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Improving Sediment Toxicity Testing for Very Hydrophobic Chemicals: Part 1—Spiking, Equilibrating, and Exposure Quantification

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Abstract: Sediment toxicity tests have applications in ecological risk and chemical safety assessments. Despite the many years of experience in testing and the availability of standard protocols, sediment toxicity testing remains challenging with very hydrophobic organic chemicals (VHOCs; i.e., chemicals with a log octanol/water partition coefficient of more than 6). The challenges primarily relate to the chemicals' low aqueous solubilities and slow kinetics, due to which several experimental artifacts may occur. To investigate the potential artifacts, experiments were performed, focusing on spiking and equilibrating (aging) sediments, as well as exposure quantification with passive sampling. The results demonstrated that generally applied, Organisation for Economic Co-operation and Development–recommended spiking (coating) methods may lead to significant chemical losses and the formation of nondissolved, nonbioavailable VHOCs. Direct spiking appeared to be the most optimal, provided that intensive mixing was applied simultaneously. Passive dosing was tested as a novel way of spiking liquid VHOCs, but the approach proved unsuccessful. Intensive postspiking mixing during sediment equilibration for 1 to 2 weeks was shown to be essential for producing a homogeneous system, minimizing the presence of nondissolved chemical (crystals or nonaqueous phase liquids; NAPLs), and creating a stable toxicological response in subsequent toxicity tests. Finally, exposure quantification of VHOCs in sediments through passive sampling was found to be feasible with different polymers, although prolonged equilibration times may be required, and determining sampler/water partition coefficients can be extremely challenging. The results of additional experiments, focusing on toxicity test exposure duration, concentrations above which NAPLs will occur, and ways to distinguish actual toxicity from false-positive results, are presented in Part 2 of this publication series. *Environ Toxicol Chem* 2024;00:1–11. © 2024 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Sediment toxicity; Spiking; Aging; *Lumbriculus variegatus*; Passive sampling; Passive dosing

INTRODUCTION

Sediment toxicity tests are frequently performed on contaminated field sediments for the purpose of ecological (retrospective) risk assessments and as part of chemical safety (prospective) assessments, as required within (inter)national chemical regulation frameworks. Sediment toxicity tests are bioassays in which benthic organisms are exposed to field-contaminated or spiked sediment, and, generally, their survival, growth, emergence, and/or reproduction are evaluated after a certain exposure duration. Sediment toxicity testing has a long tradition, and standard protocols are available for tests using species such as *Lumbriculus variegatus*, *Hyallela azteca*, and *Chironomus* sp. (Organisation for Economic Co-operation and Development [OECD], 2004, 2007; USEPA, 2000). Generally, these tests perform well and deliver useful information on chemical safety and ecological risks. However, when one is testing with very hydrophobic organic chemicals (VHOCs), that is, chemicals with an octanol/water partition coefficient (log K_{OW}) approximately above 6 (e.g., high-molecular-weight polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], petrochemicals, flame retardants, etc.), susceptibility to experimental artifacts increases due to the chemicals' relatively low aqueous

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solubilities and slow kinetics. Consequently, VHOC test results may be biased and less or not reliable (Redman et al., 2014).

In prospective testing, VHOCs generally are introduced (spiked) into sediment through one of the following methods: (1) spiking directly in a slurry with or without a carrier solvent (direct spiking); (2) spiking via a solvent on the wall of a container and subsequent evaporation of the solvent and mixing (rolling) with the sediment sample (glass coating); or (3) spiking a small subsample of sediment (sand) and subsequent evaporation of the spike solvent and mixing the subsample with the full mass of sediment (sand coating). Some of these methods are also referred to as, for example, solvent, conventional, shell, and dilution mixing (Hiki et al., 2021a; Northcott & Jones, 2000a; Picone et al., 2022). The third method is recommended by the OECD for spiking chemicals to sediment (OECD, 2004, 2007). For all methods, there is variation in the spiking solvent used and the technique, intensity, and duration of mixing applied. Because of their relatively low aqueous solubilities, spiking VHOCs may result in the occurrence of solid chemical (crystals) or so-called nonaqueous phase liquids (NAPLs), the presence of which may lead to biased toxicity results (Jonker & Diepens, 2024 [this issue]). Potentially, both false-negative and false-positive test results may occur, with the latter caused by physical effects on organisms through fouling and smothering with NAPLS (Muijs & Jonker, 2010; Redman et al., 2014). Therefore, setting proper upper limit test concentrations is crucial, but information on such concentrations is lacking (Jonker & Diepens, 2024 [this issue]). Also, although spiking methods applied for hydrophobic chemicals have been the subject of investigations and reviews for more than two decades (Murdoch et al., 1997; Northcott & Jones, 2000b; Picone et al., 2022), experimental data proving how to best spike VHOCs to sediment are lacking.

Other artifacts may be introduced due to the relatively slow kinetics of VHOCs. Chemical–sediment equilibration times and organism–sediment contact (exposure) times applied in standard fashion to less hydrophobic chemicals may not be sufficient to obtain a homogeneous test matrix, an environmentally relevant or realistic bioavailability status, and/or steady-state internal concentrations for VHOCs (Mackay et al., 2015). However, experimental data supporting this concern are limited.

Considering the above factors, a critical review of the literature on VHOC sediment toxicity testing up to 2022 suggests that in the majority of cases spiking may actually have resulted in the emergence or presence of crystals or NAPLs (see the Supporting Information, Table S1, for a compilation). For instance, glass and sand coating spiking involves evaporation of the spiking solvent, undoubtedly yielding pure undissolved chemicals, which may not (fully) dissolve and sorb to the sediment in case of insufficiently long or intensive mixing or equilibrating.

A final challenge when working with VHOCs is related to quantifying actual exposure concentrations in sediments. Traditionally, exposure quantification in sediments relies on solvent (total) extractions; however, nowadays a scientific consensus exists agreeing that this approach does not lead to realistic exposure and risk assessments (Greenberg et al., 2014; Mayer et al., 2014). Instead, passive sampling (i.e., partitioning-based, nondepletive extractions with polymers), yielding freely dissolved concentrations (C_{free}) is believed to provide essential information for understanding actual bioaccumulation and toxicological effects (Lydy et al., 2014; Mayer et al., 2014). Although standard protocols exist for passive sampling of common environmental contaminants (e.g., PAHs, PCBs; Jonker et al., 2020), information on the feasibility of the technique to other, for example, more hydrophobic or liquid chemicals is scarce (Cornelissen et al., 2008; Li et al., 2014; Muijs & Jonker, 2012).

