nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-020-0311-y

Check for updates

# 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

Michiel T. O. Jonker[1]✉, Robert M. Burgess[2], Upal Ghosh[3], Philip M. Gschwend[4], Sarah E. Hale[5], Rainer Lohmann[6], Michael J. Lydy[7], Keith A. Maruya[8], Danny Reible[9] and Foppe Smedes[10]

**The freely dissolved concentration ($C_{free}$) of hydrophobic organic chemicals in sediments and soils is considered the driver behind chemical bioavailability and, ultimately, toxic effects in benthic organisms. Therefore, quantifying $C_{free}$, although challenging, is critical when assessing risks of 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). Here, we provide a state-of-the-art passive sampling protocol for determining $C_{free}$ in sediment and soil samples. It represents an international consensus procedure, developed during a recent interlaboratory comparison study. The 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) because of prolonged equilibration times. However, hands-on time, excluding chemical analysis, is approximately 3 d for a set of about 15 replicated samples.**

## 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 have been 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 ($C_{free}$ values) 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 (femtogram–nanogram/liter range)[8–10]. Such low

[1]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands. [2]Atlantic Coastal Environmental Science Division, Office of Research and Development, U.S. Environmental Protection Agency, Narragansett, RI, USA. [3]Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland, Baltimore County, Baltimore, MD, USA. [4]RM Parsons Laboratory, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. [5]Geotechnics and Environment, Norwegian Geotechnical Institute, Oslo, Norway. [6]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA. [7]Center for Fisheries, Aquaculture and Aquatic Sciences, and Department of Zoology, Southern Illinois University, Carbondale, IL, USA. [8]Chemistry Department, Southern California Coastal Water Research Project Authority, Costa Mesa, CA, USA. [9]Civil, Environmental and Construction Engineering, Texas Tech University, Lubbock, TX, USA. [10]Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Brno, Czech Republic. ✉e-mail: m.t.o.jonker@uu.nl

concentrations are typically below the limits of detection (LODs) 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 that have attracted the attention of environmental managers seeking to assess more accurately risks associated with contaminated sediments and soils[7,11–13]. The methods use 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. When the chemical-specific affinity for the polymer is known, $C_{free}$ 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, in which 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 interlaboratory comparison study ('ring test') was recently performed on passive sampling in sediments[28]. This study demonstrated that standardization of passive sampling methods is critical for reducing interlaboratory variability. Furthermore, when performed in a unified, quality-controlled way, passive sampling yields robust and precise results, with very low inter-method variability (a factor of <1.7)[28].

Here, we present a standardized protocol that was developed and applied in the interlaboratory 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 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- or soil-to-water and sediment- or soil-to-polymer ratios) 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]; those primarily provided general recommendations. In addition, the protocol is more specific than the general SPME protocol published by Risticevic et al.[30], because it exclusively focuses on sediment and soil applications but 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 contamination, which will enable 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, Authorisation, and Restriction of Chemicals), the European Union chemical regulation)[35]. Standard protocols exist for such toxicity tests[36–38], but exposure characterization therein relies on total extractable concentrations in the matrix. 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 pore water[40]) have hampered these efforts. Overall, the current protocol may be useful to researchers, engineers, and analysts from academia, government, consultancy, and industry, including those 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 ($K_{ow}$ values) larger than ~$10^3$. 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 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 ~$10^3$, or ionized chemicals, because these have limited affinity for the polymers used as passive samplers. For metals, it is possible to determine an analogous $C_{free}$ value (e.g., free ion activity or concentration) with the help of passive sampling methods, such as diffusive gradients in thin films (DGTs) 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 $C_{free}$ values 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 the LODs of passive sampling generally are (much) lower than those of traditional analysis methods of pore water samples, passive sampling, as described in the current protocol, also has its detection limits. Whether or not $C_{free}$ values of HOCs in sediments and soils can be quantified with the current protocol 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) to 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) microgram/kilogram range, even though in the protocol the sample and polymer masses are standardized and maximized, respectively (to obtain optimal system homogenization and equilibration, as well as 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 Eqs. 3 and 4 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, because in such a case depletion of target chemicals from the sample will occur, which will result in an underestimated $C_{free}$ (discussed below). Because there is also a minimum sampler mass that can be used, the current protocol will therefore not be applicable to

samples with an $f_{oc}$ < ~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 less than the LOD, 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 markedly to enable quantification of $C_{free}$ at all (see below). Such 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 a 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 can be minimized by adding a biocide and photodegradation can be 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 the 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 of how $C_{free}$ can be used in the assessment of bioaccumulation, remediation necessity, and management of contaminated soils and sediments, as well as in the interpretation of toxicity assays, are beyond the scope of this protocol. We refer readers to experts and other literature sources for additional information[7,24,48–50].

## Experimental design

### Selection of the passive sampler

The passive samplers 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'; 25–100 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 (QA) measures, the same results (i.e., $C_{free}$ values) will be obtained with the different samplers[28]. However, in the protocol, a distinction will be made between the two groups of samplers, because their handling and other practical issues differ. In Table 1, the samplers most often applied (i.e., those also included in the recent interlaboratory comparison study[28]) are listed, along with an overview of their advantages and disadvantages. In Fig. 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 comments apply:

- 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 these aspects. For example, we recommend using a magnifying glass when cutting fibers and to measure (microscopically) the thickness of the coating. The actual coating thickness may differ from the thickness specified by the supplier[28].

**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** | | | |
| PE; 25 µm | -Easily obtainable and inexpensive<br>-Relatively fast equilibration<br>-Extraction possible with many solvents | -Relatively difficult to cut<br>-Relatively easily folds and crumples during cleaning<br>-Limited thickness can translate into larger sampler, which may be difficult to fit into an autosampler vial, which may complicate extraction<br>-Is relatively easy blown away (in a fume hood) | -Acetone<br>-Acetonitrile<br>-DCM<br>-Heptane<br>-Hexane<br>-Hexane/acetone (*x*:*y*)[b] |
| PE; 50 µm | -Easy handling (cutting and weighing)<br>-Easily obtainable and inexpensive<br>-Compared to thinner PE: more robust and rugged; easier to clean, cut, weigh and dry<br>-Extraction possible with many solvents<br>-High(est) sensitivity | -Compared to thinner PE: slower equilibration | -Acetone<br>-Acetonitrile<br>-DCM<br>-Heptane<br>-Hexane<br>-Hexane/acetone (*x*:*y*) [b] |
| POM; 77 µm | -Very easy to cut, weigh and clean<br>-Robust and rugged<br>-Obtained from a single supplier | -Relatively slow equilibration because of slow internal diffusion<br>-Intensive shaking needed during equilibration<br>-Extractable with only a limited number of solvents<br>-Compatibility with PRCs is unclear | -Acetonitrile<br>-DCM<br>-Hexane/acetone (1:1) |
| PDMS and SR; 100 µm | -Extraction possible with many solvents<br>-Easy to extract (soft polymer)<br>-Fast internal diffusion of most chemicals | -Difficult to trace in suspensions because of transparency/refractive index<br>-Dry polymer sticks quite strongly to surfaces, which complicates cutting and weighing<br>-Composition may vary from supplier to supplier and so may target contaminant partitioning to the sampler ($K_{pw}$)<br>-48-h pre-extraction (Soxhlet) recommended to remove oligomers | -Acetone<br>-Acetonitrile<br>-DCM<br>-Heptane<br>-Hexane<br>-Hexane/acetone (*x*:*y*)[b]<br>-Methanol[c] |
| **SPME fibers** | | | |
| PDMS coating; 10 µm | -Fastest equilibration for most chemicals | -Lowest sensitivity (limited polymer phase)<br>-Vulnerable sampler (fiber easily breaks)<br>-Sampler with highest variability | -Acetonitrile<br>-DCM<br>-Heptane<br>-Hexane<br>-Hexane/acetone (*x*:*y*)[b]<br>-Methanol |
| PDMS coating; 30 µm | -Second-fastest equilibration<br>-Compared to 10-µm fiber: higher sensitivity, less fragile, lower variability | -Relatively high variability in results | -Acetonitrile<br>-DCM<br>-Heptane<br>-Hexane<br>-Hexane/acetone (*x*:*y*)[b]<br>-Methanol |
| PDMS coating; 30 µm (500-µm core) | -Higher sensitivity than previous fiber due to larger polymer volume per centimeter of fiber; yet has same kinetics | -Difficult to cut<br>-Fiber length fitting in an autosampler vial insert is limited | -Acetonitrile<br>-DCM<br>-Heptane<br>-Hexane<br>-Hexane/acetone (*x*:*y*)[b]<br>-Methanol |
| PDMS coating; 100 µm | -Stiff/strong fiber. Possible to shake instead of rolling<br>-Fiber with highest sensitivity and lowest variability | -Fiber with slowest equilibration kinetics | -Acetonitrile<br>-DCM<br>-Heptane<br>-Hexane<br>-Hexane/acetone (*x*:*y*)[b]<br>-Methanol |
| Polyacrylate coating; 30 µm | -Relatively strong sorption of chemicals<br>-Also suitable for more polar chemicals | -Relatively slow equilibration because of slow internal diffusion<br>-Exact composition of polymer unknown<br>-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 the case that organochlorine pesticides are targeted and solvent boiling (after a cleanup) is performed, because several targets may degrade[87].

