each phase. For a more detailed description of the EP approach in sediment ERA, see Section 8.9.2 below. For pesticides, the toxicity trigger 21d NOEC or EC_{10} for *Daphnia* <0.1 mg/L is used, although another representative crustacean or insect may also be appropriate. However, this toxicity trigger – which is currently implemented for pesticides –focuses on invertebrates, since *Daphnia* is not representative for species such as rooted macrophytes. Furthermore, for veterinary medical products, a sediment ERA is not required if risks for pelagic aquatic invertebrates have not been demonstrated (Table 3).

This disregards the fact that the environmental risks of hydrophobic veterinary chemicals for pelagic organisms may be predominantly acute, while those for benthic organisms will more often be chronic, at least if the chemical is persistent in the sediment compartment.

Implementing a uniform set of triggers would improve harmonization between the guidance documents underlying the regulation/directives for various types of chemicals. A recent ECHA workshop recommended using a combination of triggers based on the physicochemical properties of the toxicant and the potential toxicity to benthic organisms.[20] In regulatory documents (see Table 3), hydrophobicity (log K_{ow}) and the organic carbon-water partitioning coefficient (log K_{oc}) are interchangeably used as triggers for the potential to adsorb to sediments from the water column. However, these are not equivalent; the values for log K_{oc} can deviate substantially from log K_{ow}.[80,268,439,490,491] Because log K_{oc} is a more direct measure for chemical binding to the sediment than log K_{ow}, using log K_{oc} is preferred. Considering the information presented in Table 3, a log K_{oc} (preferred) or log K_{ow} of ≥ 3 is generally used as a trigger value for sediment effect assessment. However, hydrophobic chemicals with a log K_{oc} of ≥ 3 do not necessarily need to be persistent in the sediment compartment. Therefore, we also recommend using the results of Organisation for Economic Co-operation and Development (OECD) guideline 308[492] to assess the persistence of the chemical in the sediment. For this purpose, the persistence trigger, as used for pesticides and medical products (>10% of the substance is present in sediment at or after day 14), may be adopted for other chemicals as well.

A promising approach is to evaluate the potential risks of sediment exposure to benthic organisms based on EP and available chronic toxicity data for pelagic organisms, at least if the taxonomic groups assessed for water ERA overlap with those required for sediment ERA. From Table 3 it appears that in regulatory documents for chemicals under REACH, biocides, and medical products (for veterinary and human use), the EP approach can be used as a screening method for chemicals with a log K_{ow} 3-5, and that when the EP approach is used for chemicals with a log K_{ow} >5, an extrapolation factor (EF) of 10 should be used to account for dietary uptake of the toxicant in the predicted no effect concentration for sediment based on the EP approach ($PNEC_{sed;EP}$) derivation. If the risk quotient (RQ $=PEC_{sed}/PNEC_{sed;EP}$) <1, then the environmental risks to benthic organisms are considered acceptable. The report of the ECHA workshop [20], however, states that the EP approach is not valid for chemicals classified as ionizable, perfluorinated alkylated or insoluble. For these chemicals the $PNEC_{sed}$ should be derived on the basis of spiked sediment toxicity tests with benthic organisms. In addition, this ECHA report recommended exploring the validity of the EP approach for other organic chemicals. This can be done by comparing the screening level $PNEC_{sed;EP}$ with the $PNEC_{sed}$ derived from spiked sediment toxicity tests for a number of representative chemicals. The ECHA workshop report [20] also suggested additional sediment tests for chemicals with a log K_{ow} > 5. Furthermore, for chemicals with a log K_{ow} ≥ 5, we recommend also verifying whether the EP approach and the extrapolation factor (EF) of 10 (Table 3) can be considered a realistic worst case approach to derive a PNEC for benthic organisms.

Table 3. Criteria that are currently required to trigger sediment toxicity testing as described in existing EU regulations and directives, and the guidelines accompanying these regulations.

Regulation	Trigger	Reference
Regulation EC/1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)	Sediment effect assessment is required if the chemical has a tonnage band ≥1000 tonnes per manufacturer or importer per year and a log K_{oc} or log K_{ow} of ≥3. • log K_{ow} > 3: at least a screening assessment using the equilibrium partitioning (EP) method has to be performed. • log K_{ow} 3-5: the screening assessment using EP is considered appropriate, and no further testing is required if the risk quotient ($RQ=PEC_{sed}/PNEC_{sed;EP}$) <1. • log K_{ow} >5 or a correspondingly high adsorption or binding behaviour: a more comprehensive sediment assessment is needed. If using the EP approach, the risk quotient (RQ) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment. If the RQ based on EP is >1, then a study, preferably long term, with benthic organisms using spiked sediment is recommended. For substances that are highly insoluble and for which no effects are observed in aquatic studies, the application of the equilibrium partitioning method is not possible. In this case, at least one sediment test has to be performed.	493
Regulation EC/1107/2009 concerning the placing of Plant Protection Products on the market	Sediment toxicity tests with benthic organisms are required: • if in the water-sediment fate study >10% of the applied radioactivity of the parent compound is present in the sediment at or after day 14 (OECD 308[492]), and the chronic toxicity value (EC_{10} or NOEC) derived from the 21d *Daphnia* test (or another comparable chronic toxicity tests with a relevant crustacean or insect) is <0.1 mg/L. • compounds applied more than once, with a potential for accumulation of residues in the sediment, should also be considered for sediment testing [494].	494,482
Directive 98/8/EC concerning the placing of biocidal products on the market[a]	A log K_{oc} or log K_{ow} of ≥3 can be used as a trigger value for sediment effects assessment. If the RQ (*based on EP*) is ≥1, then testing of sediment organisms is recommended. For substances with a log K_{ow} >5, the RQ (*based on EP*) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment. If the RQ based on EP is >1, then a study, preferably long-term, with benthic organisms using spiked sediment, is recommended.	495
Veterinary medicinal products	If the RQ for aquatic invertebrate is ≥1 it is recommended to estimate the RQ for benthic organisms based on EP. If this RQ (*based on EP*) is ≥1, then testing of sediment organisms is recommended. For substances with a log K_{ow} >5, the RQ (*based on EP*) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment. If the RQ based on EP is >1, then a study, preferably long term, with benthic organisms using spiked sediment is recommended.	497
Guideline on the environmental risk assessment of medicinal products for human use	If a substance is not readily biodegradable and if the results from the water sediment study (OECD 308[492]) demonstrate significant shifting of the drug substance to the sediment, effects on sediment organisms should be investigated in Tier B. The criterion for sediment studies is met if more that 10% of the substance at any time point after or at 14 days is present in sediment. A detailed strategy for further testing in order to refine the PNEC for the aquatic compartment can be found in the Technical Guidance document.[216] If the RQ (*based on EP*) is ≥1, then testing of sediment organisms is recommended. For substances with a log K_{ow} >5, the RQ (*based on EP*) is increased by an extra factor of 10 to take account of possible uptake via ingestion of sediment. If the RQ based on EP is >1, then a study, preferably long term, with benthic organisms using spiked sediment is recommended.	498

[a]Guidance documents underlying the new Biocidal Products Regulation [496] are still in preparation.

Since the validation status of the EP approach has not yet been appropriately evaluated for a sufficient number of compounds, for the time being we propose using an EF of 10 to derive a $PNEC_{sed;EP}$ for organisms that ingest sediment particles. The reasoning for this proposal is further elaborated in Section 8.9.2 below.

8.6 Data requirements for effect assessment

8.6.1 Toxicity data requirements in European regulatory frameworks

If the triggers, described in Chapter 5, are met, toxicity data for benthic organisms are required (Table 4). Hommen et al.[479] provided an overview of data requirements for aquatic ERA. Current regulations do not always specify the requirements for sediment toxicity testing. Data requirements for freshwater organisms especially concern tests with *Chironomus* sp. and *Lumbriculus variegatus*. Macrophyte tests (e.g. using the rooted macrophyte *Myriophyllum spicatum*[272]) are only required by the Plant Protection Products regulation when specific triggers are met for substances with an herbicidal mode-of-action. For marine systems, no specific test species are mentioned in regulatory documents as data requirements, although examples are given in some regulations.

From Table 4 it appears that the data requirements may concern a water-sediment test with *Chironomus* using either spiked water or spiked sediment. We suggest that the spiked sediment test should have priority in sediment ERA. Exposure via sediment in spiked water OECD toxicity tests, however, may also be considered appropriate if the concentration in the top sediment layer is measured (or adequately predicted) and the biotic activity of the test species is highest in this layer. If a chemical is not stable, then a time-weighted average (TWA) concentration for the duration of the sediment toxicity test may be required. To obtain a more realistic worst-case effect estimate, the chronic EC_{10}/NOEC value can be calculated based upon the TWA concentration of the chemical during the test and not solely on the peak concentration at the start of the test. The organic carbon (OC) content (%) of the sediment needs to be known to enable standardization of chemical concentration to OC, or to express the toxicity value in terms of a fixed OC content per unit DW sediment.

Data requirements for prospective sediment risk assessment rely on official test protocols for standard test species. Chapter 4[259] and Fojut et al.[303] provided overviews of internationally accepted sediment tests for freshwater, estuarine and marine invertebrates, as well as macrophytes. In the available protocol tests for marine/estuarine benthic organisms, amphipods seem to be overrepresented. For vertebrates, the whole-sediment toxicity test for larvae of the freshwater frog *R. pipiens* became available only in 2013[499], so little experience has been acquired in conducting and interpreting this test. No official test guidelines exist for estuarine/marine rooted macrophytes and estuarine/marine vertebrates. Furthermore, no protocol tests for sediment-dwelling microbes are currently available. Most of the experience in tiered effect assessments therefore concerns benthic invertebrates.

Table 4. Data that are currently required for sediment toxicity testing as described in existing EU regulations and directives, and the guidelines accompanying these regulations.

Regulation	**What needs to be tested?**	**Reference**
Regulation EC/1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)	- Long-term test with *Lumbriculus variegatus* using spiked sediment - Long-term test with *Chironomus* sp. using spiked sediment - Long-term tests with a further benthic species using spiked sediment. Selection of 3rd species should supplement the first 2 species in terms of habitat, feeding strategy, taxa or life-stage. For example, the amphipod *Hyalella azteca* or the nematode *Caenorhabditis elegans* could be used For the marine compartment, the same testing strategy is followed. However, for this compartment more tests may be necessary to reduce the higher assessment factor applied if only limited data are available. For possible test species, refer to available protocol tests developed for estuarine/marine species.	500
Regulation EC/1107/2009 concerning the placing of plant protection products on the market	- OECD (2004). OECD Guideline 218: Sediment – water chironomid toxicity test using spiked sediment; adopted 13 April 2004. OECD Publishing. - OECD (2007). OECD Guideline 225: Sediment – water *Lumbriculus* toxicity test using spiked sediment; adopted 16 October 2007. OECD Publishing. - OECD (2010). OECD Guideline 233: Sediment – water chironomid life – cycle toxicity test using spiked water or spiked sediment; adopted 22 July 2010. OECD Publishing. - ISO (2010). ISO/DIS 16191 Water quality - Determination of the toxic effect of sediment and soil on the growth of *Myriophyllum aquaticum*. International Organization for Standardization, Geneva. - OECD (2014). OECD guideline 239 spiked sediment test with *Myriophyllum spicatum* *Glyceria* - in preparation	482
Directive 98/8/EC concerning the placing of biocidal products on the market[a]	For freshwater ERAs, long-term sediment tests with *Chironomus* sp., *Lumbriculus variegatus* and a 3th benthic test species differing in taxonomy and/or feeding habit are required. For estuarine/marine ERAs sub-chronic and chronic sediment toxicity tests for the following species are mentioned as example: *Corophium* sp., *Leptocheirus plumulosus*, *Neanthes* (=*Nereis*) sp., *Arenicola marina*, *Echinocardium cordatum*.	495
Veterinary medicinal products	Freshwater sediment invertebrate species: OECD 219 (spiked water water-sediment *Chironomus* test) is normally used. If exposure is through sediment or adsorbed to soil in run-off, OECD 218 (spiked sediment test with *Chironomus riparius*) should be used. Marine sediment invertebrate species: seek regulatory guidance (probably the standard protocol tests are referred to).	497
Guideline on the environmental risk assessment of medicinal products for human use	Effects on a sediment dwelling organism (*Hyalella* sp; *Lumbriculus* sp. or *Chironomus* sp.) should be investigated.	498

[a]Guidance documents underlying the new Biocidal Products Regulation [496] are in preparation.

8.6.2 Recommendation for a suite of benthic test species

Sediment risk assessment should ideally include a set of sediment toxicity tests to cover a relevant number of representatives of benthic communities and focus on long-term exposure and chronic endpoints.[259] Test exposure durations should depend on the generation time of the tested species (e.g. shorter for microorganisms than for invertebrates). Preferably, a chronic toxicity test should cover the full life-cycle of the test organism, or should at least cover its most sensitive life-stage.

An important question at stake is whether the current data requirements underlying European regulations are adequate and whether currently available standard test protocols are sufficient. Sediment toxicity tests should consider the SPUs and associated ecological entities as discussed in Section 8.4, depending on the mode-of-action of the organic chemical under evaluation. The current suite of standard test species used in the European prospective sediment ERA is limited and does not cover all SPUs; benthic microbes, rooted macrophytes and vertebrates receive hardly any attention (Table 4). For instance, for pesticides in Europe the prescribed Tier-1 benthic test species are *Chironomus riparius* (insect), *Lumbriculus variegatus* (oligochaete worm), and for herbicidal compounds that accumulate in sediment *Myriophyllum* (macrophyte) species.[482] It remains to be investigated whether the current Tier-1 approach based on chronic toxicity tests with these benthic standard test species, together with the proposed assessment factor, sufficiently covers the protection of all SPUs. Furthermore, a harmonized testing strategy between freshwater and estuarine/marine environment is not yet in place. For a suite of freshwater, estuarine and marine benthic test species and methods, including microorganisms, macrophytes and invertebrates, is referred to Chapter 2[259]. Since sediment toxicity testing with benthic vertebrates was not discussed in that review, this topic is addressed briefly in Section 8.9.8.

8.7 Factors affecting exposure of sediment-dwelling organisms

Exposure plays an important role in both sediment toxicity testing (the focus of this paper) and in predicting the field exposure concentrations in sediments. In this paper, exposure is defined as the external concentration of the chemical in environmental media potentially affecting sediment-dwelling organisms, together with the processes that affect its bioaccessibility and its bioavailability, including bioaccumulation.

For any organism, exposure is the net result of chemical uptake and depuration fluxes between the organism and its direct environment (see Chapter 2[259] and references therein). For benthic invertebrates, uptake may take place through fluxes from pore water, overlying water, and particle contact and ingestion.[59,259] Transport to pore water takes place through desorption from the bulk sediment. If uptake through particle or food (prey) ingestion occurs, particle or diet composition is important. Depuration may include passive elimination, defecation, transformation and exudation. Chemical concentrations in organisms may also be reduced by growth dilution. For rooted macrophytes, partitioning

to roots and shoots, translocation between roots and shoots and growth dilution are important (Chapter 3).[307] This means that uptake is a complex, time-dependent process, because the relative importance of the individual processes varies with environmental and life-stage changes over time. In addition, the relative importance of these uptake processes may differ between chemicals and benthic organisms.

In assessing exposure of benthic organisms, four types of influential factors are particularly important: chemical, biological, spatial and temporal.[20] These factors are addressed in the subsections below.

8.7.1 Chemical factors

Traditional exposure assessment concepts use total sediment concentrations and the EP model for a first-tier screening approach to estimate exposure in field sediments.[20,259] Single sorption domain EP models, however, are known to work well only for partitioning of conventional organic substances to sediment amorphous organic matter phases. The EP model will not work for ionisable chemicals, perfluorinated alkylated substances, Non-Aqueous Phase Liquids (NAPLs), long aged sediments, or in the presence of sedimentary condensed organic matter pools like soot or black carbon (BC). Therefore, specific K_d models should be used to estimate exposure concentrations in field sediments.[20,80,259,439,491,501] If the traditional single domain EP approach is used, condensed organic matter phases may increase actual K_d values by two to three orders of magnitude, leading to a substantial overestimation of exposure.[80] A realistic worst case approach would be to use a correction of one order of magnitude on the previous EP-based K_d values. For other chemicals, Quantitative Structure Property Relationship (QSPR) models can be used. These models are based on molecular descriptors, such as the Abraham parameters,[502] and are available for many compound classes. For degradable compounds, however, exposure is dynamic in time, and it may be necessary to account for degradation products in the exposure assessment if they are also toxic. Sufficiently accurate predictive models to describe degradation in time or to translate laboratory degradation data into field-relevant rates have not been developed as yet.

8.7.2 Biological factors

Species traits such as body size, lipid content, surface area-to-volume, respiratory strategies, diet, digestive processes and dietary assimilation affect bioaccumulation[86,296] and thus internal exposure. Particle or food ingestion depends on diet and plays a dominant role for some benthic invertebrates such as *C. volutator* (Chapter 4),[346] *Lumbriculus variegatus* (Chapter 5),[62,296,426] *Arenicola marina* (Chapter 4)[300,346] and *Macoma balthica* (Chapter 4)[98,300,301,346] whereas for other species such as *Ilyodrilus templetoni* [302] water uptake is dominant. For conventional organic substances, EP-based approaches predict biota sediment accumulation factors (BSAFs) values of approximately 1 or 2. For benthic invertebrates, however, much higher values are often observed (Chapter 4 and 5),[321,325,346] which can be explained from food ingestion.[304] A recent model analysis showed how actual parameter distributions contribute to this variation [59]. On the other

hand, values much lower than 1 or 2 are sometimes observed.[80,331] This can be explained by binding to black carbon as mentioned above. In that case, the EP approach would be over-protective, unless a black carbon-inclusive EP approach is used. For organisms like benthic algae and sediment-rooted macrophytes, black carbon effects are similar, but food ingestion does not occur and thus will not add to variance in accumulation. Established models for invertebrates[81,301,315,316,346] are available to quantify biological factors on BSAFs.

Experiments with the rooted macrophytes *Elodea canadensis* and *Myriophyllum spicatum* showed that an equilibrium state is not reached within 28 days, a timeframe that is even longer than the duration (7-14 d) of a standard macrophyte test (Chapter 3).[307] This means that maximum internal exposure might not be reached and that when conducting spiked sediment toxicity tests with rooted macrophytes, test durations should be increased. Alternatively, mechanistic models might be used as extrapolation tools to calculate maximum levels of internal exposure.[159,161,307,503]

For any food web that includes the sediment compartment, exposure of sediment-associated chemicals along the food chain may occur. Whether or not a chemical will bioaccumulate and/or biomagnify depends on the hydrophobicity and persistence of the chemical, the feeding relationships and length of the chain, and the capacity to metabolise and eliminate the chemical by the respective species.[284] A novel approach to detect secondary poisoning is to directly assess the relative chemical fugacity in an organism at a certain trophic level by equilibrating its tissues with passive samplers in a closed system.

8.7.3 Spatial factors

Both contaminant concentrations and presence of benthic organisms in field sediment are patchy (horizontally heterogeneous), and 'exposure hot spots' are present, which may be identified by appropriate spatial sampling strategies and geostatistics.[20] Similarly, colonization potentials of benthic organisms are influenced by spatial factors. This information is important for the development of realistic exposure assessment goals and exposure scenarios. An exposure scenario can be defined as the set of variables determining chemical exposure.[504] These exposure scenarios will yield spatially explicit exposure assessments on which spatially explicit ERA's can be based. An alternative approach is to deal with spatial heterogeneity through probabilistic modelling.[20] This results in a point estimate of exposure for a heterogeneous region, where the heterogeneity is accounted for by the uncertainty interval in the point estimate.

Besides the abovementioned horizontal heterogeneity, vertical gradients may also affect the exposure of benthic organisms. Sediment exposure usually varies with sediment depth and, consequently, also relates to the biologically active layer, which may be different for various types of sediment-dwelling organisms. This means that vertical heterogeneity also has to be considered to in ERA.

8.7.4 Temporal factors

Sediments can act as a buffer against fluctuations of chemical concentrations in the overlying water. Flushing or run-off events may cause sudden peaks in exposure in the water column and sequentially at the sediment-water interface and in the biologically relevant sediment top layer where exposure may last longer than in the water column.[20] This indicates that chronic exposure generally is more relevant for sediment assessment than acute exposures. The buffering is stronger for pore water concentrations than for near-sediment overlying water concentrations. Chemical exposure would thus be more variable in time for benthic species that are partly or fully exposed to overlying waters and suspended solids. Furthermore, the temporal dynamics of sediment re-suspension and deposition downstream may be relevant if re-deposited sediments are heavily contaminated.