The lack of knowledge about and validated experimental approaches and protocols for sediment toxicity testing of VHOCs potentially hampers high-quality chemical safety and ecological risk assessments and consequently may lead to improper management of contaminated sediments and chemicals. Therefore, a project aiming to improve sediment toxicity testing design, performance, and data interpretation for VHOCs was performed. The present study presents the results of experiments focusing on (1) comparing spiking procedures, including a novel approach based on passive dosing; (2) investigating chemical–sediment equilibration time before test initiation; and (3) testing the applicability of passive sampling for VHOCs. Practical recommendations are provided, in an attempt to maximize the realism and value of future VHOC sediment toxicity testing. A companion experimental study (Jonker & Diepens, 2024 [this issue]) investigates the required toxicity test exposure duration for VHOCs and aims to identify upper limit test concentrations and ways to discriminate between actual toxicity and physical effects caused by NAPLs. A third study will integrate the obtained toxicity results with literature data and explicitly test the applicability of the Equilibrium Partition Theory modeling framework (Di Toro et al., 1991; Redman et al., 2014) to VHOCs.

STUDY DESIGN

First, the three generally applied spiking approaches (direct, glass coating, and sand coating spiking) were compared for five VHOCs by applying mixing conditions that were considered optimal for the chemicals in terms of intensity and duration. Mixing conditions were not varied, because this would result in an unfeasibly large experimental setup. In addition, a spiking approach based on passive dosing, using two different dosing polymers, was developed for spiking liquid VHOCs. For all methods, spiking performance was evaluated on the basis of both total concentrations in the solid phase (C_{tot}) and C_{free}. The latter concentrations are crucial for understanding chemical bioavailability, but have not been included in previous spiking performance evaluations for hydrophobic chemicals (Hiki et al., 2021a; Northcott & Jones, 2000a; Reid et al., 1998). Thus, previous work and recommendations (Hiki et al., 2021a; Murdoch et al., 1997; Northcott & Jones, 2000b; Picone et al., 2022) basically have focused solely on homogenization (chemical distribution) effectiveness, and have disregarded actual exposure, that is, key information for toxicity testing.

Next, an equilibration time experiment was performed to investigate the minimum postspiking mixing time required to obtain a test matrix yielding a stable toxicological response. In this experiment, sediments spiked with three VHOCs were equilibrated and mixed for different periods of time (2–112 days), after which toxicity tests with *L. variegatus* were performed. The rationale for this experiment was the hypothesis that the contact time between chemical and sediment after spiking may determine chemical redistribution processes within the sediment and thereby the bioavailability and finally the toxic response observed in subsequent toxicity tests (Hiki et al., 2021a; Redman et al., 2014). *Lumbriculus* was considered the organism most robust and suited for investigating any effects of equilibration time, because its close contact with the sediment matrix should best reflect any effects.

Lastly, the applicability of passive sampling to VHOCs was evaluated by studying sampling kinetics of 10 chemicals in spiked sediment, using three sampling polymers, and by determining the polymer/water partition coefficients of the chemicals. The technique was then applied to quantify exposure in sediments from the spiking and equilibration time experiments.

The emphasis in the overall study was on liquid chemicals, because of their potential to form NAPLs and cause fouling effects, which are discussed in the companion study (Jonker & Diepens, 2024 [this issue]). Therefore, 8 of the 10 test chemicals were liquids (Supporting Information, Table S2). One of these is not a VHOC by definition (1,3-dimethyladamantane; log K_{OW} <6; Supporting Information, Table S2); however, it was included to characterize the gradient from hydrophobic to very hydrophobic chemicals and as a positive control substance for the toxicity tests described in Jonker & Diepens (2024 [this issue]).

MATERIALS AND METHODS

Chemicals

The following chemicals were used as test substances: bicyclohexane (BCH; Aldrich; 99%), 1,11-dibromoundecane (DBUD; Aldrich; 98%), di(2-ethylhexyl)phthalate (DEHP; TCI; >98%), 2,7-di-isopropylnaphthalene (DIPN; TCI; >97%), 1,3-dimethyladamantane (DMA; TCI; >99%), hexachlorobenzene (HCB; Apollo Scientific; 95%), 1-hexadecene (HD; Aldrich; >98.5%), 2,2,4,4,6,8,8,-heptamethylnonane (HMN; Acros; 98%), octachloronaphthalene (OCN; Aldrich; 98%), and 2,2,4,6,6-pentamethylheptane (PMH; TCI; >98%). Solvents used were: acetone, dichloromethane, ethyl acetate, and hexane (Pesti-S grade; Biosolve), and acetonitrile (HPLC-S grade; Biosolve). Sodium azide (Merck) was used as a biocide.

Polymers for sampling and dosing

Three passive sampling materials were used: polyethylene (PE) sheet (26 μm; VWR International), polyoxymethylene (POM) sheet (77 μm; CS Hyde), and solid-phase micro-extraction (SPME) fibers, consisting of a 100-μm-thick glass core, coated with a 30-μm-thick polydimethylsiloxane (PDMS) layer (Poly Micro Industries). Passive dosing experiments were performed with silicone rubber (SR) tubing (Rubber BV; 7 mm internal diameter; 9 mm external diameter) and PE lay flat tubes (Vendrig Packaging; 35 mm wide; 50 μm thickness). Small strips were cut from the PE (2, 5, or 9 mg) and POM sheets (2 or 29 mg), and 3 or 5-cm pieces were cut from the SPME fiber. Prior to use, PE and SPME fibers were precleaned as described in Jonker (2022), and POM was washed as described in Jonker et al. (2020). Details on dimensions, washing, and preparation of the polymers used for passive dosing are described in the Supporting Information, Text S1).

Artificial sediment

Artificial sediment was prepared as prescribed by the OECD (2004, 2007). Sphagnum peat (Klasmann Deilmann Benelux) was weighed into glass jars and water was added. While stirring with a large magnetic stirrer, the pH was adjusted to 5.8 ± 0.3 by adding calcium carbonate (99.3%; Sigma-Aldrich), and the jars were placed on an orbital shaker operating at 120 rpm and 20 °C for 3 days. Then stinging nettle powder (obtained by mortaring organic-quality leaves obtained from a local tea shop), kaolin clay (type CH112H; Keramikos, Haarlem), and sand (Geba; Sibelco) were added. The final dry weight phase ratio was as follows: sand:clay:peat:stinging nettle = 0.75:0.195;0.05:0.005. Water content was 40% to 50%, depending on the experiment, and the organic carbon content was determined to be 2.18% (±0.14%; Jonker & Diepens, 2024 [this issue]). Sediment prepared for the spiking comparison and passive sampling and dosing experiments additionally received sodium azide as a biocide (300 mg/L).