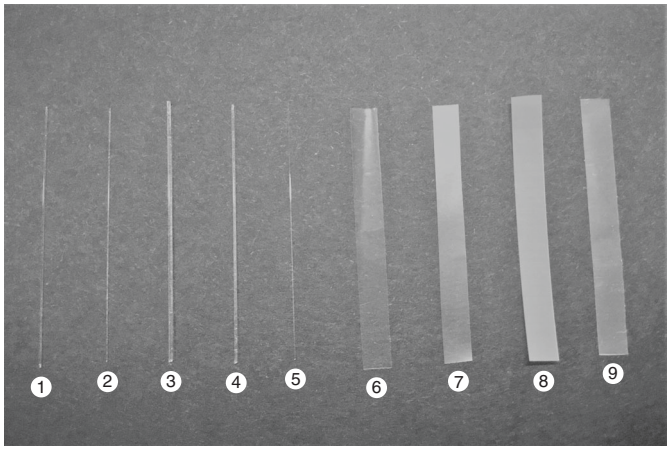


**Fig. 1 | Photograph of different passive samplers.** From left to right: (1) 10-µm PDMS-coated SPME fiber (core thickness, 200 µm), (2) 30-µm PDMS-coated SPME fiber (core thickness, 100 µm), (3) 30-µm PDMS-coated SPME fiber (core thickness, 500 µm), (4) 100-µm PDMS-coated SPME fiber (core thickness, 200 µm), (5) 30-µm polyacrylate-coated SPME fiber (core thickness, 100 µm), (6) 25-µm-thick PE, (7) 50-µm-thick PE, (8) 77-µm-thick POM, (9) 100-µm-thick PDMS. All samplers are 4 cm long. The four sheet samplers are approximately 5 mm wide; their weights are approximately 5 mg (6), 8 mg (7), 20 mg (8), and 20 mg (9).

- 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 Eq. 4 below) need to be performed for a definitive answer to the question of whether the SPME application will result in detectable concentrations. The 30-µm-coated fibers with a 500-µm core represent an exception because they have a larger overall coating volume.
- Generally, for a given polymer mass, the lowest LODs can be achieved with PE sheet samplers, because 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, because these are the easiest to cut, weigh and clean. Thinner (25-µm) PE is somewhat more difficult to cut and clean, because 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 is easiest to add to and remove from slurries, as well as to clean.
- In terms of kinetics, thin PE and SPME fibers (10- to 30-µm coatings) are superior, because 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, because of 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 at concentrations roughly >1,000 mg/kg in sediments[53–55] and may complicate $C_{free}$ determinations. Under these conditions, the use of PDMS is discouraged, because this polymer can absorb high levels of oil and thin SPME fibers may easily become fouled with NAPLs[49,55]. Fouled fibers are difficult to clean and may result in biased measurements. POM has been suggested to be an appropriate sampler in these cases[17,49,55], because it can be cleaned from NAPLs more easily and the affinity of petroleum hydrocarbons for this polymer is much lower than that for PDMS. The performance of PE in NAPL-containing samples has received little attention and needs further study, but wiping with solvent-soaked tissue has been suggested as a way to remove NAPL coatings from this polymer[56]. In any case, $C_{free}$ determinations in NAPL-containing sediments and soils are challenging and one should be cautious. The presence of high concentrations of NAPL constituents in polymer extracts may call for caution in interpreting the results.
- 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 1 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. By 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, because of their generally slow equilibration kinetics for HOCs and because their compatibility with so-called performance reference compounds (PRCs; see below) is still unclear[57–59]. Therefore, understanding the extent of equilibrium achieved with these polymers can be challenging, especially for the more hydrophobic chemicals with $K_{ow}$ values larger than ~$10^6$. 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 toward applications with PE and PDMS/SR sheets and (30- to 100-µm) PDMS-coated SPME fibers. Procedures for POM and polyacrylate are included in the Supplementary Methods.

**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 design a priori 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 into 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 sheets or 3–20 cm of SPME fiber is added. Polymer sheet samplers of <2 mg are discouraged, because the uncertainty in their actual weight is too large, and samplers weighing >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 <3 cm are discouraged, because these are more difficult to trace in dense suspensions and the uncertainty in coating volume increases with decreasing length. Lengths of >20 cm will not fit into the prescribed extraction glassware (autosampler inserts). As mentioned in the "Limitations' section, larger masses (or longer fibers) may be needed in specific cases in which concentrations of target contaminants in sediment or soil are very low. To keep the extraction non-depletive (see below) in such cases, the 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 used, the details should be spelled out and the appropriate QA test results (e.g., depletion percentage, equilibrium verification; 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 critical, because considerable uptake from the sediment or soil by the sampler depletes chemical concentrations in the sample and measurements under depletive conditions may substantially underestimate the actual $C_{free}$ value[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, we recommend setting the maximum depletion limit to 5%, but we note that this value is arbitrary and the intention should always be to keep the depletion as low as possible, because underestimation of $C_{free}$ increases with increasing depletion. To ensure not exceeding any chosen depletion percentage, a priori calculations that are both sampler and target analyte dependent are necessary. The goal of the calculations is to determine the maximum mass of polymer sheet ($M_{p(max)}$; milligrams) or length of SPME fiber ($L_{f(max)}$; centimeters). 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:

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

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 (liters/kilogram), and $K_{pw}$ the polymer–water partition coefficient of the target chemical (liters/kilogram). Note that 0.05 represents the maximum depletion criterion (5%) and it is assumed that the fraction of the total mass of the target chemical in the pore water is negligible ($M_s \times f_{oc} \times K_{oc} \gg$ volume of water). If the $f_{oc}$ is unknown, but the organic matter content ($f_{om}$) is available, $f_{oc}$ can be estimated[60] as $0.58 \times f_{om}$. Generally, $K_{oc}$ values will be unknown and will need to be estimated. Several equations are available for this purpose, commonly relating $K_{oc}$ to $K_{ow}$ (e.g., $K_{oc} = 0.63 \times K_{oc}$[61]) or to so-called Abraham descriptors[62]. It should be noted that estimating $K_{oc}$ values for sediments requires different equations than for soils, because of the difference in organic carbon nature. In addition, 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 than sorption to spiked, laboratory-contaminated samples[63–65], 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 the following equation:

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

with $\rho_p$ 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 (microliters/centimeter). 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, because $K_{ow}$ 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, to maximize detectability of target chemicals, the final volume of the sampler extract ($V_{extract}$; milliliters) and the sampler mass or volume ($M_p$ or $L_f$) should be optimized such that target chemical concentrations in the extract ($C_{extract}$; micrograms/liter) are within the calibration range of the analytical equipment (while ensuring that $M_p$ or $L_f$ remain $< M_{p(max)}$ or $L_{f(max)}$). $C_{extract}$ can be assessed a priori, albeit roughly, on the basis of the (solvent-extractable) concentration of the target HOC in the whole sediment or soil sample ($C_s$; micrograms/kilogram). This concentration is often available from initial field assessments (first-tier screenings) or, in the case of spiked samples, from nominal concentrations.

