Ex situ determination of freely dissolved concentrations of hydrophobic organic chemicals in sediments and soils: basis for interpreting toxicity and assessing bioavailability, risks and remediation necessity

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Passive sampling protocol for ex situ determination of freely dissolved concentrations of hydrophobic organic chemicals in sediments and soils: Basis for interpreting toxicity and assessing bioavailability, risks, and remediation necessity and efficiency

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Abstract

This paper describes a state-of-the-art passive sampling protocol for determining freely dissolved concentrations (C_{free}) of hydrophobic organic chemicals in sediment and soil samples. It represents an international consensus procedure, developed during a recent inter-laboratory comparison study. Quantifying C_{free}, although challenging, is crucial when assessing risks of

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AUTHOR CONTRIBUTIONS

M.T.O.J. wrote the manuscript. F.S. drafted Box 1 and the section on interpretation of PRC results, and helped fine-tuning several conceptual and methodological aspects. R.L. drafted the PRC section in the Experimental Design. All coauthors contributed to improving the manuscript by providing comments and edits.

COMPETING FINANCIAL INTERESTS

The authors declare no competing interests.

DATA AVAILABILITY

No datasets were generated or analysed during the current study.

contamination in field and spiked sediments and soils (e.g., when judging remediation necessity or interpreting results of toxicity assays performed for chemical safety assessments), as it is considered the driver behind chemical bioavailability and, ultimately, toxic effects. This protocol describes the selection and preconditioning of the passive sampling polymer; critical incubation system component dimensions; equilibration and equilibrium condition confirmation; quantitative sampler extraction; quality assurance/control issues and final calculations of C_{free}. The full procedure requires several weeks (depending on the sampler used) due to prolonged equilibration times. However, hands-on time, excluding chemical analysis, is approximately 3 days for a set of about 15 replicated samples.

Keywords

passive sampling; sediment; soil; freely dissolved concentration; toxicity; exposure; environmental risks; contamination; polymer; chemical safety assessment; PAHs; PCBs; pesticides; bioavailability; remediation

INTRODUCTION

Passive sampling in contaminated sediments and soils

Numerous sediments and soils around the world are contaminated with anthropogenic hydrophobic organic chemicals (HOCs; e.g., polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and chlorinated pesticides), which can pose a serious threat to ecosystems and human health[1,2]. To assess the associated exposure and risks, traditionally, sediment and soil grab samples are subjected to organic solvent extractions to determine the total concentrations of contaminants in the particulate phase[3,4]. However, many studies have demonstrated that this approach often misrepresents risks at contaminated sites and that actual risks are better assessed based on measured freely dissolved concentrations (C_{free}) of contaminants in interstitial (pore) water[5]. This is in agreement with the presumption that C_{free} is a good surrogate for the driving force for diffusive uptake in benthic organisms and subsequent toxic effects[3,5,6]. Therefore, C_{free} is considered the most relevant exposure metric upon which to base risk assessments in benthic systems[6,7].

Measuring C_{free} for many sediment or soil associated HOCs is particularly challenging, because these concentrations are generally very low (fg - ng/L range)[8–10]. Such low concentrations are typically below the limits of detection (LOD) of traditional analytical methods used for pore water samples, but they can be accurately determined using partitioning-based, non-depletive sampling with polymers, colloquially referred to as passive sampling. Compared to conventional (bulk) sampling methods, passive sampling methods have several additional advantages, which have attracted the attention of environmental managers seeking to assess more accurately risks associated with contaminated sediments and soils[7,11–13]. The methods employ a specific permeable polymer, which is placed in contact with the sediment or soil sample of interest. Organic contaminants present in the sample will passively diffuse into the polymer, driven by a chemical-specific affinity for this phase, after which they can be extracted from the polymer and quantitatively chemically analyzed. Knowing the chemical-specific affinity for the polymer, C_{free} can be calculated

from the concentration in the polymer[14]. Passive sampling can be applied in the field (in situ) or under controlled laboratory conditions (ex situ). Factors to consider when deciding to perform either in situ or ex situ measurements have been presented earlier[14]. The present protocol focuses on the latter application, where field collected sediment or soil samples or spiked samples are incubated with a polymer in laboratory batch experiments, resulting in a relatively simple, inexpensive, and rapid determination of C_{free}. Despite this, many steps and considerations described here are applicable to in situ deployments as well.

Over the years, researchers have applied several different passive samplers, made of various polymers and having different conformations. The materials that are most often applied as passive samplers in sediment and soil research and regulation include strips of thin polymer sheets made of low density polyethylene (PE)[13,15,16], polyoxymethylene (POM)[8,17–19], polydimethylsiloxane (PDMS) and silicone rubber (SR)[20,21]; and solid phase micro-extraction (SPME) fibers coated with PDMS[22–24]. Other samplers have also been described and applied, but these are often not commercially available and need to be custom prepared (e.g., vials coated with PDMS[25] or ethylene vinyl acetate (EVA)[26,27]). The proliferation of different passive sampling methods to determine C_{free} (i.e., methods vary per type of sampler and from laboratory to laboratory) has made it exceedingly difficult to compare results across laboratories, which has subsequently hampered regulatory acceptance of the technique. To address this issue, an international inter-laboratory comparison study (“ring test”) was recently performed on passive sampling in sediments[28]. This study demonstrated that standardization of passive sampling methods is crucial for reducing inter-laboratory variability. Furthermore, when performed in a unified, quality controlled way, passive sampling yields robust and precise results, with very low inter-method variability (< factor of 1.7) [28].

Here, we present a standardized protocol that was developed and applied in the inter-laboratory study described above[28]. This protocol represents the state of the art in passive sampling in sediments and soils, standardizes critical aspects, integrates best practices from several expert laboratories, simplifies sampler handling and extraction, and can be considered as a consensus protocol from a large group of leading international scientists in this research field. Key protocol considerations include the selection and preconditioning of the most suitable polymer and its conformation; incubation system dimensions (sediment/soil-to-water and sediment/soil-to-polymer ratio) to avoid depletion of target contaminants; achievement and confirmation of equilibrium conditions; quantitative polymer extraction with specific organic solvents; chemical analytical procedures and final (model) calculations to determine C_{free}. In terms of detail, the protocol goes well beyond previously published practical guidance for passive sampling in sediments[14,29], which primarily provided general recommendations. Also, the protocol is more specific than the general SPME protocol published by Risticevic et al.[30], as it exclusively focuses on sediment and soil applications, but also includes multiple passive sampling materials in addition to SPME.

Potential applications of the protocol

The protocol presented here has two main application areas. First, it can be applied to quantify C_{free} of HOCs in field contaminated sediments and soils. In this case, the C_{free}

value can be used to assess bioavailability, exposure, bioaccumulation, and risks of the contamination, which will allow environmental consultants, site managers, and regulators to make better science based cleanup decisions and monitor cleanup efficiency. As such, passive sampling methods provide clear benefit to the status quo in several risk assessment and remediation case studies. For example, the United States Environmental Protection Agency (US EPA) Superfund Program readily applies passive sampling information for assessing risks at contaminated sediment sites[13,31–33]. In addition, the protocol could be applied to field contaminated samples in conjunction with bioassays, to identify levels of specific stressor chemicals causing adverse effects (e.g., PAHs)[34].

Second, the protocol can be applied to determine C_{free} in laboratory spiked matrices when investigating the toxicity of chemicals in sediment or soil. Such tests are required for specific classes of chemicals as part of chemical safety assessment procedures under international regulations (e.g., REACH – Registration, Evaluation, Authorization, and restriction of CHemicals; the European chemical regulation)[35]. Standard protocols exist for such toxicity tests[36–38], but exposure characterization therein relies on total extractable concentrations in the matrix. In order to improve data interpretation and relevance of the results, C_{free} should be quantified in such cases as well[39]. However, the limitations of traditional methods used for trying to measure C_{free} (e.g., centrifugation to isolate porewater[40]) have hampered these efforts. Overall, the current protocol may be useful for researchers, engineers, and analysts from academia, government, consultancy, and industry; working in the fields of environmental chemistry, exposure sciences, risk assessment, aquatic and terrestrial ecotoxicology, remediation, and chemical safety assessment.

The protocol can be applied for the determination of C_{free} for a wide range of non-ionized organic chemicals of concern, in particular those with octanol-water partition coefficients (K_{ow}) larger than approximately 10^3. Examples of these neutral HOCs include petroleum and combustion derived chemicals (e.g., PAHs and aliphatic chemicals), organochlorine pesticides (OCPs; e.g., DDT isomers and degradation products, drin compounds (e.g., dieldrin, endrin), hexachlorocyclohexane isomers), PCBs, chlorobenzenes, chloroanilines, and several other ubiquitous chemicals of concern (persistent, bioaccumulative and toxic (PBT) compounds) that are included in the Stockholm Convention, European Commission, and the US EPA's priority pollutants lists.

Limitations

The protocol presented in this paper is not applicable to metals, chemicals with a K_{ow} less than approximately 10^3, and ionized chemicals as these have limited affinity for the polymers used as passive samplers. For metals, it is possible to determine an analogous C_{free} (e.g., free ion activity or concentration) with the help of passive sampling methods, such as diffusive gradients in thin films (DGT) and Gellyfish[41–43], but these techniques differ, mechanistically and practically, from the approach for HOCs presented here. For ionized chemicals, such as many surfactants, pharmaceuticals and munitions, passive sampling is possible, but polymers other than those described here are required for the sampling procedure. For example, polyacrylate-coated SPME fibers and fibers with a mixed-mode coating have been applied to determine C_{free} of anionic and cationic chemicals[44,45] and EVA

has shown promise with munitions[46]. However, it should be noted that sorption of such chemicals to these polymers may be concentration dependent, which greatly complicates data interpretation and calculation of C_{free}. Accompanying considerations and calculations are not part of the current protocol, which relies on linear sorption isotherms of the target chemicals to the passive sampling polymers, which have been demonstrated for the neutral HOCs listed in the previous section[8,17,47].

Although LODs of passive sampling generally are (much) lower than those of traditional analysis methods on pore water samples, passive sampling, as described in the current protocol, also has its detection limits. Whether or not C_{free} of HOCs in sediments and soils can be quantified with the current protocol, obviously depends on the concentrations present in the matrix and the LOD of the analytical equipment used for quantification. In addition, the ratio of the sampler uptake capacity (i.e., the sampler mass used multiplied by the sorption affinity of the target chemical for the sampling polymer) and the sample sorption capacity (estimated as the organic carbon fraction (f_{oc}) of the sample multiplied by the organic carbon-water partition coefficient of the target chemical) is also important. Because this ratio is sample, chemical, and polymer dependent, a universal minimum concentration in sediment or soil above which passive sampling will be able to quantify C_{free} cannot be provided. Generally, this limit will be in the (low) μg/kg range, even though in the protocol the sample and polymer masses are standardized and maximized, respectively (to warrant optimal system homogenization and equilibration, and sampler extraction). However, a priori calculations are needed to assess whether application of the protocol to a certain sediment or soil sample will result in detectable concentrations (see equations 3 and 4; discussed below).

In the case of low estimated concentrations, one might tend to increase (maximize) the sampler mass to lower the passive sampling LOD. However, this may cause an overly large sampler uptake capacity, which overwhelms the sample sorption capacity. This should be avoided, as in such a case depletion of target chemicals from the sample will occur, which will result in an underestimated C_{free} (discussed below). Since there is also a minimum to the sampler mass that can be used, the current protocol will therefore not be applicable to samples with a f_{oc} < approximately 0.002 (i.e., very sandy samples).

Although very low concentrations often do not imply risks and do not call for remediation, there are cases in which such low concentrations are toxicologically relevant (e.g., dibenzo-*p*-dioxins, brominated flame retardants, or pyrethroid pesticides). If calculations indicate that passive sampling according to the current protocol will yield results < LODs or very sandy samples are under investigation, but C_{free} quantification is desirable, system dimensions (mass of sample, system volume, and possibly mass of sampler) would have to be increased drastically to be able to quantify C_{free} at all (see below). These conditions are challenging to standardize and are outside the application and scope domains of the current protocol. Very low concentrations do usually not occur in connection with toxicity tests for chemical safety assessment purposes, and thus the above limitation generally does not apply to samples prepared in the laboratory. Hence, here the chemical applicability domain is also broader, as long as the above mentioned chemical criteria (i.e., chemical is neutral and $K_{ow} > 10^3$) are met.