Spiking methods

Artificial sediment (4.6 kg; 40% water content), was prepared in three 2.5-L glass jars, as described in the previous *Artificial sediment* section. The jars were initially equilibrated at 60 rpm for 2 days on a roller bank (New Brunswick Scientific; model RC-42; upgraded with a heavy duty, variable-speed electric motor), after which they were spiked with five VHOCs (HMN, DIPN, DBUD, HCB, and OCN) according to the three generally applied spiking methods, that is, (1) directly adding the chemicals dissolved in solvent into the sediment slurry; (2) adding the chemicals dissolved in solvent to the wall of a rolling jar, followed by a solvent evaporation step and addition of the sediment; and (3) adding the chemicals dissolved in solvent to a small portion of sand, followed by a solvent evaporation step and addition of the remainder of the sediment.

The test chemicals were dissolved at a concentration of 100 or 200 mg/L acetone in three spike solutions. For all three spiking approaches, spiking resulted in nominal concentrations of 1 mg/kg dry weight for DIPN and DBUD; and 2 mg/kg dry weight for HMN, HCB and OCN. Details on the actual spiking procedures are presented in the Supporting Information, Text

S2. After spiking, the jars were immediately placed on a roller bank operating at 50 rpm. From each jar, five random-spot subsamples of approximately 12 g wet weight were then taken after 1, 3, 7, 14, and 28 days on the roller bank. The samples were placed in 20-mL glass vials and stored at −20 °C. Once all samples had been collected, they were thawed and homogenized, and 1.67 g wet weight (1 g dry wt) samples were taken from each vial for a C_{tot} determination of the test chemicals. In addition, 7 g wet weight (4.2 g dry wt) samples were taken for a C_{free} determination with SPME (two 5-cm fibers; 5-week equilibration time), according to a standard protocol (Jonker et al., 2020) and as explained in the next paragraph.

In addition to the three spiking approaches just described, a sediment spiking method for liquid VHOCs based on passive dosing was developed and tested. The goal was to saturate sediment with the VHOCs, that is, load the sediment up to its maximum sorption capacity, to obtain upper limit toxicity test concentrations of the test chemicals; see Jonker & Diepens (2024 [this issue]). These saturated samples could then be diluted stepwise with clean sediment, to create a concentration range. Two passive dosing approaches were investigated, one using SR tubing and one using sealed PE lay flat tubes. Both approaches were applied to HMN and DIPN. Briefly, SR and PE tubing was filled with the liquid substances, sealed, and equilibrated in sediment slurries on a roller bank for different lengths of time (1–12 weeks), after which C_{tot} and C_{free} were determined. A detailed method description is provided in the Supporting Information, Text S1.

Sediment equilibration time experiment

Separate batches of artificial sediment (45% water content) were spiked with six different concentrations (0, 30, 100, 300, 1000, and 3000 mg/kg dry wt) of either BCH, DIPN, or PMH. Direct spiking without a carrier solvent was performed (i.e., with the pure, liquid chemicals, because applying a carrier solvent would require adding very large solvent aliquots, due to solubility constraints of the test substances in solvent), while intensively stirring with a mechanical mixer at approximately 700 rpm for in total 5 min/system. The systems were then closed with aluminum-lined lids and equilibrated for five different periods: 2 days, 1 week, 2 weeks, 1 month, or 4 months (i.e., 2, 7, 14, 28, and 112 days), after which sediment toxicity tests were started. Sediment equilibration took place by continuous mixing on a roller bank at 60 rpm, but the sediments to be equilibrated for 4 months were removed from the roller bank after 1 month and left in a box at room temperature for the remaining 3 months, because some jars had started to leak. Spiking of the systems was performed on different days and such that all toxicity experiments could be performed in two subsequent batches, 3 days apart. This way, potential experimental and biological variability was minimized. When the toxicity assay exposure systems were being prepared, sediment samples were taken for the determination of C_{tot} and C_{free}, (~2 and 60 g wet wt, respectively) with the latter determined using PE as the passive sampler (2-mg samplers; 6-week equilibration time).

The toxicity tests were performed with the deposit feeding Annelida *L. variegatus* (Müller) according to OECD (2007) test guideline 225 with some small adaptations, as described in the Supporting Information, Text S3. Ten individuals were added to the test systems, and each system was replicated three times. Tests lasted for 28 days, and the endpoints were total number of individuals, reproduction, and total *Lumbriculus* dry weight at the end of the test. Total dry weight appeared to be the most robust metric and was plotted as a function of either C_{tot} or C_{free} to obtain concentration–response curves. Concentrations at which the endpoints were affected for 50% (EC50) were determined by fitting a dose–response model to the data, using the DRC package in *R* software (see the Supporting Information, Text S3, for details).

Passive sampling of VHOCs

The C_{free} of the test substances in sediment from the above experiments were determined with PE or SPME, according to a standard passive sampling protocol for ex situ measurements (Jonker et al., 2020). Prior to the actual application in the experiments, sampling kinetics for PE, SPME, and POM were investigated by determining test chemical concentrations in the polymers as a function of time (1, 2, 4, 6, 9, and 12 weeks) after exposure to artificial sediment spiked with all 10 test substances. Further details on the preparation of this sediment and the kinetics experiment can be found in the Supporting Information, Text S4. Polymer/water partition coefficients (K_{PW}) required for the final calculation of C_{free} were determined according to a previously described batch-shake method (Jonker, 2022). Briefly, samplers were equilibrated for 18 weeks at 150 rpm and 20 °C in dishwasher- and solvent-cleaned 250-mL amber-colored glass bottles, filled with Milli-Q water containing 100 mg/L of sodium azide and spiked with a mixture solution of the test chemicals in acetone. The PE and POM samplers were fixed on a thin stainless steel rod, locked between the bottom and the stopper of the bottles, to improve the hydrodynamics around the samplers (i.e., reducing the aqueous boundary layer and speeding up kinetics). After equilibration was ended, water phases were liquid–liquid extracted with hexane, and samplers were extracted with acetonitrile in autosampler vials (Jonker, 2022). The K_{PW} determinations for PE and POM were replicated 5 times, and for SPME 10 times (spiking at two different levels because of solubility concerns). In addition, the K_{PW} values for all polymers were derived by cross-calculations from the results of the sampling kinetics experiment, in which all polymers had been equilibrated simultaneously in the same sediment. This was done as follows: first, using the equilibrium concentrations in one polymer and this polymer's K_{PW} values (values judged reliable from the batch-shake experiment), C_{free} values were calculated. Then, using these calculated C_{free} values and the equilibrium concentrations measured in another polymer, the K_{PW} values for this other polymer were calculated. This approach allowed us to calculate missing K_{PW} values, and also to validate values that had been determined in the batch-shake setup.