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

or:

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

with $M_p$ in units of milligrams and $L_f$ in centimeters. 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 versus 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. 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- to 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 to 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 can 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 is important to consider here (Eqs. 3 and 4), because 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 Eqs. 3 or 4, is as high as possible (within calibration limits), enabling the concentration to drop by a factor of 10 (due to a factor of 10 stronger sorption), yet still be above the lowest calibration concentration. For PAHs in field samples, sorption can be a factor of up to about 1,000 times stronger (because of strong association with carbonaceous geosorbents[65]). Therefore, for these compounds, a 'safety margin' of 100, if possible, is recommended in the calculations.

**Preparation of the samplers**

Before 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 liquid chromatography (LC) tubing or pre-column (oligomers), or by contaminating the gas chromatography (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 to remove oligomers and additives[66] but is unnecessary and discouraged for PE, because it 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, whereas 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, to provide a generally applicable pre-extraction procedure that is compatible with most solvents used for extraction and to ensure 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 Supplementary Methods.

**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 ($t_{eq}$) and is dependent on several chemical- and polymer-related factors and incubation conditions. First, $t_{eq}$ increases with target chemical hydrophobicity[20,23,28,67]. For example, chlorobenzenes will equilibrate faster than dibenzodioxins and three-ring PAHs will reach equilibrium sooner than six-ring PAHs. Second, $t_{eq}$ is dependent on the type and thickness of polymer. As mentioned above, 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[25,28,68,69]. 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- to 30-μm coating/100-μm core) cannot be shaken vigorously because of their fragile nature but are preferably equilibrated on a 'rock and roller' apparatus, which does result in sufficient mixing. However, SPME fibers with thicker coatings (e.g., 100 μm) can be shaken vigorously, with an intensity similar to that for sheet samplers. A 1-dimensional, reciprocal table shaker, with an amplitude of ~3–5 cm and

operating at 150–180 r.p.m. is recommended in these cases. Mixing on an orbital shaker is discouraged for all samplers used in dense suspensions, because this causes insufficient mixing of such sediment or soil suspensions. Finally, because diffusion kinetics increase with temperature, $t_{eq}$ will be shorter at higher temperatures. It should be noted, however, 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, we recommend performing 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[70].

Generally, under the above-mentioned conditions (intensive shaking and 20 °C), $t_{eq}$ for thin SPME fibers (10- to 30-μm coatings) and PE samplers (25 μm) are up to about 4 weeks for chemicals with $K_{ow}$ values up to ~$10^8$, such as three- to six-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 4–6 weeks[28]. Although one may rely on these rules of thumb for well-studied chemicals (e.g., PAHs, PCBs) and samples on the basis of experience, $t_{eq}$ for other chemicals and uncharacterized samples may deviate. In particular, for strongly hydrophobic chemicals, equilibration may take longer. In addition, for specific sediments and soils, longer equilibration times may be needed[71], owing to very slow desorption of bound chemicals to the aqueous phase, which is often related to the extent of depletion and the presence of specific carbonaceous geosorbents (e.g., black carbon, tar, coal)[65,71]. To prevent any biodegradation during the equilibration phase, a biocide (i.e., sodium azide) should always be added to the system.

If full equilibrium has not been attained, measurements generally will be inaccurate by 10–20%, although higher percentages have been observed in specific cases[71]. 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 $C_{free}$ by, for example, using test durations of 2, 4, 6, 8, and 10 weeks[28,71,72]. 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)[10,25,73,74]. 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 samplers of different thicknesses, equilibrium conditions are verified.

3 Incorporate PRCs. These are chemicals added to passive samplers before 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.[75] to better characterize the uptake of HOCs into semi-permeable membrane devices, 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) analogs of the target analytes. Similarity to the target compounds ensures that the release process of PRCs from 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 incorporated into the passive sampler before the deployment in the sediment or soil slurry, through partitioning from a solvent–water mixture, and the initial and final concentrations 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, because 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 for doing this and extrapolating PRC results to all target compounds of interest are available in the literature[59]. There is no firm rule about the number of PRCs that is needed (i.e., use of more PRCs is better, but this 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, particularly at the higher end). In addition, they should not interfere analytically with the target compounds or any other compounds used for QA/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 to correct for non-equilibrium conditions (for stable isotope analog 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 have rarely been industrially produced (e.g., PCBs 29, 69, 155, 192), and $^{13}$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 additional 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)**

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, because quantitative extraction of the target compounds is required and not all solvents are practically capable of meeting this criterion for all polymers. 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 Supplementary Methods). 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 have not been tested and would need to be verified before application of the respective solvent. Because 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 as compared to solvent extracts of sediment, soil and biological tissue samples. Therefore, a cleanup of passive sampler extracts obtained using the current protocol often may not be necessary and is discouraged here, because 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, for example, when assessing very complex, heavily contaminated field samples, cleanup may prove necessary and protocols are available. If cleanup 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 the 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 interlaboratory 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 with regard to 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], because inaccurate calibration will cause a systematic bias. Therefore, we strongly recommend the inclusion of a sample or standard with known target chemical concentrations (e.g., a certified analytical standard). Further, calibration should be 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 ~1–500 or ~1–1,000 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 standards, that is, a chemical(s) that is not natively present in the samples under investigation and is not applied as a PRC(s) but is included at the same concentration (e.g., ~50–200 μg/L) as the concentration that is added to the extracts. Internal standards are incorporated to correct for variations in extract and injection volumes and cannot be used to adjust for extraction recovery. Commonly used internal standards include PCBs 30, 121, 198, 204, 205 and 209; $^{13}$C-labeled PCBs; *p*-terphenyl; and deuterated PAHs.

**Calculation of $C_{free}$: polymer–water partition coefficients**

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 ($K_{pw}$ values). 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, with which 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[70,76,77], and specific experimental expertise is required. Therefore, we recommend making use of quality-controlled literature $K_{pw}$ values. These are available for PAHs and PCBs for both PE[78] and PDMS[79]. If literature values are not available for the chemicals in question and experimental determination is required, one should consult the literature[70,78,80] (and/or with an expert) for practical guidance on measuring $K_{pw}$ values.

**Quality assurance/control**

In the above sections, several QA/quality control (QA/QC) steps and concerns are 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 QA/QC 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 closely adhering to the protocol. Relative standard deviations in such cases may generally be <5%[28]. However, for soils, which usually are much more heterogeneous, we recommend at least quadruplicate determinations, and relative standard deviations will often be (much) higher than 5%. Variability in the results depends not only 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, we highly recommend the analysis of a reference sample. 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

interlaboratory 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 M.T.O.J.).