8.8 Exposure concentration in sediment ERA

8.8.1 The ecotoxicologically relevant concentration for sediment-dwelling organisms

In a prospective risk assessment, predicted no effect concentrations (PNEC) are evaluated against predicted environmental exposure concentrations (PECs), where the PEC/PNEC ratio often is used as an indicator of risk. Lack of a clear conceptual basis for the interface between the exposure and effect assessment may lead to a low overall scientific quality of the risk assessment.[14] This interface is defined by EFSA[24] and Boesten et al.[14] as the concentration that correlates appropriately with ecotoxicological effects; it is called the ecotoxicologically relevant concentration (ERC). In prospective ERA, the ERC must be consistently applied so that sediment exposure estimates (PEC_{sed}) and effect estimates for sediment-dwelling organisms (such as $PNEC_{sed}$) can be compared. More specifically, the 'C' in the PEC_{sed} estimate should be consistent with the 'C' in the $PNEC_{sed}$ estimate. From a theoretical point of view, the internal concentration (body burden) at the target site in the benthic organism under evaluation would be the most appropriate ERC. Concentrations are hard to measure directly at the target site, especially for small animals. Therefore, whole body internal concentrations can be used.[505] In the vast majority of toxicity studies with benthic organisms, however, internal concentrations are not measured[13] and in none of the regulatory guidelines is it given as a recommended measurement endpoint in ecotoxicological studies. Consequently, the 'C' in the PEC_{sed} and $PNEC_{sed}$ estimates usually refers to external exposure concentrations.

An important question is whether the PEC_{sed} and $PNEC_{sed}$ estimates should be expressed in freely dissolved chemical concentration in pore water, ingested particles or total sediment concentration. Since the bioavailability of organic toxicants may be affected by the OC content of the sediment, an additional question is whether the total sediment concentration should be normalized to standard sediment or expressed in terms of OC content of the dry sediment.

The current OECD sediment test protocols (OECD 218[76], 219[75], 225[77], 233[78]) advocate the use of artificial sediments containing 4-5% peat, while EPA OPPTS 850.173.5[506] advocates the use of clean, field-collected sediments. All protocols require the determination of OC content of the sediment, enabling the recalculation of effect concentrations based on OC content. In toxicity tests retrieved from the literature, different types of sediments varying in OC are used, hampering a direct comparison of test results. To allow comparison of sediment toxicity data from different sources, sediment toxicity data may be standardized to concentrations normalized on sediment OC content. An alternative approach might be to standardize all toxicity data to sediment with an organic matter content of 5% (which equals approximately 2.5% of OC), an approach often followed in Europe. The basic principle, however, is the same. To appropriately link exposure and effects, the PEC_{sed} and $PNEC_{sed}$ estimates should be expressed either in terms of mg/kg DW standard sediment with a fixed OC content (=$PEC_{sed\text{-}tot}$ or $PNEC_{sed\text{-}tot}$) or in terms of mg/kg OC in dry sediment (=$PEC_{sed\text{-}oc}$ or $PNEC_{sed\text{-}oc}$). In our paper we have normalized the total concentration of the organic chemical in the sediment to organic carbon ($PEC_{sed\text{-}oc}$ and $PNEC_{sed\text{-}oc}$).

The sediment-water chironomid tests using spiked sediment (OECD Guidelines 218[76] and 233[78]) specify that – as a minimum – the concentrations in overlying water, pore water, and sediment should be measured. According to OECD guideline 218, effect concentrations should be expressed as concentrations in sediment, based on dry weight, at the beginning of the test. OECD Guideline 233, however, does not explicitly specify on what basis the concentration in the $L(E)C_x$ or NOEC values should be expressed, although in daily practice the concentration in the sediment at start of the test is generally used. The *L. variagatus* toxicity test using spiked sediment (OECD Guideline 225) specifies that the concentration in sediment and overlying water should be verified by measurement. The guideline also outlines a method for isolation and subsequent measurement of the chemical in pore water. The effect concentration should be expressed in mg/kg sediment on dry weight basis.[77]

The United States Environmental Protection Agency (EPA) OPPTS 850.1735 Guideline (whole sediment acute toxicity invertebrates, freshwater) states that 'Concentrations of spiked chemicals may be measured in sediment, interstitial water, and overlying water ...', but does not specify on what basis effect concentrations should be expressed, other than 'In some cases it may be desirable to normalize sediment concentrations to factors other than dry weight, such as OC for non-ionic organic compounds or acid volatile sulfides for certain metals'.[506] The various guidelines lack clarity and are mutually inconsistent on these aspects.

The EFSA has recently published a Scientific Opinion on the assessment of exposure of organisms to pesticides in soils.[507] They recommend that the ERC should be reported both in concentration units of mass of pesticide per mass of dry soil and as a concentration in pore water.[507,508] If the rationale behind the recommended use of both measures of exposure would also apply to sediment, which seems likely, then this would suggest

that toxicity data generated for sediment organisms should also be reported along with concentrations in pore water and in sediment mass or in sediment OC mass. This is not in line with OECD and EPA guidelines, where the most common recommendation is to report effect concentrations on the basis of sediment mass only. If the pore concentration is not measured, or is difficult to measure, then we propose an appropriate modelling approach to estimate pore water concentrations.

In a toxicity test the final response of the test organism in most cases will be influenced by the dynamics in exposure concentration during the test. We therefore propose as a minimum requirement to always measure exposure at the start and the end of the experiment. For organic chemicals that are expected to rapidly dissipate from sediment, we recommend measuring exposure concentrations, including ecotoxicologically relevant metabolites, at different time intervals during the test. Measurement of dynamics in exposure concentrations in pore water, total sediment, overlying water, and test organisms is advisable if chemical equilibrium is not reached between the different environmental compartments during the test period.

In conclusion, the PEC_{sed} and $PNEC_{sed}$ used in the RQ should be expressed in the same type of concentration. Ideally, internal concentrations should be measured during the experiment. As a minimum, concentration in pore water and total sediment (in units of mass of organic chemical per mass of dry sediment) and the organic matter content (%) of the dry sediment should be measured, as well as the concentration in the overlying water. Models may be used to calculate chemical concentrations in environmental compartments in which data is lacking.

8.8.2 Overview of fate and exposure models

Fate models are essential for understanding and evaluating the required time for chemical equilibrium between sediment and pore water and to optimize other aspects of the tests, such as the water-sediment ratio, water renewal and pre-equilibration after spiking. There is a need for approaches to translate biodegradation process parameters obtained from lab tests to parameters that are relevant in the field. The development of passive samplers for more classes of chemical can provide more accurate input for such models.

Exposure models have been reviewed[509-511] and three basic approaches have been identified: multiple box models, numerical solutions to advection-dispersion transport models and meta-models. Geographic information system (GIS)-based modelling was proposed as a convenient fourth approach.[511] Single point multi-media models typically provide average concentrations in environmental compartments for a region or country using emission data and mass balance equations or material flow analysis (e.g. EUSES[512], SIMPLEBOX[513]). However, spatially and temporally explicit models use more detailed and realistic process descriptions to simulate concentrations in aquatic systems as a function of place and time (e.g. DUFLOW[514], TOXSWA[435], GREAT-ER[515]). In exposure modelling of aquatic systems, single point multi-media models can be considered as a lower tier approach and spatially and temporally explicit models as a higher tier approach. For

prospective ERAs, however, the development of exposure scenarios is a prerequisite to successfully apply exposure models. Consequently, more realistic exposure models are needed for emerging chemical classes like ionizable organics and polar substances; such models should also take degradation processes into account.

8.8.3 Linking exposure to effects in sediment ERA

For exposure in chronic risk assessment, either the peak concentration (max) in total sediment normalized to organic carbon content ($PEC_{sed\text{-}oc;max}$) or pore water ($PEC_{sed\text{-}pw;max}$), or the TWA concentration in total sediment ($PEC_{sed\text{-}oc;TWA}$) or pore water ($PEC_{sed\text{-}pw;TWA}$) can be used to compare with the predicted no effect concentration for sediment based on chronic toxicity data (either $PNEC_{sed\text{-}oc;ch}$ or $PNEC_{sed\text{-}pw;ch}$). In the text below, when referring to PEC_{sed} and $PNEC_{sed}$ estimates, this may be either in pore water or the concentration in total sediment normalized to OC.

In principle, the $PEC_{sed;max}$ or $PEC_{sed;TWA}$ should be lower than the $PNEC_{sed;ch}$. However, if using the $PEC_{sed;TWA}$ in the risk assessment, the time window for the $PEC_{sed;TWA}$ estimate should be equal to or shorter than the time window for the chronic effect estimate that drives the risk (i.e. the duration of tests delivering the critical chronic EC_{10} values that drive the $PNEC_{sed;ch}$). In addition, proof of reciprocity in toxicity tests should be provided in order to use the $PEC_{sed;TWA}$ in the risk assessment. Reciprocity refers to Haber's law, which assumes that toxicity depends on the product of concentration and time.

We recommend that the effect estimate derived from sediment toxicity tests be expressed in terms of TWA or mean exposure concentration during the test. However, in current sediment toxicity tests the effect estimate (such as EC_x and NOEC) is usually expressed in terms of initial exposure concentration. If the effect estimate is expressed in terms of initial exposure concentration, it should be shown that the exposure profile in the toxicity test is worst-case relative to that in the field. If the effect estimates on which the PNEC is based are expressed in terms of the initial test concentration, then the $PEC_{sed;max}$ concentration should always be used in the risk assessment to assure a more realistic worst-case risk assessment.

8.9 Tiered effect assessment for benthic test species and spiked sediments

8.9.1 Tiered approach

Tiered approaches start with a simple conservative assessment and do additional, more complex work only if necessary. This provides a cost-effective procedure, both for industry and regulatory agencies. The tiered system as a whole should be (i) appropriately protective, (ii) internally consistent, (iii) cost-effective and it should (iv) address the problem with a higher degree of realism and complexity when going from lower to higher tiers (see Figure 3).[14-16] Furthermore, a tiered ERA scheme must be developed for each specific protection goal. An additional advantage of the tiered approach is that higher

tiers can be used to calibrate the lower tiers.[516] Appropriate field observations may be used to verify the tiered effect assessment approach based on experimentation.

Below, a tiered ERA scheme for benthic invertebrates and rooted macrophytes is presented and discussed. Most data and experience with spiked sediment tests is available for these taxa. Despite the scarcity of spiked sediment toxicity tests with microorganisms and vertebrates, in this paper we also discuss sediment ERA approaches for these organisms. In principle, however, all tiers can be used for different groups of sediment organisms.

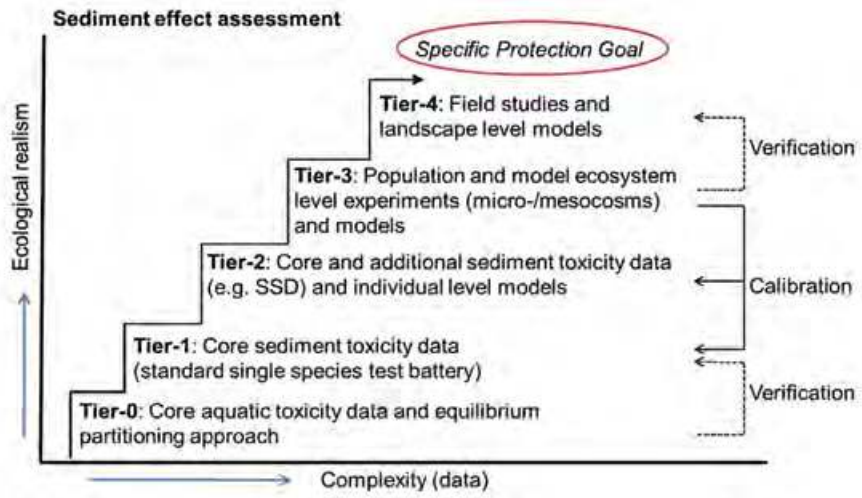


Figure 3. Schematic overview of a tiered approach in prospective risk assessment. In each tier an assessment factor (AF) may be necessary to derive a predicted no effect concentration (PNEC). The higher tiers can be used to calibrate the lower tiers (adapted from Chapter 2[259]).

8.9.2 Tier-0 effect assessment based on equilibrium partitioning

Di Toro et al. [517] showed that the bioavailability of non-ionic organic chemicals is a function of their distribution between environmental phases (e.g. organic matter and interstitial water). This understanding was the foundation for using EP to derive mechanistic sediment quality guidelines. Assuming that the toxicity of a non-ionic organic chemical is proportional to its concentration in water, then the sediment concentration of this chemical that will cause toxicity can be estimated if the relationship between the chemical concentration in the pore water and that in sediment is understood. The partitioning of a chemical between OC phase in the sediment and pore water can be represented by a simple equilibrium equation[518]:

$$C_{sed\text{-}oc}=C_{pw}*K_{oc} \quad (1)$$

In which $C_{sed\text{-}oc}$ is the concentration of the chemical in the sediment per unit mass of OC (µg/kg OC), C_{pw} is the concentration of the chemical in pore water (µg/L) and K_{oc} is the partition coefficient of the chemical to sediment OC (L/kg OC). When replacing C_{pw} by

the predicted no effect concentration for surface water based on chronic toxicity data ($PNEC_{sw;ch}$) derived for pelagic water organisms on basis of water toxicity tests, the $C_{sed\text{-}oc}$ becomes the $PNEC_{sed;ch\text{-}EP}$.

An essential step in the application of the EP approach is the derivation of an appropriate K_{oc}, such as with OECD 106[519]. Because reported K_{oc} values may have a high variability, we recommend using the geometric mean value, since K_{oc} values usually show a log-normal distribution[520]. If no K_{oc} is available, then this value can be estimated from K_{ow} using quantitative structure-activity relationship (QSAR) models.[216]

Research in the past decade has shown that the EP theory does not accurately predict in situ partitioning. This is because field K_{oc} values typically are two to three orders of magnitude higher than those in the laboratory due to the ubiquitous presence of condensed carbon phases, such as black carbon.[80,223,331] Consequently, the chemical concentration in sediment that causes toxicity also will be two to three orders of magnitude higher. When the used K_{oc} value is based on sediment lacking a condensed carbon phase we recommend a worst case approach in Tier-0. This approach accounts for the effect of black carbon by using a K_{oc} value in Eq. 1, which is only ten times higher than the K_{oc} values traditionally used in the EP approach. This means that Eq. 1 will return toxic thresholds for sediments that are a factor of ten higher. Another shortcoming of the EP approach is that it neglects sediment ingestion as a relevant uptake pathway. EP also neglects specific species traits and is adequate only as long as the chemical transfer occurs through passive organic matter-water-lipid partitioning. EP-based approaches predict BSAF values of approximately 1 or 2. However, this has been shown to be inadequate for organisms such as the mayfly *Hexagenia* sp. with a BSAF up to 20 for PCB153,[59] the annelid *L. variegatus* with a BSAF up to 99 for chlorpyrifos,[521] the marine amphipod *C. volutator* with a BSAF ranging from 16 to 218 for PCBs (Chapter 4),[346] the marine polychaete worm *A. marina* with a BSAF ranging from 10 to 40 for PCBs (Chapter 4)[321,346] and the marine decapod *Chasmagnathus granulata* with a BSAF ranging from 0.1 to 44 for a range of organochlorine pesticides.[522] These organisms thus accumulate up to two orders of magnitude higher concentrations than EP theory predicts. Therefore, to be protective, a Tier-0 approach should take this into account, and produce a toxic threshold in sediment that is a factor of 100 lower than calculated by the original Eq. 1. The two effects –the black carbon effect and the sediment ingestion effect – act in the opposite direction, and thus partly compensate for each other, but still yield a net effect of 100/10=10 as an extra safety factor to be applied to the effect threshold calculated for a Tier-0 for invertebrates and vertebrates that ingest sediment. For microorganisms, benthic algae and sediment-rooted macrophytes, ingestion does not play a role – but these organisms may have the capacity to extract a fast-desorbed fraction of the organic contaminant. For these organisms, we propose that the extra safety factor is not needed when using the EP approach.

8.9.3 Tier-1 effect assessment based on protocol tests for benthic invertebrates and macrophytes

The following approach can be used to derive a chronic Tier-1 PNEC value based on sediment toxicity tests with the freshwater, estuarine and marine standard test species that were described in Section 8.6:

1) For the chemical of concern, collect the Tier-1 and additional toxicity data for (pelagic) water organisms in the compartment overlying water.
2) Identify the taxonomic group(s) of water organisms that is/are likely to be most sensitive.
3) Collect the available spiked sediment toxicity data for benthic freshwater and estuarine/marine standard test species (see sections above).
4) Determine whether the most sensitive taxonomic group for Tier-1 water column organisms is likely to be represented in the core data set of benthic test species according to standard protocols.
5) If so, use Table 5 to conduct the Tier-1 effect assessment for benthic organisms in freshwater and estuarine/marine ecosystems. If not, determine whether the most sensitive taxonomic group is also represented in the additional toxicity data, which can then be added to the core data set of benthic test species, or try other approaches (such as the EP approach).

Table 5. Proposal for assessment factors (AF) to be applied to the lowest sediment toxicity value for standard tests with spiked sediment and benthic organisms (adapted from EFSA [482], ECHA [493] and European Commission [518]). To extrapolate semi-chronic toxicity data a range in AF is proposed to acknowledge differences in toxic mode-of-action and associated differences in time to onset-of-effects. An AF in the lower range may be selected for compounds with a short time to onset-of-effects and an AF in the higher range if latent effects likely will occur (informed by toxicity data of pelagic organisms and read across using data for compounds with a similar mode of action).

Available data	**AF**
Three chronic EC_{10}/NOEC values for different taxonomic/feeding groups, of which at least two test species, including the most sensitive, are representative for the ecosystem under evaluation (freshwater or marine/estuarine)	10[a]
Three chronic EC_{10}/NOEC values for different taxonomic/feeding groups, of which only the most sensitive is representative for the ecosystem under evaluation (e.g. freshwater test species for an marine/estuarine ERA)	30[a]
Three chronic EC_{10}/NOEC values for different taxonomic/feeding groups, of which one is representative for the ecosystem under evaluation (e.g. freshwater test species for an marine/estuarine ERA), but this species is not the most sensitive	50[a]
Two chronic EC_{10}/NOEC values for different taxonomic/feeding groups and representative for the ecosystem under evaluation (freshwater or marine/estuarine)	50
Two chronic EC_{10}/NOEC values for different taxonomic/feeding groups of which one value each is representative for respectively freshwater and marine/estuarine ecosystems	100
Three chronic EC_{10}/NOEC values for different taxonomic/feeding groups and not representative for the ecosystem under evaluation	100[a]
Three semi-chronic (10 d) L(E)C_{10}/NOEC values for different taxonomic/feeding groups and for standard benthic test species typical for the ecosystem (freshwater or marine/estuarine) under evaluation	30-100[a]
Two chronic EC_{10}/NOEC values for different taxonomic/feeding groups that are not representative for the ecosystem under evaluation (e.g. freshwater test species for an marine/estuarine ERA)	200
Three semi-chronic (10 d) L(E)C_{50} values for different taxonomic/feeding groups and for standard benthic test species typical for the ecosystem (freshwater or marine/estuarine) under evaluation	100-300[a]
Three semi-chronic (10 d) L(E)C_{50} values for different taxonomic/feeding groups and not all test species are typical for the ecosystem (freshwater or marine/estuarine) under evaluation, but the most sensitive test species is typical.	200-500[a]
Two semi-chronic (10 d) L(E)C_{50} values for different taxonomic/feeding groups and for standard benthic test species typical for the ecosystem (freshwater or marine/estuarine) under evaluation	200-500

[a] For substances with a specific toxic mode of action (e.g. insecticides and herbicides) it may suffice to test two representative species of the potentially sensitive taxonomic group(s). This is demonstrated when the representative test species of the sensitive taxonomic group(s) that drive the risk are an order of magnitude more sensitive than the other test species in the chronic aquatic effect assessment for pelagic species.