Total concentration determinations

The C_{tot} of the test substances was determined as follows: sediment samples were dried and homogenized with $NaSO_4$ (Emsure; Merck; dried at 250 °C for 4 h). The resulting samples were extracted with a weighed volume of 5 mL of acetonitrile by subsequently vortexing for 1 min, sonication for 30 min, shaking overnight on a reciprocal shaker at 180 rpm, sonication for 30 min, vortexing for 1 min, and finally centrifugation at 1250 g for 10 min. Then 2.5 mL of supernatant of each sample was weighed into a pointed glass tube and concentrated under a gentle stream of N_2 to a volume of 0.9 mL. Finally, 100 µL of internal standard solution (10 mg/L PCB-209 in acetonitrile) was added, and the extracts were transferred to autosampler vials. Three blanks ($NaSO_4$) and three procedural recovery determinations ($NaSO_4$ spiked with 50 µL of a 100-mg/L test chemical mixture spike in acetone) were included in each extraction series. Blanks were always below the detection limits; recoveries were 85% to 100%, depending on the chemical. Final concentrations were corrected for these values.

Instrumental analysis

All extracts were analyzed with an Agilent gas chromatography–mass spectrometry (GC–MS) system. System and method specifications are provided in the Supporting Information, Text S5. All resulting chromatograms were manually integrated with MassHunter software. Quantification occurred on the basis of at least five calibration standard levels, which were analysed at least four times within an analysis sequence.

RESULTS AND DISCUSSION

Passive sampling of VHOCs

The results of the passive sampling kinetics experiment indicate that passive sampling basically is possible with all three samplers for most of the VHOCs, although equilibration may take several weeks (Supporting Information, Figure S1). Generally, SPME application yielded the highest data variability, in particular for the relatively volatile chemicals, that is, PMH, DMA, HMN, and BCH (see the Supporting Information, Figure S2). This sampler is generally considered the fastest passive sampler, but 26-µm-thick PE appeared to be equally fast for the current test compounds, although SPME obviously was the fastest sampler for OCN. On the other hand, SPME was the slowest sampler for DEHP (equilibrium not yet attained after 12 weeks), equilibrium was not reached for HD after 12 weeks, and equilibrium conditions for HMN after 12 weeks were questionable. Therefore, for most of the investigated chemicals, SPME is not the preferred sampler. In general, PE appeared to be as fast as SPME, although it performed more slowly for OCN and faster for DEHP. However, the data variability for this sampler was generally much lower. The lowest data variability (highest precision) was, however, obtained with POM (Supporting Information, Figure S2). On the other hand, POM appeared to be the slowest sampler (e.g., equilibrium not attained for OCN after 12 weeks), an observation that agrees with previous findings (Jonker et al., 2020). Still, equilibrium was reached for DEHP and HMN after 9 weeks, although this was not the case for SPME; overall, for most of the test chemicals, equilibrium conditions for POM were attained within 9 weeks. All in all, equilibrium passive sampling of all test VHOCs in sediment therefore is possible within 9 weeks, with the exception of HD, for which determination of C_{free} would require more than 12 weeks. Which would be the best polymer to use for sampling depends on the chemical and, assuming polymer–water partition coefficients are available, will be a trade-off between the time available for determining C_{free} and the required or acceptable data variability. Furthermore, sampler choice depends on the potential presence of NAPLs, as is discussed in the companion paper (Jonker & Diepens, 2024 [this issue]). The presence of NAPLs in the present experiment was highly unlikely (if not impossible), considering the low concentrations and the very intensive equilibration (Jonker & Diepens, 2024 [this issue]). Therefore, the increased variability and slow uptake observed for some chemicals cannot be related to fouling of samplers.

Interestingly, several of the above observations do not agree with the general perception of passive sampling in sediments (Jonker et al., 2020) and suggest that simple log K_{OW}-equilibrium sampling time relationships may not apply. Hence, for VHOCs that have not been investigated before, it is recommended to determine sampling kinetics prior to actual sampling to confirm successful application and equilibration.

Determining the C_{free} of chemicals requires the K_{PW} for each target chemical, which needs to be measured experimentally. Although these measurements are very challenging for VHOCs due to the chemicals' extremely low aqueous solubilities (Jonker et al., 2015), the combination of the two applied methods (batch-shake and cross-calculation) in the present study yielded reliable K_{PW} values for all polymers and VHOCs, except for HD (aqueous concentrations of this chemical in the batch-shake experiments could not be determined due to background issues during GC analysis). The cross-calculation approach was used to derive missing values and overall validated the batch-shake–derived K_{PW} values by yielding values generally agreeing within 0.2 log units. The major exception was OCN, for which the batch-shake $K_{PE/w}$ presumably was biased (underestimated), leading to similarly underestimated PE-based cross-calculated values. The values obtained by the different approaches are listed in the Supporting Information, Table S3. These values were compared, and final recommended values were selected for each chemical/polymer combination; these are presented in Table 1.