## Materials

### Reagents

- Acetone (GC grade for residue analysis; Merck, cat. no. 1006581000) **! CAUTION** Acetone is flammable; avoid inhalation, ingestion and skin contact.
- *n*-Hexane (GC-MS grade for residue analysis; Merck, cat. no. 1007951000) **! CAUTION** *n*-Hexane is flammable; avoid inhalation, ingestion and skin contact.
- Organic solvent of choice (GC or LC-MS grade for residue analysis) to be used as extraction and injection solvent during chemical analysis (e.g., heptane (Merck, cat. no. 1043602500), iso-octane (Merck, cat. no. 1154401000), acetonitrile (Merck, cat. no. 1006651000), methanol (Merck, cat. no. 1008371000), dichloromethane (Merck, cat. no. 1006681000)) **! CAUTION** These organic solvents are flammable; avoid inhalation, ingestion and skin contact.
- (Optional) Ethyl acetate (GC-MS grade for residue analysis; Merck, cat. no. 1007891000) **! CAUTION** Ethyl acetate is flammable; avoid inhalation, ingestion and skin contact. **▲ CRITICAL** Ethyl acetate is required only when using PDMS or SR sheets as passive samplers.
- (Optional) Methanol (GC-MS grade for residue analysis; Merck, cat. no. 1008371000) **! CAUTION** Methanol is flammable; avoid inhalation, ingestion and skin contact. **▲ CRITICAL** Methanol is required only when applying PRCs.
- Milli-Q water (18.2 MΩ × cm; Merck Millipore)
- Sodium azide ($NaN_3$; Merck, cat. no. S2002) **! CAUTION** Sodium azide is highly toxic; avoid ingestion, inhalation and skin or eye contact. Avoid contact with metal surfaces; explosive metal azides may be formed.
- Soil or sediment sample(s) containing chemicals for which $C_{free}$ needs to be determined (user supplied) **! CAUTION** Field and spiked sediments and soils may contain high concentrations of toxic chemicals. Avoid skin contact (wear gloves) and ingestion or inhalation of any vapors.

### Equipment

- Balance with at least two decimal places when weighing grams (0.00 g)
- Clean fume hood
- Air-conditioned room at 20 ± 3 °C
- Autosampler vials (amber colored, 1.5 mL, short-thread; BGB, cat. no. 080401-XLW) with screw caps (BGB, cat. no. 090300)
- Autosampler vial box
- Metal spoons for transferring sediment and/or soil
- Razor blade (or scalpel)
- Metal tweezers without ribs (two pair)
- Scintillation vial(s) with metal foil-lined caps (20 mL; Fisher Scientific, cat. no. 10079010)
- Clean glass beakers (250/500 mL)
- Ultraclean Erlenmeyer flasks with ground-glass stopper 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
- Glass Pasteur pipettes and small pipette bulbs
- Clean syphon with freshly drawn Milli-Q water
- Clean syphon with high-purity acetone
- Waste receptacle for sediment/soil waste
- Permanent marker (fine-tipped)
- Blank self-adhesive laboratory labels
- Vortex mixer(s)
- Chemical-analytical equipment for the detection of the target compounds (typically occurring in the microgram/liter range) in solvent extracts: GC-MS, HPLC-FLD and/or LC-MS system

**Equipment used only for polymer sheet samplers**

- Polymer sheet: low-density polyethylene (PE) sheet, 25-µm thickness (VWR), PE sheet, 50-µm thickness (Brentwood Plastics or Carlisle Plastics), or polydimethylsiloxane (PDMS) or silicone rubber (SR) sheet, 100-µm thickness (Specialty Silicone Products or Shielding Solutions)
- Bottles (amber colored, 120 mL (4 oz.); Brocacef Supplies & Services, cat. no. FLEGL7661, or Uline, cat. no. S-15649) with polypropylene screw cap containing PTFE liner (Fisher Scientific, cat. no. 10536934)
- Small, wide-neck glass funnel fitting into the mouth of the 120-mL bottles
- Analytical balance with five decimal places (two decimal places when weighing milligrams (0.00 mg))
- Shaker table (reciprocal, 1-dimensional, with an amplitude of 3–5 cm; capable of shaking at 180 r.p.m.; Gerhardt, model no. Laboshake LS500)
- Laboratory aluminum foil (thick (30–50 µm); The Lab Warehouse, cat. no. AL202-35)
- Scissors
- Tea sieve (or regular geological sieve; mesh ~1 or 2 mm; with bowl)
- Pipette for pipetting volumes of 100–1,000 µL and clean tips
- (Optional) Soxhlet extraction equipment ▲ **CRITICAL** Soxhlet extraction equipment is required only when using PDMS or SR sheets as passive samplers.

**Equipment used only for SPME fibers**

- 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 or Fiberguide)
- Vials (amber-colored, 15 mL; Sigma-Aldrich, cat. no. 27088-U) with black screw caps with aluminum liner (Sigma-Aldrich, cat. no. 27164)
- Small, wide-neck glass funnel fitting into the mouth of the 15-mL vials
- Rock and roller shaker capable of rolling vials at a speed of ~33 r.p.m. (Stuart, model no. SRT9)
- Autosampler vial inserts (conical, 300 µL; BGB, cat. no. 110502)
- Wire cutter for cutting fibers
- Tissues (soft; e.g., Kleenex)
- Adhesive tape (transparent)
- Pipette for pipetting volumes of 20–200 µL or 100–1,000 µL and clean tips (Pipette choice depends on extract volume.)
- (Optional) Tea sieve or large glass Petri dish (10-cm diameter) ▲ **CRITICAL** Tea sieve or large glass Petri dish is required only when fibers <3 cm are applied for sampling.

### Reagent setup

**Standard aqueous solution**

Prepare standard aqueous solution (SAS) by dissolving 200 mg of $NaN_3$ per liter of Milli-Q water in a clean glass bottle with a plastic cap. Shake to dissolve and homogenize. This highly toxic solution should be used within a week after preparation. Keep closed and store at room temperature until use.

### Equipment setup

**Preconditioning of passive sampling polymers**

Passive sampling polymers should be preconditioned as described in the 'Experimental design' section 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.

## 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 or 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 a PDMS or SR sheet as a sampler, the sheet should be Soxhlet-extracted[66] for 48 h with ethyl acetate and air-dried in a fume hood before cutting. The number of strips should be equal to the number of systems that will be

prepared plus ~10 additional ones, to be used as blanks, controls and for 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, because 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; Supplementary Fig. 1) because 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 a screw cap. The total mass of the strips per vial should not exceed 1,000 mg.

■ **PAUSE POINT** Samplers can be stored in closed vials at room temperature 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, place the fiber on a glass plate underneath or on top of which a ruler is fixed (Supplementary Fig. 2). Cut lengths of maximally 5.00 cm and cut several (10–20) extra (sets of) fibers to be used as blanks, controls, and for 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, because 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 a screw cap. The maximum number of fibers per vial should not exceed 300.

■ **PAUSE POINT** Fibers can be stored in closed vials at room temperature 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, as well as for Soxhlet-pre-extracted (ethyl acetate) PDMS and SR sheet samplers.

- Add about 18 mL of high-purity *n*-hexane to the 20-mL vial(s) containing the samplers; cap the vial(s) tightly and put it(them) on its(their) side (in horizontal position) on a reciprocal (1-dimensional) shaker, operating at about 180 r.p.m., 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 acetone. After the last acetone wash, discard all acetone.

! **CAUTION** Adding and exchanging solvents as described above 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 given above is critical because of the water and methanol miscibility of the last solvent.

3 Prepare the samplers. For samplers (sheets and fibers) that will be loaded with PRCs, use option A; for sheets that will not be loaded with PRCs, use option B; and for fibers that will not be loaded with PRCs, use option C:

(A) **Samplers that will be loaded with PRCs**

(i) After the last acetone wash in Step 2, samplers can be loaded with PRCs. Refer to Box 1 for the loading procedure.