Another limitation of the protocol is that it does not allow a quick determination of C_{free}. The metric is most easily and accurately determined under equilibrium conditions[6,14] and equilibration takes days to months (depending on the target chemical, sampler, and conditions). Consultants or regulators in charge of managing contaminated field sites often prefer receiving information on potential risks and remediation necessity as soon as possible, but the long equilibration times do not permit rapid decisions. Although decision urgency will vary from case to case, one should realize that (i) the contamination has often been present for many years and waiting additional weeks will not particularly worsen the situation, (ii) sometimes, toxicological or bioaccumulation bioassays are also performed and these will require several weeks to complete as well, and (iii) waiting for sampler equilibrium will benefit the accuracy of the results and increase confidence in the final risk assessment. In the case of investigating spiked samples for research or chemical safety assessment purposes, the prolonged sample processing time prescribed by the current protocol generally will not be problematic. Admittedly, during prolonged equilibration times, degradation of target compounds could occur; however, biodegradation may be minimized by adding a biocide and photodegradation avoided by equilibrating in the dark. Unfortunately, chemical degradation cannot be prevented, but chemically degradable (unstable) compounds are inherently not those that are persistent in the environment or those that will prompt remediation. Therefore, C_{free} determinations for such compounds have a limited use in risk assessments.

Finally, it should be stressed that C_{free} values, as determined with the current protocol, do not provide a direct answer to the question of whether the sediment or soil under investigation presents a human or environmental risk. The translation from C_{free} to risks requires information on effect concentrations (e.g., Environmental Quality Standards for pore water, such as Maximum Permissible Concentrations (MPCs) or US EPA's Final Chronic values (FCVs)), as well as expert judgment and possibly modeling of chemical transfer to the potential receptors. These aspects, as well as a detailed discussion on how C_{free} can be used in the assessment of bioaccumulation, remediation necessity, and management of contaminated soils and sediments; and in the interpretation of toxicity assays, is beyond the scope of this protocol. The reader is referred to experts and other literature sources for additional information[7,24,48–50].

EXPERIMENTAL DESIGN

Selection of the passive sampler

The passive samplers, which are available for determining C_{free} of HOCs in sediments and soils can be broadly divided into two groups: thin polymer sheets (i.e., 'sheet samplers'; 25 to 100 μm thick) and SPME fibers (i.e., glass fibers coated with a 10 to 100 μm thick polymer layer). If the experimental protocol presented below is strictly adhered to along with all its quality assurance measures, the same results (i.e., C_{free}) will be obtained with the different samplers[28]. However, in the protocol, a distinction will be made between the two groups of samplers, as their handling and other practical issues differ. In Table 1, the most often applied samplers (i.e., those also included in the recent inter-laboratory comparison study[28]) are listed, along with an overview of their advantages and disadvantages. In Figure

1, a photograph of the different samplers is shown. Table 1 can be used as a guide when selecting the most appropriate sampler for a specific sampling activity. The following general remarks can be presented:

- Accuracy and precision of SPME fiber results depend on the exactness of fiber length and coating thickness. When selecting SPME fiber as a sampler, attention should be paid to this issue. For example, it is recommended to use a magnifying glass when cutting fibers and to measure (microscopically) the thickness of the coating. The actual coating thickness may differ from the thickness as specified by the supplier[28].
- The thinner the SPME fiber coating, the faster the equilibration, but the lower the sensitivity and the higher the variability of the results[28]. The use of 10 and 30 μm coated fibers is therefore discouraged when investigating samples with known or anticipated very low HOC concentrations, but a priori calculations (see below; equation 4) need to be performed for a definitive answer to the question if the SPME application will result in detectable concentrations. The 30 μm coated fibers with a 500 μm core are an exception as they have a larger overall coating volume.
- Generally, for a given polymer mass, the lowest LODs can be achieved with PE sheet samplers, as the affinity of most HOCs for PE is higher than for PDMS.
- In terms of practical handling, 50 μm thick PE and POM are preferable for ex situ measurements, as these are the easiest to cut, weigh, and clean. Thinner (25 μm) PE is somewhat more difficult to cut and clean, as it folds and crumples relatively easily. PDMS and SR sheets are difficult to trace in suspensions and tend to stick to glass and metal surfaces (only when dry). POM is the polymer that can be added, removed from slurries, and cleaned the easiest.
- In terms of kinetics, thin PE and SPME fibers (10–30 μm coatings) are superior, as they generally equilibrate the fastest. The thinner a specific polymer and the lower the affinity of a chemical for this polymer, the faster the equilibration. However, equilibration kinetics also depend on diffusion rates inside the polymer. Consequently, POM and polyacrylate generally equilibrate the slowest for most commonly studied HOCs, due to these chemicals' slower internal diffusion within these two polymers[28,51,52].
- When investigating field samples that contain high levels of petroleum hydrocarbons (oil), a pure petroleum phase (droplets or films) may be present. Such so-called non-aqueous phase liquids (NAPLs) start to form roughly above 1000 mg/kg in sediments[53–55] and may complicate C_{free} determinations. Under these conditions, the use of SPME fibers is discouraged, as PDMS can absorb high levels of oil and the thin fibers may easily get fouled with NAPLs[49,55]. Fouled fibers are difficult to clean and may result in biased measurements. POM has been suggested to be a more appropriate sampler in these cases[17,49,55], as it can be cleaned from NAPLs more easily and the affinity of petroleum hydrocarbons for this polymer is much lower than for PDMS and PE. In any

case, C_{free} determinations in NAPL-containing sediments and soils are challenging and one should be wary.

- The costs per sampler are low for all passive samplers (less, or much less, than $1/sampler), with PE probably being the least expensive. SPME fibers are not as easily obtained as sheet samplers and fiber suppliers generally have a minimum ordering length of several hundred meters to one km of fiber, which requires a substantial financial investment.

Altogether, sampler selection will depend on the skills/experience and preference of the practitioner, time restraints, budget, the presence of NAPLs, and target chemical concentrations in the sediment/soil samples under investigation. However, the first choice may often be PE, because of its relatively low detection limits, fast equilibration, low costs, easy handling, and good availability (of different thicknesses). For the practitioner without any passive sampling experience, the use of PE sheet samplers is also recommended, as it is a convenient sampler with which to work. In contrast, the more fragile SPME fibers typically require more experience and care in handling. Despite some of their advantages, POM and polyacrylate-coated fibers are not recommended, owing to their generally slow equilibration kinetics for HOCs and because their compatibility with so-called Performance Reference Compounds (PRCs; see below) is still unclear[56–58]. Therefore, understanding the extent of equilibrium achieved with these polymers can be challenging, especially for the more hydrophobic chemicals with K_{ow} larger than approximately 10^6. SPME fibers with a very thin (e.g., 10 μm) PDMS coating are also not recommended, specifically for field contaminated samples and for volatile HOCs, because of their inherent low sensitivity and relatively high measurement variability across replicates. Taking all of this into consideration, the current protocol is therefore primarily directed towards applications with PE and PDMS/SR sheets and (30–100 μm) PDMS-coated SPME fibers. Procedures for POM and polyacrylate are included in the Supplementary Information (SI).

Determining the sampler mass/volume and the sampler extract volume

Passive sampling needs to be performed such that only a negligible amount of the target chemical(s) is sampled from the sediment or soil under investigation, keeping the extraction ‘non-depletive’[14], while maximizing the ability to detect measurable concentration(s) of the target chemical(s) in the final extracts. Therefore, it is necessary to a priori design passive sampling measurements by determining the system components (sediment or soil mass, system volume, and sampler mass or volume) and the volume of the final extract.

In the current protocol, the mass of sediment or soil is standardized at 30 g dry weight (in 100 mL of water) for measurements with sheet samplers and 4.2 g dry weight (in 14 mL of water) for SPME fibers. These bulk material masses fit well in the prescribed equilibration system glassware and, after adding dilution water, yield slurries with a density that allows both effective homogenization by shaking and relatively fast equilibration kinetics[20]. To the slurries, typically 2–30 mg of polymer sheet or 3–20 cm of SPME fiber is added. Polymer sheet samplers of less than 2 mg are discouraged, as the uncertainty in their actual weight is too large, and samplers weighing more than 30 mg may not easily fit into the extraction vials. In addition, the extraction of larger polymer masses may not be exhaustive in the

current setup. Similarly, fiber lengths of less than 3 cm are discouraged, as these are more difficult to trace in dense suspensions and the uncertainty in coating volume increases with decreasing length. Lengths of more than 20 cm will not fit in the prescribed extraction glassware (autosampler inserts). As mentioned in the "Limitations' section, larger masses (or longer fibers) may be needed in specific cases, where concentrations of target contaminants in sediment or soil are very low. In order to keep the extraction non-depletive (see below), here bulk material mass and system dimensions would have to be scaled-up. However, it can be challenging to properly shake systems larger than those prescribed here and to achieve equilibrium conditions (see below). Scaling up would also imply that a different sampler extraction method is needed, potentially including solvent evaporation steps and the use of recovery (surrogate) standards. The required procedures for scaled-up systems are not discussed here. If deviations from the current protocol are utilized, the details should be spelled out and the appropriate quality assurance test results (depletion percentage, equilibrium verification, etc.; see below) should be provided to document the effectiveness of the altered methodology.

The mass or volume of the passive sampler is tuned to fulfill the non-depletion criterion. This is critically important, as considerable uptake from the sediment or soil by the sampler depletes chemical concentrations in the sediment and measurements under depletive conditions may significantly underestimate the actual C_{free} [20]. Therefore, a sampler with an overly large uptake capacity (i.e., sampler mass or volume multiplied by the sorption affinity of a chemical for the sampler) should be avoided. Here, it is recommended to set the maximum depletion limit at 5 %, but it is noted that this value is arbitrary and the intention should always be to keep the depletion as low as possible, as underestimation of C_{free} increases with increasing depletion. In order to insure not exceeding any chosen depletion percentage, a priori calculations are necessary, which are both sampler and target analyte-dependent. The goal of the calculations is to determine the maximum mass of polymer sheet ($M_{p(max)}$; mg) or SPME fiber length ($L_{f(max)}$; cm). Keeping the actual sampler mass or volume below this maximum is intended to prevent depletion from occurring. For sheet samplers, the calculation can be performed according to the following equation:

$$M_{p(max)} = \frac{10^6 \cdot M_s \cdot f_{oc} \cdot K_{oc}}{K_{pw} \cdot (\frac{1}{0.05} - 1)} \quad (1)$$

with M_s being the standardized dry mass of sediment or soil sample (0.03 kg) in the system, f_{oc} the fraction of organic carbon in the sample, K_{oc} the organic carbon-water partition coefficient of the target chemical (L/kg), and K_{pw} the polymer-water partition coefficient of the target chemical (L/kg). Note that 0.05 represents the maximum depletion criterion (5%) and it is assumed that the mass of the target chemical in the pore water is negligible.

If the f_{oc} is unknown, but the organic matter content (f_{om}) is available, f_{oc} can be estimated[59] as $0.58 \cdot f_{om}$. Generally, K_{oc} values will be unknown and need to be estimated. Several equations are available for this purpose, commonly relating K_{oc} to K_{ow} (e.g., $K_{oc} = 0.63\ K_{oc}$ [60]) or to so-called Abraham descriptors[61]. It should be noted that estimating K_{oc} values for sediments requires other equations than for soils, due to the difference in organic carbon

nature. Also, many relationships have been derived based on sorption data for neutral HOCs in spiked, laboratory contaminated samples. Because sorption of these chemicals to field contaminated samples has often been observed to be stronger[62–64], maximum polymer masses calculated with the above equation will be conservative (i.e., on the safe side) when studying field contaminated sediments and soils.

Similarly and under the same assumptions, the maximum SPME fiber length can be calculated according to:

$$L_{f(max)} = \frac{10^6 \cdot M_s \cdot f_{oc} \cdot K_{oc}}{K_{pw} \cdot \left(\frac{1}{0.05} - 1\right) \cdot (\rho_p \cdot V_{pc})} \quad (2)$$

with ρ_p being the density of the polymer (i.e., 0.97 kg/L for PDMS) and V_{pc} the volume of the polymer coating per unit of length (μL/cm). Here, M_s is fixed at 0.0042 kg dry weight.