Comparing spiking methods

The C_{tot} data for the different spiking methods are presented as replicate-averaged values relative to the nominal concentrations (Figure 1) and thus provide information on the effectiveness of the spiking, as well as the variability in the data as a function of time, the spiking approach, and the chemical. The C_{free} data are presented as concentrations in the passive sampling polymer (PDMS) and not in porewater (Figure 1),

TABLE 1: Logarithmic polydimethylsiloxane/water ($K_{PDMS/w}$), polyethylene/water ($K_{PE/w}$), and polyoxymethylene/water ($K_{POM/w}$) partition coefficients for the chemicals tested in the present study

Chemical	log $K_{PDMS/w}$	log $K_{PE/w}$	log $K_{POM/w}$
Bicyclohexane (BCH)	6.04	5.96	4.73
1,11-Dibromoundecane (DBUD)	5.30	5.51	4.97
Di(2-ethylhexyl)phthalate (DEHP)	—	6.20	5.77
2,7-Di-isopropylnaphthalene (DIPN)	5.69	5.57	5.16
1,3-Dimethyladamantane (DMA)	5.04	5.23	3.79
2,2,4,4,6,8,8,Heptamethylnonane (HMN)	7.26	7.19	5.79
Hexachlorobenzene (HCB)	4.92	5.32	5.27
Hexadecene (HD)	—	—	—
Octachloronaphthalene (OCN)	6.50	7.29	6.62
2,2,4,6,6-Pentamethylheptane (PMH)	5.77	6.38	4.86

Dashes (—) indicate cases in which values could not be obtained.

because one of the chemicals (HMN) was sampled in the kinetic mode, and equilibrium concentrations in porewater thus could not be calculated. For the other four chemicals, the data do reflect equilibrium concentrations in the porewater, but these are not presented for consistency reasons. The trends and variability in the presented data will, however, be identical to those in the actual C_{free} data.

The average relative standard deviation of the C_{tot} determinations generally was <5%, which demonstrates that the homogeneity within the spiked systems, as well as the precision of the analytical determinations, was high. Therefore, the current mixing (rolling) conditions were sufficiently intensive to produce homogeneous slurries, even after only a day of rolling. Obviously, at lower mixing speeds, this conclusion may not necessarily apply.

The glass and sand coating methods resulted in very large losses for HMN (95% and 99.7%, respectively; Figure 1A). Losses during direct spiking were lower, but still substantial (~45%). Most probably, the losses are caused by evaporation of the test chemical. Although with the sand coating approach this C_{16} compound had the possibility to evaporate during 1 h in the fume hood, the evaporation phase during glass coating only lasted for less than 10 min. Therefore, it may not be possible to avoid large losses of relatively volatile chemicals with these approaches. In contrast, with direct spiking, losses may be reduced by applying a shorter stirring duration. In the present study, the sediment became somewhat warmer due to the intensive and prolonged stirring. Therefore, in subsequent experiments (Jonker & Diepens, 2024 [this issue]), the stirring time during spiking was reduced to only several minutes. Also for the less volatile liquid chemical DIPN losses were observed for all spiking methods (Figure 1B), although less substantial, showing the same trend: approximately 30%, 20%, and 15% for sand coating, glass coating, and direct spiking, respectively. These results suggest that for relatively volatile chemicals, sand and glass coating are not appropriate methods and that direct spiking is the preferred way of spiking. Alternatively, it might be worth investigating spiking the peat instead of the sand fraction, because this fraction provides a sorption domain that may absorb the chemicals after soaking by the spiking solvent.

Another conclusion that can be drawn from Figure 1 is that also for the nonvolatile compound OCN, sand and glass coating appear to be inappropriate. The C_{tot} data for this chemical (Figure 1C) demonstrate that actual concentrations after spiking are close to nominal concentrations, and that losses thus did not occur for all of the spiking methods. However, the C_{free} data (Figure 1H) suggest that this very poorly soluble chemical dissolves extremely slowly after administration as a solid, that is, with the glass and sand coating methods, where crystals will have formed after evaporation of the spiking solvent. After all, the concentration of OCN in PDMS is more or less stable from a mixing duration of 1 day on with the direct spiking approach, but is observed to increase with equilibration time post spiking with the coating methods.

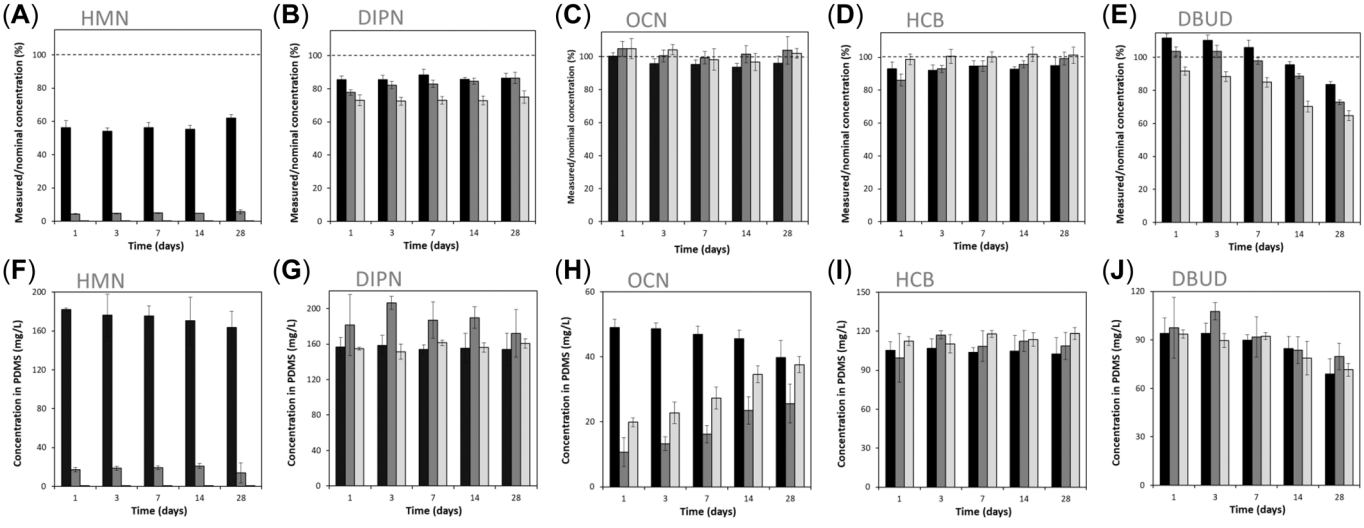

FIGURE 1: (**A–J**) Comparison of direct spiking (black bars), glass coating (gray bars), and sand coating (light gray bars) in terms of the ratio measured/nominal total concentrations (upper row) and concentrations in the passive sampling polymer polydimethylsiloxane (PDMS; lower row) as a function of sediment equilibration time (1–28 days) for the chemicals 2,2,4,4,6,8,8,-heptamethylnonane (HMN), 2,7-di-isopropylnaphthalene (DIPN), octachloronaphthalene (OCN), hexachlorobenzene (HCB), and 1,11-dibromoundecane (DBUD). Dotted lines in the upper row graphs indicate the 100% value (measured = nominal concentration). Error bars represent standard deviations ($n = 5$).