8.9.4 Tier-2 approach on basis of laboratory toxicity data for standard and additional benthic invertebrates and/or rooted macrophytes

Geometric mean approach

If valid toxicity data from several species are available, but this number is too low to apply the species sensitivity distribution (SSD) approach, EFSA[24,482] proposed the option of the geometric mean-AF approach. In this approach, the geometric mean toxicity value is calculated for species from the same taxonomic group (e.g. crustaceans, insects, annelids, nematodes, bivalves) and the same measurement endpoint (e.g. LC_{50} values). The lowest geometric mean value for the various taxonomic groups is selected, and the same AF normally used in the Tier-1 effect assessment is applied. For the acute aquatic effect assessment of pelagic species exposed to insecticides, the geometric mean approach was recently calibrated by Van Wijngaarden et al.[516] with threshold concentrations for effects derived from aquatic micro/mesocosm tests. This study demonstrated that the geometric mean approach proposed by EFSA for acute effect assessment of insecticides provides sufficient protection to water organisms.

Given the requirements described above, the geometric mean approach could also be applied to sediment ERA that uses acute and/or semi-chronic LC_x values for benthic species of the same taxonomic group and that have the same feeding strategy. However, in the chronic effect assessment based on spiked sediment toxicity data, the geometric mean approach might be more difficult to use. This is because the chronic toxicity data for different species within the same taxonomic and/or feeding group in the majority of cases concern different measurement endpoints – such as mortality, growth, biomass and emergence – in tests with different durations. Furthermore, the evaluation of the predictive value of the geometric mean approach by EFSA[24] was predominantly based on acute toxicity data. Consequently, for the time being, we propose restricting the geometric mean approach for deriving a $PNEC_{sed;ch}$ on the basis of (10d) semi-chronic $L(E)C_{50}$ values for benthic species of the same taxonomic group and with the same feeding strategy. For this purpose, an AF of 100 - 300 (if at least three taxa representative for the system under evaluation are available) or 200 – 500 (if less than three taxa representative for the system under evaluation are available) as proposed in Table 5 should be applied to the geometric mean $L(E)C_{50}$ value for comparable semi-chronic toxicity of all species belonging to the most sensitive taxonomic group. An AF in the lower range may be selected for compounds with a short time to onset-of-effects and an AF in the higher range if latent effects likely will occur (informed by toxicity data of pelagic organisms and read across using data for compounds with a similar mode of action). In the future, when more chronic spiked sediment laboratory toxicity data become available for organic chemicals and benthic organisms of the same taxonomic group, as well as appropriate semi-field experiments to evaluate the ecological relevance of these laboratory data, the geometric mean approach to derive chronic sediment PNECs based on chronic toxicity data and sub-lethal endpoints can be reconsidered.

Species sensitivity distribution (SSD) approach

The use of the SSD approach in ERA is described in Posthuma et al.[224]. In current prospective ERA for pelagic water organisms, toxicity data for at least 8 species (for pesticides[482]) and 10 species (for other toxicants[215]) – but preferably more – are needed to apply the SSD approach. Given the limited number of test protocols currently available for benthic species, as well as the limited published sediment toxicity data for organic chemicals, it will be difficult to collect chronic toxicity data for more than 10 benthic species. For sediment ERA, we propose – as a minimum – toxicity data for 8 benthic species representing at least 5 different taxonomic/feeding groups, except when the ERA based on water organisms shows that a specific taxonomic group is at least an order magnitude more sensitive that other taxonomic groups. For example, this may be the case for toxicants with a specific toxic mode-of-action such as insecticides, for which arthropods (insects and crustaceans) are particularly sensitive, and herbicides, for which algae and macrophytes usually are the most sensitive groups. In case of organic toxicants with a specific toxic mode-of-action, the 8 species with toxicity data to construct the SSD should preferably be selected from the sensitive taxonomic group(s).[222,482,523] We consider this minimum number of 8 toxicity values as a reasonable and pragmatic solution to derive a chronic $PNEC_{sed;ch}$ when using the SSD approach, but we also recommend applying an AF to the hazardous concentration to 5% of the species tested as calculated from the SSD (HC_5) to address the remaining uncertainty.

Since benthic species of freshwater and marine/estuarine ecosystems have many traits in common, we assume that sediment toxicity data for both freshwater and marine/estuarine benthic species can be combined to construct the SSD curve. Again, an AF may be applied to address the remaining uncertainty in deriving a $PNEC_{sed;ch}$ for marine/estuarine benthic species based on an HC_5 calculated from an SSD curve largely constructed with toxicity data from freshwater species and the other way around when deriving a $PNEC_{sed;ch}$ for freshwater species mainly based on marine/estuarine data. Guidance for criteria that can be used to select the size of the AF are shown in Table 6. The use of the SSD approach is valid only if it has been verified that the selected toxicity data show an appropriate fit with the model used to calculate the SSD curve (e.g. the Anderson-Darling test for goodness-of- fit is accepted).[524-526]

Preferably, to derive a $PNEC_{sed:ch}$ based on the SSD approach, the SSD should be constructed with chronic EC_{10}/NOEC data addressing sub-lethal endpoints. However, if for an essential taxon, such as the 8th species in the SSD, a valid chronic toxicity value is missing but a valid semi-chronic toxicity value is available, then the approach described in Table 7 may be an option to derive the corresponding chronic EC_{10}/NOEC. The size of the extrapolation factor (EF) to be applied should be based on read-across information on toxicity data for pelagic and benthic species and compounds with a similar toxic mode-of-action. EFs in the lower range may be appropriate for compounds with a short time to onset-of-effects (e.g. pyrethroid insecticides) while EFs in the higher range may be more appropriate for compounds with more latent effects e.g. if they have hormone disruptive properties (e.g. tributyltin). We recommend using this extrapolation approach for no more

than two species in the chronic SSD curve, which means that minimal six species with chronic data is available. Another approach is to use semi-chronic data (e.g. 10d $L(E)C_{50}$ values) separately to construct an SSD and to calculate a corresponding semi-chronic HC_5. A $PNEC_{sed;ch}$ can be estimated with the approach described in Table 6 (but using semi-chronic instead of chronic toxicity data in the SSD) as well as an extra AF of 5 - 10. An AF in the lower range may be selected for compounds with a short time to onset-of-effects and an AF in the higher range for compounds with latent effects (read across).

Table 6. Criteria, based on European guidance documents[215,482], that can be used to select the size of the assessment factor (AF) to be multiplied with the median HC_5 (SSD approach) to derive a $PNEC_{sed;ch}$ for benthic organisms.

AF	**Criteria**
1	• ≥ 10 chronic toxicity data (spiked sediment) • ≥ 8 different taxonomic/feeding groups[a] • ≥ 5 taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine) • Lower limit HC_5 is less than a factor of 5 lower than the median HC_5
2	• ≥ 10 chronic toxicity data (spiked sediment) • ≥ 8 different taxonomic/feeding groups[a] • ≥ 5 taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine) • Lower limit HC_5 is more than a factor of 5 lower than the median HC_5 but less than a factor of 10
3	• ≥ 8 chronic toxicity data (spiked sediment) • ≥ 5 different taxonomic/feeding groups[a] • ≥ 4 taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine) • Lower limit HC_5 is less than a factor of 10 lower than the median HC_5
4	• ≥ 8 chronic toxicity data (spiked sediment) • ≥ 5 different taxonomic /feeding groups[a] • ≥ 4 taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine) • Lower limit HC_5 is more than a factor of 10 lower than the median HC_5
5	• ≥ 8 chronic toxicity data (spiked sediment) • ≥ 5 different taxonomic /feeding groups[a] • < 4 taxa from the type of ecosystem under evaluation (freshwater or marine/estuarine)

[a] The default option is to select taxa belonging to different phylogenetic phyla or orders, unless (a) evidence is provided that a second benthic species selected for the same Phylum/Order has another feeding strategy, or (b) a specific taxonomic group is most sensitive (e.g. Arthropoda for insecticides). If (b), it suffices to select the required number of taxa from different Genera within the specific sensitive taxonomic group unless the second benthic species selected within a Genus has another feeding strategy (e.g. deposit feeder, suspension feeder, predator).

Table 7. Proposed extrapolation factor to be applied to an individual semi-chronic or chronic toxicity value to estimate the corresponding chronic NOEC/EC_{10} to be used in the SSD curve.

Available toxicity value	Extrapolation factor
10d LC_{50}	10-30
10d EC_{50}	5-15
10d NOEC	3-10
≥ 21-28d L(E)C_{50}	2-5

8.9.5 Tier-3 approach based on semi-field experiments

An important requirement for the use of micro/mesocosm test systems to derive a chronic PNEC value for sediment-dwelling organisms is that the concentration-response relationships for benthic organisms are expressed in terms of exposure concentrations measured in the sediment compartment. Lipophilic organic chemicals that enter aquatic ecosystems via the water compartment will easily sorb to sediment particles in the upper sediment layer. In addition, many benthic invertebrates can be found in this layer, because of more favourable food and oxygen conditions. Consequently, the measurement and/or calculation of exposure concentrations in micro/mesocosm test systems to derive concentration-response relationships for benthic organisms should focus on the upper sediment layer of these test systems. However, it may be useful to measure the dynamics in exposure concentrations in different sediment layers because of variations in the habitat occupied by different benthic taxa. We propose measuring the dynamics in exposure concentration (freely dissolved pore water concentration; total concentration in sediment normalised on the basis of OC content) in different sediment layers, for example 0-1 cm, 1-2.5 cm, 2.5-5 cm and 5-10 cm. Depending on the habitat preference of the benthic organism at risk, the exposure concentration in the appropriate sediment layer can be selected (e.g. the 0-1 cm layer for epi-benthos or 0-10 cm layer for rooted macrophytes).

Ideally, the sediment used to construct the micro/mesocosm experiments is spiked with the contaminant. The advantage of using spiked sediments when constructing micro/mesocosm test systems is that the contaminant under investigation is homogeneously distributed in the sediment compartment, at least initially. A possible disadvantage of such a design is that the benthic community is not yet established when exposure starts. However, spiked sediment micro/mesocosm tests can be used to study the impact of different sediment concentrations on the colonization of the sediment compartment by benthic organisms (seeded or spontaneous) and on their dynamics in population densities. Since the exposure regime of organic chemicals that accumulate in sediments, and for which an ERA has to be performed, is long term, the duration of spiked sediment micro/mesocosm tests can be long as well, allowing a sufficiently long colonization period for most benthic invertebrates and rooted plants.

Alternatively, micro/mesocosm test systems with a well-established aquatic community can be used by spiking the water compartment with the contaminant. The advantage of this approach is that benthic populations already present in the test systems become exposed. A disadvantage, however, is that initially the benthic organisms are primarily exposed via the overlying water, while in later phase sediment exposure becomes more important. In addition, this experimental design requires a more detailed assessment of the dynamics in exposure concentrations in different sediment layers and the overlying water. Expressing the treatment-related responses of benthic organisms in terms of sediment exposure concentrations most likely will result in a relatively worst-case assessment for epi-benthic taxa in particular, since the initial high exposure via overlying water will also affect these organisms. Note that in spiked water micro/mesocosm tests, the peak concentration of the organic contaminant in the sediment compartment is usually measured days to weeks after the application.[527]

8.9.6 Tier-4 approach based on field studies

Currently, too little data and experience are available to give specific recommendations for a Tier-4 approach based on field studies. However, chemical and biological monitoring studies in the sediment compartment of aquatic ecosystems may be used as a quality check of prospective ERA procedures for sediment organisms.

8.9.7 Effect models to supplement the experimental tiers

Current ERA schemes focus largely on toxicity and bioaccumulation at the individual level, while specific protection goals as proposed in Section 8.4 focus mainly on the population level. Effect models can be used to extrapolate results of experimental tiers, amongst others, in linking spatial-temporal variability in exposure to effect, in predicting concentration-response relationships at different levels of biological organisation and different spatial and temporal scales, and in addressing ecological recovery times, bioaccumulation in food-webs and food-web interactions in ecosystems.[428,479,509,528,529] Despite their ability to include and extrapolate effects that cannot be captured by the experimental tiers, effect models are rarely recommended in technical documents of ERA.[479,529]

Although a wide variety of effect models have been developed,[509,530,531] most of these models address specific scientific research questions and are not directly suitable in ERA. The use of effect models in ERA and their potential to address the requirements of protection goals in EU directives have been assessed previously.[479,529] Recently, EFSA[532] published a scientific opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products in which critical steps to implement the use of effect models in ERA were identified. *First*, a clear problem formulation is needed that defines one or more specific questions according to the available data and specific protection goals and consider how the output matches with the specific protection goal. *Second*, the application domain of the model, and thus its predictive power, must to be considered to validate the broader conclusions based

on model output. This means that either sufficient data should be available for model validation, or there is the potential to generate this data. *Third*, focal species must be selected, as not all species present in the ecosystem under evaluation can be modelled. Logically, these focal species should be vulnerable representatives of the main taxonomic groups of benthic organisms at risk. *Fourth*, realistic worst case environmental scenarios must be defined in relation to the specific protection goal and problem definition. An environmental scenario is a conceptual and quantitative description of the environmental system relevant to ERA, and has been defined by EFSA[532] as a combination of abiotic, biotic and agronomic parameters, thus including both exposure and effect. Scenarios from exposure models should be in line with those of the effect models, as they may share common variables.[504] EFSA[532] recommends that several scenarios be considered, including a control/baseline and a toxic standard. A future research activity would be to develop and link scenarios in exposure and effect models that include the sediment compartment. For ERA, a set of freely available scientific sound robust models with a user friendly interface and a well-defined set of scenarios are needed.[532]

Currently, most effect models used in ERA focus on pelagic organisms and freshwater ecosystems, while marine systems,[529] benthic organisms and the sediment compartment in general are usually disregarded. Below, we discuss effect models at the individual, population, ecosystem and spatial explicit level, which include benthic invertebrates and/or the sediment compartment or have the potential to do so.

Individual level models

Individual level models can be used as an addition to Tier 2. Given the characteristics of spatial and temporal variable exposure in the heterogeneous sediment compartment and the role of different exposure routes (e.g. exposure via pore water and food), the simplest models to use for linking exposure to effect at the individual level are TKTD models (e.g. GUTS).[445,533] TKTD models mechanistically account for time-varying exposure and effects of chemicals on individuals. More complex models that can be used are dynamic energy budget (DEB) models,[534] which embed individual growth and development to account for growth dilution. For some freshwater benthic invertebrates (*Asellus aquaticus*, *Gammarus pulex*, and *C. riparius*), models that link exposure and effect have been developed and parametrized, while for other benthic species such as *M. balthica*,[301] uptake models exist but have not yet been linked to effect. Uptake, elimination and effects of contaminants are complicated for aquatic macrophytes because roots in the sediment as well as leaf and stem surfaces in the water layer contribute to these processes (Chapter 3).[307,503] A model describing these processes has been developed for *E. canadensis* and *M. spicatum* in Chapter 3[307] and for *M. spicatum* exposure has been coupled to effects.[503]

Population-level models

Population models can be divided into three types: Lotka-Volterra type models, matrix models and individual-based models (IBM),[529] and can be used as an addition to the experimental Tier-3. IBMs are a convenient approach to deal with the complexity arising from complex life cycles of the organisms, seasonality and small- and large-scale spatial

heterogeneity.[535] Relevant population endpoints are recovery times after a peak exposure and population growth rate in case of chronic exposure and sub-lethal effects.[536] In the latter case, a more analytical approach to model structured populations is possible. Individual models can be connected to population models to link individual responses to chemical exposure.[421] For the freshwater (epi)benthic species (*Asellus aquaticus* and *Gammarus pulex*) and sediment dwelling species (*C. riparius*), models have been developed previously.[23,424] However, these models disregard sediment exposure via direct contact and ingestion of food and sediment particles. Because this may lead to an underestimation of actual exposure,[60,346] these models should be extended with exposure via this additional pathway. In Chapter 7, sediment uptake was explicitly added to a TKTD model integrated in an IBM to assess effect of sediment ingestion on the population level for *C. riparius*.[537] This study showed that simultaneous exposure via water and ingestion of contaminated organic matter leads to a larger impact and a delayed recovery compared to exposure via water only. This highlights the importance of sediment and food ingestion as an exposure pathway for benthic invertebrates and underpins the need for sediment toxicity tests in environmental risk assessment. For marine and estuarine organisms, *C. volutator* is the only benthic species for which a simple Leslie-matrix population model has been presented,[221] which has not yet been linked to exposure. This could be a possibility for future research. Another possibility is to integration the existing TKTD models for *M. spicatum* with an existing population model, such as that from Best and Boyd.[538]

Ecosystem level models

Ecosystem level models can be used as an addition to the experimental Tier 3. Only a few models have included higher levels of biological organisation, and mainly freshwater ecosystem models, such as AQUATOX,[539] have been applied in ERA.[529] Food web accumulation modelling is a good approach to assess secondary poisoning. Such models are flexible, usually well calibrated and have been evaluated. Several of these models, some including benthic organisms, have been confirmed and recommended for use in the regulatory context.[509]

Spatially explicit models

Spatially explicit models can be used as an addition to field studies in Tier 4. Depending on the combination of exposure pattern and species at hand, it may be important to explicitly consider spatiotemporal dynamics of both exposure and populations by modelling spatially-structured populations. This approach is relevant when there is a spatial differentiation in the exposure patterns, with some parts of the system being exposed to higher concentrations than others. Clearly, dealing with this heterogeneity becomes more urgent when larger systems, such as watersheds, are being considered. Also, the species at hand should have limited mobility relative to the scale of the system.[421] At the lowest level of spatial complexity, we may deal with relatively simple uniform systems representing streams, ditches and ponds, as in the FOCUS surface water scenarios [540] used for edge-of-field evaluation of plant protection products, or patches of estuarine and

marine ecosystems. Ultimately, the larger spatial scale can be considered, for instance addressing both exposure and population dynamics in a complex ditch system,[541] a larger watershed or interconnected patches of an estuarine/marine ecosystem. For *Chironomus*, landscape-level approaches can be developed, possibly based on Galic et al.[424] and Focks et al.[541]. In those studies, however, the focus was on the overlying water compartment. A future activity could be to integrate exposure via the sediment into the landscape/watershed level, for example by using the, in Chapter 7, developed sediment-including IBM model for *C. riparius*.[537]

8.9.8 Effect assessment for vertebrates

European Directive 2010/63/EU states that in the Member States of the European Union, testing with vertebrates should be minimized because of ethical considerations such as animal welfare. Therefore, as an animal friendly first-tier approach, cell line assays of vertebrate species can be used, such as the activated luciferase gene expression (CALUX) assay.[542] These tests are designed to assess the sensitivity of a chemical for a specific mode of action such as dioxin-like activity or estrogenic activity.[543,544] However, we consider the cell line assays not yet appropriate for use in prospective ERA, since there is a lack of established cell lines. In addition, knowledge about the relationship between toxicant-induced cell line responses and effects on individuals and populations of vertebrates is insufficient.[545-549] Therefore, an important topic for future research is the development of in vitro cell line assays and the evaluation of their ecotoxicological relevance. An alternative for cell line assays could be the sediment contact assay using zebrafish embryos.[550] As a more conventional Tier-1 assessment, the 10-day single species test (ASTM E2591 – 07[499]) for amphibians may be used. Considering the very limited experience with benthic vertebrates, we will not provide a tiered ERA scheme for this group in this paper. However, the Tier-0 EP approach might provide a sufficiently conservative $PNEC_{sed;ch}$ estimate for benthic vertebrates.

8.9.9 Effect assessment for microorganisms

Although advanced molecular techniques to determine functional and community responses exist, none have yet been ring tested and described as standard tests (Chapter 2).[259] Moreover, experience with microorganisms in prospective sediment tests is limited. Several issues must be considered in a tiered ERA for microorganisms. Microorganisms might be negatively affected or stimulated by contaminants. Furthermore, functional redundancy is high among microorganisms. Consequently, even if there is a clear effect on the community composition, this may not result in an effect on their function.[163] This challenges the interpretation of the test outcomes, depending on the specific protection goal adopted. Another challenge is to link exposure and effect, as microorganisms affect exposure by degradation and transformation of the contaminant. However, such feedback loops between toxicity and exposure play a role in all sediment tests, as it is very difficult to exclude microorganisms from a test system (Chapter 6).[323]

Although, single species microbial tests do exist (e.g. *Vibrio fischeri*) their ecological relevance requires support. Nevertheless, the *V. fischeri* test has been proposed within the first tier in retrospective risk assessment[231] and could also serve in a first tier in sediment ERA. As a higher tier option, simple laboratory microcosm tests with spiked sediment in which functional endpoints of microbes are determined, such as nitrification and denitrification. These microcosm tests also allow consideration of the community composition of microorganisms. For the terrestrial ERA, the nitrogen transformation test (OECD 216[551]) is currently recommended. Ideally, a set of standard functional endpoints should be tested, guided by knowledge about the mode of action of the chemical. Another higher-tier option could be a mesocosm study in which benthic invertebrates, macrophytes and microorganisms are tested simultaneously. For microorganisms, the same endpoints as in the laboratory microcosm can be used.