If C_{free} needs to be determined for multiple target chemicals simultaneously (e.g., a series of PAHs or PCBs), $M_{p(max)}$ or $L_{f(max)}$ should be calculated (e.g., in Microsoft Excel) for each individual chemical, since K_{ow} and K_{pw} are chemical specific. The smallest polymer mass or fiber length resulting from these calculations should be applied in the resulting C_{free} determination test to ensure non-depletive conditions for all target chemicals.

Next, in order to maximize detectability of target chemicals, the final volume of the sampler extract ($V_{extract}$; mL) and the sampler mass or volume (M_p or L_f) should be optimized such that target chemical concentrations in the extract ($C_{extract}$; μg/L) are within the calibration range of the analytical equipment (while assuring M_p or L_f stay below $M_{p(max)}$ or $L_{f(max)}$). $C_{extract}$ can be assessed a priori, albeit roughly, based on the (solvent-extractable) concentration of the target HOC in the whole sediment or soil sample (C_s; μg/kg). This concentration is often available from initial field assessments (first tier screenings) or, in the case of spiked samples, from nominal concentrations.

$$C_{extract} = \frac{10^{-3} \cdot K_{pw} \cdot C_s \cdot M_p}{f_{oc} \cdot K_{oc} \cdot V_{extract}} \quad (3)$$

or:

$$C_{extract} = \frac{10^{-3} \cdot K_{pw} \cdot C_s \cdot L_f V_{pc}}{f_{oc} \cdot K_{oc} \cdot V_{extract}} \quad (4)$$

with M_p in units of mg and L_f in cm. If the calculated $C_{extract}$ exceeds the calibration range, the variable M_p or L_f (numerator) should be reduced and/or the variable $V_{extract}$ (denominator) should be increased. If $C_{extract}$ is too low, the opposite can be performed. Obviously, when multiple chemicals are targeted simultaneously, the two variables should be tuned such that concentrations of all chemicals are expected to be within the calibration range. If this is not possible, either later dilutions of the extract or starting separate systems for different (e.g., high vs. low concentrations) groups of chemicals could be considered. The current protocol allows $V_{extract}$ to range between 0.5 and 1.5 mL for sheet samplers and

0.2 and 1.5 mL for SPME fibers. As such, the highest $C_{extract}$ captured with polymer sheets can be obtained by extracting the samplers in 0.5 mL of organic solvent, in which maximally 30 mg of sampler should be placed. For 10–30 μm thick coated SPME fibers, this volume is 0.2 mL of organic solvent (in an autosampler insert), in which maximally 20 cm (30 μm coating/100 μm core) to 30 cm (10 μm coating) can be fitted. In the case of very high anticipated concentrations, the sampler mass or volume can be set at 2 mg (sheets) or 3 cm (fibers), minimally, which can be extracted in 1.5 mL of organic solvent maximally. If in such a case expected concentrations still exceed the calibration range, the extract could be diluted later in the procedure.

As mentioned above, most K_{oc} estimation models are based on laboratory studies and may underestimate sorption in field contaminated samples. This phenomenon is important to consider here (equations 3 and 4), as stronger sorption will imply a lower $C_{extract}$. In such cases, $V_{extract}$ and M_p should be set such that $C_{extract}$, as calculated according to equation 3 or 4, is as high as possible (within calibration limits), allowing the concentration to drop by a factor of 10 (due to a factor of 10 stronger sorption), yet still being above the lowest calibration concentration. For PAHs in field samples, sorption can be a factor of up to about 1000 times stronger (because of strong association with carbonaceous geosorbents[64]). Therefore, for these compounds a ‘safety margin’ of 100, if possible, is recommended in the calculations.

Preparation of the samplers

Prior to use as a passive sampler, polymer sheets or SPME fibers should be pre-extracted with organic solvent(s) to remove any background contaminants that may exist in the material, including organic additives, monomers, and oligomers generated during polymer synthesis. Such compounds may interfere with subsequent analysis, either at the level of peak integration, by clogging LC tubing or pre-column (oligomers), or by contaminating the GC liner or (pre-) column.

Pre-extraction with appropriate solvents can be performed at room temperature (i.e., 20 ± 3 °C) through shaking or at elevated temperature (Soxhlet extraction). The latter is required for PDMS and SR sheets, in order to remove oligomers and additives[65], but is unnecessary and discouraged for PE, as this may damage the polymer. PE should be extracted at room temperature with, as a minimum, the solvent (or its equivalent) specified for extracting target compounds from the exposed sampler and that will be used as the injection solvent for instrumental analysis. This will minimize background contamination when extraction of the exposed sampler is also performed at room temperature. Alternatively, different solvents can be used, combining polar and nonpolar ones, aiming to remove as many different interfering substances as possible and to minimize analytical issues. When pre-extracting with different solvents, mutual miscibility should be considered, as well as the water miscibility or volatility of the solvent to be used last. Water miscibility is important if samplers are kept in water after pre-extraction and/or will be loaded with PRCs in a polar solvent/water mixture, while volatility is important if sheet samplers are subsequently air-dried.

Apart from the warm solvent (Soxhlet) extraction requirement for PDMS and SR, the choice of subsequent pre-extraction solvents for these polymers and those for PE is not critical.

Still, in order to provide a generally applicable pre-extraction procedure, which is compatible with most solvents used for extraction; and to assure full removal of solvent after pre-extraction, the protocol prescribes washing steps with *n*-hexane and acetone for the recommended samplers. Pre-extraction procedures for POM and polyacrylate-coated fibers can be found in the SI.

Equilibration and verification of equilibrium conditions

Uptake of chemicals from the sediment or soil sample into the polymer is controlled by diffusion and therefore requires time[14]. The uptake will continue until a thermodynamic equilibrium among all phases in the system (sediment/soil, passive sampler, and pore water) has been reached. The time required to reach this point is referred to as the time to equilibrium (t_{eq}), which is dependent on several chemical and polymer related factors and incubation conditions. First of all, t_{eq} increases with target chemical hydrophobicity[20,23,28,66]. For example, chlorobenzenes will equilibrate faster than dibenzodioxins and 3-ring PAHs will reach equilibrium sooner than 6-ring PAHs. Second, t_{eq} is dependent on the type and thickness of polymer. As mentioned before, PDMS and PE generally equilibrate faster than POM and polyacrylate, because diffusion of target chemicals in the former polymers is faster; and t_{eq} increases with increasing polymer thickness for a specific polymer[25,28,67,68]. Third, mixing speeds up equilibration. Although the results of static equilibrations (no mixing) can match those of dynamic (intensive mixing) tests[28], mixing increases equilibration kinetics and simplifies the test, as for static equilibrations, PRCs and modeling are required to calculate a final C_{free} if equilibrium conditions have not been attained (see below). Thin SPME fibers (10–30 μm coating/100 μm core) cannot be shaken vigorously due to their fragile nature, but are preferably equilibrated on a rock and roller apparatus, which does result in sufficient mixing. SPME fibers with thicker coatings (e.g., 100 μm) can be shaken vigorously though, with an intensity similar to that for sheet samplers. A one-dimensional, reciprocal table shaker, with an amplitude of about 3–5 cm and operating at 150–180 rpm is recommended in these cases. Mixing on an orbital shaker is discouraged for all samplers used in dense suspensions, as this causes insufficient mixing of such sediment or soil suspensions. Finally, since diffusion kinetics increase with temperature, t_{eq} will be shorter at higher temperatures. It should be noted though, that C_{free} is calculated using a polymer-water partition coefficient (K_{pw}; see below), which is also temperature-dependent and which is commonly determined at 20–25°C. Moreover, most sediment and soil toxicity tests and bioaccumulation studies are performed at room temperature. Therefore, it is recommended to perform ex situ passive sampling measurements at 20 ± 3 °C. If equilibration is performed at an alternative temperature, K_{pw} values used for calculation of C_{free} should reflect the measurement-specific temperature. One should realize however, that K_{pw} determinations, as well as temperature corrections for K_{pw} are very challenging[69].

Generally, under the above mentioned conditions (intensive shaking and 20 °C), t_{eq} for thin SPME fibers (10–30 μm coatings) and PE samplers (25 μm) are up to about 4 weeks for chemicals with K_{ow} values up to approximately 10^8, such as 3–6 ring PAHs and tri to heptachlorinated biphenyls[14,23,28]. For PDMS/SR sheets (100 μm), fibers with a thick PDMS coating (100 μm), and thick PE (50 μm), t_{eq} for these chemicals is extended to 4 to 6

weeks [28]. Although one may rely on these rules of thumb for well-studied chemicals (e.g., PAHs, PCBs) and samples based on experience, t_{eq} for other chemicals and uncharacterized samples may deviate. For instance, for more hydrophobic chemicals, equilibration may take longer. Also, for specific sediments and soils longer equilibration times may be needed[70], due to very slow desorption of bound chemicals to the aqueous phase, which is often related to the presence of specific carbonaceous geosorbents (e.g., black carbon, tar, coal)[64,70]. Because of the required long equilibration times, a biocide (i.e., sodium azide) should always be added to the sample suspension to prevent any biodegradation.

If full equilibrium has not been attained, measurements generally will be inaccurate by 10–20%, although higher percentages have been observed in specific cases[70]. When measurement goals deem it essential for C_{free} to reflect full equilibrium conditions, these conditions should be verified. This can be accomplished in three different ways:

1. Perform a time series determination of C_{free}, e.g., employing a test duration of 2, 4, 6, 8, and 10 weeks[28,70,71]. When a stable C_{free} is established (i.e., no statistically different C_{free} concentrations are detected) for at least the last two time points, equilibrium conditions are supported.

2. Determine C_{free} using passive samplers of different polymer thickness (but of the same polymer type and having the same mass or volume)[10,25,72,73]. For instance, simultaneously use equal weight PE strips of 25 and 50 μm; or SPME fibers of the same length, but with different PDMS coating thicknesses. In the case that the same C_{free} is determined with both samplers of different thicknesses, equilibrium conditions are verified.

3. Incorporate PRCs. These are chemicals added to passive samplers prior to starting the C_{free} determination. During the equilibration phase, they are released from the polymer into the sediment or soil suspension. The PRC mass remaining in the polymer at the end of the exposure can be used to assess the sample depletion percentage and the state of equilibration reached by the target compounds during the laboratory incubation. This concept was first introduced by Booij et al.[74] to better characterize the uptake of HOCs into semi-permeable membrane devices (SPMDs), an early version of a passive sampler. Ideal PRCs are chemically similar to the target compounds of interest, such as mass-labeled (^{13}C or deuterated) analogues of the target analytes. Similarity to the target compounds ensures that the release process of PRCs out of the passive sampler mirrors the uptake process of target compounds from the sediment or soil into the passive sampler. For example, when a target compound reaches a 33 % equilibrium between the sediment or soil pore water and the polymer, 33 % of the corresponding PRC will have been released from the passive sampler. PRCs are impregnated into the passive sampler prior to the deployment in the sediment or soil slurry, through partitioning from a solvent-water mixture, and the initial and final concentration after equilibration are measured. Equilibrium of a target chemical is commonly assumed if the remaining concentration of its matching PRC (or one(s) with a higher K_{pw}) in the passive sampler is < 5%. Under these conditions, non-depletion (< 5%) is also confirmed, as the depletion percentage

mirrors the decrease percentage. If PRCs remain at a greater percentage in the passive sampler, they can potentially be used to correct for non-equilibrium and to estimate full equilibrium C_{free}. Several approaches are available in the literature about how to do this and on how to extrapolate PRC results to all target compounds of interest[58]. There is no firm rule about the number of PRCs that is needed (i.e., more PRCs is better, but can be prohibitive analytically and financially), but PRCs should encompass the range of properties of the target analytes (i.e., they should cover the K_{pw} range of the target chemicals, in particular at the higher end). In addition, they should not interfere analytically with the target compounds and any other compounds used for quality assurance/control (i.e., internal standards) during the chemical analysis. Finally, the polymer-water partition coefficients of the PRCs at 20 ± 3 °C should be known, when aiming to use PRCs for correcting for non-equilibrium conditions (for stable isotope analogue PRCs, the K_{pw} of the target analytes can be used). Typical PRCs include deuterated PAHs (e.g., pyrene-D_{10}, chrysene-D_{12}, dibenz[*ah*]anthracene-D_{12}), ^{13}C-PCBs (e.g., congeners 28, 52, 101, 153, 180), PCB congeners that were rarely industrially produced (e.g., PCBs 29, 69, 155, 192), or ^{13}C-*p,p*-DDT or ^{13}C-DDD.