Probably, during mixing the OCN crystals are released easily from the glass wall of the jar (glass coating) and rapidly physically distributed homogeneously throughout the system (glass and sand coating), yielding relatively low variation in the C_{tot} data (Figure 1C). However, several months appear to be required to dissolve the crystals, because even after 4 weeks of intensive mixing on the roller bank, concentrations in PDMS-coated SPME fibers (which had equilibrated in the sediments for an additional 5 weeks) did not reach the same levels as with direct spiking. These data therefore demonstrate that (1) spiking through coating approaches, as recommended by the OECD (2004, 2007), is also unsuitable for very poorly soluble chemicals, and (2) spiking performance for VHOCs cannot be evaluated on the basis of C_{tot} alone, as has been done so far (Murdoch et al., 1997; Northcott & Jones, 2000a; Reid et al., 1998). For this purpose, it is essential to include C_{free}, that is, the exposure metric describing actual uptake and effects in subsequent bioassays.

Although also a solid, HCB apparently dissolved faster (its aqueous solubility is 100–1000 times higher than that of OCN; Supporting Information, Table S2), because the above-mentioned issues are not observed for this compound (Figure 1D and I). Concentrations in PDMS are relatively stable with equilibration time post spiking, and no major differences were observed for the different spiking methods. Therefore, all spiking methods seem to be appropriate for this chemical, although for glass coating, 1 day of intensive mixing on the roller bank may be too short, because the resulting C_{tot} is reduced compared with the data for longer mixing times.

Finally, the data in Figure 1E and J demonstrate that DBUD degrades in sediment. Both the C_{tot} and C_{free} data show a decreasing trend with mixing time. Because a biocide was added, this decrease most probably is due to chemical degradation. Obviously, for this and other unstable compounds, relatively short mixing times would be required. Applying the current mixing conditions, for DBUD, 1 to 3 days would be the optimal mixing time, although it should be noted that degradation will continue and may become substantial during the subsequent toxicity tests (lasting for up to 4 weeks), which would complicate interpretation of the test results and require quantification of metabolite concentrations.

Although a recent meta-analysis of sources of variation in sediment toxicity could not identify an effect of the spiking method on toxic effect concentrations (Hiki et al., 2021a), overall, our results clearly demonstrate that the spiking approach can have a substantial impact on these concentrations, when expressed on a C_{tot} basis. Spiked chemicals may either be lost from the system or be unavailable for uptake when spiked through specific methods. Expressing effect concentrations on a C_{free} basis should remove differences between different methods (Hiki et al., 2021b).

Passive dosing

Passively dosing sediment with liquid VHOCs appeared to be unsuccessful. First of all, the PE bags burst open, possibly because the polymer was not fully resistant to the test chemicals or the mixing-induced shear stress, or the seals did not hold. Second, concentrations in the solid phase dosed through silicone tubing continued to increase up to (and presumably beyond) 12 weeks of mixing (Figure 2A and C), reaching concentrations far above NAPL-forming concentrations (Jonker & Diepens, 2024 [this issue]). In addition, accompanying C_{free} values, as represented by concentrations in the passive sampling polymer (PDMS), were more or less stable and at the solubility level (see Supporting Information, Text S6) already from 1 to 2 weeks of mixing on (Figure 2B and D). Most probably, the last two observations can be explained by excessive absorption of the liquid VHOCs in and swelling of the silicone dosing polymer, and subsequent continuous chemical sweating from the polymer to the medium under dynamic conditions. This would imply release of a liquid phase rather than passive diffusion or partition-driven dosing. More details on the passive dosing results are presented in the Supporting Information, Text S6. To further investigate the swelling and sweating hypothesis, 15-mg pieces of silicone rubber tubing and PDMS sheet (250-µm-thick) were submerged in different liquid chemicals in autosampler vials for 1 week on a rotation table shaker, after which they were dried with lint-free tissue and weighed again. The results showed that both polymers indeed can take up high weight percentages (over 150%) of certain liquid substances, thus causing substantial swelling (Supporting Information, Table S4). This hypothesis may also explain the lack of success of previous passive dosing attempts in water, trying to dose (liquid) dodecylbenzene from silicone tubing (Stibany et al., 2017).

Sediment equilibration

Although our results suggest that intensive mixing on a roller bank for a few days following direct spiking is sufficient for obtaining a chemically homogeneous system, these conditions do not guarantee a toxicologically stable system per se. After all, the passive sampling analyses conducted to judge the homogeneity required several additional weeks of equilibration. Previously, it has been hypothesized that prolonged equilibration of sediments can reduce chemical bioavailability (Alexander, 2000; Landrum et al., 1992; Redman et al., 2014). However, the results of the present dedicated equilibration time experiment, as shown in Figures 3 and 4, do not suggest such an effect. Generally, the EC50 values for *Lumbriculus* derived from concentration–response curves (expressed on an actual C_{tot} or a C_{free} basis; Figure 3 and Supporting Information, Figure S3) obtained for the three tested VHOCs were not significantly different for sediments that had been equilibrated for varying lengths of time. Several exceptions exist (Supporting Information, Text S7), but these are exposure metric, endpoint, and chemical dependent, and trends do not exist. This is illustrated in Figure 4, in which the EC50 values expressed on a C_{tot} and C_{free} basis derived for the different equilibration times are plotted graphically. These results demonstrate that the length of the postspiking equilibration period does not have a significant impact on the toxic response