8.10 Sediment effect assessment: Case studies

In this section we present three case studies with ivermectin, chlorpyrifos and tributyltin to investigate the tiered approach in sediment risk assessment as described above, with a focus on benthic invertebrates. In the subsections below, a distinction is made between semi-chronic toxicity tests (test duration usually 10 d), and chronic toxicity tests (test duration usually ≥21-28 d). However, not all tests reported in the literature as chronic considered sub-lethal endpoints and/or covered the whole life cycle (or the most sensitive life-stage) of the test organisms. All sediment toxicity data provided in the cases are expressed in µg/g OC, based on the OC of the sediment as reported in the original papers and/or assuming an OC content of 2.5% in standard OECD sediment with a peat content of 4-5%.

8.10.1 The pharmaceutical ivermectin

Evaluation of standard and additional toxicity data for pelagic organisms and ivermectin
The laboratory toxicity data for typical pelagic organisms and the pharmaceutical ivermectin are shown in Table 8.

It can be concluded from the information in Table 8 that invertebrate populations most likely are the most sensitive taxonomic group on which a chronic effects assessment for sediment-dwelling organisms should focus. Note that the reported toxicity values for the crustacean *Daphnia magna* are at least two orders of magnitude more sensitive than for the green alga and the fish. Another striking phenomenon is the high acute-to-chronic ratio that is reported for *Daphnia magna*. The Tier-1 $PNEC_{sw;ch}$ ($3x10^{-5}$ ng/L) is based on the application of an AF of 10 to the lowest chronic toxicity value (for *D. magna*).

Table 8. Toxicity data for typical water column organisms and the pharmaceutical ivermectin.

Test species	Acute toxicity	Chronic toxicity	Reference
Pseudokirchneriella subcapitata *(green alga)*		72h EC_{50} = > 4 mg/L 72h NOEC = 391 µg/L	[552]
Daphnia magna (Crustacea)	48h EC_{50} = 5.7 ng/L	21d NOEC = 0.0003 ng/L	[552]
Oncorhynchus mykiss *(fish)*	96h LC_{50} = 3.0 µg/L		[553]
Salmo salar *(fish)*	96h LC_{50} = 17 µg/L		[554]
Tier-1 $PNEC_{sw;ch}$		0.0003/10 = 0.00003 ng/L	
Invertebrate community in mesocosms		10-97d NOEC = <30 ng/L	[555]

Tier-0 effect assessment for ivermectin on basis of Equilibrium Partitioning

The following equation is used to calculate the $PNEC_{sed;ch;EP}$:

$$PNEC_{sed;ch;EP} = PNEC_{sw;ch} * K_{oc} * 0.1 \quad (2)$$

In which $PNEC_{sed;ch;EP}$ is the concentration of the chemical in the sediment per unit mass of OC (µg/kg OC), $PNEC_{sw;ch}$ is the concentration of the chemical in pore water (µg/L) and K_{oc} is the partition coefficient of the chemical to sediment OC (L/kg OC). We selected the tier-1 $PNEC_{sw;ch}$ of $3x10^{-5}$ ng/L (Table 8) and a K_{oc} geometric mean of 12497 L/kg (n=5) from a values range of 4000 – 25800 L/kg[556]. The geometric mean K_{oc} value, resulting in $PNEC_{sed;ch;EP}$ value of $3.75x10^{-5}$ ng/g OC.

Tier-1 effect assessment for benthic organisms and ivermectin

Chronic sediment toxicity data for three standard benthic freshwater organisms are available (insect, oligochaete, and nematode) (Table 9). In addition, the tests were conducted largely in accordance with internationally accepted guidelines: *C. riparius* (OECD 218), *L. variegatus* (OECD 225) and *C. elegans* (ISO 10872). In the chronic effect assessment, 28 d EC_{10} values are preferred over 28 d NOEC values.

In Table 9*, C. riparius* shows lower toxicity values than *L. variegatus* and *C. elegans*. Selecting the 28d EC_{10} of 0.14 µg/g OC of *C. riparius* and the application of an assessment factor of 10 (Table 5) results in a Tier-1 $PNEC_{sed;ch}$ of 0.014 µg/g OC for sediment-dwelling organisms in freshwater ecosystems. This Tier-1 PNEC value is lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 9, but is considerably higher than the Tier-0 $PNEC_{sed;ch;EpP}$ calculated above (Figure 4).

Table 9. Sediment toxicity data for benthic organisms and the pharmaceutical ivermectin. The values in bold concern the standard toxicity data used in the Tier-1 effect assessment and were acquired in accordance with internationally accepted guidelines (see Table 2 in Chapter 2[259]).

Species and test protocol	Effect endpoint	Toxicity endpoint	Toxicity value µg/g OC	Reference
Chironomus riparius *Insecta (freshwater; OECD 218)*	Mortality	10d LC_{50}	2.75	
	Mortality	10d LC_{10}	1.46	
	Mortality	10d NOEC	1.07	
	Individual dry weight	10d NOEC	0.13	557
	Female emergence	28d EC_{50}	0.39	
	Female emergence	**28d EC_{10}**	**0.14**	
	Female emergence	28d NOEC	0.27	
Lumbriculus variegatus *Oligochaeta (freshwater: OECD 225)*	Total dry weight	28d EC_{50}	131.86	
	Total dry weight	**28d EC_{10}**	**28.76**	557
	Total dry weight	28d NOEC	7.08	
Caenorhabditis elegans *Nematoda (freshwater; ISO/CD 10872)*	**Reproduction**	**4d NOEC**	**4.31**	**558**
Arenicola marina *Polychaeta (marine; non-standard test with field collected sediment)*	Mortality	10d LC_{50}	16.48[a]	112,559
	Mortality	10d NOEC	12.50	559
	Mortality	100d LC_{50}	15.56	
	Casting	10d EC_{50}	5.19	
	Casting	10d NOEC	2.16	112
	Casting	100d EC_{50}	6.41	
	Casting	100d NOEC	<0.43	
Corophium volutator *Crustacea (marine; test with field collected sediment)*	**Mortality**	**10d LC_{50}**	**10.68[a]**	**559,560**
	Mortality	10d NOEC	1.67	560
	Mortality	28d LC_{50}	14.56	112
Asterias rubens *Echinodermata (marine; non-standard test with field collected sediment)*	Mortality	10d LC_{50}	11800	560
	Mortality	10d NOEC	2500	

[a] Geometric mean

For marine benthic organisms, toxicity data are available but the tests were not conducted according to standard test protocols, with the possible exception of the test with the crustacean *C. volutator*. The Tier-1 $PNEC_{sed;ch}$ for marine/estuarine benthic organisms can be derived on the basis of Table 5 in different ways. To demonstrate the concept of the table, we will show all possibilities. One option is to use the three chronic toxicity data for standard freshwater test species by applying an AF of 100 to the lowest chronic NOEC/EC_{10} (Table 5). Applying an AF of 100 to the 28d EC_{10} of 0.14 µg/g OC of *C. riparius* results in a Tier-1 $PNEC_{sed;ch}$ of 0.0014 µg/g OC for sediment-dwelling organisms in marine/estuarine ecosystems. A second option is to use three semi-chronic toxicity

data for marine organisms by applying and AF of 30-100 to the lowest semi-chronic 10d L(E)C_{10}/NOEC (Table 5). In this case we selected an AF of 100 since the acute to chronic ratio for *Daphnia magna* was very large (Table 8). Applying an AF of 100 to the 10d NOEC of 1.67 µg/g OC of *C. volutator* results in a Tier-1 $PNEC_{sed;ch}$ of 0.0167 µg/g OC for sediment-dwelling organisms in marine/estuarine ecosystems. A third option is to use three semi-chronic toxicity data for marine organisms by applying an AF of 100 - 300 to the lowest semi-chronic 10d L(E)C_{50} (Table 5). Again we selected an AF in the higher range since the acute to chronic ratio for *Daphnia magna* was very large (Table 8). Applying an AF of 300 to the 10d EC_{50} of 5.19 µg/g OC of *C. volutator* results in a Tier-1 $PNEC_{sed;ch}$ of 0.0173 µg/g OC for sediment-dwelling organisms in marine/estuarine ecosystems. Each of the Tier-1 PNEC values is lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 9 and again is considerably higher than the Tier-0 $PNEC_{sed;ch;EP}$ calculated above. Options 2 and 3 based on marine species are very similar, but these options are an order of magnitude higher than the Tier-1 $PNEC_{sed;ch}$ derived for marine/estuarine ecosystems from the freshwater chronic toxicity data (due to the extra factor of 10 for the freshwater – marine extrapolation) (Fig. 4). To assess the $PNEC_{sed;ch}$ for estuarine/marine benthic species, it is logical to prefer options 2 and 3, since these options use toxicity data for marine/estuarine benthic organisms.

Tier-2 effect assessment based on standard and additional test species for ivermectin

Geometric mean approach

When analysing the toxicity data presented in Table 9, the geometric mean approach cannot be used since all toxicity data concern test species from different taxonomic groups.

Species Sensitivity Distribution approach

When analysing the toxicity data presented in Table 9, the SSD approach cannot be used since semi-chronic or chronic toxicity values for fewer than 8 benthic species are available.

Tier-3 effect assessment based on micro/mesocosm experiments for ivermectin

The effects of ivermectin exposure was investigated in indoor freshwater microcosms using ivermectin-spiked sediments, with a focus on the response of the nematode community [561]. An overall microcosm NOEC for Nematoda was observed at 0.4 µg/g OC. This value is approximately a factor of 10 lower than the 4d NOEC observed for the nematode *C. elegans* in a laboratory test. To date, it remains a research question whether this NOEC for the populations of Nematoda is representative for populations of other potentially sensitive taxonomic groups (e.g. arthropods, Oligochaeta and Polychaeta).

Conclusions from the ivermectin toxicity data for benthic organisms

- Applying the concept of EP to the $PNEC_{sw;ch}$ (based on water toxicity data for pelagic organisms) results in a very conservative estimate of the $PNEC_{sed;ch;EP}$ (Tier 0) (Figure 4)
- The semi-chronic sediment toxicity data for freshwater and marine benthic organisms overlap
- The derived $PNEC_{sed;ch}$ based on the Tier-1 approach (Table 5) was remarkably similar for freshwater and marine/estuarine species, at least when using the corresponding toxicity data
- In microcosms, the overall NOEC of the Nematode community was approximately a factor of 10 lower than the NOEC of the standard test nematode *C. elegans*

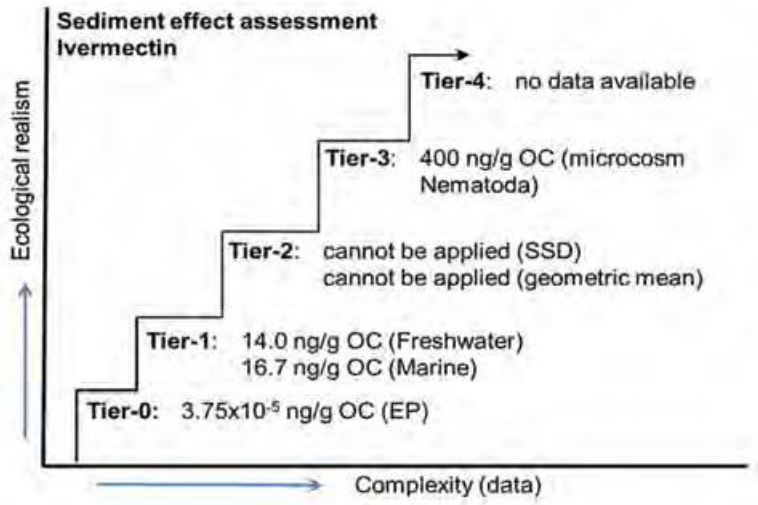


Figure 4. Predicted no effect concentration (ng/g OC) for ivermectin derived for different tiers.

8.10.2 The insecticide chlorpyrifos

Evaluation of standard and additional toxicity data for pelagic organisms and chlorpyrifos

The laboratory toxicity data for typical pelagic organisms and the insecticide chlorpyrifos are shown in Table 10.

It can be concluded from the information in Table 10 that invertebrate populations, and arthropods in particular, are probably the most sensitive taxonomic group on which a chronic effects assessment for sediment-dwelling organisms should focus. Note that the reported toxicity values for aquatic arthropods are at least one to two orders of magnitude lower than for algae and fish. The acute-to-chronic ratio for aquatic arthropods is approximately a factor of 10.

Table 10. Toxicity data for typical water column organisms and the insecticide chlorpyrifos.

Test species	Acute toxicity	Chronic toxicity	Reference
Skeletonema costatum *(marine diatom)*		EC50 = 403 µg/L	Alterra database
Daphnia magna *(Crustacea)*	48h EC_{50} = 0.4 µg/L	21d NOEC = 0.057 µg/L	Alterra database
Chironomus riparius *(Insecta)*	96h EC_{50} = 0.09 µg/L		Alterra database
Americamysis bahia *(Crustacea)*	96h EC_{50} = 0.04 µg/L	35d NOEC = 0.0046 µg/L	Alterra database
Oncorhynchus mykiss *(fish)*	96h LC_{50} = 3.0 µg/L	21d NOEC = 0.51 µg/L	Alterra database
Tier-1 $PNEC_{sw;ch}$		0.00046 µg/L	Application of AF of 10 to the chronic NOEC of *A. bahia*
SSD aquatic arthropods	Acute HC_5 = 0.042 µg/L (n =42)		Alterra database
Lowest NOEC micro/mesocosm	0.033 – 0.10 µg/L for arthropods(pulsed exposure)	0.01 µg/L for arthropods (more or less constant exposure)	Alterra database
Higher tier $PNEC_{sw;ch}$		0.005 µg/L	Application of AF of 2 to threshold level of 0.01 µg/L in chronic micro/ mesocosm study

Tier-0 effect assessment for chlorpyrifos based on Equilibrium Partitioning

K_{oc} values reported for chlorpyrifos have a geometric mean of 10617 L/kg (n=7) in the range of 3000–25,565 L/kg.[441] Initially we selected the lower tier $PNEC_{sw;ch}$ of 0.00046 µg/L (see Table 10) and the abovementioned geometric mean K_{oc} value, resulting in a $PNEC_{sed;ch;EP}$ value of 0.00049 µg/g OC using Eq. 2. We then selected the higher-tier $PNEC_{sw;ch}$ of 0.0033 µg/L (see Table 10) and the abovementioned geometric mean K_{oc} value, resulting in a $PNEC_{sed;ch;EP}$ value of 0.0035 µg/g OC using Eq. 2. We consider this latter value to be more realistic, since it is based on higher-tier information.

Tier-1 effect assessment for benthic organisms and chlorpyrifos

For one freshwater benthic insect species (*C. riparius*) a chronic sediment toxicity value is available (21d NOEC of 0.32 µg/g OC), although this value was not derived according to standard guidelines (Table 11). Furthermore, 10d LC_{50} values are available for the freshwater insect *C. dilutus*, for the freshwater/marine amphipod *H. azteca*, for the estuarine amphipod *E. estuarius* and for the marine amphipod *A. abdita*. These tests were conducted essentially in accordance with USA guidelines.

Table 11. Sediment toxicity data for benthic organisms and the insecticide chlorpyrifos. The values in bold concern the toxicity data acquired essentially in accordance with internationally accepted guidelines (see Table 2 in Chapter 2[259]).

Species and test protocol	Effect endpoint	Toxicity endpoint	Toxicity µg/g OC	Reference
Chironomus riparius *Insecta (Freshwater; field collected sediment)*	Mortality	4d LC_{50}	1.58[a]	433
	Mortality	21d LC_{50}	0.43[a]	
	Mortality	21d NOEC	0.32[a]	
Chironomus dilutus *Insecta (freshwater; ASTM E1706*	**Mortality**	**10d LC_{50}**	**7.19[a]**	**562,563**
Hyalella azteca *Crustacea; Amphipoda (fresh/ estuarine: ASTM E1706)*	**Mortality**	**10d LC_{50}**	**2.8[a]**	**564-566**
Ampelisca abdita *Crustacea; Amphipoda (marine: ASTM E1367)*	**Mortality**	**10d LC_{50}**	**15.9**	**567**
Eohaustorius estuarius *Crustacea; Amphipoda (estuarine: ASTM E1367)*	**Mortality**	**10d LC_{50}**	**13.2**	**567**
Amphiascus tenuiremus *Crustacea; Copepoda; field collected sediment*	Mortality	4d LC_{50}	1.74	568

[a] Geometric mean

The freshwater invertebrate species listed in Table 11 comprise only two taxonomic groups (insects and crustaceans) and the species *C. riparius*, *C. dilutus* and *H. azteca*. The insect *C. riparius* showed the lowest toxicity values (21d NOEC of 0.32 µg/g OC; 21d LC_{50} of 0.43 µg/g OC) but this test was not conducted according to standard test guidelines. However, the semi-chronic tests conducted with *C. dilutus* and *H. azteca* can be considered standard ASTM tests. Because of the specific mode of action of chlorpyrifos, two species are sufficient. Following the Tier-1 effect assessment according to Table 5, an AF of 100 - 300 has to be applied to the lowest 10d LC_{50} value of *C. dilutus* and *H. azteca*. In this case we selected an AF of 100 since the toxicity data for pelagic organisms showed a relatively low acute to chronic ration, suggesting a fast time to onset-of-effects. The amphipod *H. azteca* (geomean 10d LC_{50} of 2.8 µg/g OC) is the most sensitive, resulting in a Tier-1 $PNEC_{sed;ch}$ of 0.028 µg/g OC for sediment-dwelling organisms in freshwater ecosystems. This Tier-1 $PNEC_{sed;ch}$ value is substantially lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 11. Furthermore, this Tier-1 $PNEC_{sed;ch}$ value is higher than the Tier-0 $PNEC_{sed;ch;EP}$ calculated from the lower tier $PNEC_{sw;ch}$ and higher tier $PNEC_{sw;ch}$ (Figure 5).

In Table 11, semi-acute toxicity data for three marine/estuarine benthic organisms are shown. These data were acquired according to ASTM guidelines using the amphipods *H. azteca*, *A. abdita* and *E. estaurius*. These taxa comprise only one taxonomic/feeding group. However, when the 4d LC_{50} value for the marine copepod *A. tenuiremus* is included in the Tier-1 core data set, the marine toxicity data then comprise two feeding strategies and two

taxonomic groups. The Tier-1 $PNEC_{sed;ch}$ for marine/estuarine benthic organisms can be derived by applying an AF of 100 - 300 to the lowest LC_{50} for the combination *H. azteca*, *A. abdita*, *E. estaurius* and *A. tenuiremus.* Again we selected and AF in the lower range because of the relatively low acute to chronic ration for pelagic organisms. Although not a standard test species, the marine benthic copepod has the lowest LC_{50} value (1.74 µg/g OC), resulting in a Tier-1 $PNEC_{sed;ch}$ of 0.0174 µg/g OC for sediment-dwelling organisms in marine/estuarine ecosystems. This Tier-1 $PNEC_{sed}$ value is substantially lower than all toxicity values reported for freshwater and marine benthic organisms presented in Table 11. Again, this Tier-1 $PNEC_{sed;ch}$ value is higher than the Tier-0 $PNEC_{sed;ch;EP}$ calculated from the lower tier $PNEC_{sw;ch}$, but equals Tier-0 $PNEC_{sed;ch;EP}$ values calculated from the higher tier $PNEC_{sw;ch}$ (Figure 5).

Tier-2 effect assessment based on standard and additional test species for chlorpyrifos

Geometric mean approach

When analysing the data presented in Table 11, the geometric mean approach is only possible for the 10d LC_{50} values for the amphipods *H. azteca*, *A. abdita* and *E. estuarius*. The geometric mean LC_{50} for these taxa is 8.4 µg/g OC. This value is higher than the 10d LC_{50} of 2.8 µg/g OC for *H. azteca* (the most sensitive species in the freshwater data set) and the 4d LC_{50} of 1.74 µg/g OC for *A. tenuiremus* (the most sensitive species in the marine/estuarine data set). Applying the geometric mean approach (AF of 100 as used in Tier-1 and the geometric mean LC_{50} of 8.4 µg/g OC), results in a Tier-2 $PNEC_{sed;ch}$ values of 0.084 µg/g OC. This value can be used for both freshwater and marine taxa since for both types of organisms sufficient semi-chronic toxicity data are available.