The first two options for verifying equilibrium conditions are relatively simple, but require additional systems and samplers and thus chemical analyses to be performed, adding costs to the base procedure. For the second approach, part of this disadvantage could be negated if samplers of different thicknesses can be added to the same system (i.e., if $M_{p(max)}$ is not exceeded by adding the additional polymer mass). The PRC approach requires additional materials (PRC standards), sampler processing, and calculations; and thus costs as well. However, it avoids an increase in the number of incubations and provides the additional advantages of being able to verify equilibrium and non-depletion conditions and to estimate equilibrium C_{free} by way of model calculations for compounds for which equilibrium was not attained.

Selection of the extraction solvent(s)

In order to recover the target chemicals that have accumulated within the passive sampler during exposure to the sample suspension, an organic solvent extraction step is needed. Solvent choice is critical, as quantitative extraction of the target compounds is required and not all solvents are practically capable of meeting this criterion for all polymers. As such, choosing a less efficient solvent may lead to an underestimation of C_{free}. However, this primarily applies to POM, which is the most difficult polymer to extract (see SI). Solvent choice is less restrictive for PE, PDMS, and SR. The last two are soft polymers (elastomers), which swell in most solvents[52]; consequently, it is simple to extract chemicals from these samplers. Swelling of PE is not considerable[52], yet chemicals can be extracted effectively from this polymer with most common organic solvents. A list of solvents that can be used for the final extraction of equilibrated samplers, yielding full recoveries of the target compounds, is provided in Table 1. Note that in this respect, target compounds are those nonpolar HOCs mentioned in the *Introduction*; extraction recoveries of other, more polar chemicals (e.g., fluorinated chemicals, modern pesticides, and hormones) using these

solvents has not been tested and would need to be verified prior to application of the respective solvent.

As the polymers described herein act as a ‘chemical sieve’ and only ‘selectively’ extract chemicals from the sample matrix in question, the resulting solvent extracts of exposed passive samplers are relatively ‘clean’, at least when compared to solvent extracts of sediment, soil, and biological tissue samples. Therefore, a clean-up of passive sampler extracts obtained using the current protocol often may not be necessary and as such is discouraged here, as this additional step tends to be relatively labor-intensive, may cause loss of target chemicals, and thereby may increase uncertainty and variability in the results. Still, in certain cases, e.g., when assessing very complex, heavily-contaminated field samples, clean-up may prove necessary and protocols are available. If clean-up is being performed, the procedural recovery of the target chemicals and any PRCs through this additional processing step should be determined and corrected for in the calculations.

Chemical analysis, calibration, and internal standards

Typically, organic chemicals in passive sampler extracts are analyzed using gas chromatography-mass spectrometry (GC-MS), gas chromatography-electron capture detection (GC-ECD), high performance liquid chromatography-fluorescence detection (HPLC-FLD), or liquid chromatography-mass spectrometry (LC-MS) instrumentation, depending on the type of target chemicals. It is beyond the scope of this publication to describe specific analytical methods, instrument settings and conditions, and analytical consumables needed. Yet, chemical analysis of the extracts is a very important step in determining the precision and overall accuracy of the final C_{free} results. The previous inter-laboratory passive sampling comparison study demonstrated that about half of the variability in passive sampling results obtained by research laboratories was caused by differences in analytical methods and techniques, primarily compound identification and instrument calibration[28]. The largest incidental variability was introduced by misidentifications of target contaminants. Although the complexity of passive sampler extract chromatograms depends on the level and diversity of contamination present in the samples studied, it is generally low when compared to solvent extracts of sediments or soils, in particular when spiked artificial sediments or soils are studied. However, solely relying on retention times for the identification of target chemicals in field samples, as is performed with GC-ECD, may not be sufficient. Therefore, GC-MS is recommended for compounds such as PCBs, with the inclusion of at least two unique qualifier ions for each target chemical. For non-GC-MS analyses, the application of two different (GC) separation columns, with stationary phases that differ in their polarity, may be helpful in minimizing target chemical identification errors.

Irrespective of the instrumentation used, proper calibration is of paramount importance[28], as inaccurate calibration will cause a systematic bias. Therefore, the inclusion of a sample or standard with known target chemical concentrations (e.g., a certified analytical standard) is strongly recommended. Further, calibration should be performed based on at least five calibration standards containing the target chemicals in the same solvent as used for the extraction. The calibration standards should cover the relevant concentration range (typically

about 1 – 500 or 1000 μg/L; preferably in the linear range of the instrument), and should be analyzed at least in triplicate (preferably quadruplicate) during an analysis series. In addition, the calibration standards should contain one or more internal standard(s), i.e., a chemical(s) that is not natively present in the samples under investigation and is not applied as PRCs, but is included at the same concentration (e.g., about 50 to 200 μg/L) as added to the extracts. Internal standards are incorporated to correct for variations in extract- and injection volume, and cannot be used to adjust for extraction recovery. Commonly-used internal standards include PCBs 30, 121, 198, 204, 205, 209, ^{13}C-labeled PCBs, *p*-terphenyl and deuterated PAHs.

Calculation of C_{free}: polymer-water partition coefficients (K_{pw})

Concentrations quantified in the extracts are ultimately converted to concentrations in the sampling polymer, which in turn are used to calculate C_{free} in the pore water of the investigated sediment or soil sample. This last step requires polymer-water partition coefficients (K_{pw}). These coefficients are both chemical- and polymer-specific (i.e., they are unique for a specific chemical-polymer combination); however, they are independent of the sample studied. They are quantified in separate laboratory experiments with polymers incubated in water, where the distribution of a spiked chemical between the aqueous phase and the polymer is determined. Such determinations are practically and analytically very challenging for hydrophobic chemicals, because of the chemicals' very low aqueous solubilities[69,75,76], and specific experimental expertise is required. Therefore, making use of quality-controlled literature K_{pw} values is recommended. These are available for PAHs and PCBs for both PE[77] and PDMS[78]. If literature values are not available for the chemicals in question and experimental determination is required, one should consult the literature[69,77,79] (and/or with an expert) for practical guidance on measuring K_{pw}s.

Quality assurance/control

In the above sections, several quality assurance and control (QA/QC) steps were discussed, including system component dimensioning, creating and (approaches for) verifying non-depletive and equilibrium conditions; the use of internal standards, the number and replication of calibration standards, and the analysis of a certified analytical standard. The protocol described below also contains several additional quality assurance steps, such as cleaning steps for the samplers, tools, and glassware; the inclusion of blanks; the use of amber glassware to minimize any photodegradation of target contaminants; and accurate and precise cutting of the samplers. In addition to these steps and precautions, two additional QA/QC aspects are mentioned here. First, C_{free} determinations need to be replicated. Generally, for well-mixed sediments under ex situ conditions, triplicate measurements suffice if they are performed by experienced personnel. Relative standard deviations in such cases may generally be < 5%[28]. However, for soils, which usually are much more heterogeneous, at least quadruplicate determinations are recommended and relative standard deviations will often be (much) higher than 5%. It should be stressed that variability in the results not only depends on the heterogeneity of the samples, but also on the passive sampler and associated methods applied[28]. As mentioned in Table 1, (thin) SPME fibers carry the highest variability; whereas, the thicker sheet samplers generally produce results with the lowest variability[28]. Second, the analysis of a reference sample is highly recommended.

Inclusion of a certified analytical standard provides insight into the accuracy of the chemical analysis, but inclusion of a reference sediment or soil sample will yield information on the quality of the overall procedure. Unfortunately, no certified sediment or soil samples are currently available for this purpose, but one of the sediments investigated in the previous inter-laboratory passive sampling comparison study has been dried, homogenized, and disseminated in portions sufficient for triplicate C_{free} determinations with either SPME or sheet samplers. These are available until stocks are exhausted (contact MTOJ).

MATERIALS

Reagents

- Acetone (GC grade for residue analysis) (Merck, cat. no. 1000121000 or equivalent)

  ! CAUTION Flammable; avoid inhalation, ingestion and skin contact.

- *n*-Hexane (GC-MS grade for residue analysis) (Merck, cat. no. 1007951000 or equivalent)

  ! CAUTION Flammable; avoid inhalation, ingestion and skin contact.

- Organic solvent to choose (GC or LC-MS grade for residue analysis) to be used as extraction- and injection solvent during chemical analysis (e.g., heptane, iso-octane, acetonitrile, methanol, dichloromethane).

  ! CAUTION Flammable; avoid inhalation, ingestion and skin contact.

- Ethyl acetate (GC-MS grade for residue analysis) (Merck, cat. no. 1007891000 or equivalent) (optional – required only when using PDMS or SR sheets as passive sampler).

  ! CAUTION Flammable; avoid inhalation, ingestion and skin contact.

- Methanol (GC-MS grade for residue analysis) (Merck, cat. no. 1008371000 or equivalent) (optional – required only when applying PRCs).

  ! CAUTION Flammable; avoid inhalation, ingestion and skin contact.

- Millipore water (18.2 MΩ·cm).

- Sodium azide (NaN_3; Merck, cat. no. S2002 or equivalent)

  ! CAUTION Highly toxic; avoid ingestion, inhalation and skin and eye contact. Avoid contact with metal surfaces; explosive metal azides may be formed.

Equipment

- Balance with minimal 2 decimals when weighing grams (0.00 g).

- Clean fume hood.

- Air-conditioned room at 20 ± 3 °C.

- Amber-colored 1.5 mL short thread autosampler vials (BGB; part no.080401-XLW or equivalent) with screw caps (BGB; part no. 090300 or equivalent).
- Autosampler vial box.
- Metal spoons for transferring sediment and/or soil.
- Razor blade (or scalpel).
- Metal tweezers without ribs (two pair).
- 20 mL scintillation vial(s) with metal foil-lined caps (Fisher Scientific; art. no. 10079010 or equivalent).
- Clean glass beakers (250/500 mL).
- Ultraclean Erlenmeyer flasks with ground-glass stopper or alike (e.g., a 20 mL vial) for storing the different high purity solvents used for extraction of the samplers.
- GC syringe or calibrated (micro) pipette for adding internal standard solution to sampler extracts (syringe/pipette volume depending on extract volume).
- Thick, lint-free laboratory tissue.
- Disposable, powder-free, nitrile gloves.
- Pasteur’s pipettes + small ‘balloon’.
- Clean syphon with freshly drawn Millipore water.
- Clean syphon with high purity grade acetone.
- Waste receptacle for sediment/soil waste.
- Permanent marker (fine-tipped).
- Labels.
- Vortex mixer(s).
- Chemical-analytical equipment for the detection of the target compounds in solvent extracts, typically occurring down to the μg/L range: GC-MS, HPLC-FLD, and/or LC-MS.

Optional – used for polymer sheet samplers only

- Polymer sheet: (1) low density polyethylene (PE) sheet, 25 μm thickness (VWR International Ltd., Leicestershire, UK); or (2) PE sheet, 50 μm thickness (Brentwood Plastics, Inc., Brentwood, MO, USA; Carlisle Plastics, Inc., Minneapolis, MN, USA); or (3) polydimethylsiloxane (PDMS) or silicone rubber (SR) sheet, 100 μm thickness (Specialty Silicone Products Inc., Ballston Spa, NY, USA or Shielding Solutions Ltd., Great Notley Essex, UK).
- Amber-colored 120 mL (4 oz.) bottles (Brocacef Supplies & Services; art. no. FLEGL7661 or Uline; art. no. S-15649) with polypropylene screw cap containing PTFE liner (Fisher Scientific; art. no. 10536934).