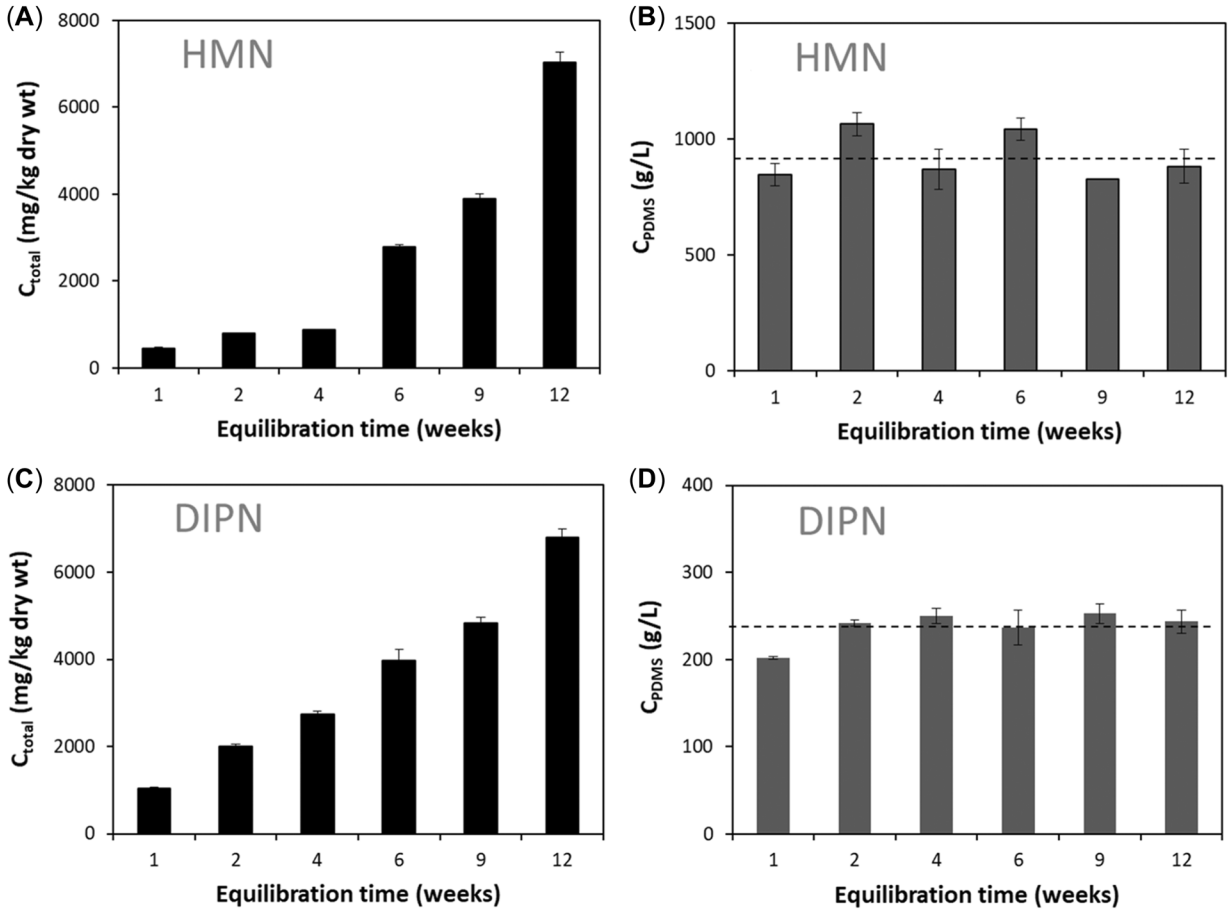

FIGURE 2: Concentrations of 2,2,4,4,6,8,8,-heptamethylnonane (HMN) and 2,7-di-isopropylnaphthalene (DIPN) in sediment (**A** and **C**, respectively) and in a passive sampling polymer (polydimethylsiloxane [PDMS]; **B** and **D**, respectively), as a function of passive dosing time with silicone rubber tubes filled with the pure chemicals. The dashed lines in graphs **B** and **D** indicate the presumed saturation concentration of the respective chemical in PDMS (see the Supporting Information, Text S6, for explanation). Error bars represent standard deviations ($n = 3$).

of *Lumbriculus* to the three test chemicals. This finding suggests that the bioavailability of the VHOCs in spiked sediment did not substantially change from 2 to 112 days. To investigate this possibility in more detail, organic carbon–normalized sediment/water partition coefficients (K_{OC} values) were calculated, using measured C_{tot} and C_{free} values and the organic carbon content. The results are presented in the Supporting Information, Figure S4, and show that log K_{OC} values are independent of the chemical–sediment contact time, which indeed implies that the bioavailability of these chemicals was

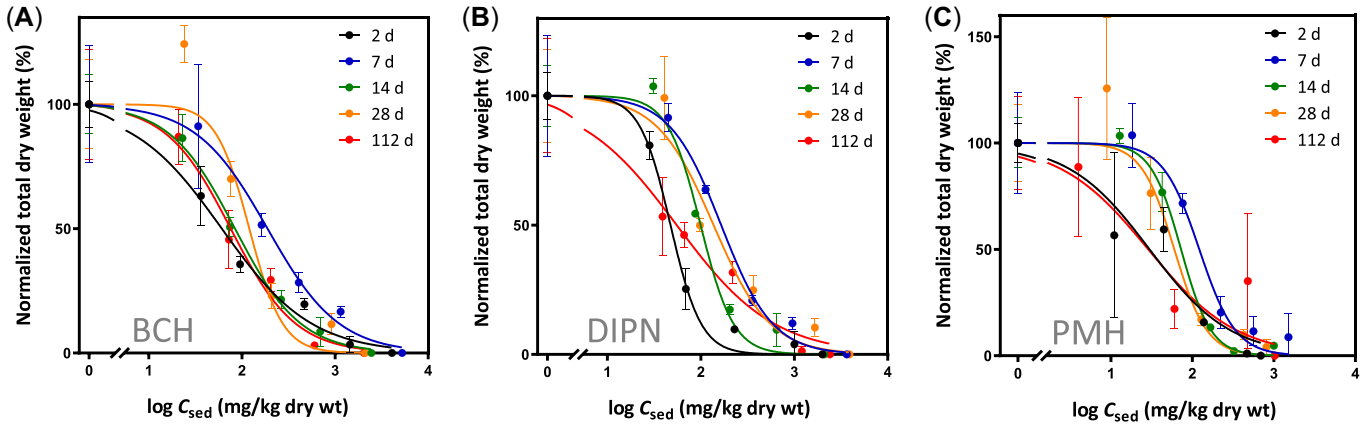

FIGURE 3: Concentration–response curves for the effects of bicyclohexane (BCH; **A**), 2,7-di-isopropylnaphthalene (DIPN; **B**), and 2,2,4,6,6-pentamethylheptane (PMH; **C**) on the dry weight of *Lumbriculus* exposed to spiked artificial sediment that had been equilibrated for 2, 7, 14, 28, or 112 days. Concentrations are expressed on a total sediment concentration (C_{sed}, mg/kg) basis. Responses (total dry wt) were normalized to the averaged response in the control systems. Error bars represent standard deviations ($n = 3$). Data for log $C_{sed} = 0$ represent control systems.