(B) **Sheets that will not be loaded with PRCs**

(i) 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 tissue; 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**

## 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 an 80:20 methanol/water mixture. The approach allows for a relatively fast equilibration of PRCs in the polymer phase, because of the high methanol content, which causes very low sampler–solvent partition coefficients. The loading is not designed to be quantitative, because 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 $V_L$ (milliliters) and the total passive sampler polymer phase with mass $M_{p(tot)}$ (grams) (for SPME fibers, the total volume of the PDMS phase applies), according to the ratio of the uptake capacities ($V_L/M_{p(tot)} \times K_{p80}$; with $K_{p80}$ being the passive sampler–methanol/water (80:20) partition coefficient (liters/kilogram)). To calculate the mass of each individual PRC that should be added to the loading system ($N_{add}$, nanograms) to achieve the desired concentration in the passive sampler polymer ($C_p$, nanograms/gram), the following equation can be applied (adapted from Booij et al.[88]):

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

In Supplementary Table 1, 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 <5% ($C_{p(min)}$) is estimated according to the following equation:

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

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

**Procedure**

1 Following Step 3A(i) 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: for PRCs available as neat material (solid standards), use option A and for PRCs obtained as standards dissolved in a nonpolar solvent (e.g., pentane, hexane, nonane, dichloromethane), use option B.

(A) **PRCs available as neat standards**

(i) 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.

(ii) 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 the samplers are then added.

(B) **PRCs dissolved in a nonpolar solvent**

(i) 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.

(ii) Purge the vial in a fume hood with a gentle stream of nitrogen, such that the nonpolar solvent(s) is/are slowly evaporated.

(iii) 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).

(iv) 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 r.p.m.

4 Add 1.75 mL (or 0.1 $V_L$) of Milli-Q water and continue shaking for another 8 h. Add another 1.75 mL (or 0.1 $V_L$) of Milli-Q 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 Milli-Q water by shaking at 180 r.p.m. on the reciprocal table shaker. After washing, discard the water and store the samplers 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 can also be stored in their loading solution in the dark for prolonged times, but then step 5 should be performed just before deploying the samplers.

6 Confirm (preferably before deployment) that the PRC concentrations are in the target range and their heterogeneity is acceptable (e.g., <10%) by analyzing multiple individual samplers, extracted in the appropriate (extraction and injection) solvent, having a volume of $V_{extract}$. Note that these analyses also need to be performed when analyzing the final samples, because they are needed to determine the reference (100%) PRC level in the samplers.

(ii) Place the open 20-mL-vial(s) next to the polymer pieces with the mouth facing the fume hood window (the cap can be placed on the tissue too).

(iii) 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(s) and cap it (them).

(C) **Fibers that will not be loaded with PRCs**

(i) Remove the remaining acetone from SPME fibers that will not be loaded with PRCs by washing them twice with Milli-Q water for 30 min while shaking on a reciprocal shaker. After the last wash, discard the water, fill the vial with freshly drawn Milli-Q 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 containers (bottles or vials), using well-sticking labels and a permanent marker or printed labels (during equilibration on a shaker (Steps 13 and 14), deterioration of labels may occur). Use option A for polymer strips and option B for SPME fibers.

(A) **Polymer strips**

(i) Wash and label 120-mL bottles for polymer strip samplers.

(B) **SPME fibers**

(i) Wash and label 15-mL vials for SPME fibers. Because 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 SAS in an ultraclean bottle (washed with soap and rinsed with high-purity acetone and Milli-Q water). The volume should equal ~90 (mL) × the number of containers to prepare for polymer strip sampler tests, or ~12 (mL) × the number of systems (containers) to prepare for SPME fibers.

**! CAUTION** SAS is a 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 minutes with a metal spoon. If field samples are studied, manually remove larger objects (e.g., leaves, twigs and stones) before mixing or use a coarse (>2-mm) sieve. The dry weight percentage and organic carbon fraction of the samples should be known ('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 vapors.

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:

- For polymer strip samplers: 30 g of dry-weight sample in a 120-mL bottle
- 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., less than ~30–40%), it may not be possible to fit the intended dry-weight mass into the equilibration system, because the wet-weight mass to be added is too large (e.g., for a sample containing 90% water, the required 300 g of wet-weight sample does not fit into a 120-mL system). Such samples could be centrifuged first and then the supernatant can be discarded, which will yield a sample with a higher dry-weight content (to be quantified). Alternatively, if the sample has a high organic carbon content (e.g., >10%), the sample mass can be reduced (as long as the final design is still according to Eqs. 1 or 2) or possibly a larger bottle could be used (assuming such systems can be homogenized sufficiently on an appropriate shaker).

8 Place a small, wide-neck, glass funnel in the mouth of the bottle or vial, position it on the balance (two- or three-decimal balance; Supplementary Fig. 3) and tare it. Use a metal spoon to transfer an appropriate sample mass to the respective glass system. 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 (for polymer strips; option A) or vial (for SPME fibers; option B).

(A) **Bottle (polymer strips)**

(i) 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).
**? TROUBLESHOOTING**

(B) **Vial (SPME fibers)**

(i) Because only a few milliliters 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). When adding SAS, lift the funnel and raise the end above the suspension level, allowing the solution to enter the system.
**? TROUBLESHOOTING**

10 Close the bottle or vial with the appropriate cap and leave it 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 before filling the next system. Clean the spoon and funnel with water and acetone before changing samples (not necessary between 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, we recommend proceeding right away or the next day.

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

(A) **Polymer strips**

(i) If polymer strips were loaded with PRCs and put into the freezer (Box 1), remove the loading/storage vial from the freezer and place it 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 it in four, and place it 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 has been placed (Supplementary Fig. 1). Record on paper the weight of the sampler in milligrams to two decimal places (e.g., 5.98 mg) and add it to the corresponding bottle.
**? TROUBLESHOOTING**

(ii) 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.

(iii) 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, making sure that the foil touches the bottle mouth completely and shows no creases (Supplementary Fig. 4), and very tightly screw the cap onto the bottle.
**▲ CRITICAL STEP** Application of aluminum foil is critical. 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) **SPME fibers**

(i) 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.

(ii) Perform this step for all systems.

### Equilibrating the systems ● Timing 4–6 weeks

13 Equilibrate the systems, using option A for polymer strips or option B for SPME fibers.
(A) **Polymer strips**
(i) Place bottles with polymer strips in horizontal position on a 1-dimensional (reciprocal) table shaker, operating at 150–180 r.p.m. and 20 ± 3 °C in the dark. When a large number of systems need to be equilibrated, the bottles can be stacked and fixed in a box.
(B) **SPME fibers**
(i) Place 15-mL vials with SPME fibers in horizontal position on a rock and roller shaker, operating at ~33 r.p.m. and 20 ± 3 °C in the dark.
▲ **CRITICAL STEP** Shaking at the specified intensity is critical, particularly when not verifying equilibrium conditions with PRCs.

14 Equilibrate for 4–6 weeks, depending on the sampler and the sample, as described in the 'Experimental design' section. Frequently check the systems and the ambient temperature. When equilibrating 15-mL vials, frequently tighten the caps, because 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 (option A) or SPME fibers (option B).
(A) **Polymer strips**
(i) Obtain a vial box with the appropriate number of autosampler vials, a clean syphon with freshly drawn Milli-Q 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 four layers (hereafter referred to as 'mat tissue'), a stock of the lint-free tissue, a 1- to 2-mL pipette with tip, an Erlenmeyer flask with the solvent intended for extracting the polymer samplers (Experimental design) and a waste receptacle for the discarded sediment/soil suspensions. An overview of these materials is presented in Supplementary Fig. 5. Using a tea sieve is most convenient (Supplementary Fig. 7), but a regular geological sieve (mesh ~1 or 2 mm) is also possible (use a receiving bowl underneath).
(B) **SPME fibers**
(i) Obtain a vial box with autosampler vials (containing 300-µL inserts, if needed), a box with tissues, a clean syphon with freshly drawn Milli-Q water, a beaker in which 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 suspensions and an optional tea sieve or large glass Petri dish (this is needed only when fibers <3 cm long are applied). An overview of these materials is presented in Supplementary Fig. 6.