- Small, wide-neck glass funnel fitting in the mouth of the 120 mL bottles.
- Analytical balance with 5 decimals; thus 2 decimals when weighing milligrams (0.00 mg).
- Reciprocal, 1 dimensional shaker table with an amplitude of 3 – 5 cm.
- Thick (30 – 50 μm) laboratory aluminum foil (The Lab Warehouse; cat. no. AL202–35 or equivalent).
- Scissors.
- Tea sieve (or regular sieve - mesh > 1 or 2 mm - with bowl).
- Pipette for pipetting volumes of up to 1.00 mL (e.g., Gilson P1000) and clean tips.
- Soxhlet extraction equipment (optional – required only when using PDMS or SR sheets as passive sampler).

Optional – used for SPME fibers only

- PDMS-coated disposable SPME fiber, e.g., 30 μm coating thickness on a 100 or 500 μm-thick glass fiber core; or 100 μm coating thickness on a 200 μm-thick core (Poly Micro Industries, Phoenix, AZ, USA or Fiberguide, Stirling, NJ, USA).
- Amber-coloured 15 mL vials (Sigma-Aldrich; cat. no. 27088-U) with black screw caps with aluminum liner (Sigma-Aldrich; cat. no. 27164).
- Small, wide-neck glass funnel fitting in the mouth of the 15 mL vials.
- Rock and roll shaker capable of rolling vials at a speed of approximately 33 rpm (Stuart roller mixer SRT9 or equivalent).
- Autosampler vial inserts; conical, 300 μL (BGB; part no. 110502 or equivalent).
- Wire cutter for cutting fibers (see Figure S10).
- Tissues (soft; e.g., Kleenex).
- Adhesive tape (transparent).
- Pipette for pipetting volumes of up to 200 μL (e.g., Gilson P200) or 1.00 mL (e.g., Gilson P1000) and clean tips. Pipette choice depending on extract volume.
- Tea sieve or large glass petri dish (10 cm diameter) (optional – used only when fibers < 3 cm are applied for sampling).

Reagent setup

Standard aqueous solution (SAS) is prepared by dissolving 200 mg of NaN_3 per liter of Millipore water in a clean glass bottle with plastic cap. Shake to dissolve and homogenize. This highly toxic solution should be used within a week after preparation. Keep closed until use.

Equipment setup

Passive sampling polymers should be preconditioned as described under ‘Experimental design’ and steps 2–3 of the procedure. The analytical balance should be recently serviced and/or externally calibrated. Just before performing the actual measurements, it should be leveled, cleaned, and internally calibrated, respectively.

PROCEDURE

Sizing the samplers • TIMING 0.5–5 min/sampler

1. Follow the appropriate option for the sampler type of choice: option A for PE/PDMS/SR sheet samplers; option B for SPME fibers.

   A. Polymer strips

   i. Cut small strips from an untreated/uncleaned polymer sheet, using a razor blade and ruler or a sharp pair of scissors. When using PDMS or SR sheet as a sampler, the sheet should be Soxhlet-extracted for 48 h with ethyl acetate and air-dried in a fume hood prior to cutting.

   The number of strips should be equal to the number of systems that will be prepared, plus about 10 additional ones, to be used as blanks, controls, and any PRC recoveries, as well as to compensate for any losses that may occur during later washing or handling steps.

   ▲CRITICAL STEP The width of the strips should be 4–6 mm, as the strips will finally be placed in autosampler vials and wider strips will not fit. The length should be adapted such that the desired mass is obtained, as recorded by weighing on an analytical balance.

   ii. Cut the pieces to an accuracy of maximally ± 0.20 mg. The exact weights do not need to be noted yet. Place a small object on the analytical balance first (e.g., an upside down metal lid or cup; Figure S1) as it is not easy to get tweezers underneath the small polymer pieces when trying to pick them up from the balance.

   iii. Place the strips together in a 20 mL (scintillation) vial with screw cap. The total mass of the strips per vial should not exceed 1000 mg.

   ■ PAUSE POINT Samplers can be stored in closed vials in the dark for years.

   B. SPME fibers

   i. Cut the required number of pieces of fiber from the roll as obtained from the supplier, using a razor blade (or scalpel).

For example, do so on a glass plate underneath or on top of which a ruler is fixed (Figure S2). Cut lengths of maximally 5.00 cm and cut several (10–20) extra (sets of) fibers to be used as blanks, controls, and any PRC recoveries, as well as to compensate for any losses that may occur during later washing or handling steps.

▲CRITICAL STEP The length of the fibers should be as accurate as possible, as this, among other things, will determine the accuracy of the final results. Therefore, the use of a magnifying glass is highly recommended.

ii. Place the fibers in a 20 mL (scintillation) vial with screw cap. The maximum number of fibers per vial should not exceed 300.

■ PAUSE POINT Fibers can be stored in closed vials in the dark for years.

Preconditioning the samplers • TIMING 2–3 h

2. Follow the washing procedure below for untreated PE sheet samplers and PDMS-coated SPME fibers; and Soxhlet-pre-extracted (ethyl acetate) PDMS and SR sheet samplers.

! CAUTION Adding and exchanging solvents as described below should be performed in a fume hood - solvent vapors should not be inhaled; wear disposable gloves to avoid skin contact and safety glasses for eye protection.

▲CRITICAL STEP The order of application of the specific solvents listed below is critical because of the water and methanol miscibility of the last solvent.

- Add about 18 mL of high purity grade *n*-hexane to the 20 mL vial(s) containing the samplers, cap tightly, put it on its side (in horizontal position) on a reciprocal (1-dimensional) shaker, operating at about 180 rpm, and shake for 30 min.
- Remove the cap, carefully pour off the *n*-hexane, leaving all samplers in the vial; replace with 18 mL of fresh *n*-hexane, and repeat the above washing step (i.e., shake the samplers for 30 min on the shaker).
- Again, remove the cap, discard the *n*-hexane and subsequently wash the samplers another two times for 30 min, but now with high purity grade acetone. After the last acetone wash, discard all acetone.

3. The next steps are different for samplers (sheets and fibers) that will be loaded with PRCs (A), sheets that will not be loaded with PRCs (B), and fibers that will not be loaded with PRCs (C):

A. After the last acetone wash in step 2, samplers can be loaded with PRCs. Refer to Box 1 for the loading procedure.

B. Air-dry sheet samplers that will not be loaded with PRCs (this is not possible for SPME fibers). Perform the drying on lint-free laboratory tissue in a clean fume hood after the last acetone washing step. Use clean (wiped with acetone) tweezers without ribs to place the acetone-containing polymer strips on four layers of the tissue and separate clotted strips in order to optimally expose them to air. Make sure the air flow is sufficiently gentle, such that no strips are blown away.

? TROUBLESHOOTING

Place the open 20 mL vial(s) next to the polymer pieces with the mouth facing the fume hood window (the cap can be put on the tissue too). After 30 min, turn the strips upside down with clean tweezers. After another 30 min, use tweezers to transfer the strips to the dry 20 mL vial and cap it.

C. Remove the remaining acetone from SPME fibers that will not be loaded with PRCs by washing them twice with Millipore water for 30 min while shaking on a reciprocal shaker. After the last wash, discard the water, fill the vial with freshly-drawn Millipore water, and cap the vial.

■ PAUSE POINT Washed and/or dried samplers (either dry or in water) without PRCs can be stored in tightly closed vials in the dark at room temperature for up to six months. For PRC-loaded samplers, refer to Box 1.

Preparing the equilibration systems • TIMING 5–10 min/system

4. Wash and label the required number of amber-colored glass systems, using well-sticking labels and a permanent marker or printed labels (during equilibration on a shaker (steps 13–14) deterioration of labels may occur). Option A applies to polymer strips; option B to SPME fibers.

a. Use 120 mL bottles for polymer strip samplers.

b. Use 15 mL vials for SPME fibers. As these will be equilibrated on a rock and roller shaker, it is necessary to additionally fix the labels with thin transparent adhesive tape, to prevent deterioration of the labels during rolling.

5. Prepare the standard aqueous solution (SAS) in an ultraclean bottle (washed with soap and rinsed with high purity grade acetone and Millipore water, respectively). The volume should equal approximately 90 (mL) times the number of systems to prepare for polymer strip sampler tests, or about 12 (mL) times the number of systems to prepare for SPME fibers.

! CAUTION Highly toxic solution. Avoid skin contact (wear gloves) and ingestion.

■ PAUSE POINT The solution can be stored in a closed bottle in the dark at room temperature for up to a week.

6. Thoroughly homogenize the sediment or soil sample(s) under study. Preferably mix mechanically (e.g., using an electric drill mixer), but if this is not possible, thoroughly mix manually for several of minutes with a metal spoon. If field samples are studied, manually remove larger objects (e.g., leaves, twigs, and stones) prior to mixing or use a coarse (1 or 2 mm) sieve. The dry weight percentage and organic carbon fraction of the samples should be known (see Experimental Design).

! CAUTION Field and spiked sediments and soils may contain high concentrations of toxic chemicals. Avoid skin contact (wear gloves), ingestion, and inhalation of any vapours.

7. Calculate the mass of wet weight sample that needs to be added to the equilibration systems, by using the dry weight content of the sample: divide the intended dry weight mass by the dry weight fraction of the sample. The intended dry weight should represent:

- **A.** For polymer strip samplers: 30 g of dry weight sample in a 120 mL bottle.
- **B.** For SPME fibers: 4.2 g of dry weight in a 15 mL vial.

For example, if the dry weight content of a sediment is 60% by weight, 50 g of wet sample (i.e., 30/0.6) should be added to a 120 mL bottle to add 30 g of dry weight sediment.

If the dry weight content of a sample is low (i.e., roughly < 30 – 40 %), it may not be possible to fit the intended dry weight mass in the equilibration system, as the wet weight mass to be added is too large (e.g., for a sample containing 90 % of water, the required 300 g of wet weight sample does not fit in a 120 mL system). Such samples should be centrifuged first and the supernatant be decanted, which will yield a sample with a higher dry weight content (to be quantified).

8. Place a small, wide-neck, glass funnel in the mouth of the bottle or vial, position on the balance (2 or 3 decimal; Figure S3), and tare. Transfer the appropriate sample mass to the respective glass system with the help of a metal spoon. If the funnel is too small to accommodate the entire mass, add part of the total mass and tap the bottle-funnel or vial-funnel combination on the tabletop to push the sample through the funnel neck. Then continue to add the rest of the sample.

? TROUBLESHOOTING

9. When the desired mass is added to the system, use a Pasteur’s pipette and SAS (kept in a clean 250 or 500 mL beaker) to flush the remaining sample from the funnel into the bottle (A) or vial (B):

A. Fill bottles up to 100 mL with SAS, leaving sufficient headspace to allow thorough homogenization (not possible without headspace). Use a spare bottle and fill with 100 mL of water from a volumetric cylinder to mark the 100 mL level. The headspace should be about 20 mL, starting at about the point where the glass bottle's wall converges into the neck. Use a beaker for adding the SAS and a Pasteur's pipette for the last milliliters (remove the funnel).

B. Because only a few mL are available for flushing in the case of 15 mL vials, this should be performed with a 'powerful' jet. The vials should be filled to a total volume of 14 mL, after removing the funnel. Use an empty 15 mL vial to check the height/position of this level (about 2 mm below the point where the glass wall of the vial starts to converge into the neck).

? TROUBLESHOOTING

When adding SAS, lift the funnel and raise the end above the suspension level, allowing the solution to enter the system.

10. Close the bottle or vial with the appropriate cap and leave without shaking.

11. Continue to fill all the other equilibration systems, according to steps 7 – 10. Manually homogenize the sediment or soil sample stock thoroughly each time prior to filling the next system. Clean the spoon and funnel with water and acetone before changing samples (not necessary in between preparing replicates of the same sample).

■ PAUSE POINT Closed systems containing sample and SAS can be stored at 4 °C for up to a week. However, when investigating sediments or soils containing or spiked with chemicals, which are known or suspected to be degradable, it is recommended to proceed right away or the next day.

12. Once all systems have received the required amount of sample and SAS, add either the polymer strips (A) or SPME fibers (B):

A. If polymer strips were loaded with PRCs and put in the freezer (Box 1), remove the loading/storage vial from the freezer and place in the dark (in a cupboard or box) for 2 h to allow the samplers to reach room temperature. Take a large piece of lint-free laboratory tissue, fold in four, and place next to the analytical balance. Calibrate the balance. Use clean tweezers to collect a sampler strip from the glass system in which the samplers were loaded with PRCs and place it on one half of the tissue. Fold the tissue in two and firmly press the upper half on top of the wet sampler. Swipe back and forth, making sure any water associated with the samplers will be absorbed by the tissue. Use the tweezers to take the first sampler and place it on the analytical balance, on which an acetone-cleaned metal object is placed (Figure S1). Record on paper the weight of the sampler in mg to two decimal places (e.g., 5.98 mg) and add to the corresponding bottle.