Species Sensitivity Distribution approach

When analysing the toxicity data presented in Table 11, the SSD approach cannot be used since sediment toxicity data are available for fewer than 8 benthic species.

Tier-3 effect assessment based on micro/mesocosm experiments

An appropriate micro/mesocosm test that allowed concentration-response relationships for benthic organisms and sediment exposure concentrations to be derived could not be found in the open literature.

Conclusions from the chlorpyrifos toxicity data for benthic organisms for chlorpyrifos

- Applying the concept of EP to the higher-tier $PNEC_{sw;ch}$ (based on a microcosm test with a chronic exposure regime) results in a lower $PNEC_{sed;ch;EP}$ (Tier-0) estimate when compared with the Tier-1 $PNEC_{sed;ch}$ estimates for both freshwater and marine/estuarine ecosystems (Figure 5)
- The available sediment toxicity data are limited to arthropods and are predominantly semi-chronic in nature
- The sediment toxicity data for freshwater and marine benthic arthropods overlap

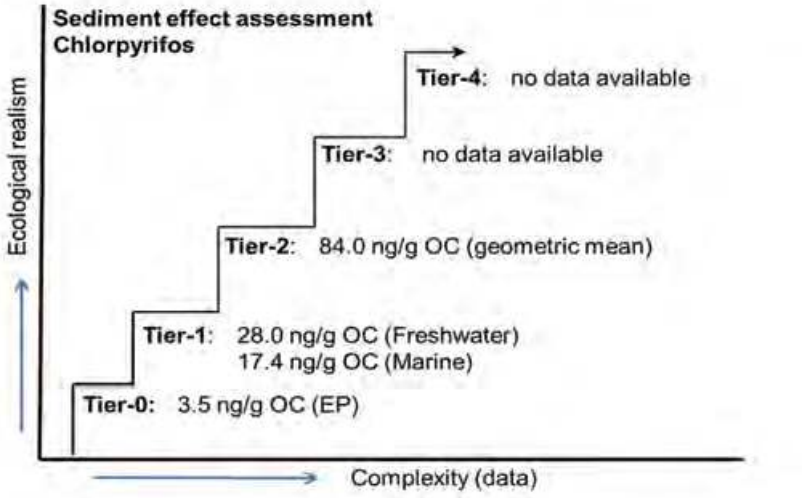


Figure 5. Predicted no effect concentration (ng/g OC) for chlorpyrifos derived for different tiers.

8.10.3 The biocide tributyltin

Evaluation of standard and additional toxicity data for pelagic organisms and tributyltin

The laboratory toxicity data for water organisms and long-term water exposure to the biocide tributyltin are shown in Table 12.

It can be concluded from the information in Table 12 that Mollusca are probably the most sensitive taxonomic group. However, the chronic toxicity values for aquatic arthropods are reported to be relatively low as well. The $PNEC_{sw;ch}$ for pelagic organisms can be derived by applying an AF of 10 to the chronic NOEC of *Nucella lapillus*, resulting in a value of 0.0002 µg/L. This value is remarkably similar to the annual average quality standard (AA-QS) (0.0002 µg/L) derived for tributyltin compounds as part of the Water Framework Directive[569].

Tier-0 effect assessment for tributyltin based on Equilibrium Partitioning

K_{oc} values reported for tributyltin compounds have a geometric mean of 1317 L/kg (n=16) with a range of 188–2814.[573] We selected the $PNEC_{sw;ch}$ of 0.0002 µg/L (see Table 12) and the geometric mean K_{oc} value of 1317 L/kg, resulting in a $PNEC_{sed;ch;EP}$ value of 2.63×10^{-5} µg/g OC using Eq. 2.

Table 12. Chronic toxicity data for water organisms and the biocide tributyltin (data from IPCS[570]; EPA[571]; and Hall et al.[572]).

Test species	**Criterion**	**Chronic toxicity**
Algae	IC_{50} (primary production)	0.92 – 320 µg/L
Daphnia magna *(Crustacea; Cladocera)*	21d NOEC (life cycle test)	0.14 – 0.25 µg/L
Acartia tonsa *(Crustacea; Copepoda)*	6 d geometric mean of NOEC/LOEC	0.014 µg/L
Euryptemora affinis *(Crustacea; Copepoda)*	13d geometric mean of NOEC/LOEC (Life Cycle test)	<0.088 and 0.15 µg/L
Acanthomysis scuppta *(Crustacea; Mysidae)*	63d geometric mean of NOEC/LOEC (Life Cycle test)	0.13 µg/L
Mytilus edulus *(Mollusca; Bivalvia)*	33d geometric mean of NOEC/LOEC	0.017 µg/L
Crassostrea gigas *(Mollusca; Bivalvia)*	geometric mean of NOEC/LOEC Shell thickening	0.02 µg/L
Nucella lapillus *(Mollusca; Gastropoda)*	2 year geometric mean of NOEC/LOEC (imposex)	0.002 µg/L
Oncorhynchus mykiss *(fish)*	110d; 20% growth reduction	0.2 µg/L
Pimephales promelas *(fish)*	33d geometric mean of NOEC/LOEC (Early life stage test)	0.26 µg/L
$PNEC_{sw;ch}$	Application of AF of 10 to the chronic NOEC of *Nucella lapillus*	0.0002 µg/L

Tier-1 effect assessment for benthic organisms and tributyltin

An overview of the toxicity data for benthic invertebrates and spiked sediment tests with tributyltin is presented in Table 13. Note that in several of the studies reported in this table, toxicity values were expressed in terms of ng Sn/g DW sediment. These values were converted to µg TBT/g OC with a factor of 2.6 (=118.7/307.06), derived by the division of the molecular mass of tin by the molecular mass of tributyltin.

Table 13. Sediment toxicity data for benthic organisms and the biocide tributyltin. The values in bold concern toxicity data acquired essentially in accordance with internationally accepted guidelines (see Table 2 in Chapter 2[259]). Note that in several of the studies reported in this table, toxicity values were expressed in terms of ng Sn/g DW sediment.

Species and test protocol	Effect endpoint	Toxicity endpoint	Toxicity µg TBT/g OC	Reference
Chironomus riparius	Mortality	28d LC_{50}	227.9	574
Insecta (Fresh; artificial sediment;	Mortality	28d NOEC	76.0	
semi-artificial sediment)	**Male emergence time**	**28d EC_{10}[a]**	**14.7**	
	Growth	10d EC_{50}	750.3	575
	Growth	10d NOEC	296.6	
Hexagenia	Mortality	21d LC_{50}	296.6	575
Insecta (fresh: semi-artificial	Growth	21d EC_{50}	104.7	
sediment)	**Growth**	**21d NOEC**	**52.3**	
Tubifex tubifex	Mortality	28d LC_{50}	2320.8	575
Oligocheate (fresh: semi-artificial	Growth	28d EC_{50}	279.2	
sediment)	**Growth**	**28d NOEC**	**122.1**	
Hyalella azteca	Mortality	28d LC_{50}	189.8	576
Crustacea; Amphipoda (fresh/	Mortality	70d LC_{50}	121.3	
estuarine: field collected sediment;	**Mortality**	**70d LC_{10}[a]**	**26.0**	
semi-artificial sediment)	Reproduction	70d EC_{50}	30.9	
	Growth	14d EC_{50}	244.3	575
	Growth	14d NOEC	139.6	
Potamopyrgus antipodarum	Mortality	28d LC_{50}	58.5	577
Mollusca; Gastropoda	Mortality	56d LC_{50}	44.8	
(freshwater; artificial sediment)	Total embryos development	28d EC_{50}	18.0	
	Total embryos development	56d EC_{50}	9.8	
	Total embryos development	28d EC_{10}	1.103	
	Total embryos development	56d EC_{10}	0.365	
Corophium volutator	**Mortality**	**10d LC_{50}**	**5.7**	108
Crustacea; Amphipoda				
(marine; field collected sediment)				
Eohaustorius washingtonianus	Mortality	9d LC_{50}	170	578
Crustacea; Amphipoda (marine:	Mortality	41d LC_{50}	78	
field collected sediment)				
Rhepoxynius abronius	Mortality	10d LC_{50}	3500	578
Crustacea; Amphipoda (marine;				
field collected sediment)				
Armandia brevis	Mortality	10d LC_{50}	930	578
Polychaeta	Mortality	42d LC_{50}	158.2	
(marine: field collected sediment)	Growth	42d EC_{50}	38.7	579
	Growth	42d EC_{10}	5.9	
Echinocardium cordatum	Mortality	14d LC_{50}	10.5	108
Echinodermata (marine; field	Mortality	28d LC_{50}	4.1	
collected sediment)	Mortality	28d NOEC	2.94	
Ruppia maritima	Relative growth rate	21d EC_{10}[a]	0.692	123
Seagrass (marine: field collected				
sediment)				

[a] Estimated value from graph

The chronic NOEC/L(E)C_{10} toxicity values for standard freshwater benthic invertebrates concern the insect *C. riparius* (28d EC_{10} of 14.7 µg TBT/g OC), the insect *Hexagenia* (21d NOEC of 52.3 µg TBT/g OC), the crustacean *H. azteca* (28d LC_{10} of 26.0 µg TBT/g OC) and the oligocheate *T. tubifex* (28d NOEC of 122.1 µg TBT/g OC) (Table 13). Another chronic toxicity value for a freshwater benthic organism concerns the freshwater snail *Potamopyrgus antipodarum* (56d EC_{10} of 0.365 µg TBT/g OC) (Table 13). Although the latter species is not a standard test species, it is considered a relevant Tier-1 test species, since the information presented in Table 12 shows that molluscs in particular are the most sensitive taxonomic group.

Following the Tier-1 effect assessment according to Table 5, an AF of 10 has to be applied to the lowest chronic NOEC/EC_{10} value for the combination *C. riparius*, *Hexagenia*, *H. azteca*, *P. antipodarum* and *T. tubifex*. The snail *P. antipodarum* (56d EC_{50} of 0.365 µg TBT/g OC) is the most sensitive, resulting in a Tier-1 $PNEC_{sed;ch}$ of 0.0365 µg TBT/g OC for sediment-dwelling organisms in freshwater ecosystems. This value is considerably higher than the Tier-0 $PNEC_{sed;ch;EP}$ value mentioned above based on the EP concept (Figure 7).

In Table 13, chronic NOEC/EC_{10} values are available for four marine/estuarine benthic organisms: the amphipod *H. azteca*, the polychaete *Armandia brevi*, the echinoderm *E. cordatum* and the seagrass *Ruppia maritime*. Only *H. azteca* is a standard test species. Furthermore, for one standard test species (the amphipod *C. volutator*) a 10d LC_{50} is available. However these taxa do not comprise Mollusca, the most sensitive taxonomic group mentioned in Table 13 (water exposure tests). Consequently, the freshwater snail *P. antipodarum* (56d EC_{10} of 0.365 µg TBT/g OC) was also considered when deriving a Tier-1 $PNEC_{sed;ch}$ for marine/estuarine ecosystems. Following the Tier-1 effect assessment according to Table 5, an AF of 10 has to be applied to the lowest chronic NOEC/EC_{10} for the combination *H. azteca*, *A. breva*, *E. cordatum*, *Ruppia maritime* and *P. antipodarum.* The snail *P. antipodarum* (56d EC_{10} of 0.365 µg TBT/g OC) is the most sensitive, resulting in a Tier-1 $PNEC_{sed;ch}$ of 0.0365 µg TBT/g OC for sediment-dwelling organisms in estuarine/marine ecosystems. Again, this value is considerably higher than the Tier-0 $PNEC_{sed;ch;EP}$ value mentioned above based on the EP concept (Figure 7). Alternatively, a Tier-1 $PNEC_{sed;ch}$ for marine/estuarine ecosystems can be derived by using the semi-chronic toxicity data for the amphipods *H. azteca* (14d EC_{50} of 244.3 µg TBT/g OC), *C. volutator* (10d LC_{50} of 5.7 µg TBT/g OC), *E. washingtonianus* (9d LC_{50} of 170 µg TBT/g OC) and *R. abronius* (10d LC_{50} is 3500 µg TBT/g OC), the polychaete *A. brevis* (10d LC50 is 930 µg TBT/g OC) and the echinoderm *E. cordatum* (14d LC_{50} is 10.5 µg TBT/g OC). These marine taxa comprise three taxonomic groups, so that an AF of 100 - 300 (see Table 5) can be applied to the lowest semi-chronic L(E)C_{50} to derive a $PNEC_{sed;ch.}$ We selected an AF of 300 since the available toxicity data reveal latent effects and hormone-disrupting properties of TBT. Applying and AF of 300 to the lowest 10d LC_{50} (5.7 µg TBT/g OC for *C. volutator*) results in a $PNEC_{sed;ch}$ of 0.019 µg TBT/g OC for marine/estuarine benthic organisms. Note that this $PNEC_{sed;ch}$ value is lower than the Tier-1 $PNEC_{sed;ch}$ of 0.0365 µg TBT/g OC for sediment-dwelling organisms in estuarine/marine ecosystems derived on the basis of chronic toxicity data. However, the chronic Tier-1 $PNEC_{sed;ch}$ was selected in

the effect assessment, since an assessment based on chronic toxicity data overrules that based on semi-chronic toxicity data.

Tier-2 effect assessment based on standard and additional test species for tributyltin

Geometric mean approach

Considering the data presented in Table 13, and the criteria for the geometric mean approach mentioned in section 8.9.4, this approach seems possible only for the 9-10d LC_{50} values for the marine amphipods *C. volutator*, *E. washingtonianus* and *R. abronius*, resulting in a geometric mean LC_{50} of 150.2 µg TBT/g OC for these marine amphipod taxa. For two other marine taxonomic groups, a single semi-chronic LC_{50} value is available: for the polychaete *A. brevis* (10d LC_{50} of 930 µg TBT/g OC) and the echinoderm *E. cordatum* (14d LC_{50} of 10.5 µg TBT/g OC). The value for *E. cordatum* is lower than the geometric mean LC_{50} for marine amphipods, so this value has to be selected for the Tier-2 $PNEC_{sed;ch}$ derivation according to the geometric mean approach, although only a single value is available for Echinodermata. To derive a $PNEC_{sed;ch}$, an AF of 100 - 300 (see Table 5) can be applied to the geometric mean semi-chronic $L(E)C_{50}$ value of the most sensitive taxonomic group. We selected an AF of 300 since the available toxicity data reveal latent effects and hormone-disrupting properties of TBT. Applying an AF of 300 (see Table 5) to the LC_{50} of 10.5 µg TBT/g OC for *E. cordatum* results in a $PNEC_{sed;ch}$ estimate of 0.035 µg TBT/g OC for marine/estuarine benthic organisms. Note that for estuarine/marine benthic organisms this Tier-2 $PNEC_{sed;ch}$ (based on semi-chronic toxicity data) is somewhat higher that the Tier-1 $PNEC_{sed;ch}$ value of 0.019 µg TBT/g OC based on semi-chronic toxicity data. Since the Tier-2 $PNEC_{sed;ch}$ value based on the geometric mean approach is somewhat lower than the Tier-1 $PNEC_{sed;ch}$ of 0.0365 µg TBT/g OC for sediment-dwelling organisms in estuarine/marine ecosystems derived on basis of chronic toxicity data, the geometric mean approach in this case does not help to refine the effect assessment (Figure 7).

Species Sensitivity Distribution approach

Table 14 gives an overview of the $PNEC_{sed;ch}$ derivation based on the SSD approach and by using the chronic or semi-chronic toxicity values presented in Table 14. Since chronic EC_{10}/NOEC are available for only seven species, the procedure described in Table 7 was used to estimate the chronic NOEC/EC_{10} based on chronic $L(E)C_{50}$ values. To illustrate the SSD approach as recommended in Section 8.9.4, several SSDs were constructed. Two SSDs were constructed with chronic toxicity data, one with 9 species (A in Table 14) and the other with 8 species (B in Table 14). In addition, three SSDs were constructed with semi-chronic toxicity data for 10 species (C in Table 14), 9 species (D in Table 14) and 8 species (E in Table 14). For all the SSDs constructed and summarized in Table 14, the Anderson-Darling test for normality was accepted at all levels, indicating that the curves fitted the toxicity data well. Figure 6 presents the SSD curve constructed with chronic toxicity data for 9 species of benthic freshwater and marine/estuarine organisms (A; upper panel) as well as the SSD curve constructed with semi-chronic toxicity data for 10 species (B; lower panel).

The median HC_5 values for tributyltin based on semi-chronic data are in most cases more than a factor 10 higher than the HC_5 values based on chronic data. We proposed that a $PNEC_{sed;ch}$ can be estimated using the semi-chronic HC_5 by applying an AF according to the criteria mentioned in Table 6, as well as an extra AF of 5 – 10. Because of the hormone-disruptive properties of TBT we propose to select the extra AF in the high range (10).

The $PNEC_{sed;ch}$ estimates based on the SSD approach as presented in Table 14 are remarkably similar between procedures that use the same number of species with chronic and semi-chronic toxicity data. For example the procedure using 8 species with chronic toxicity data resulted in a $PNEC_{sed;ch}$ of 0.055 µg TBT/g OC, while the procedure using 8 species with semi-chronic toxicity data resulted in a $PNEC_{sd;ch}$ of 0.048 µg TBT/g OC for freshwater taxa and 0.064 µg TBT/g OC for marine taxa (Table 14). This suggests that the SSD approach as proposed in Section 8.9.4 works well. However, a $PNEC_{sed;ch}$ preferably should be derived based on chronic toxicity data and a $PNEC_{sed;ch}$ thus obtained overrules a $PNEC_{sed;ch}$ derived based on semi-chronic toxicity data. The preferred chronic $PNEC_{sd;ch}$ of 0.074 µg TBT/g OC is higher the PNEC derived in Tier-0, Tier-1 and in the geometric mean approach in Tier-2 (Table 6).

The data presented in Table 14 also show that the median HC_5 value increases and its confidence interval decreases if a larger number toxicity data is used to construct the SSD. This indicates that it may be rewarding in the Tier-2 effect assessment to generate spiked sediment toxicity data for a higher number of benthic taxa.

Table 14. Overview of $PNEC_{sed;ch}$ values (µg TBT/g OC) for tributyltin derived by means of SSDs constructed with chronic or semi-chronic toxicity data for benthic organisms (see Table 13). **A**: SSD constructed with chronic toxicity data for 9 species as presented in Figure 6. **B**: SSD constructed with chronic toxicity data for 8 species similar to those presented in Figure 6, except *Eohautorius washingtonianus*. **C**: SSD constructed with semi-chronic toxicity data for 10 species similar to those presented in Figure 6. **D**: SSD constructed with semi-chronic toxicity data for 9 species similar to those presented in Figure 7 except Hexagenia. **E**: SSD constructed with semi-chronic toxicity data for 8 species similar to those presented in Figure 6 except Hexagenia and *Tubifex tubifex*.