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 (Experimental design) with an appropriate pipette and tip pre-rinsed with solvent.
! **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 in the 'Experimental design' section (Table 1).

17 Calibrate the solvent volume to the expected concentrations of the target analytes (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 the expected target chemical concentrations in the extracts of SPME fibers with a coating/core thickness of 10/100 or 30/100 µm 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, because the volume of the latter solution should be proportional to $V_{extract}$).

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

19 Include the following vials and mark each with a code:
- A solvent blank vial: add only the solvent to this autosampler vial (volume minus the internal standard volume to be added)

- A sampler blank vial: add a sampler with a representative mass (sheet) or length (fiber) as obtained in Step 3B(iii) or 3C(i), respectively, to this vial and add a representative volume of solvent (minus the volume of internal standard to be added)
- If PRCs are being used: add (at least) three vials containing PRC-loaded samplers, as obtained in Step 3A(i) (Box 1). Add solvent as described (minus the volume of internal standard to be added)
- 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(iii), or 3C(i), respectively. Later, add to this vial the second-lowest calibration standard (including the internal standard), which will be used for chemical quantification in Step 25.

20 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 acetone (wipe with acetone-wetted tissue).

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

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

**? TROUBLESHOOTING**

(iv) Pour the contents of the bottle onto the sieve (which should be positioned on a beaker) and find the polymer strip. Use tweezers without ribs to pick up the polymer strip and rinse it clean with Milli-Q water from the syphon (Supplementary Fig. 7).

**? TROUBLESHOOTING**

(v) Place the sampler on the mat tissue (i.e., four 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. Use scissors to cut pieces with a length of ≤7 mm from the strip. The pieces will 'fall' into the vial (Supplementary Fig. 8). Long sampler strips can first be folded in two and the 'closed end' cut open, twice if necessary, while lying on the mat tissue.

(ix) Carefully check (i) the scissors, (ii) the tweezers, (iii) the tissue, and (iv) the vial box for the presence of polymer pieces, because 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). Ensure that 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 20A(i–xi) for all the other equilibration systems. Take a new piece of thick lint-free, 'wiping' tissue (folded in four) for each new sampler and a new mat tissue once the current one no longer has any dry areas (about every three to four samples). Clean the scissors and tweezers with acetone when necessary (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 Milli-Q 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 contents onto the tea (or geological) sieve (which should be positioned on a beaker) or into an empty 10-cm Petri dish. Water may be needed to remove the entire vial contents. Use tweezers and any additional water to find and retrieve the fiber(s) from the sieve or the Petri dish and use tweezers to place the fiber(s) on the wetted half of the tissue.

(v) Check the number of fibers and wipe it/them three times with the wet tissue while holding it/them between thumb and forefinger/middle finger (Supplementary Fig. 9). Then grip the fiber(s) at the other end and wipe the other side(s) 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.
▲ **CRITICAL STEP** When sampling volatile chemicals, perform this step as quickly as possible (i.e., within 30 s) and/or return the fibers that are not being cleaned at that moment to the 15-mL vial or leave them in the Petri dish.
**? TROUBLESHOOTING**

(vi) Open the corresponding autosampler vial and stick the fiber(s) into its opening. Use the wire cutter/scissors to cut pieces with a length of ≤1.3 cm from the fiber(s) (Supplementary Fig. 10) and ensure 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 it in another vial box.

(ix) Repeat steps 20B(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

21 Remove the cap from the first vial and add internal standard solution (Experimental design) with a GC syringe or calibrated (micro) pipette. Tightly cap the vial again. Repeat these steps for all vials, including the vials prepared in Step 19. As mentioned (in Step 17), 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.

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

23 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. If the chemical analysis described in Step 25 is to be performed more than 1 d later, place the vials in the freezer.
■ **PAUSE POINT** Tightly closed and vortexed vials can be stored at −20 °C for up to 2 months.

24 Analyze the extracts as described in Steps 25–28. If vials were frozen for storage, take them from the freezer several hours before starting the analysis and vortex each vial again for 30 s 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

25 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 five 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.

26 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 that the presence of the sampler does not affect peak shape or surface, or yield any interfering background noise.

27 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 ($C_{extract}$; micrograms/liter). This step can also be performed automatically by the instrument data processing software, but the calculations should be checked manually in a couple of cases.

28 If calculated concentrations exceed the highest calibration level, the extracts should be diluted and reanalyzed. This can be performed by taking, for example, 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.

## Troubleshooting

Troubleshooting advice can be found in Table 2.

**Table 2 | Troubleshooting table**

| Step | Problem | Possible reason | Solution |
|---|---|---|---|
| 3B(i) | Polymer strips are blown away and land 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 into 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 |
| 9A (i),9B(i) | Too much mass is added, leaving insufficient headspace | Miscalculation | Remove superfluous slurry with a Pasteur's pipette to obtain the desired weight/headspace volume |
| | A fraction of sample remains in the funnel | More SAS is 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) |
| 12A(i) | 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 it dry again by firmly pressing the other half of the tissue on top of the sampler and manually swiping back and forth on the upper half of the tissue |
| 20A(iii) | Cap is dirty on the inside | The aluminum foil might be broken, and the polymer in the cap may have absorbed part of the chemical pool, causing the extraction to be depletive | Record observation. This may be a reason to dismiss the results for this sample |
| 20A(iv) | Sampler stays in the bottle | Sampler sticks to glass wall or is stuck in remaining sample mass | Add a small volume of Milli-Q water, shake firmly, and try to pour the sampler out again |
| 20A(vi) | Sampler cannot be fully cleaned; certain stains remain | Stains probably concern NAPL spots (e.g., oil) | Make a note (and take a 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 |
| 20B(v) | 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 $C_{free}$ |

## Timing

Step 1, sizing samplers: 0.5–5 min per sampler
Steps 2 and 3, preconditioning samplers: 2–3 h
Step 3, (optional) loading samplers with PRCs: 3 d
Steps 4–12, preparing the equilibration systems: 5–10 min/equilibration system
Steps 13 and 14, equilibrating the systems: 4–6 weeks
Steps 15–20, collecting and cleaning samplers: 5–10 min/equilibration system
Steps 21–24: extraction: 25 h
Steps 25–28, chemical analysis: dependent on the analysis run time, the number of samples, and the number of target compounds; typically 1–4 d

## 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 ($C^*_{extract}$; micrograms/liter). Note that the results are not corrected for the *sampler* blank. This blank serves only QC 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 ($C_p$; micrograms/kilogram) by multiplying $C^*_{extract}$ by 1,000 and the extract volume ($V_{extract}$; milliliters); and subsequently dividing the result by the sampler weight ($M_p$; milligrams):

$$C_p = \frac{1,000 \times C^*_{extract} \times V_{extract}}{M_p} \quad (5)$$

For SPME fibers, calculate the concentration in the polymer coating ($C_{pc}$; micrograms/liter) by multiplying $C^*_{extract}$ by 1,000 and the extract volume ($V_{extract}$; milliliters) and subsequently dividing the result by the product of fiber length ($L_f$; centimeters) and coating volume per centimeter ($V_{pc}$; microliters/centimeter):

$$C_{pc} = \frac{1,000 \times C^*_{extract} \times V_{extract}}{L_f \times V_{pc}} \quad (6)$$

Finally, calculate $C_{free}$ (nanograms/liter) by multiplying $C_p$ or $C_{pc}$ by 1,000 and dividing the result by the chemical-specific polymer–water partition coefficient ($K_{pw}$; liters/kilogram) for the polymer used as the passive sampler:

$$C_{free} = \frac{1,000 \times C_p}{K_{pw}} \quad (7)$$

or

$$C_{free} = \frac{1,000 \times C_{pc}}{K_{pw}} \quad (8)$$

### 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[8,28,71,72]. 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 Eqs. 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 *unexposed* 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:

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

and in fibers:

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

Three scenarios are possible for $f_{\mathrm{PRC}}$:

1 $f_{\mathrm{PRC}} < 0.05$ 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 ($K_{\mathrm{pw}}$) within the $K_{\mathrm{pw}}$ range covered by the applied PRCs.
2 $f_{\mathrm{PRC}} > 0.05$ 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 $f_{\mathrm{PRC}} < 0.05$ only for PRCs with a relatively low $K_{\mathrm{pw}}$, whereas $f_{\mathrm{PRC}}$ increases (>0.05) with increasing $K_{\mathrm{pw}}$ of the PRCs. This indicates non-equilibrium conditions for the latter group of PRCs and consequently for target compounds having a similar $K_{\mathrm{pw}}$ range.