? TROUBLESHOOTING

If the samplers were air-dried and not loaded with PRCs, take them from the storage vial one by one and weigh them directly, without placing and drying them on a tissue.

After a sampler has been added to an equilibration bottle, place an acetone-cleaned 5×5 cm piece of thick aluminum foil on the mouth of the bottle, with the dull side facing the inside of the bottle. Carefully crimp the foil around the neck, make sure that the foil touches the bottle mouth completely and shows no creases (Figure S4), and very tightly screw the cap on the bottle.

▲CRITICAL STEP Application of aluminum foil is crucial. Omitting the foil will expose the sediment or soil slurry to the plastic cap, which may result in a depletive extraction of the sample and an underestimation of C_{free}.

B. Handling SPME fibers requires some practice. Use clean tweezers and clean disposable gloves. After the washing procedure and/or the PRC loading, the fibers will be stuck in a tight bundle in water. Use tweezers to carefully take a thin bundle from the washing vial and peel off the required number of fibers. Open the respective 15 mL vial and add the fiber(s). Carefully push them down, such that they do not stick out of the vial. Tightly close the vial with the aluminum-lined cap.

Perform this step for all systems.

Equilibrating the systems • TIMING several (4–6) weeks

13. Equilibrate the systems:

A. Place bottles with polymer strips in horizontal position on a 1-dimensional (reciprocal) table shaker, operating at 150–180 rpm and 20 ± 3 °C in the dark. When a large number of systems needs to be equilibrated, the bottles can be stacked and fixed in a box.

B. Place 15 mL vials with SPME fibers in horizontal position on a rock and roller shaker, operating at approximately 33 rpm and 20 ± 3 °C in the dark.

▲CRITICAL STEP Shaking at the specified intensity is critical, in particular when not verifying equilibrium conditions with PRCs.

14. Equilibrate for 4 to 6 weeks, depending on the sampler and the sample, as described under ‘Experimental Design’. Frequently check the systems and the ambient temperature. When equilibrating 15 mL vials, frequently tighten the caps, as these may come loose while rolling.

Collecting and cleaning the samplers • TIMING 5–10 min/system

15. Following the equilibration period, collect the bottles or vials from the shaker and place them on a clean laboratory table. Place them upright and in a logical order, with replicates grouped together and in order. Collect the materials required for the sampling of either polymer strips (A) or SPME fibers (B):

A. Polymer strips

A vial box with the appropriate number of autosampler vials, a clean syphon with freshly drawn Millipore water, a beaker to collect sediment waste, a tea sieve or geological sieve, tweezers and scissors on a clean piece of tissue, a large piece of laboratory lint-free tissue folded in 4 layers (hereafter referred to as “mat tissue”), stock of the lint-free tissue, a 1–2 mL pipette with tip, an Erlenmeyer flask with the solvent intended for extracting the polymer samplers (see Experimental Design), and a waste receptacle for the discarded sediment/soil suspensions are needed. An overview of these materials is presented in Figure S5. Using a tea sieve is most convenient (Figure S7), but a regular geological sieve (mesh > 1–2 mm) is also possible (use a receiving bowl underneath).

B. SPME fibers

A vial box with autosampler vials (containing 300 μL inserts if needed), a box with tissues, a clean syphon with freshly drawn Millipore water, a beaker to collect sediment waste, tweezers and a wire cutter/scissors on a clean piece of tissue, a large waste beaker for the discarded sediment/soil suspension, and a tea sieve or large glass petri dish (optional; only needed when fibers < 3 cm long are applied) are needed. An overview of these materials is presented in Figure S6.

16. Code the autosampler vials, place them in a logical order (same as the equilibration systems in step 15), and add to each the appropriate volume of the selected solvent (see Experimental Design) with an appropriate pipette and solvent-pre-rinsed tip.

! CAUTION Avoid inhalation, ingestion and skin contact of/with the solvent.

▲CRITICAL STEP The solvent should be carefully calibrated to the polymer and the chemical analysis, as discussed under ‘Experimental Design’ (see also Table 1).

Calibrate the solvent volume to the expected concentrations of the target analytes (see Experimental Design) and subtract from this volume the volume of the internal standard solution that will be added later on (e.g., if the desired final volume is 1.0 mL, and the volume of internal standard solution to be added is 100 μL, the vials need to be filled with 0.9 mL). When for SPME fibers with a coating/core thickness of (10/100 or) 30/100 μm the expected target chemical concentrations in the extracts are low, solvent volume should be kept small and

the use of 300 µL inserts placed in the autosampler vials is recommended. The final solvent volume in this case should be 200 µL (which according to the above calculation example implies adding 180 µL of solvent and adding 20 µL of internal standard solution later on, as the volume of the latter solution should be proportional to $V_{extract}$).

Close the vial with a screw cap immediately after the solvent has been added.

17. Include and code:

1. a solvent blank vial: only add the solvent to this autosampler vial (volume minus the internal standard volume to be added);

2. a sampler blank vial: add a sampler with a representative mass (sheet) or length (fiber) as obtained in step 3B or 3C to this vial and add a representative volume of solvent (minus the volume of internal standard to be added);

3. In case where PRCs are being used: (at least) three vials containing PRC-loaded samplers, as obtained in step 3A (Box 1). Add solvent as described (minus the volume of internal standard to be added).

4. a sampler/standard vial: add to this vial a cleaned sampler with a representative mass (sheet) or length (fiber) as obtained in step 2, 3B, or 3C. Later, add the second lowest calibration standard (including the internal standard) as will be used in step 23 to this vial.

Add internal standard solution to vials (1) through (3) later on (step 19), along with the addition of internal standard solution to the sampler extracts.

18. Collect and clean the samplers according to option A for polymer strips or option B for SPME fibers:

A. Polymer strips • TIMING 3–6 min/system

i. Clean the tweezers and scissors with high purity grade acetone (wipe with acetone-wetted tissue).

ii. Wet part of a piece of thick lint-free laboratory tissue (folded in four) with Millipore water (spray with the syphon). This will be used to clean the strips.

iii. Take the first bottle and shake intensively for about 10–15 seconds. Remove the screw cap and the aluminum foil after inspection.

? TROUBLESHOOTING

iv. Pour the content of the bottle onto the sieve (which is positioned on a beaker) and find the polymer strip. Use tweezers without ribs to pick-up the polymer strip and rinse it clean with Millipore water from the syphon (Figure S7).

? TROUBLESHOOTING

v. Place the sampler on the mat tissue (i.e., 4 layers of lint-free laboratory tissue), unfold it if necessary, hold it with tweezers at one side, and wipe it with the wet tissue, five times. Turn the sampler around and wipe the reverse side five times with a clean spot of the wet tissue. The tissue should be considerably wet.

vi. Take hold at the other end of the sampler and repeat the above wiping procedure with clean spots of the tissue (i.e., wipe both sides of the other end of the sampler).

? TROUBLESHOOTING

vii. Dry the cleaned sampler by wiping or patting with a dry piece of the tissue, while it is lying on a dry spot on the mat tissue. Water should not enter and contaminate the solvent in the vial.

viii. Separate the corresponding autosampler vial from the others, open it, and place the sampler strip in its opening, while holding it with tweezers at the other end. Using the scissors, cut pieces with a length of ≤ 7 mm from the strip. The pieces will ‘fall’ into the vial (Figure S8). Long sampler strips may first be folded in two and the ‘closed end’ cut open; twice if necessary; while lying on the mat tissue.

ix. Carefully check (a) the scissors, (b) the tweezers, (c) the tissue, and (d) the vial box for the presence of polymer pieces, as they may jump away or stick to surfaces.

x. Tightly close the autosampler vial with its screw cap and tap the vial several times on the tabletop (do not shake). Insure all pieces are submerged in the solvent. The optimal situation is that all pieces are lying flat at the bottom of the vial.

xi. Separate the vial from the other vials, preferably by placing it in another vial box.

xii. Repeat steps (i)-(xi) for all the other equilibration systems. Take a new piece of thick lint-free, ‘wiping’ tissue (folded in 4) for each new sampler, and a new mat tissue once the current one no longer has any dry areas anymore (about each 3–4 samples). Clean the scissors and tweezers when necessary with acetone (i.e., between processing samplers that were exposed to different sediments; not between replicates).

B. SPME fibers • TIMING 2–6 min/system

i. Wipe the tweezers and wire cutter (or scissors) with a (high purity) acetone-wetted tissue.

ii. Wet half of a soft tissue (e.g., Kleenex) folded in four with Millipore water from a syphon. The tissue should be damp, not soaking wet.

iii. Put on clean gloves.

iv. Open the first 15 mL vial and pour-off a couple of milliliters of suspension. If the fiber length is > 4 cm, the fiber(s) will stand out above the suspension and can be retrieved directly. If the fiber length is < 4 cm, shake the vial first and transfer the content onto the tea (or geological) sieve (which is positioned on a beaker) or to an empty 10 cm petri dish. Water may be needed to remove the entire vial content. Use tweezers and any additional water to find and retrieve the fiber(s) from the sieve or the petri dish, and place the fiber(s) with the help of tweezers on the wetted half of the tissue.

v. Check the number of fibers and wipe it/them while holding between thumb and fore/middle finger, three times with the wet tissue (Figure S9). Then, grip the fiber(s) at the other end and wipe the other side(s) also three times with the wet tissue. For each wipe, use a new, clean position on the tissue. If the fibers appear dirty, they should be separated and cleaned individually.

? TROUBLESHOOTING

▲CRITICAL STEP When sampling volatile chemicals, perform this step as fast as possible (i.e., within 30 sec) and/or return the fibers that are not being cleaned at that moment in the 15 mL vial or leave in the petri dish.

vi. Open the corresponding autosampler vial and stick the fiber(s) in its opening. By using the wire cutter/scissors, cut pieces with a length of ≤ 1.3 cm from the fiber(s) (Figure S10) and insure they enter the vial by pushing them in with a finger or the wire cutter/scissors.

vii. Check for the presence of pieces of fiber on the cutter/scissors, gloves, and in the vial box.

viii. Tightly close the autosampler vial with a screw cap and tap it several times on the tabletop (do not shake). Confirm all pieces are under the solvent surface. Separate the vial from the other vials, preferably by placing in another vial box.

ix. Repeat steps (i)-(viii) for all the other systems. Use a clean tissue for each system and clean the tweezers/cutter with acetone before starting with a new system. Make sure the

gloves remain clean; change if necessary or wipe them with an acetone-wetted tissue if they are dirty.

Extracting the samplers • TIMING 25 h

19. Remove the cap from the first vial and add internal standard solution (see Experimental Design) with a GC syringe or calibrated (micro) pipette. Tightly cap the vial again. Repeat these steps for all vials. As mentioned (in step 16), the volume of internal standard solution to be added is proportional to $V_{extract}$, such that the internal standard concentration will be the same in all vials.

20. Leave the vials at room temperature in the dark for at least 24 h. This is the first step of the extraction procedure.

21. Vortex each vial for exactly 1 min at the highest speed. Use a timer and vortex two samples at the same time, preferably on two vortex machines.

■ PAUSE POINT Tightly closed and vortexed vials can be stored at −20 °C for up to 2 months.

22. Analyze the extracts as described below. If the analysis is performed more than a day later, place the vials in the freezer. Get the vials from the freezer several hours before starting the analysis and vortex each vial again for 30 sec when it has reached room temperature.

Chemical analysis • TIMING dependent on the chemical analysis run time per sample, the number of samples, and the number of target compounds – typically 1–4 d

23. Analyze the extracts with the appropriate GC or LC equipment and quantify the target compounds with the help of calibration solutions, containing the target compounds dissolved in at least 5 different concentrations in the same solvent as applied in step 16 (extraction of the sampler). In addition, each calibration solution should contain exactly the same concentration of internal standard compound(s) as present in (added to) the extracts.