	Endpoints	No of species	No of taxonomic groups	Lower limit HC_5 (µg TBT/g OC)	Median HC_5 (µg TBT/g OC)	Upper limit HC_5 (µg TBT/g OC)	HC_5/ Lower HC_5	AF[a] Fresh	AF[a] marine	SSD-$PNEC_{sed;ch}$ Fresh (µg TBT/g OC)	SSD-$PNEC_{sed;ch}$ Marine (µg TBT/g OC)
A	Chronic	9	7	0.024	0.296	1.145	12	4	4	0.074	0.074
B	Chronic	8	7	0.012	0.220	0.999	19	4	4	0.055	0.055
C	Semi-chronic	10	6	0.334	4.124	17.087	12	4*10[b]	4*10[b]	0.103	0.103
D	Semi-chronic	9	6	0.185	3.401	16.556	18	4*10[b]	4*10[b]	0.085	0.085
E	Semi-chronic	8	5	0.078	2.424	14.355	31	5*10[b]	4*10[b]	0.048	0.061

[a]For criteria see Table 6, [b]An additional AF of 10 is applied to account for the extrapolation of semi-chronic toxicity data to chronic toxicity data

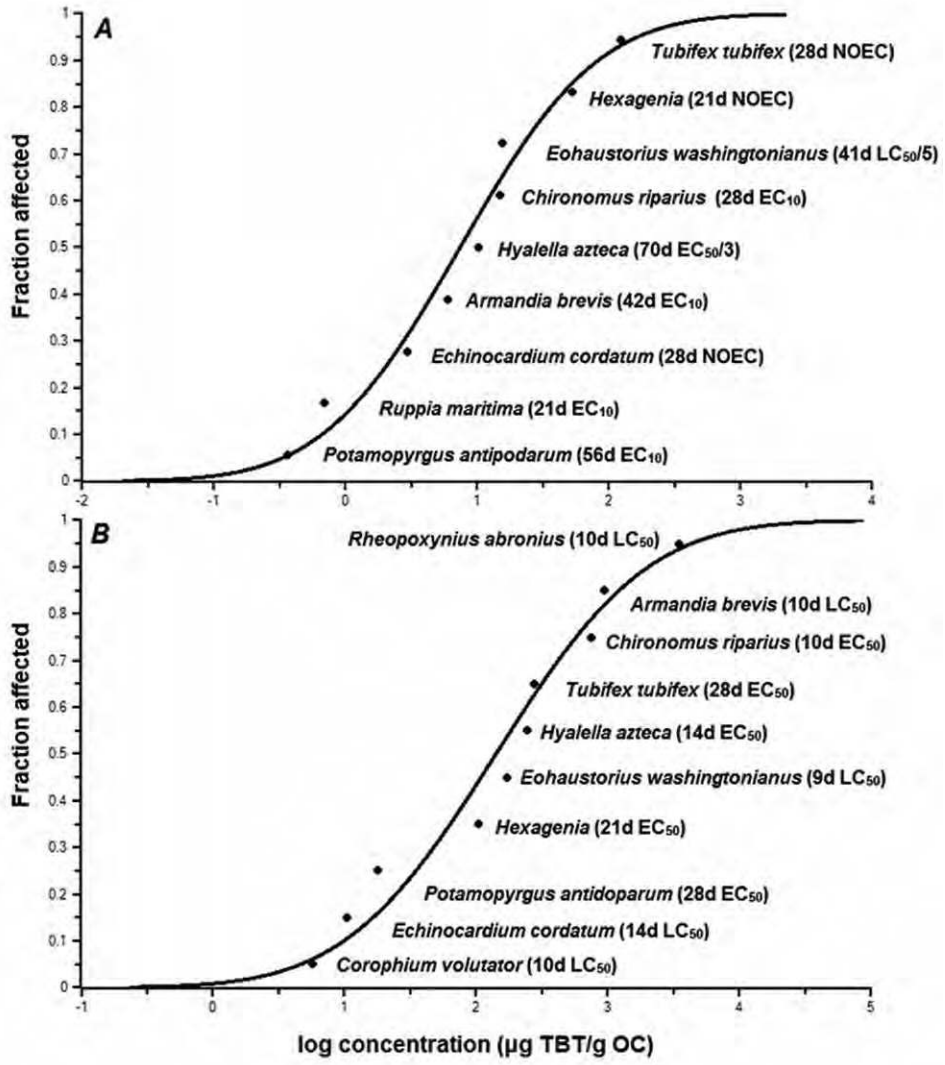


Figure 6. Species Sensitivity Distribution (SSD) for tributyltin constructed with (A) (estimated) chronic EC_{10}/NOEC values for freshwater and marine benthic invertebrates (n=9) and (B) semi-chronic $L(E)C_{50}$ values for freshwater and marine benthic invertebrates (n=10) (data from Table 14).

8

Tier-3 effect assessment based on micro/mesocosm experiments for tributyltin

Appropriate spiked sediment micro/mesocosm tests could not be found.

Conclusions from the tributyltin toxicity data for benthic organisms

- Applying the concept of EP to the $PNEC_{sw;ch}$ (based on water toxicity data for pelagic organisms) results in a conservative estimate of the $PNEC_{sed;ch;EP}$ (Tier-0) (Figure 7)
- The chronic NOEC/EC_{10} value (spiked sediment test) was lowest for a mollusc, which is in accordance with available toxicity data for water organisms and water exposure tests

- The sediment toxicity data for freshwater and marine arthropods overlap
- The toxicity data for both freshwater and marine benthic organisms can be used to construct an SSD with an appropriate fit
- The $PNEC_{sed;ch}$ value for tributyltin derived on the basis of the SDD approach is approximately a factor of 2 higher than the Tier-1 $PNEC_{sed;ch}$

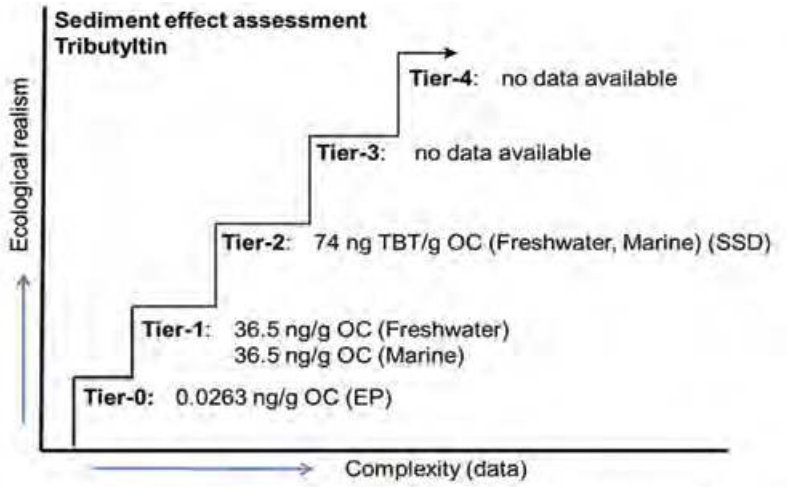


Figure 7. Predicted no effect concentration (ng/g OC) for tributyltin for different tiers.

8.10.4 Main outcomes from the case studies

In general, it can be concluded that the available sediment toxicity data are limited and the reported measurement endpoints are variable. Sediment toxicity data for freshwater and marine/estuarine benthic organisms often overlapped. Available data were mainly limited to arthropods and were predominantly sub-chronic in nature. For the insecticide chlorpyrifos, however, the focus on benthic arthropods is logical considering its specific toxic mode-of-action and the extensive dataset for water column organism, which indicates that aquatic arthropods are the sensitive taxonomic group

Applying the concept of EP to the $PNEC_{sw;ch}$ (based on water toxicity data for pelagic organisms) results in a very conservative estimate of the $PNEC_{sed;ch;EP}$ (Tier-0) for ivermectin and a conservative estimate for chlorpyrifos and tributyltin. For chlorpyrifos, however, by using the higher tier $PNEC_{sw;ch}$ (on basis of a chronic micro/mesocosm study) in the equation then the Tier-0 $PNEC_{sed;ch;EP}$ resembles the Tier-1$PNEC_{sed;ch}$ estimate for estuarine and marine species, but is a factor of 2-3 lower than the Tier-1 $PNEC_{sed;ch}$ for freshwater species. Aquatic data can provide good indicators for the most sensitive species group, as was shown for tributyltin, where the chronic $NOEC/EC_{10}$ value (spiked sediment test) was lowest for a mollusc, which is in accordance with available toxicity data for water organisms and water exposure tests.

The case studies illustrate that the geometric mean approach is of limited value in the chronic effect assessment for benthic organisms. However, these studies also show that toxicity data for both freshwater and marine benthic organisms can be used to construct an SSD with an appropriate fit. For tributyltin, the $PNEC_{sed;ch}$ values derived on the basis of the SDD approach are approximately a factor of 2 higher than the Tier-1 $PNEC_{sed;ch}$.

In microcosms in which the sediment was spiked with ivermectin, the overall NOEC of the nematode community was approximately a factor of 10 lower than the NOEC of the standard test nematode *C. elegans*.

8.11 Outlook

Sediments are often contaminated with a mixture of chemicals. Therefore, future efforts should be made to move from the current ERA, which is based on single substance exposure, to an approach that deals with multiple chemicals. The TKTD approach may be a good tool to deal with multiple exposures. Exposure to multiple stressors requires clear scenarios that combine exposure and ecology related elements.[580]

Overall, a holistic approach that combines experimental work and fate and effect modelling is needed to develop better and more cost-effective prognostic tools for sediment risk assessment.

8.12 Summary

Benthic organisms provide important ecosystem services and functions, and should therefore be protected. However, a broadly accepted framework for prospective ERA of sediment-bound organic chemicals is currently lacking. Such a framework requires clear protection goals, evidence-based concepts that link exposure to effects and a transparent tiered effect assessment. SPUs identified based on the ecosystem service concept are microorganisms, benthic algae, sediment-rooted macrophytes, benthic invertebrates and benthic vertebrates for both freshwater and marine sediments, which are similar to SPUs derived for the aquatic system. The proposed SPUs and their specific protection goals should be generally accepted and implemented to operationalize sediment risk assessment schemes.

There is an urgent need for harmonization of data requirements test protocols, and risk assessment frameworks between regulations/directives. The first step is to determine and agree on a set of harmonized triggers for sediment testing. These triggers should consist of a combination of chemical properties and toxicity triggers. When testing is required, sediment-spiked laboratory toxicity tests with standard test species should focus on long-term tests with chronic endpoints. The range of standard test species for sediment testing currently in use in Europe should be extended with species that differ in taxonomy, feeding traits and ecosystem, such as estuarine and marine species.

When defining guidance for both prospective exposure and effect assessment, chemical, biological, spatial and temporal factors should be taken into account in experimental and model approaches. For fate models there is a need for approaches to translate biodegradation process parameters obtained from lab tests to parameters that are relevant in the field. The development of passive samplers for more classes of chemical can provide more accurate input for such models. For prospective exposure modelling, more realistic exposure models are needed for emerging chemical classes like ionizable organics and polar substances; these models should also take degradation processes into account. Development of realistic exposure scenarios is a prerequisite to successfully apply exposure models.

To correctly link exposure and effect, the ERC for the PEC_{sed} and $PNEC_{sed}$ used in the RQ should be expressed in the same type of concentration. Ideally, internal concentrations should be measured during the experiment. As a minimum, the concentration in pore water and in total sediment (in units of mass of organic chemical per mass of dry sediment) and the organic matter content (%) of the dry sediment should be measured, as well as the concentration in the overlying water. Model approaches may be used to calculate chemical concentrations in environmental compartments in which data is lacking. For exposure in chronic risk assessment, either the $PEC_{sed;max}$ or $PEC_{sed;TWA}$ can be used to compare with the $PNEC_{sed;ch}$. Guidelines should give a clear and uniform description of the concentration that should be used both in exposure and effect assessment. They should also specify where (organism, water and sediment compartments, sediment layer) and when the exposure concentration should be measured.

For the first step in effect assessment, prior to actual testing, a cost-effective Tier-0 screening based on aquatic toxicity data and EP with an extra factor of 10 that accounts for BC and ingestion is recommended. This approach gives important information on the most sensitive groups and in some cases provides conservative protection levels. The case studies showed that this approach is moderately to very conservative for these chemicals.

In the Tier-1 approach to derive a $PNEC_{sed}$, spiked sediment laboratory toxicity testing with standard benthic test species and the application of an appropriate assessment factor (AF) is common practice. The size of the proposed AF to be applied depends on the number of available species with chronic and semi-chronic toxicity data and the taxonomy, feeding traits and ecosystem preference of the test species used.

Possible Tier-2 options are the geometric mean approach and Species Sensitivity Distribution (SSD) approach. Freshwater, estuarine and marine species can be combined in the Tier-2 approaches. For the time being, we recommend using the geometric mean approach only to conduct effect assessments based on acute/semi-chronic toxicity data (e.g. 10d $L(E)C_{50}$'s) for test species in the same taxonomic group (e.g. benthic insects, crustaceans, oligochaetes or polychaetes). Whether the geometric mean approach can also be applied to chronic toxicity data of the same taxonomic group addressing different measurement endpoints is still a topic for research. We propose that the SSD approach be used if toxicity data are available for eight or more benthic species. The SSD curve should

be constructed with either chronic or acute/semi-chronic toxicity data. The derivation of a $PNEC_{sed}$ based on the SSD approach is done by applying an appropriate AF to the HC_5. We propose basing the size of this AF on the number of species and quality of the available toxicity data used in the SSD. The proposed assessment factors to derive PNECs for the Tier-1 and Tier-2 should be officially accepted and implemented in the ERA by regulators in a uniform way between the different directives.

Microcosm and mesocosm experiments with spiked sediment are proposed as a 3rd experimental tier, although only limited experience is available with these types of tests. Effect models can be used to complement experimental data to link exposure to effect at different levels of biological organization and at different spatial and temporal scales. In a regulatory context, scenarios relevant for aquatic ecosystems in different EU Member States using patterns of organic chemicals that integrate exposure and effects are a prerequisite. An important future research activity, therefore, would be to develop and link scenarios in exposure and effect models that include the sediment compartment and selected standard and appropriate vulnerable benthic species.

To evaluate the consistency of the tiered approach as described in this paper for the effect assessment of sediment exposure, the higher tiers (e.g. spiked sediment microcosm tests) should be used to calibrate the lower tiers. However, hardly any data for calibration of the tiered approach is currently available. Moreover, there is an urgent need to derive tiered ERA schemes for vertebrates and microorganisms, as insufficient data, methods and experience are currently available to do so.

Acknowledgements

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Supporting Information

Table S1. List of workshop participants

Name	Surname	Affiliation
Gertie	Arts	Alterra Wageningen UR
Hans	Baveco	Alterra Wageningen UR
Theo	Brock	Alterra Wageningen UR
Eric	Bruns	Bayer
Noël	Diepens	Wageningen University
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Malyka	Galay-Burgos	Ecetoc
Mick	Hamer	Syngenta
Bruno	Hubesch	CEFIC
Bart	Koelmans	Wageningen University
Stuart	Marshall	Unilever
Andreu	Rico	Wageningen University
Mauricio	Rocha Dimitrov	Wageningen University
Cor	Schipper	Deltares
Livia	Sidney	Wageningen University
Hauke	Smidt	Wageningen University
Ariadna	Szczybelski	Wageningen University
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Bert	Van Hattum	VU University Amsterdam
Erik	Verbruggen	RIVM
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Chapter 9

Synthesis and general discussion

"…man, far from being the overlord of all creation, is himself part of nature, subject to the same cosmic forces that control all other life. Man's future welfare and probably even his survival depend upon his learning to live in harmony, rather than in combat, with these forces."

- **Rachel Carson** -

"Essay on the Biological Sciences"

Good Reading, 1958

Introduction

Aquatic ecosystems have been contaminated with xenobiotic organic chemicals for decades due to increasing anthropogenic activities worldwide. Aquatic sediments are an important part of the aquatic ecosystem, providing critical ecosystem services and processes.[3]

Sediments act as a sink for hydrophobic organic chemicals,[342] which poses direct risks to benthic organisms and indirect to other organisms through the food chain and shifts in interactions between populations of the benthic community. This can affect the services provided by the aquatic ecosystem. Despite the observed development in sediment toxicity testing over the past two decades, sediment test methods and prospective environmental risk assessment (ERA) schemes are currently insufficiently developed to adequately predict the risk of sediment-bound organic chemicals in the environment.[19-21] In addition, there is a need for harmonization of data requirements, test protocols and risk assessment frameworks between regulations and directives. This was already recognized 24 years ago by Burton and Scott[19] and highlighted recently during the ECHA workshop "Principles for environmental risk assessment of the sediment compartment" in 2013.[20] Four major research needs for sediment toxicity tests and sediment risk assessment frameworks were identified in **Chapter 1** as:

1. Need for sediment toxicity test methods for a broader range of benthic species potentially at risk.
2. Need for a unifying and overarching conceptual basis for toxicant- and species-specific exposure mechanisms in sediment toxicity tests.
3. Need for validated population models for typical benthic species in prospective ERA to predict effects sediment-bound chemicals.
4. Need for a risk assessment framework that is based on clearly defined specific protection goals and that unifies the different types of test results in a transparent tiered risk assessment procedure for sediment organisms and processes.

This thesis aimed to address these needs by providing recommendations for improved test methods and by increasing mechanistic understanding to assess potential effects of organic chemicals in sediments on macrophytes (**Chapter 3**), invertebrates (**Chapters 4** and **5**) and microorganisms (**Chapter 6**), across different taxonomic groups and levels of biological organisation (**Chapter 7**), in freshwater, estuarine and marine ecosystems. The overall aim was to support the development of whole sediment toxicity tests and the prospective risk assessment of sediment-bound chemicals (**Chapter 8**).

This synthesis discusses each major research need in the following sections. The first section starts with a discussion on challenges in sediment toxicity testing e.g. selection of test species and microorganism testing. In the second section, the roles of species traits, exposure pathways and bioaccumulation are discussed, in order to understand toxicity by understanding exposure. From the mechanistic understanding of exposure, recommendations for test methods are given, which benefits the development of toxicity testing. The third section discusses exposure and effect models for benthic species

as a complementary tool to experimental tests in the tiered effect assessment in the context of ERA. Finally, the fourth section focusses on prospective ERA for sediment-bound chemicals. Ten key points, based on findings of this thesis, are suggested to be implemented for a transparent tiered risk assessment procedure for sediment organisms and processes.

Challenges in sediment toxicity testing

Toxicity tests are essential for prospective ERA. Tiered effect assessment approaches are often used to evaluate the effect of chemicals on the environment (Figure 1). A first tier in the effect assessment usually starts with simple single species tests. When going to higher tiers, tests increase in complexity and ecological realism. Accordingly, lower tiers are more conservative than higher tiers.[14-16] **Chapter 2** critically reviewed the state of science with respect to sediment toxicity testing for single organic compounds in the context of prospective ERA. This chapter summarized the technical literature on whole-sediment toxicity tests for microorganisms, benthic invertebrates, macrophytes and benthic communities and concluded that the test approaches are currently still too heterogeneous. This hampers the translation of single species test results between freshwater, estuarine and marine ecosystems and their extrapolation to the population and community level. There is thus an urgent need to develop chronic sediment tests with a representative selection of species and endpoints that cover different trophic levels, taxonomic groups and exposure pathways and unify dose metrics and exposure assessment methodologies.

For the development of the tiered effect assessment, important steps are the formal selection of species for testing and sequential development of test methods for macrophytes, invertebrates and microorganisms at different levels of biological organisation. In the next subsection, the set of macrophytes and invertebrates species proposed for toxicity testing in **Chapter 2** is discussed based on findings in **Chapter 3**, **4** and **5**. Then, a closer look is given on how to deal with microorganisms in sediment tests, using findings from **Chapter 2** and **6**. The last subsection discusses microcosm and mesocosm test that look at higher levels of biological organisation.

Selection of test species for sediment toxicity tests

The effect assessment of sediment-bound chemicals is still mainly based on a few taxonomic groups of benthic species, despite some recent developments with respect to standard testing, e.g. the OECD protocol (239)[582] for the submerged macrophyte *Myriophyllum spicatum* and ASTM protocol (E2591 – 07)[499] for amphibians. Tests with macrophytes, microorganisms, estuarine and marine invertebrates, other than amphipods, and freshwater invertebrates, other than insects, are relatively rare.[17-20,28,126,148,303,583,584] The current set of test species poorly represents the wide range of species present in the sediment. A balanced suite of test species covering different taxonomic groups and species-specific traits was proposed for prospective assessment of sediment-bound chemicals (**Chapter 2**). Together with optimised standard protocols for long-term tests that account

for latent effects, these selected species can form the basis of the first tier of sediment toxicity risk assessment once they are formally approved in the regulatory context.