In the latter two cases, the results do not meet the equilibrium and/or depletion quality criterion, and the resulting $C_{\mathrm{free}}$ 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 can be used to correct the results for non-equilibrium conditions. Corrections for depletive conditions are generally not possible, because 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-labeled analog) as the PRCs (e.g., target compound is PCB52 and the PRC is $^{13}$C-PCB52). The two compounds will have (nearly) the same $K_{\mathrm{pw}}$, sediment– or soil–water partition coefficient, and (given that the exchange is isotropic) exchange kinetics[81]. In such a case, a 'full-equilibrium' $C_{\mathrm{free}}$ ($C_{free}^{\infty}$; nanograms/liter) of the target chemical can be calculated according to the following equation.

$$C_{\mathrm{free}}^{\infty} = \frac{1,000 \times C_{\mathrm{p}}^{(e)}}{K_{\mathrm{pw}} \times (1 - f_{PRC})} \quad (11)$$

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

For target compounds for which no isotopic analogs were applied as PRC, correction requires additional model calculations. For static exposures, several correction models have been developed; these are based on diffusive mass transfer of chemicals between the passive sampler and sediment particles close to and further removed from the sampler surface[69,81,82]. 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 ($f_{\mathrm{eq}}$) value for each target compound, based on the behavior of the PRCs[81,83,84]. This fraction can then be used to adjust target compound $C_{p}^{(e)}$ values to a $C_{free}^{\infty}$ value. Diffusive mass transfer models are particularly mathematically intensive, but online calculators are available for performing the calculations (https://www.serdp-estcp.org/Tools-and-Training/Tools/PRC-Correction-Calculator). 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 similarly 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:

$$k_{e}^{\mathrm{PRC}} = ln\left(\frac{C_{\mathrm{p}}^{\mathrm{PRC}(0)}}{C_{\mathrm{p}}^{\mathrm{PRC}(e)}}\right) \times \left(\frac{1}{t}\right) \quad (12)$$

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 the $K_{\mathrm{ow}}$, the $K_{\mathrm{pw}}$, the molar volume, or a combination of molecular weight and $K_{\mathrm{pw}}$ can be constructed[59,85,86]. The $k_{\mathrm{e}}$ values for the target chemicals can subsequently be estimated from these regressions through inter- or extrapolation. On the basis of the resulting $k_{\mathrm{e}}$ values, $C_{free}^{\infty}$ of the target compounds can be calculated according to the following equation:

$$C_{\mathrm{free}}^{\infty} = \frac{1,000 \times C_{\mathrm{p}}^{(e)}}{K_{\mathrm{pw}} \times (1 - e^{-ke \times t})} \quad (13)$$

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

Although the different models have been compared for static systems[59], 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 the case that depletion and non-equilibrium both occur, the exchange rates depend on the sampler-to-sediment/soil capacity ratio[20], which will complicate the modeling.

## Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

No datasets were generated or analyzed during the current study.

## References


1. Schwarzenbach, R. P. et al. The challenge of micropollutants in aquatic systems. *Science* **313**, 1072–1077 (2006).
2. Dickson, K. L., Maki, A. W. & Brungs, W. A. *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems* (SETAC Special Publication, Pergamon, 1987).
3. Di Toro, D. M. et al. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* **10**, 1541–1583 (1991).
4. US Environmental Protection Agency. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. Report No. SW-846 (USEPA, 2015).
5. Lydy, M. J. et al. Passive sampling methods for contaminated sediments: state of the science for organic contaminants. *Integr. Environ. Assess. Manag.* **10**, 167–178 (2014).
6. Mayer, P. et al. Passive sampling methods for contaminated sediments: scientific rationale supporting use of freely dissolved concentrations. *Integr. Environ. Assess. Manag* **10**, 197–209 (2014).
7. Greenberg, M. S. et al. Passive sampling methods for contaminated sediments: risk assessment and management. *Integr. Environ. Assess. Manag.* **10**, 224–236 (2014).
8. Hawthorne, S. B. et al. Measuring picogram per liter concentrations of freely dissolved parent and alkyl PAHs (PAH-34), using passive sampling with polyoxymethylene. *Anal. Chem.* **83**, 6754–6761 (2011).
9. Cornelissen, G. et al. Freely dissolved concentrations and sediment-water activity ratios of PCDD/Fs and PCBs in the open Baltic sea. *Environ. Sci. Technol.* **42**, 8733–8739 (2008).
10. Jahnke, A., Mayer, P. & McLachlan, M. S. Sensitive equilibrium sampling to study polychlorinated biphenyl disposition in baltic sea sediment. *Environ. Sci. Technol.* **46**, 10114–10122 (2012).
11. Parkerton, T. F. & Maruya, K. A. Passive sampling in contaminated sediment assessment: building consensus to improve decision making. *Integr. Environ. Assess. Manag* **10**, 163–166 (2014).
12. Booij, K. et al. Passive sampling in regulatory chemical monitoring of nonpolar organic compounds in the aquatic environment. *Environ. Sci. Technol.* **50**, 3–17 (2016).
13. Apell, J. N. & Gschwend, P. M. In situ passive sampling of sediments in the Lower Duwamish Waterway Superfund site: replicability, comparison with ex situ measurements, and use of data. *Environ. Pollut.* **218**, 95–101 (2016).
14. Ghosh, U. et al. Passive sampling methods for contaminated sediments: practical guidance for selection, calibration, and implementation. *Integr. Environ. Assess. Manag* **10**, 210–223 (2014).
15. Fernandez, L. A., Macfarlane, J. K., Tcaciuc, A. P. & Gschwend, P. M. Measurement of freely dissolved PAH concentrations in sediment beds using passive sampling with low-density polyethylene strips. *Environ. Sci. Technol.* **43**, 1430–1436 (2009).
16. Khairy, M. A. & Lohmann, R. Using polyethylene passive samplers to study the partitioning and fluxes of polybrominated diphenyl ethers in an urban river. *Environ. Sci. Technol.* **51**, 9062–9071 (2017).
17. Jonker, M. T. O. & Koelmans, A. A. Polyoxymethylene solid phase extraction as a partitioning method for hydrophobic organic chemicals in sediment and soot. *Environ. Sci. Technol.* **35**, 3742–3748 (2001).
18. Gomez-Eyles, J. L., Jonker, M. T. O., Hodson, M. E. & Collins, C. D. Passive samplers provide a better prediction of PAH bioaccumulation in earthworms and plant roots than exhaustive, mild solvent, and cyclodextrin extractions. *Environ. Sci. Technol.* **46**, 962–969 (2012).
19. Cornelissen, G., Arp, H. P. H., Pettersen, A., Hauge, A. & Breedveld, G. D. Assessing PAH and PCB emissions from the relocation of harbour sediments using equilibrium passive samplers. *Chemosphere* **72**, 1581–1587 (2008).
20. Smedes, F., Van Vliet, L. A. & Booij, K. Multi-ratio equilibrium passive sampling method to estimate accessible and pore water concentrations of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in sediment. *Environ. Sci. Technol.* **47**, 510–517 (2013).