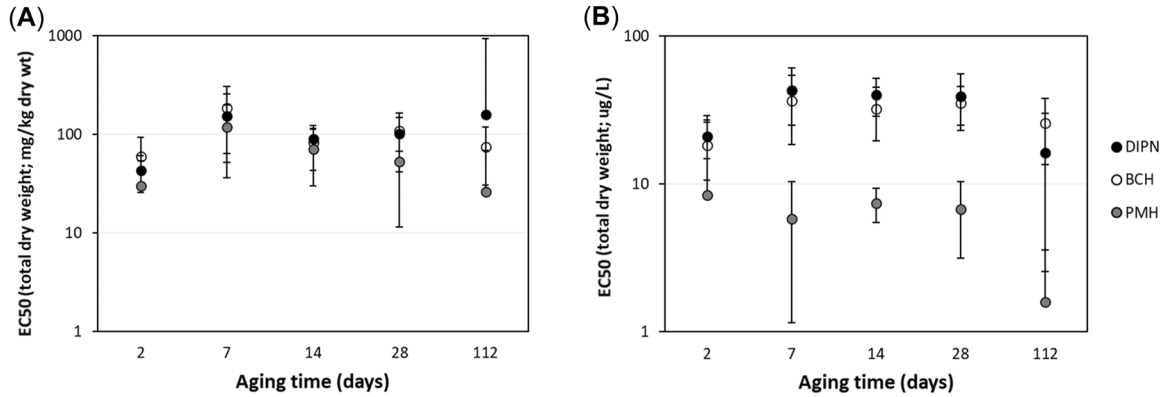

FIGURE 4: The median effective concentration (EC50) values of 2,7-di-isopropylnaphthalene (DIPN), bicyclohexane (BCH), and 2,2,4,6,6-pentamethylheptane (PMH) as a function of the sediment postspiking equilibration time. The EC50 values were derived by curve-fitting the data in Figures 3 and Supporting Information S3, with the error bars representing their 95% confidence intervals. The EC50s are expressed on a total sediment concentration (C_{sed}, mg/kg dry wt; **A**) or freely dissolved concentration (C_{free}, µg/L; **B**) basis and effects are quantified on the basis of *Lumbriculus* total dry weight.

not affected by equilibration up to 4 months. In contrast, *Lumbriculus*' reproduction and dry weight in the control systems appeared to decrease with equilibration time (see the Supporting Information, Text S7, for details) down to values just below the OECD test validity criteria. Because additionally EC50s obtained after 2 days were somewhat, although not significantly, lower than the other values, the present results would suggest that a conservative equilibration time of 7 to 14 days is recommended. This agrees with a previous recommendation to equilibrate VHOC-spiked sediments and soils for at least 7 days (Redman et al., 2014). Hence, for standardization purposes, sediments for follow-up toxicity assays (Jonker & Diepens, 2024 [this issue]) were all equilibrated for 14 days, during which the spiked systems were continuously rolled on a roller bank at 60 rpm.

The observation that equilibration time did not significantly affect EC50s in the present study may be explained by the intensive mixing applied during equilibration. The resulting system dynamics will have sped up dissolution and sorption kinetics, and we therefore consider these crucial for ensuring that poorly soluble VHOCs are homogenized, dissolved, and sorbed to the fullest degree. In contrast, in static systems, dissolution and sorption kinetics are fully determined by diffusion rates, which are extremely low for VHOCs. Hence, overall equilibration kinetics in static or mildly mixed systems will be much slower and may be insufficient for VHOCs. Although the present study was not designed to test this hypothesis, it may actually explain the previously observed effects of equilibration time (Hiki et al., 2021a; Redman et al., 2014), as well as the lack of toxicity and slow bioaccumulation kinetics observed for certain VHOCs, as is discussed in the companion paper (Jonker & Diepens, 2024 [this issue]). However, it should be stressed that only artificial sediment was tested in the present study and that any effects of equilibration time may, to some extent, be dependent on the type of sediment (You et al., 2009). Interestingly, the above plea for intensive mixing clearly contradicts the recommendation by Picone et al. (2022) to perform equilibrations statically so as not to disturb partitioning. Likewise, the USEPA (2000) recommends an equilibration period of at least 2 months for VHOCs (i.e., chemicals with log K_{OW} greater than 6), but if performed statically, 2 months may actually even be too short, in particular at low temperatures (4 °C), at which aqueous solubilities and diffusion rates are further reduced.

CONCLUSIONS AND RECOMMENDATIONS

The results of the present study demonstrate that the application of glass and sand coating approaches (OECD, 2004, 2007) for spiking relatively volatile and very poorly soluble VHOCs to sediments can result in unacceptable losses or the formation of nonbioavailable crystals. Hence, the use of these methods is discouraged; instead, direct (drop-wise) spiking is recommended, either through a water-miscible carrier solvent (preferable for solid VHOCs, if feasible) or without (liquid VHOCs). During spiking, the use of a mechanical stirrer operating at high speed is crucial. Omitting such intensive stirring may result in nonoptimal distribution/homogenization and the presence of crystals or NAPLs. For volatile chemicals, stirring time should be limited to several minutes. Immediately after spiking, mixing on a roller bank at high rpm (40–60) for 1 to 2 weeks is recommended for optimal equilibration (distribution, dissolution, and sorption) and producing a stable response in subsequent toxicity assays. Static equilibrations or less intensive mixing may result in the presence of crystals or NAPLs, which may cause either false-negative or false-positive effects in toxicity tests (Jonker & Diepens, 2024 [this issue]). For degradable VHOCs, the mixing period should be reduced, for example, to 2 days, if feasible.

The passive sampling experiments have demonstrated that it is possible to determine C_{free} for VHOCs. Therefore, application of passive sampling to quantify C_{free} in addition to C_{tot} is recommended, because C_{free} is an important exposure metric for benthic invertebrates. Universal recommendations regarding sampler choice and equilibration time cannot be made, as these are chemical specific. However, the use of PDMS-coated SPME fibers is discouraged for more volatile and liquid VHOCs in particular. Because equilibration may require

prolonged exposures, sampling kinetics should be investigated for new chemical–sampler combinations. If this is not feasible, equilibration times should be at least 6 to 9 weeks. Preferably, C_{free} measurements should be performed with sediment samples from the start and the end of the exposures, the results of which will provide an indication of test chemical stability and allow the calculation and application of a time-weighted average C_{free} value. Determining sampler–water partition coefficients, as required for calculating C_{free}, is very challenging and sometimes impossible for VHOCs. In case the experimenter has no (successful) experience in determining partition coefficients for VHOCs, an expert should be consulted.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5820.

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