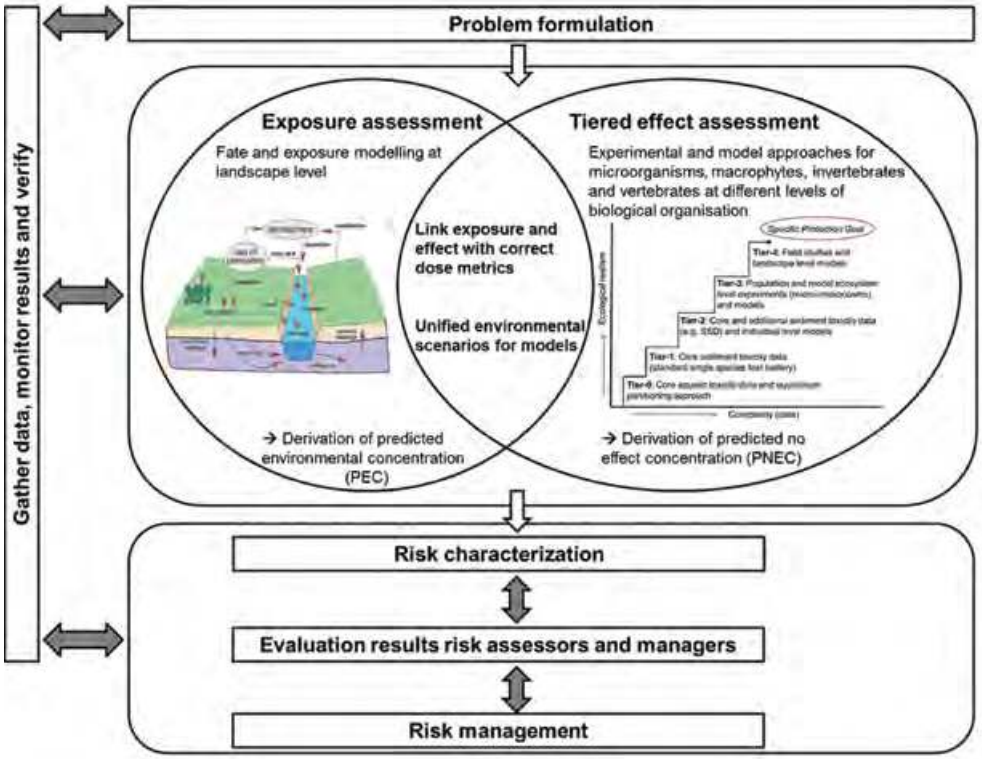

Figure 1. General framework for prospective environmental risk assessment. Grey arrows reflect interaction between risk assessors and managers and the continuous evaluation of information during the whole process. Adapted from Environmental Protection Agency (EPA)[581].

This research tested nine out of the 15 proposed species (**Chapter 2**) for bioaccumulation and exposure pathways from spiked artificial sediments, including two freshwater macrophytes, four freshwater invertebrates and three marine invertebrates (Figure 2) (**Chapter 3, 4** and **5)**. These species seem promising candidates for sediment toxicity testing as they bioaccumulate chemicals from the sediment compartment. Moreover, they are suitable based on criteria such as species collection time, laboratory handling and covering different taxonomic group and traits, whereas other proposed species *Echinocardium cordatum* and *Zostera* sp. were more difficult to collect and maintain. Nevertheless, they are still valuable candidate test species representing marine ecosystems. Currently, estuarine and marine standardized test protocols are only available for few taxonomic groups and mainly North American species. Therefore, effort should be made to develop tests for such species, especially because it is still unclear whether ERA based on traditional freshwater test species is protective for species in estuarine and marine ecosystems.

Microorganisms in sediment tests

The importance of microorganisms is often overlooked in standard testing (**Chapter 2**). Several studies address parts of the complex processes and interactions by which microorganisms can interact with organic chemicals in sediment tests.[40,162,163,166,273,328,366-377] Nevertheless, many questions on how to deal with microorganisms in standard testing are unanswered and the complexity of the many interactions is not fully understood. **Chapter 6** added several pieces of the puzzle by showing how bacterial communities and functions changed over time at different concentrations of organic matter in a 28 days bioaccumulation experiment with OECD artificial sediment and four marine benthic species.

To develop an approach that properly includes and deals with microorganisms in ERA it is important to make a clear distinction between two types of tests: tests with the focus on a) single non-microbial species (e.g. invertebrate or macrophyte) and b) tests with the focus on microorganisms only.

a) A test with either a single macrophyte or invertebrate species has the aim to determine the effects of a toxicant to the tested species. As argued in **Chapter 6,** 'pure' single species tests are difficult to perform. Sterile test systems are possible for macrophytes by sterilizing the plants (as done in the e.g. OECD test 238[585]) and for invertebrates by using germfree invertebrates.[586] However, an important question is how ecologically relevant these tests would be, especially in the light of symbiotic relationships with microorganisms. For example, the availability of essential inorganic nutrients for rooted macrophytes depends on microbial processes in the sediment compartment especially in the rhizosphere[587] and invertebrates need their gut flora for digestion. Besides, the microbial community forms a part of the diet of many benthic invertebrates.[163,166,366,367] Thus, microorganisms and macrophytes or invertebrates can interact with each other and the chemicals present in the test system in a complex way, as was shown in **Chapter 6**.[352,371-373,382] When targeting scientific questions only, it might be beneficial to work under sterile conditions, whereas in standard toxicity testing this will not be beneficial as it increases time, costs and probably control mortality. Consequently, tests can be performed following current standard guidelines using non-sterile sediments. However, more research is needed regarding the development of the microbial community in the (artificial) sediment of sediment toxicity tests, as well as the direct and indirect effects of microorganisms on test outcomes. Such research may include monitoring community composition and microbial functioning, for which functional gene abundance can be used as a proxy during the course of the experiment, and may address correlations between physicochemical variables in the water and sediment and microbial functions. Biodegradation of the tested chemical is a clear example of interaction between microbial function and test outcomes. Additionally, it was recommended in **Chapter 6** to always use a pre-equilibration period prior to the start of the experiment as this is also essential for growth and stabilization of the bacterial community in the test sediment. Moreover, it is suggested to measure chemical exposure over the experimental period to detect possible (bio)degradation.

b) A test with the focus on microorganisms has the aim to determine the effects of a toxicant to a single microbial strain, microbial functional endpoints and/or the entire microbial community. One main priority would be the development and approval of a standard test assessing community and functional endpoints of microbes. Such water sediment-spiked tests could be performed according to OECD guidelines for invertebrates leaving the invertebrates out and focusing on microbial endpoints as described in **Chapter 2**.

Before microorganisms and single species macrophyte and invertebrate tests can be used in risk assessment, several questions and issues need to be addressed. A first question should address if, and if so, what type of inoculum is preferred and how to standardize this. Either inoculum from natural sediment or a defined mixture of microbial cells created *in vitro* - a so called mock community[588] - could be used. When using an inoculum from a natural source the main question is how to standardize this, because environmental conditions change over time and thus change the inoculum. In terms of standardization and comparability among test outcomes and laboratories, a mock community representative for the ecosystem under evaluation would be preferred. This would mean that communities need to be defined for these ecosystems first, which will be a challenge as communities change over time and space. Defining communities for ecosystems would involve characterisation of microorganisms and understanding their interactions. In case an inoculum is used, the question remains how to proceed with the test itself to avoid contamination with microorganisms during different phases of the experimental procedures. As discussed above, microorganism tests can be done with initially sterile sediment inoculated with a well-described community of microbes under sterile conditions to prevent contamination, but for invertebrate single species tests this might be difficult. Another important issue is the identification of biological variability in community dynamics and function in the ecosystem under evaluation, in order to extrapolate standard laboratory tests results to field settings.

When effects on microorganisms are expected, a time and cost effective option would be to measure microorganism endpoints in single species tests with macrophytes or invertebrates, to simultaneously identify effects on both organism groups. This may require renaming ‘single species’ tests to ‘multi-functional groups’ tests i.e. one invertebrate or macrophyte with a community of microorganisms. Such a set-up would move towards a more ecological realistic and holistic approach. Bioaccumulation tests including higher organisms, as proposed in **Chapter 4** and **5,** could also be used. This set up led to effective test systems with equal exposure for all species tested.

Microcosm and mesocosms testing

In ERA, microcosm and mesocosms (cosms) are model ecosystems used to study the effects of stressors and subsequent recovery at the population level. These tools have often been used as higher tier tests. They include more realistic exposure patterns and ecological processes such as aerial recolonization and interactions between species than first tier single species tests and can be used to calibrate lower tiers.[22,201,259,589] Although much experience has been gained with aquatic systems, only a few cosm studies

focussed on the sediment compartment.[259] Therefore, there is insufficient knowledge about the impact of sediment-bound contaminants in cosm tests and the causal relationships between effects on benthic organisms in single-species and cosm tests. As it has been shown in **Chapter 2,** guidance for conducting and interpreting higher-tier sediment cosm tests is highly needed as such tests are crucial for the calibration of tests in lower tiers of the risk assessment (**Chapter 8**). Moreover, there is a clear need to validate the tiered effect assessment approach for benthic organisms and sediment exposure as has been done for pelagic organisms and water exposure.[46,516,590-592]

Understanding toxicity by understanding exposure: role of species traits, exposure pathways and bioaccumulation

Bioaccumulation and hence the internal exposure and effects depend on species,[49,59,86,296,298,300,347] chemical,[59,284,297] sediment and environmental characteristics. For a relevant effect assessment and test development it is important to first understand the mechanisms of these characteristics on effects so that the effect assessment is not merely based on a black box approach.[59,345] It is thus essential to assess the relative importance and characteristic time scales of exposure pathways and to assess the differences in bioaccumulation for a range of species with different taxonomy and traits. Previous research, however, mainly addressed effects of sediment type or chemical characteristics on bioaccumulation, whereas variability among species with different traits received less attention.[300]

For a comprehensive effect assessment, it is important to link the exposure concentration with the observed ecotoxicological effects.[14,24,25,126] Ideally, the internal concentration at the target site in the benthic organism is used. However, it is difficult to measure, laborious and expensive and therefore not common in toxicity studies.[13] Moreover, in none of the regulatory guidelines it is recommended to measure internal concentrations. Consequently, external exposure concentrations are usually linked to effects. An important question is whether this external exposure concentration should be expressed as free chemical concentration in the pore water, as concentration in ingested particles or as total sediment concentration. An additional question is whether the total sediment concentration should be normalized to the organic carbon content of the dry sediment.

The freely dissolved chemical concentration (C_{free}) in the pore water is often considered as a good estimate of external exposure concentration for bioavailability[517,593] e.g. by the SETAC workshop “guidance on passive sampling methods to improve management of contaminated sediments” (2012) and a recently published series of reviews on the topic.[594] C_{free} can adequately be measured with the aid of passive samplers, which is a cost effective method. C_{free} is ideally measured in equilibrated systems. The original equilibrium partitioning theory (EPT) by Di Toro et al.[517] assumes that for non-ionic organic chemicals, equilibrium between amorphous organic matter, biota lipids and water is governed by the chemical affinity of each phase. EPT is used to calculate C_{free} from the concentration in

the passive sampler.[595] EPT is also often used to derive mechanistic sediment quality guidelines, in a screening assessment for the toxicity trigger and to predict biota sediment accumulation factors (BSAFs).

In the past decade research has shown limitations when using C_{free} and EPT to assess bioaccumulation and effects of sediment-bound chemicals. First, EPT does not always accurately predict in situ partitioning due to the presence of condensed carbon phases in field sediments leading to different field and laboratory K_{oc} values and possible disequilibrium between the phases.[80,223,304,331] Especially, in the field and in test systems with additional phases i.e. macrophytes or invertebrates, equilibrium or steady state may not be reached as was shown in **Chapter 3**, **4** and **5**. Disequilibrium between the phases in a test system is of importance for the interpretation of the test results. Non-equilibrium might either lead to under or overestimation of the actual effect. Standard chronic toxicity tests normally have a duration of 28 days. For benthic invertebrates this test period seems to be sufficient except for annelids (**Chapter 4** and **5**) and macrophytes (**Chapter 3**). This implies that for species for which equilibrium is not reached within 28 days the standard test period needs to be extended. For example, the new OECD protocol (239)[582] for the submerged macrophyte *M. spicatum*, which is also suitable for other submerged and emergent species, prescribes a test period for 14 days. This period is probably not enough to reach equilibrium in the test system (**Chapter 3**). Additionally, this test period does not cover the full life-cycle of rooted macrophytes. It remains necessary to evaluate whether the proposed sediment-spiked toxicity tests with macrophytes can be used in the chronic effect assessment as proposed in **Chapter 8**. It is thus recommended that during the development of standard test methods, species are evaluated for their time to steady state for a range of chemicals and that this period is linked to the species' life cycle and sensitive life stages so that a chronic test period is guaranteed.

Second, EPT only accounts for chemical transfer through passive organic matter-water-lipid partitioning thereby neglecting active uptake such as through sediment ingestion and other species specific traits.[596] Important species-specific traits for bioaccumulation include body size, lipid content, diet, digestive processes and dietary assimilation.[49,59,86,296,298,300,347] Depending on the species feeding mode and hydrophobicity of the chemical, particle ingestion can be a major uptake pathway for benthic invertebrates,[62,98,296,300,301] which was also shown in **Chapter 4** and **5** for most of the tested benthic invertebrates. Ingestion may lead to bioaccumulation exceeding the levels predicted by EPT.[304] EPT predicts BSAF values of approximately 1 to 2,[284] whereas many studies show orders of magnitude variation in BSAF values across a range of benthic species.[59,297,304,321,355-359,521,522,596] Despite the numerous papers criticizing the 'C_{free}' and EPT approach, the SETAC workshop (2012) "guidance on passive sampling methods to improve management of contaminated sediments" and special series[594] only briefly mentioned the limitations of the use of C_{free} with regard to uptake via ingestion, without further discussion or recommendations.

Methods and sediments used to assess bioaccumulation vary widely and therewith the exposure in the test system, which may introduce variability in test outcomes and difficulties

in comparing results. This thesis showed that BSAFs varied widely among species when exposed to exactly the same artificial sediment, pore water and overlying water because a novel test set up was applied that assured equal exposure across all tested species (Figure 2). Additionally, chemical characteristics influenced BSAF, e.g. for invertebrates BSAF generally increased with increasing hydrophobicity. BSAF values ranged from 0.6 to 6.7 for freshwater sediment-rooted macrophytes (**Chapter 3**), which agrees fairly with EPT because macrophytes do not ingest sediment. However, BSAF values for benthic invertebrates ranged from 3 to 318 with an overlap between freshwater and marine species (**Chapter 4** and **5**). The high BSAF values and their concomitant variability across the species challenges approaches for exposure and risk assessment based on C_{free} and EPT only as was mentioned above. This also demonstrates again that particle ingestion cannot be ignored for benthic invertebrates and vertebrates. However, EPT might be suitable for macrophytes[161] and other organisms for which uptake from water, including pore water, is dominant. In **Chapter 8**, it was recommended to at least measure the chemical concentration in the pore water (C_{free}) and in total sediment (in units of mass of chemical per mass of dry sediment) and the organic matter content (%) of the dry sediment as well as to measure the concentration in the overlying water. The combination of C_{free} measured with passive samplers, whole sediment measurements, biota concentrations and bioaccumulation models accounting for additional sediment uptake pathways is a promising and powerful approach to estimate internal concentrations and bioaccumulation potential of a chemical (**Chapter 4** and **5**).

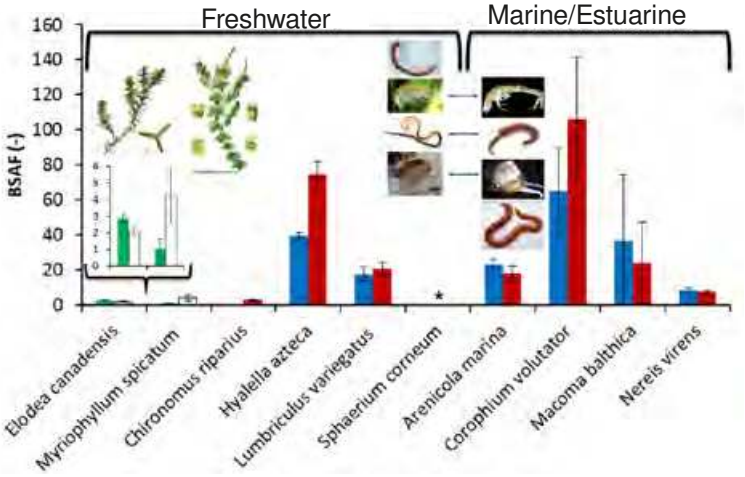

Figure 3. Average Biota Sediment Accumulation Factor (BSAF; -) for ten benthic species. Two freshwater submerged macrophytes: *Elodea canadensis* and *Myriophyllum spicatum* for PCB 29 (green bars, $logK_{ow}$ 5.58) and PCB 155 (white bars, $logK_{ow}$ 6.5). Four freshwater benthic invertebrates: *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus* and *Sphaerium corneum* and four marine/estuarine benthic invertebrates: *Arenicola marina*, *Corophium volutator*, *Macoma balthica* and *Nereis virens* for PCB 28 (blue bars, $logK_{ow}$ 5.58) and PCB 118 (red bars, $logK_{ow}$ 6.5). For *S. corneum* biomass was too low to detect analyses chemical concentration in tissue, indicated by asterisk (*). Error bars represent standard deviation.

Despite the disadvantages, EPT can be used as a cost effective screening tool in Tier-0 using the available aquatic dataset as proposed in **Chapter 8**. The advantage of EPT as a screening tool is that all available information about a chemical is effectively taken into account in ERA[597] and unnecessary testing with benthic organisms can be prevented. Due to the shortcomings of EPT, as discussed above, it was recommended to always use an extrapolation factor of 10 to derive a $PNEC_{sed;EP}$ to account for presence of condensed carbon phases (e.g. black carbon) and ingestion (**Chapter 8**) for sediment ingesting organisms. The case studies in **Chapter 8** showed that this approach led to very conservative to conservative estimates for $PNEC_{sed;EP}$ when compared with PNECs derived from higher tiers, which at least shows that this approach is protective. Note however that the case studies only addressed three chemicals.

Models as a tool for ERA

Current ERA schemes mainly focus on toxicity and bioaccumulation at the individual level, whereas specific protection goals focus mainly on the population level. Effect models can be used to extrapolate results of experimental tiers by linking spatial-temporal variability in exposure to effect, by predicting concentration-response relationships at different levels of biological organisation and at different spatial and temporal scales and by addressing ecological recovery times, bioaccumulation in food-webs and food-web interactions in ecosystems.[428,479,509,528,529] Models are suitable tools in ERA to mechanistically interpret toxicokinetic processes and to assess parameters and can be used complementary to experimental tests in tiered effect assessments. Nevertheless, models are hardly mentioned in technical documents concerning ERA.[479,529] Recently, the European Food Safety Authority (EFSA)[532] identified critical steps to implement the use of effect models in ERA, including a clear problem formulation, consideration of the application domain of the model, selection of focal species and definition of environmental scenarios as discussed in the last subsection.

A wide range of effect and bioaccumulation models have been developed,[509,530,531] including single-species models that predict species responses at the population and landscape/watershed level.[23,221,421-425] Nevertheless, most of them do not take the sediment compartment into account. However, several bioaccumulation models exist that account for accumulation of sediment bound chemicals into aquatic food webs and for ingestion of prey and/or sediment.[69,81,83,279,301,304,315,316,427-429] It is unclear, however, to what extend effects of sediment-bound chemicals are important at the individual level and how this translates to effects on the population level.

Individual level

Mechanistic individual level models that include the sediment compartment and that describe toxicokinetic processes were developed as described in **Chapters 3, 4** and **5** for a range of macrophytes and benthic invertebrates including the regulatory species *Chironomus riparius*, *Hyalella azteca*, *Lumbriculus variegatus* and *Myriophyllum spicatum*

as proposed in **Chapter 2**. These models linked mechanisms and parameters to species-specific traits and were able to sufficiently predict bioaccumulation. Model output showed that bioaccumulation highly depended on chemical characteristics and species traits (**Chapter 3, 4** and **5**). These models provided insight in the relative importance and characteristic time scales of exposure pathways and showed that species-specific traits (e.g. ingestion) and differentiation of food sources were important factors for bioaccumulation from sediment. This can be used to a priori optimize test methods, including duration and conditions, as part of a prospective ERA framework.

These models are generic and can be applied to other species. For further parametrization, measuring bioaccumulation and species-specific traits will be important, especially for true marine benthic invertebrates e.g. *Echinocardium cordatum*, estuarine/marine macrophytes e.g. *Zostera* sp. and emergent macrophytes like G*lyceria maxima*. For emergent macrophyte species, toxicokinetic processes might be different from submerged species due to differences in exposure and transport routes. Emergent macrophytes may share features with terrestrial plants for which the transpiration stream plays a role.[157,598-600]

Population level

Chapter 7 showed an example for the benthic invertebrate *C. riparius* of how the bioaccumulation model including exposure via sediment and food ingestion developed in **Chapter 4** and parameterized in **Chapter 5**, can be linked to effects at the individual and population level. The model showed that sediment exposure via ingestion is important at the population level. Exposure via ingestion substantially influenced the mortality and herewith the recovery times of the population following a pulsed exposure to a pesticide. This model framework is general and can easily be implemented for other benthic invertebrate species with similar life cycles, by defining the species-specific toxicokinetic and toxicodynamic (TKTD) parameters and ingestion rates.