24. Integrate the peaks of all target chemicals, internal standard compound(s), and any PRCs in the extracts, blanks, controls, and calibration standards, using the instrument integration software. Do not rely only on automatic integrations, but (re-)integrate or check the integrations manually. Overlay the chromatograms of the sampler-standard control and a standard of the same calibration level and verify the presence of the sampler does not affect peak shape or surface, or yields any interfering background noise.

25. Divide the peak area of each target chemical by the peak area of the internal standard in the respective sample (use the instrument software or Microsoft Excel). Do this for all extracts, blanks, controls, and calibration standards. Construct a calibration curve for each target chemical by plotting the averaged ratios for the calibration standards against the target chemical concentrations in the respective calibration standards. Interpolate the ratios calculated for the extracts, blanks, and controls in these calibration curves, using regression lines,

and calculate the concentrations of the target chemicals in the extracts ($C_{extract}$; μg/L). This step may also be performed automatically by the instrument data processing software, but the calculations should be checked manually in a couple of cases.

26. If calculated concentrations exceed the highest calibration level, the extracts should be diluted and re-analyzed. This can be performed by taking e.g., 100 μL of extract and adding it to 900 μL of pure (the same) solvent present in another autosampler vial (1:10 dilution). The internal standard solution does not need to be added, but the peak(s) of this/these chemical(s) after dilution should be sufficiently large to allow accurate integration.

• TIMING

Step 1, sizing samplers: 0.5–5 min per sampler

Step 2–3, preconditioning samplers: 2–3 h

Step 3, loading samplers with PRCs (optional): 3 d

Steps 4–12, preparing the equilibration systems: 5–10 min/equilibration system

Steps 13–14, equilibrating: several weeks (4–6)

Steps 15–18, collecting and cleaning samplers: 5–10 min/equilibration system

Steps 19–21: extraction: 25 h

Steps 22–25, chemical analysis: dependent on the analysis run time, the number of samples, and the number of target compounds – typically 1–4 d

? TROUBLESHOOTING

Troubleshooting advice can be found in Table 2.

ANTICIPATED RESULTS

Calculation of C_{free}

Ultimate results of passive sampling analyses are freely dissolved concentrations of target chemicals in sediment or soil pore water (C_{free}). These are calculated as follows.

Subtract from each target chemical concentration in the extracts the concentration of this chemical determined in the *solvent* blank, yielding a blank-corrected concentration ($C^*_{extract}$; μg/L). Note that the results are not corrected for the *sampler* blank. This blank only serves quality control purposes; if concentrations are high or higher than $C^*_{extract}$ one should investigate the cause.

For polymer strips, calculate the concentrations of the target chemicals in polymer strips (C_p; μg/kg) by multiplying $C^*_{extract}$ by 1000 and the extract volume ($V_{extract}$; mL); and subsequently dividing the result by the sampler weight (M_p; mg):

$$C_p = \frac{1000 \cdot C^*_{extract} \cdot V_{extract}}{M_p} \quad (5)$$

For SPME fibers, calculate the concentration in the polymer coating (C_{pc}; μg/L) by multiplying $C^*_{extract}$ by 1000 and the extract volume ($V_{extract}$; mL) and subsequently dividing the result by the product of fiber length (L_f; cm) and coating volume per cm (V_{pc}; μL/cm):

$$C_{pc} = \frac{1000 \cdot C^*_{extract} \cdot V_{extract}}{L_f \cdot V_{pc}} \quad (6)$$

Finally, calculate C_{free} (ng/L) by multiplying C_p or C_{pc} by 1000 and dividing the result by the chemical-specific polymer-water partition coefficient (K_{pw}; L/kg) for the polymer used as the passive sampler:

$$C_{free} = \frac{1000 \cdot C_p}{K_{pw}} \quad (7)$$

or

$$C_{free} = \frac{1000 \cdot C_{pc}}{K_{pw}} \quad (8)$$

Assessing equilibrium conditions

The equilibrium status of the C_{free} values can be assessed in three ways. First, if a time series is determined, the time profile can be used to qualitatively judge whether C_{free} has stabilized over time[8,28,70,71]. Quantitatively, C_{free} reflects equilibrium conditions if consecutive determinations in the time series yield statistically indistinguishable values. Second, equilibrium has been reached if the application of different samplers with the same mass (sheet samplers) or length (fibers), but with different sheet- or coating thicknesses results in statistically identical C_{free} values. Third, PRC data can be used to assess both the equilibration and depletion state of the system after deployment as described below.

Interpretation of PRC results

Calculate the concentrations of the individual PRCs in all exposed samplers, either polymer sheets or fibers, according to equation 5 or 6, respectively. This yields $C_p^{PRC(e)}$ or $C_{pc}^{PRC(e)}$. Similarly, calculate the concentrations of the individual PRCs in the *un*exposed samplers (i.e., the PRC reference samplers), which were extracted immediately after the PRC loading step (Box 1), but analyzed together with the samples. Per individual PRC, average the values obtained for the replicate samplers. This yields $C_p^{PRC(0)}$ or $C_{pc}^{PRC(0)}$.

The fraction of each PRC, which is retained in exposed polymer strip samplers (f_{PRC}) is then calculated according to:

$$f_{\mathrm{PRC}} = \frac{C_p^{PRC(e)}}{C_p^{PRC(0)}} \tag{9}$$

and in fibers:

$$f_{\mathrm{PRC}} = \frac{C_{pc}^{PRC(e)}}{C_{pc}^{PRC(0)}} \tag{10}$$

Three scenarios are possible for f_{PRC}:

1. $f_{PRC} < 0.05$ for all PRCs. This indicates that equilibrium conditions were (sufficiently) attained and the sampling was non-depletive for the chemicals that have sampler-water partition coefficients (K_{pw}) within the K_{pw} range covered by the applied PRCs.
2. $f_{PRC} > 0.05$ for all PRCs. This observation suggests that the sampler capacity was too large and the sampling was depletive or that insufficient time for system equilibration was applied.
3. $f_{PRC} < 0.05$ only for PRCs with a relatively low K_{pw}, while f_{PRC} increases (>0.05) with increasing K_{pw} of the PRCs. This indicates non-equilibrium conditions for the latter group of PRCs and consequently for target compounds having a similar K_{pw} range.

In the latter two cases, the results do not meet the equilibrium and/or depletion quality criterion, and the resulting C_{free} of (some of) the target chemicals will be underestimated. This information should be included in the reporting or the sampling should be re-designed and repeated. Alternatively, the PRC data may be used to correct the results for non-equilibrium conditions. Corrections for depletive conditions are generally not possible, as they require the (unknown; sample-specific) sediment- or soil-water partition coefficients of the target chemicals.

Correction for non-equilibrium conditions is straightforward if performed for a specific target compound using an identical isotopic surrogate (deuterated or ^{13}C-labelled analogue) as the PRCs (e.g., target compound is PCB52 and the PRC is ^{13}C-PCB52). Both compounds will have (nearly) the same K_{pw}, sediment- or soil-water partition coefficient, and (given that the exchange is isotropic) exchange kinetics[80]. In such a case, a 'full-equilibrium' C_{free} (C_{free}^{∞}; ng/L) of the target chemical can be calculated according to:

$$C_{free}^{\infty} = \frac{1000 \cdot C_p^{(e)}}{K_{pw}(1 - f_{PRC})} \tag{11}$$

with $C_p^{(e)}$ being the concentration of the target chemical in the sampler after exposure (μg/kg). If SPME fibers were applied, this term should be replaced with $C_{pc}^{(e)}$ (μg/L).

For target compounds for which no isotopic analogues were applied as PRC, correction requires additional model calculations. For static exposures, several correction models have been developed, which are based on diffusive mass transfer of chemicals between the passive sampler and sediment particles close to and further removed from the sampler surface[68,80,81]. The models use compound-specific diffusivities within both the passive sampler polymer and the aqueous phase being sampled (i.e., interstitial water) and can generate a degree or fraction of equilibrium (f_{eq}) value for each target compound, based on the behavior of the PRCs[80,82,83]. This fraction can then be used to adjust target compound $C_p^{(e)}$ values to a C_{free}^{∞}. Diffusive mass transfer models are particularly mathematically-intensive, but on-line calculators are available for performing the calculations[84]. The current protocol, however, prescribes well-mixed systems, which implies homogeneous concentrations in the sample and relatively simple exchange kinetics. Such kinetics can be modeled similar to passive sampling kinetics in an aqueous phase, using a first order kinetic model[85]. The accompanying first order PRC exchange rate constants (k_e^{PRC}; d^{-1}) are calculated from the PRC data according to:

$$k_e^{PRC} = ln\left(\frac{C_p^{PRC(0)}}{C_p^{PRC(e)}}\right)\left(\frac{1}{t}\right) \quad (12)$$

with t the deployment time (d). Because the exchange rate constants are inversely related to the sampler uptake capacity and the diffusion kinetics through the aqueous phase, linear relationships between k_e^{PRC} and either the K_{ow}, the K_{pw}, the molar volume, or a combination of molecular weight and K_{pw} can be constructed[58,85,86]. The k_e values for the target chemicals can subsequently be estimated from these regressions through inter- or extrapolation. Based on the resulting k_e values, C_{free}^{∞} of the target compounds can be calculated according to:

$$C_{free}^{\infty} = \frac{1000 \cdot C_p^{(e)}}{K_{pw}\left(1 - e^{-ke \cdot t}\right)} \quad (13)$$

If SPME fibers were applied, $C_p^{(e)}$ and $C_p^{(0)}$ in equations 12 and 13 should again be replaced with $C_{pc}^{(e)}$ and $C_{pc}^{(0)}$.

Although the different models have been compared for static systems[58], no comparisons are available for dynamic exposures; hence it is not yet evident which of the models is superior for well-homogenized ex situ passive sampler exposures, as described in the current protocol. Finally, it should be noted that in case depletion and non-equilibrium both occur, the exchange rates depend on the sampler-to-sediment/soil capacity ratio[20], which will complicate the modeling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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BOX 1 |

LOADING PASSIVE SAMPLERS WITH PRCs • TIMING 3 d

This procedure describes the loading of passive samplers (PE or PDMS; sheet strips or fibers) with PRCs in a 80/20 methanol/water mixture. The approach allows for a relatively fast equilibration of PRCs in the polymer phase, due to the high methanol content, which causes very low sampler–solvent partition coefficients. The loading is not designed to be quantitative, as the only critical factor is the final ratio of the PRC concentrations before and after deployment with both concentrations being determined analytically. Yet, the loading needs to be designed with care, such that the concentration level of the PRCs in the samplers will reflect (as much as possible) the concentration levels of the target compounds in the sediment or soil sample and will fit the calibration concentration range (both before and after equilibration).

Designing the loading

The PRCs will distribute between the solvent phase with volume V_L (mL) and the total passive sampler polymer phase with mass $M_{p(tot)}$ (g) (for SPME fibers, the total volume of the PDMS phase applies), according to the ratio of the uptake capacities (V_L/$M_{p(tot)} \cdot K_{p80}$; with K_{p80} being the passive sampler-methanol/water (80/20) partition coefficient (L/kg)). To calculate the mass of each individual PRC that should be added to the loading system (N_{add}, ng) in order to achieve the desired concentration in the passive sampler polymer (C_p, ng/g), the following equation can be applied (adapted from Booij et al.[88]):

$$N_{add} = C_p M_{p(tot)} \left(1 + \frac{V_L}{M_{p(tot)} K_{p80}}\right)$$

In Table S1 of the Supplementary Information, a list with indicative K_{p80} values for a series of potential PRCs in PE and PDMS is provided. Preferably, the samplers are loaded with PRCs in the 20 mL washing vials. However, for an adequate equilibration, all samplers should be able to freely move around in the loading solvent and V_L may be increased if needed by using a larger vial or bottle.

The PRC concentration in the passive sampler polymer which is *minimally* required to be able to check if depletion is below 5% ($C_{p(min)}$) is estimated according to:

$$C_{p(min)} = 20 \cdot \left(\frac{V_{extract} \cdot LOQ}{M_{p(low)}}\right)$$

with $V_{extract}$ being the volume of the final extract (mL), LOQ the limit of quantification (ng/mL), and $M_{p(low)}$ the mass of the smallest passive sampler deployed (g).

Procedure

1. Following step 3A of the main procedure, shake the vial(s) with the samplers for 30 min with 18 mL of methanol to remove the acetone. Fully discard the methanol.

2. Add the desired mass of PRCs (N_{add}), as designed. Two options are possible: (A) for PRCs available as neat material (solid standards) and (B) for PRCs obtained as standards dissolved in a nonpolar solvent (e.g., pentane, hexane, nonane, dichloromethane).

   A. Dissolve the solid PRCs as a mixture in methanol, each at an appropriate concentration. The concentration should be such that a small spike volume (e.g., 100–200 μL) contains N_{add}. If the chemicals are not expected to or do not dissolve at the intended high concentration, ethyl acetate can be used as alternative solvent. Add 14 mL (or $0.8\,V_L$) of methanol to the washing vial, containing the samplers. To this methanol, add the required small volume of the spike solution (containing N_{add}) and close the vial with an aluminum foil-lined cap. Alternatively, the PRCs can be dissolved directly in 14 mL (or $0.8\,V_L$) of methanol present in a vial, to which then the samplers are added.

   B. Add a volume of the nonpolar solvent containing the required mass of PRC(s) to the bottom of an empty, clean 20 mL vial. If PRCs were obtained as individual standards, add aliquots of each solution to the vial. Purge the vial in a fume hood with a gentle stream of nitrogen, such that the nonpolar solvent(s) is/are slowly evaporated. Add 14 mL (or $0.8\,V_L$) of methanol and ensure full dissolution of the PRCs (e.g., sonicate the closed vial for 15–30 min). Upon visual confirmation of full dissolution (i.e., crystals are no longer visible), use clean tweezers to transfer the passive samplers from the washing vial to the PRC solution. Close the vial with an aluminum foil-lined cap.

3. Place the vial in horizontal position on a reciprocal (1-dimensional) table shaker and shake for (at least) 16 h at 180 rpm.

4. Add 1.75 mL (or $0.1\,V_L$) of Millipore water and continue shaking for another 8 h. Add another 1.75 (or $0.1\,V_L$) of Millipore water and shake for 40 h.

5. Discard the 80/20 methanol/water loading solution and wash the samplers twice for 30 min with 18 mL (or V_L) of Millipore water by shaking at 180 rpm on the reciprocal table shaker. After washing, discard the water and store the samplers as such in a freezer at −20 °C until use.

   ■ PAUSE POINT Passive samplers loaded with PRCs can be stored in a closed vial in the freezer for prolonged times. Alternatively, PRC-loaded samplers may also be stored in their loading solution in the dark for prolonged times, but then step 5 should be performed just prior to deploying the samplers.

6. Confirm (preferably before deployment) that the PRC concentrations are in the target range and heterogeneity is acceptable (e.g., below 10%), by analyzing multiple individual samplers, extracted in $V_{extract}$ of the appropriate

(extraction and injection) solvent. Note that these analyses also need to be performed when analyzing the final samples, as they are needed to determine the reference (100%) PRC level in the samplers.

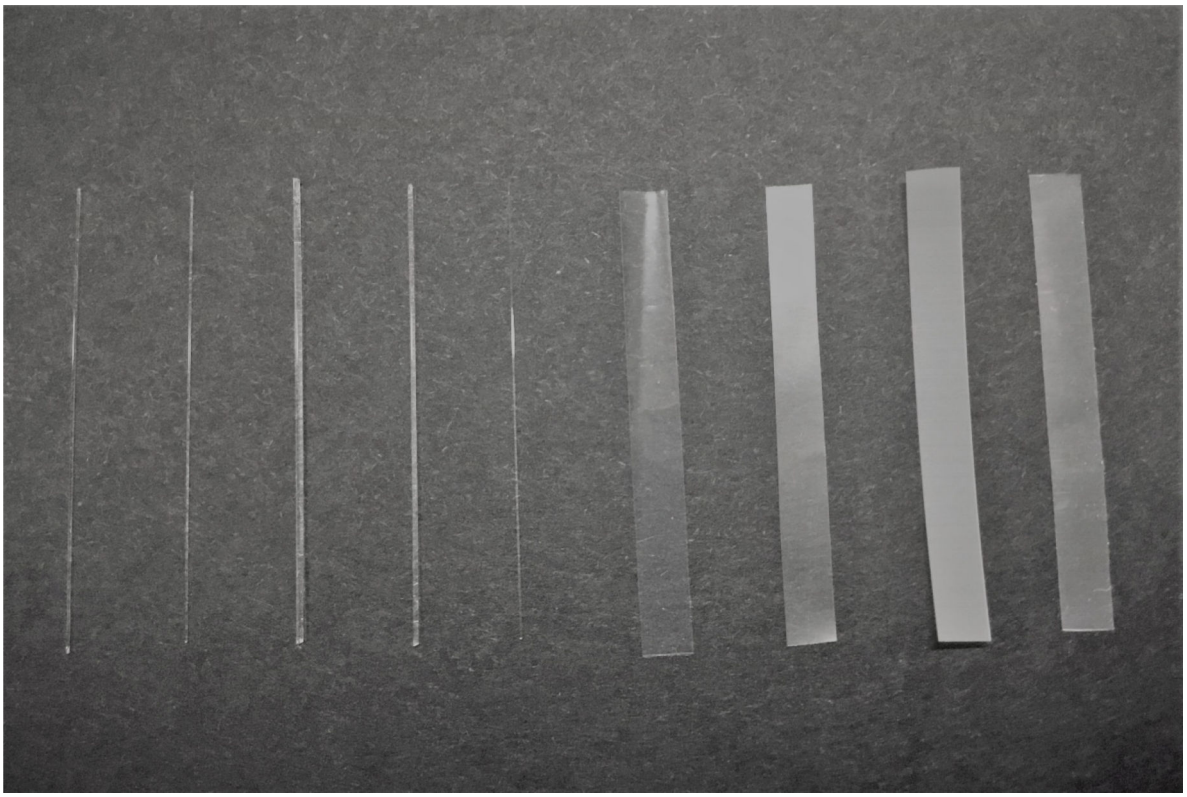

FIGURE 1.

Photograph of different passive samplers (from left to right): (1) 10 μm PDMS-coated SPME fiber, (2) 30 μm PDMS-coated SPME fiber, (3) 30 μm PDMS-coated SPME fiber with a 500 μm core, (4) 100 μm PDMS-coated SPME fiber, (4) 30 μm Polyacrylate-coated SPME fiber, (6) 25 μm thick PE, (6) 51 μm thick PE, (6) 77 μm thick POM, (6) 100 μm thick PDMS. All samplers are 4 cm in length.

TABLE 1

Commonly-used passive samplers for the ex situ determination of C_{free} in sediments and soils: advantages, disadvantages and recommended solvents for the final extraction of deployed samplers.

Sampler	Advantages	Disadvantages	Extraction solvents [a]
Sheet samplers			
Polyethylene (PE); 25 μm	- Easily obtainable and inexpensive - Relatively fast equilibration - Extraction possible with many solvents	- Relatively difficult to cut - Relatively easily folds and crumples during cleaning - Limited thickness can translate into larger sampler, which may be difficult to fit in an autosampler vial, which may complicate extraction - Gets relatively easy blown away (in a fume hood)	- Acetone - Acetonitrile - DCM - Heptane - Hexane - Hexane:acetone (x:y) [b]
Polyethylene (PE); 50 μm	- Easy handling (cutting and weighing) - Easily obtainable and inexpensive - Compared to thinner PE: more robust and rugged; easier to clean, cut, weigh, and dry - Extraction possible with many solvents - High(est) sensitivity	- Compared to thinner PE: slower equilibration	- Acetone - Acetonitrile - DCM - Heptane - Hexane - Hexane:acetone (x:y) [b]
Polyoxymethylene (POM); 77 μm	- Very easy to cut, weigh, and clean - Robust and rugged - Obtained from a single supplier	- Relatively slow equilibration due to slow internal diffusion - Intensive shaking needed during equilibration - Extractable with only a limited number of solvents - Compatibility with PRCs unclear	- Acetonitrile - DCM - Hexane:acetone (1:1)
Polydimethylsiloxane (PDMS) and silicone rubber (SR); 100 μm	- Extraction possible with many solvents - Easy to extract (soft polymer) - Fast internal diffusion of most chemicals	- Difficult to trace in suspensions due to transparency/refractive index - Dry polymer sticks quite strongly to surfaces, which complicates cutting and weighing - Composition may vary from supplier to supplier and so may target contaminant partitioning to the sampler (K_{pw}) - 48-hour pre-extraction (Soxhlet) recommended to remove oligomers	- Acetone - Acetonitrile - DCM - Heptane - Hexane - Hexane:acetone (x:y) [b] - Methanol [c]
SPME fibers			
PDMS coating; 10 μm	- Fastest equilibration for most chemicals	- Lowest sensitivity (limited polymer phase) - Vulnerable sampler (fiber easily breaks) - Sampler with highest variability	- Acetonitrile - DCM - Heptane - Hexane - Hexane:acetone (x:y) [b] - Methanol
PDMS coating; 30 μm	- Second fastest equilibration - Compared to 10 μm fiber: higher sensitivity, less fragile, lower variability	- Relatively high variability in results	- idem
PDMS coating; 30 μm (500 μm core)	- Higher sensitivity than previous fiber due to larger polymer volume per cm of fiber; yet same kinetics	- Difficult to cut - Fiber length fitting in an autosampler vial insert is limited	- idem
PDMS coating; 100 μm	- Stiff/strong fiber. Possible to shake instead of rolling	- Fiber with slowest equilibration kinetics	- idem

Sampler	Advantages	Disadvantages	Extraction solvents [a]
	- Fiber with highest sensitivity and lowest variability		
Polyacrylate coating; 30 μm	- Relatively strong sorption of chemicals - Also suitable for more polar chemicals	- Relatively slow equilibration due to slow internal diffusion - Exact composition of polymer unknown - Compatibility with PRCs unknown	- Acetonitrile

[a] Use of other solvents for the extraction of deployed samplers is discouraged, unless extraction recovery determinations demonstrate full extraction.

[b] Different ratios are possible (i.e., x and y can be 1, 2 or 3).

[c] Methanol is not a suitable solvent in case organochlorine pesticides are targeted and solvent boiling (after a clean-up) is performed, as several targets may degrade[87].

TABLE 2

Troubleshooting table.

Step	Problem	Possible reason	Solution
3(B)	Polymer strips blown away and landed on dirty surface (e.g., fume hood surface/floor)	Fume hood air flow to high	Open fume hood window further. Wash respective strip(s) once more with acetone for 30 min and restart the drying period
8	Entire mass of sample does not fit in the funnel and/or sample will not pass into bottle	Mouth of funnel too narrow	Push sample through with spatula and/or SAS. Replace funnel with a wider-mouth version
8	Too much mass is added, leaving insufficient headspace	Miscalculation	Remove superfluous slurry with a Pasteur's pipette to the desired weight/headspace volume
9(B)	A fraction of sample remains in the funnel	More SAS required, but system is already full	If the mass fraction is negligible as compared to the mass that entered the system, this is not a problem (discard extra sample)
12(A)	Weight of the sampler is not stable/drifts	Sampler may still carry some water or is not at room temperature	Make sure the samplers are at room temperature and place the sampler on a dry spot of the tissue. Dab dry again by firmly pressing the other half of the tissue on top of the sampler and manually swipe back and forth on the upper half of the tissue
18(A)	Aluminum foil is broken and the cap is dirty on the inside		Record observation. This may be a reason to dismiss the results for this sample. The polymer in the cap may have sorbed part of the chemical pool, causing the extraction to be depletive
18(A)	Sampler stays in the bottle.	Sampler sticks to glass wall or is stuck in remaining sample mass	Add a small volume of Millipore water, shake firmly, and try to pour the sampler out again
18(A)	Sampler cannot be fully cleaned – certain stains remain	Stains probably concern non-aqueous phase liquid (NAPL) spots (e.g., oil)	Make a note (and picture). This may be a reason to dismiss the final results if they differ widely from the other replicates for this particular sample. Wipe thoroughly with clean, wet tissue, trying to remove the stains, without damaging the sampler
18(B)	Fiber appears/is shorter than at the start of the equilibration	Fiber may be/is broken	Measure length and/or try to find the other piece(s) and determine the length of the separate pieces. Use the recovered length for calculating C_{free}.