Additional ecological realism can be added to the model by exposing each life stage to the relevant environmental compartment (e.g. adults to exposure via the air), by considering the sensitivity of different life stages and by including bioturbation in the exposure model. Moreover, due to a lack of data, parameters for *Chaoborus obscuripes* were used for the TKTD modelling of *C. riparius*. Although both life cycles have similar stages it would be better to use a parameter set specifically for *C. riparius*.

For macrophyte populations, various models with different complexity are available for a range of species. These models often include environmental parameters such as light intensity, temperature, pH, dissolved oxygen and species specific parameters, such as overwintering strategies and reproduction. They can also include turbidity, herbivorous grazing, wave action, mechanical control, competition and may have a spatial component (e.g. Best and Boyd[538]; van Nes et al.[601]). Additional competition effects and feedback loops can be captured in population models when two or more species are modelled. Recovery of affected systems might occur by drift dispersal of seeds or plant parts. Macrophytes can also contribute to a decrease of chemical exposure by joint detoxification and/or growth dilution,[71] which is potentially an important positive feedback mechanism in recovery.

Little is known, however, about the recovery of macrophyte populations from chemical stress. There is a need to develop a model that links sediment exposure to individual and population effects of macrophytes and incorporates macrophyte recovery.

Environmental scenarios for modelling

When coupling exposure and effect models an important requirement is the use a common model scenario, to prevent a mismatch between exposure model output e.g. sediment exposure in a river and effect model e.g. population dynamics in a stagnant pond. A mismatch can lead to under- or overestimation of effects. EFSA[532] identified the definition of realistic worst case environmental scenarios in relation to the specific protection goals and problem definition underlying ERA as one of the main pending research needs. An environmental scenario is a conceptual and quantitative description of the environmental system relevant to ERA, and has been defined by EFSA[532] as "a combination of abiotic, biotic and agronomic parameters, thus including both exposure and effect". Recently, Rico et al.[602] proposed to adjust this definition to allow for better integration of exposure and biological characteristics of the ecosystem under evaluation. It was defined as "the combination of biotic and abiotic parameters (including agronomic practises and properties of agricultural landscapes), and their input values, that are required to provide a realistic worst-case representation of the pesticide exposure, effects and recovery for the ecological entities that are to be evaluated". Thus, exposure scenarios obtained from exposure models should be in line with those of the effect models, as they may share common variables (Figure 1).[504] **Chapter 7** shows an example of how the same environmental scenario can be used in exposure and effect modelling including the sediment compartment.

Ideally, fate models with flexible landscape features are combined in one model with detailed effect models at the landscape level including processes for the recovery of vulnerable key species. This would require one environmental scenario including agronomical practise in the landscape that is considered.

9.1 Prospective ERA for sediment-bound chemicals

The past years, progress has been made in the field of prospective ERA for sediment-bound chemicals. The ECHA workshop, organised in Finland in 2013,[20] and recently established workgroups like the EFSA sediment workgroup are meant to raise attention on sediment topics and discuss questions still to be addressed. However, our understanding of mechanisms and processes governing exposure and effects that are the basis for ERA are still far from complete. Especially more complex scenarios including metabolites, mixture toxicity and effects at landscape scale are missing in ERA, let alone the issue of multiple stressors. Prospective ERA usually concerns individual chemicals and their possible metabolites. However, it is important to know how potential additional stressors might impact the threshold concentration for the assessed chemical. Recently, a SETAC PELSTON workshop on mixture toxicity, organized in Valencia in 2015, aimed to generate guidance on "how generalized decision trees can be used in forecasting where chemical

exposure may represent a potential concern". Although the focus of this workshop was on retrospective ERA, the findings are important for prospective ERA to improve assessment factors and integrate mixture toxicity testing for products that are marketed with a mixture of chemicals.

In **chapter 8** we provided guidance to establish a prospective ERA framework for organic chemicals in sediments of freshwater, estuarine and marine ecosystems (Figure 1) and ten key points to implement in ERA were identified (Table 1). To realize these key points, the new information from the previous chapters can be used. Recommendations about test methodologies in **Chapter 2, 3, 4, 5** and **6** together with the gained insight in characteristic time scales of exposure pathways and bioaccumulation for a range of species with different taxonomy and traits, as explored in **Chapters 3, 4** and **5**, and proposed population model approaches in **Chapter 7**, provide a strong basis for further development of cost effective and widely accepted test methods for microorganisms, macrophytes, invertebrates and vertebrates. Moreover, these chapters provide tools for the translation of results between ecosystems and different levels of biological organization.

This work contributes to the development of a transparent holistic sediment ERA approach for organic chemicals that is based on mechanistic understanding and combines experimental work and fate and effect modelling using smarter and more cost-effective prospective tools. A well-developed sediment ERA will protect species currently not covered by the aquatic ERA. As science is evolving, it is important to assure that ERA is continuously updated with the latest technology and the newest scientific knowledge. The guidance in **Chapter 8** mainly focusses on Europe. It would be best, however to unify guidelines and regulations across chemical groups and geographic units worldwide. Future steps can be taken to include metabolites, mixture toxicity and approaches at the landscape level. This calls for an interdisciplinary approach to improve our scientific understanding and to communicate findings with all stakeholders involved in ERA.

Table 1. Ten key points to implement in prospective sediment ERA (**Chapter 2-8**).

10 key points to implement in prospective sediment ERA
1. Set specific protection goals defined as service providing units (key species) with ecological entity and attribute
2. Define and agree on a set of harmonized triggers for sediment testing
3. Define and harmonize data requirements
4. Select and formally approve a set of standard test species
5. Develop chronic standard test protocols
6. Develop protocols for conducting and interpreting higher-tier sediment microcosm and mesocosm tests
7. Develop tiered schemes for vertebrates and microorganisms
8. Calibration and validation of the tiered approach
9. Link exposure and effect with correct dose metrics
10. Develop models and model scenarios on all levels of biological organisation

Commonly used abbreviations

AF	Assessment factor
ASTM	American Society for Testing and Materials
BSAF	Biota sediment accumulation factor
C_{free}	Freely dissolved chemical concentration
CPF	Chlorpyrifos
C_{pw}	Concentration of the chemical in pore water
$C_{sed;oc}$	Concentration of the chemical in the sediment per unit mass of organic carbon
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DW	Dry weight
ECHA	European Chemicals Agency
EC_x	Effect concentration x percent
EFSA	European Food Safety Authority
EMEA	European Medicines Agency
EPT	Equilibrium partitioning theory
EPA	United States Environmental Protection Agency
ERA	Environmetnal risk assessment
ERC	Ecotoxicologically relevant concentration
GIS	Geographic information system
HC_5	Hazardous concentrations to 5% of the test species
HOC	Hydrophobic organic chemical
IBM	Individual-based modelling
ISO	International Organization for Standardization
K_d	Sediment-water partitioning coefficient
K_{oc}	Organic carbon-water partitioning coefficient
K_{om}	Organic matter-water partitioning coefficient
K_{ow}	Octanol-water partition coefficient
LC_X	Lethal concentration x percent
LIN	Linuron
NOEC	No observed effect concentration
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
OM	Organic matter
PEC	Predicted environmental exposure concentrations
PEC_{sed}	Sediment exposure estimates
$PEC_{sed;max}$	Sediment exposure estimates based on peak concentration
$PEC_{sed;twa}$	Sediment exposure estimates based on time-weighted average concentration
PCB	Polychlorinated biphenyl
PNEC	Predicted no effect concentration
$PNEC_{sed}$	Effect estimates for sediment-dwelling organisms

$PNEC_{sed;ch}$	Predicted no effect concentration for sediment based on chronic toxicity data
$PNEC_{sed;ch;EP}$	Predicted no effect concentration for sediment based on chronic toxicity data calculated by equilibrium partitioning
$PNEC_{sw;ch}$	Predicted no effect concentration for surface water based on chronic toxicity data
PPP	Plant protection products
qPCR	Quantititave polymerase chain reaction
QSPR	Quantitative structure property relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ	Risk Quotient (RQ=PEC/PNEC)
rRNA	Ribosomal ribonucleic acid
SD	Standard deviation
SPU	Service providing units
SSD	Species sensitivity distribution
TKTD	Toxicokinetic toxicodynamic
TWA	Time-weighted average
VICH	Veterinary International Conference on Harmonization
WW	Wet weight

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Summary

Aquatic sediments are an important part of the aquatic ecosystem providing critical ecosystem services. Sediments form a major sink for hydrophobic organic compounds (HOC). HOCs may affect ecosystem services, pose long term risks to benthic organisms and may accumulate in the food chain. Sediment test methods and prospective environmental risk assessment (ERA) schemes are currently inadequately developed to sufficiently predict the risk of sediment-bound HOCs on the environment. For the development of an unified ERA framework it is essential to harmonize data requirements, test protocols and risk assessment frameworks between regulations and directives.

The overall aim of this thesis was to support the development of whole sediment toxicity tests and the prospective risk assessment of sediment-bound chemicals. This included providing recommendations for improved test methods for macrophytes, invertebrates and microorganisms, across different taxonomic groups and levels of biological organization in freshwater, estuarine and marine ecosystems and increase mechanistic understanding to assess potential effects of organic chemicals in sediments on species and populations.

Chapter 2 started with critically reviewing the state of science of and gave recommendations on sediment toxicity test protocols for microorganisms, macrophytes, benthic invertebrates and benthic communities. This review concluded that current methods in sediment toxicity testing are fragmentary and diverse, hindering the read across of single species test results between freshwater, estuarine and marine ecosystems and to higher levels of biological organisation. It was concluded that there is a need to develop chronic sediment tests with a representative selection of species and endpoints covering different trophic levels, taxonomic groups and exposure pathways and to unify dose metrics and exposure assessment methodologies for a balanced strategy for sediment toxicity testing of single organic compounds in the context of prospective ERA.

For a relevant effect assessment, it is crucial to understand exposure for a range of species with different taxonomy and traits. **Chapters 3**, **4** and **5** assess the relative importance and characteristic time scales of exposure pathways and variances in bioaccumulation for freshwater sediment-rooted macrophytes and marine and freshwater benthic invertebrates, using experimental and model approaches.

Chapter 3 investigated bioaccumulation in two sediment-rooted macrophytes: *Elodea canadensis* and *Myriophyllum spicatum* by tracking and modelling chemical flows of chlorpyrifos, linuron and six polychlorinated biphenyls (PCBs) in water-sediment-macrophyte systems. Biota sediment accumulation factor (BSAF) values ranged from 0.6 to 6.7 for both species, which fairly agrees with predictions by the traditional equilibrium partitioning theory (EPT). Chemical fluxes across the interfaces between pore water, overlying water, shoots and roots were modelled using a novel multicompartment model. The modelling generated the first mass transfer parameter set described for bioaccumulation by sediment-rooted macrophytes, with satisfactory narrow confidence limits for more than half of the estimated parameters. Exposure via the water column led to rapid uptake by shoots followed by

transport to the roots, after which tissue concentrations gradually declined. Translocation played an important role in the exchange between shoots and roots. Exposure via spiked sediment led to gradual uptake by the roots, but subsequent transport to the shoots and overlying water remained limited for the chemicals studied. These contrasting patterns showed that exposure is sensitive to the test set up, chemical properties and species traits. This work concluded that an exposure period of 28 days might not be sufficient for sediment spiked toxicity tests with sediment-rooted macrophytes as the uptake from sediment and translocation to shoots is a slow chemical- and species specific process and equilibrium is only reached after 28 days.

In **Chapter 4** and **5** the causal links between species traits and bioaccumulation were assessed by measuring and modelling PCB bioaccumulation for four marine and four freshwater invertebrates. Uniformity of exposure was achieved by testing each species in the same aquarium, separated by enclosures, to ensure that the observed variability in bioaccumulation was due to species traits. The relative importance of chemical uptake from pore water or food (i.e. organic matter (OM)) ingestion was manipulated by using artificial sediment with different OM contents and by using sediment that had been pre-equilibrated with the chemicals for different aging times. For the marine species, BSAFs ranged from 5 to 318, in the order *Nereis virens* <*Arenicola marina* ≈*Macoma balthica* <*Corophium volutator* (**Chapter 4**). For the freshwater species BSAFs ranged from 3 to 114, in the order *Chironomus riparius* < *Sphaerium corneum* ≤ *Lumbriculus variegatus* ≤ *Hyalella azteca* (**Chapter 5**). An overlap was shown between freshwater and marine species. The high BSAF values and their concomitant variability across the species challenges the presumed value of 1-2 typically employed in ecological risk assessment schemes based on pore water concentration analysis and EPT. The dynamic bioaccumulation model with species-specific bioaccumulation parameters fitted well to the experimental data. The model included species-specific parameters representing key traits, which illustrates how models provide an opportunity to read across benthic species with different feeding strategies. Key traits included species-specific differentiation between a) ingestion rates, b) ingestion of suspended and settled OM and c) elimination rates. It was proposed that combining multi-enclosure testing and mechanistic modelling will substantially improve exposure assessment in sediment toxicity tests.

Although sediment microbial communities play a crucial role in ecosystems, the importance of microorganisms is often overlooked in standard testing (**Chapter 2**). Moreover, it is not clear to what extent changing microbial community composition and associated functions affect sediment test results. In **Chapter 6**, the development of bacterial communities in artificial sediment was assessed during the 28 d bioaccumulation test described in **Chapter 4**, with PCBs, chlorpyrifos and four marine benthic invertebrates. Denaturing gradient gel electrophoresis (DGGE) and 454-pyrosequencing of polymerase chain reaction (PCR)-amplified 16S ribosomal ribonucleic acid (rRNA) genes were used to characterize the bacterial community. Abundances of total bacteria and selected genes encoding enzymes involved in important microbial mediated ecosystem functions were measured by quantitative PCR. Community composition and diversity responded most to

the time course of the experiment, whereas OM content showed a low but still significant effect on community composition, biodiversity and two functional genes tested. Moreover, OM content had a higher influence on bacterial community composition than invertebrate species. Medium OM content had the highest gene abundance and is preferred for standard testing. This chapter also indicated that a pre-equilibration period is essential for growth and stabilization of the bacterial community. Changes in microbial community might affect results of bioaccumulation and effect studies in the context of ERA by affecting general water quality and chemical exposure.

The previous **Chapters** (**3**, **4** and **5**) focused on single species. However, to assess risks of sediment-bound contaminants, larger temporal and spatial scales have to be addressed than can be covered in single species laboratory tests. Although population models can address these scales, so far they lacked the coupling between chemical fate in the sediment, toxicokinetics and toxicodynamics of the chemical within individuals as well as propagation of individual-level effects to the population level. In **Chapter 7**, an individual-based population model was developed that for the first time included all these processes. The model was used to assess the importance of chemical uptake routes on the impact and recovery rates of a *C. riparius* population after pulsed exposure to chlorpyrifos. Effects of aqueous exposure only, combined exposure from water and sediment, food intake, varying thickness of the exposure layer, chemical sorption affinity and sediment organic matter content were assessed on the population, through scenario studies. This chapter showed the importance of particle ingestion as an additional exposure pathway for *C. riparius* population dynamics and recovery. Accounting only for pore water exposure based on EPT could underestimate the risks of sediment-bound chemicals at the population level, which was also shown for the individual level in for benthic invertebrates (**Chapters 4** and **5**). Additional scenario studies showed the importance of selecting the biologically relevant sediment layer and long term data output for population modelling and further illustrate the usefulness of the model approach as a tool in prospective risk assessment.

A broadly accepted framework for prospective ERA of sediment-bound HOCs requires clear protection goals, evidence-based concepts that link exposure to effects and a transparent tiered-effect assessment. **Chapter 8** provided guidance to establish such a framework for freshwater, estuarine and marine ecosystems, with a focus on the applicable European regulations and the underlying data requirements. Using the ecosystem services concept, specific protection goals were derived for ecosystem service providing units: microorganisms, benthic algae, sediment-rooted macrophytes, benthic invertebrates and benthic vertebrates. Triggers for sediment toxicity testing should consist of a combination of chemical properties and toxicity triggers. When testing is required a tiered approach was recommended (Tier-0 through Tier-3). The Tier-0 approach is a cost-effective screening based on chronic water-exposure toxicity data for pelagic species and EPT. The Tier-1 approach can be based on long-term spiked sediment laboratory toxicity tests with standard benthic test species and protocol test methods focussing on chronic endpoints. If chronic toxicity data for both standard and additional benthic test species are available, the Species Sensitivity Distribution (SSD) approach is a more viable Tier-2 option than the

geometric mean approach. Criteria for the application of the SSD approach in sediment risk assessment are discussed. Microcosm and mesocosm experiments with spiked sediment were proposed as a Tier-3 approach. Ecological effect models can be used to supplement the experimental tiers. A strategy for unifying information from various tiers by experimental work and exposure and effect modelling is provided.

The final **Chapter (9)** summarizes and discusses outcomes from previous chapters and puts them in the context of the developments in prospective ERA. Ten key points to implement in ERA were identified: 1) Set specific protection goals defined as service providing units (key species) with ecological entity and attribute, 2) define and agree on a set of harmonized triggers for sediment testing, 3) define and harmonize data requirements, 4) select and formally approve a set of standard test species, 5) develop chronic standard test protocols, 6) develop protocols for conducting and interpreting higher-tier sediment microcosm and mesocosm tests, 7) develop tiered schemes for vertebrates and microorganisms, 8) calibration and validation of the tiered approach, 9) link exposure and effect with correct dose metrics and 10) develop models and model scenarios that account for all levels of biological organisation. To realize these key points, the new information and recommendations from the previous chapters can be used. This work contributes to the development of a transparent holistic sediment ERA approach for HOCs that is based on mechanistic understanding and combines experimental work and fate and effect modelling using smarter and more cost-effective tools.

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Noël

Curriculum vitae

Noël J. Diepens (Eindhoven, the Netherlands, 22-02-1987) obtained her Bachelor degree in Aquatic Ecotechnology from the Applied University of Zeeland, The Netherlands. This degree included a variety of topics, from waste water treatment, civil engineering, hydrology, watershed management, toxicology to ecology. Although enjoying this variety, she was most interested in aquatic ecology, water quality and ecotoxicology. Therefore, she decided to get a Master's degree in Aquatic Ecology and Water Quality Management at Wageningen University, the Netherlands. During her Master, she had a strong focus on ecotoxicology especially tropical ecotoxicology and toxicokinetic and dynamic modelling. In 2011, after finishing her master she started a PhD in the same group. The PhD focused on sediment toxicity tests in the context of prospective environmental risk assessment of organic chemicals. This work contributes to the mechanistic understanding of potential effects of organic chemicals in sediments on macrophytes, invertebrates and microorganisms, across different taxonomic groups, levels of biological and ecosystems. During her PhD she co-supervised six BSc and MSc students, participated in international workshops, presented at several international conferences, is member of the Student Advisory Council of the Society of Environmental Toxicology and Chemistry (SETAC) and was member of the scientific committee of the Young Environmental Scientist meeting in Serbia in 2015. In the future, she will continue working in the field of aquatic and sediment ecotoxicology and environmental risk assessment.

In her free time she enjoys rock and ice climbing, mountaineering, biking, reading, travelling, making interactive theatre, scuba diving and sailing.

List of publications

Diepens NJ, AA Koelmans, H. Baveco, PJ Van den Brink, MJ Van den Heuvel-Greve, TCM Brock. Under revision. *Prospective environmental risk assessment for sediment-bound organic chemicals: A proposal for tiered effect assessment.* Reviews of Environmental Contamination and Toxicology

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Diepens NJ, MJ Van den Heuvel-Greve, AA Koelmans. Under revision. *Model-supported bioaccumulation assessment by battery testing allows read across among marine benthic invertebrate species.* Environmental Science and Technology

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- *ECO17 - Evaluation of test methods for measuring toxicity to sediment organisms*. 15th Cefic-LRI Annual Workshop, 20-21 November 2013, Brussel, Belgium
- *Bioaccumulation assessment by battery testing allows read across among marine benthic invertebrate species*. SETAC Europe 24th Annual Meeting, 11 - 15 May 2014, Basel, Switzerland
- *Prospective environmental risk assessment for sediment-bound organic chemicals: A proposal for tiered effect assessment*. SETAC Europe 25th Annual Meeting, 3-7 May 2015, Barcelona, Spain

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