21. Tuikka, A. I. et al. Predicting the bioaccumulation of polyaromatic hydrocarbons and polychlorinated biphenyls in benthic animals in sediments. *Sci. Total Environ.* **563-564**, 396–404 (2016).
22. Ter Laak, T. L., Agbo, S. O., Barendregt, A. & Hermens, J. L. M. Freely dissolved concentrations of PAHs in soil pore water: measurements via solid-phase extraction and consequences for soil tests. *Environ. Sci. Technol.* **40**, 1307–1313 (2006).
23. Maruya, K. A., Zeng, E. Y., Tsukada, D. & Bay, S. M. A passive sampler based on solid-phase microextraction for quantifying hydrophobic organic contaminants in sediment pore water. *Environ. Toxicol. Chem.* **28**, 733–740 (2009).
24. Thomas, C., Lampert, D. & Reible, D. Remedy performance monitoring at contaminated sediment sites using profiling solid phase microextraction (SPME) polydimethylsiloxane (PDMS) fibers. *Environ. Sci. Process. Impacts* **16**, 445–452 (2014).
25. Reichenberg, F., Smedes, F., Jönsson, J. A. & Mayer, P. Determining the chemical activity of hydrophobic organic compounds in soil using polymer coated vials. *Chem. Cent. J.* **2**, 8 (2008).
26. Golding, C. J., Gobas, F. A. P. C. & Birch, G. F. Characterization of polycyclic aromatic hydrocarbon bioavailability in estuarine sediments using thin-film extraction. *Environ. Toxicol. Chem.* **26**, 829–836 (2007).
27. St. George, T., Vlahos, P., Harner, T., Helm, P. & Wilford, B. A rapidly equilibrating, thin film, passive water sampler for organic contaminants; characterization and field testing. *Environ. Pollut.* **159**, 481–486 (2011).
28. Jonker, M. T. O. et al. Advancing the use of passive sampling in risk assessment and management of sediments contaminated with hydrophobic organic chemicals: results of an international ex situ passive sampling interlaboratory comparison. *Environ. Sci. Technol.* **52**, 3574–3582 (2018).
29. US Environmental Protection Agency/Strategic Environmental Research and Development Program/ Environmental Security Technology Certification Program. *Laboratory, Field, and Analytical Procedures for Using Passive Sampling in the Evaluation of Contaminated Sediments: User's Manual. Report No. EPA/600/R-16/357* (Office of Research and Development, 2017).
30. Risticevic, S., Lord, H., Górecki, T., Arthur, C. L. & Pawliszyn, J. Protocol for solid-phase microextraction method development. *Nat. Protoc.* **5**, 122–139 (2010).
31. US Environmental Protection Agency. *Guidelines for Using Passive Samplers to Monitor Nonionic Organic Contaminants at Superfund Sediment Sites. Sediment Assessment and Monitoring Sheet. Report No. OSWER Directive 9200.1-110 FS* (Office of Superfund Remediation and Technology Innovation/Office of Research and Development, 2012).
32. Fernandez, L. A., Lao, W., Maruya, K. A. & Burgess, R. M. Calculating the diffusive flux of persistent organic pollutants between sediments and the water column on the Palos Verdes Shelf Superfund site using polymeric passive samplers. *Environ. Sci. Technol.* **48**, 3925–3934 (2014).
33. Burgess, R. M. et al. Application of passive sampling for measuring dissolved concentrations of organic contaminants in the water column at three marine Superfund sites. *Environ. Toxicol. Chem.* **34**, 1720–1733 (2015).
34. Jahnke, A., Witt, G., Schäfer, S., Haase, N. & Escher, B. I. Combining passive sampling with toxicological characterization of complex mixtures of pollutants from the aquatic environment. *Adv. Biochem. Eng. Biotechnol.* **157**, 225–261 (2017).
35. European Chemicals Agency. Guidance on Information Requirements and Chemical Safety Assessment. R.7b: Endpoint Specific Guidance. Version 4.0 (ECHA, 2017).
36. US Environmental Protection Agency. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants With Freshwater Invertebrates. Report No. EPA 600-R-99-096* (Office of Research and Development, 2000).
37. Organisation for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals. Test No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment (OECD, 2004).
38. Organisation for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals. 225: Sediment-water *Lumbriculus* toxicity test using spiked sediment (OECD, 2007).
39. Ortega-Calvo, J. J. et al. From bioavailability science to regulation of organic chemicals. *Environ. Sci. Technol.* **49**, 10255–10264 (2015).
40. Carr, R. S. & Nipper, M. *Porewater Toxicity Testing: Biological, Chemical, and Ecological Considerations* (SETAC Press, 2003).
41. Zhang, H., Davison, W., Miller, S. & Tych, W. In situ high resolution measurements of fluxes of Ni, Cu, Fe, and Mn and concentrations of Zn and Cd in porewaters by DGT. *Geochim. Cosmochim. Acta* **59**, 4181–4192 (1995).
42. Senn, D. B. et al. Equilibrium-based sampler for determining Cu2+ concentrations in aquatic ecosystems. *Environ. Sci. Technol.* **38**, 3381–3386 (2004).
43. Dong, Z., Lewis, C. G., Burgess, R. M. & Shine, J. P. The Gellyfish: an in situ equilibrium-based sampler for determining multiple free metal ion concentrations in marine ecosystems. *Environ. Toxicol. Chem.* **34**, 983–992 (2015).
44. Haftka, J. J. H., Hammer, J. & Hermens, J. L. M. Mechanisms of neutral and anionic surfactant sorption to solid-phase microextraction fibers. *Environ. Sci. Technol.* **49**, 11053–11061 (2015).
45. Haftka, J. J. H., Scherpenisse, P., Jonker, M. T. O. & Hermens, J. L. M. Using polyacrylate-coated SPME fibers to quantify sorption of polar and ionic organic contaminants to dissolved organic carbon. *Environ. Sci. Technol.* **47**, 4455–4462 (2013).

46. Warren, J. K., Vlahos, P., Smith, R. & Tobias, C. Investigation of a new passive sampler for the detection of munitions compounds in marine and freshwater systems. *Environ. Toxicol. Chem.* **37**, 1990–1997 (2018).
47. Ter Laak, T. L., Barendregt, A. & Hermens, J. L. M. Freely dissolved pore water concentrations and sorption coefficients of PAHs in spiked, aged, and field-contaminated soils. *Environ. Sci. Technol.* **40**, 2184–2190 (2006).
48. US Environmental Protection Agency. *Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Procedures for the Determination of the Freely Dissolved Interstitial Water Concentrations of Nonionic Organics. Report No. EPA-600-R-02-012* (Office of Research and Development, 2012).
49. Van der Heijden, S. A. & Jonker, M. T. O. PAH bioavailability in field sediments: comparing different methods for predicting in situ bioaccumulation. *Environ. Sci. Technol.* **43**, 3757–3763 (2009).
50. Giesen, D., Jonker, M. T. O. & van Gestel, C. A. M. Development of QSARs for the toxicity of chlorobenzenes to the soil dwelling springtail *Folsomia candida*. *Environ. Tox. Chem.* **31**, 1136–1142 (2012).
51. Ahn, S. et al. Phenanthrene and pyrene sorption and intraparticle diffusion in polyoxymethylene, coke, and activated carbon. *Environ. Sci. Technol.* **39**, 6516–6526 (2005).
52. Rusina, T. P., Smedes, F., Klanova, J., Booij, K. & Holoubek, I. Polymer selection for passive sampling: a comparison of critical properties. *Chemosphere* **68**, 1344–1351 (2007).
53. Jonker, M. T. O., Sinke, A. J. C., Brils, J. M. & Koelmans, A. A. Sorption of polycyclic aromatic hydrocarbons to oil contaminated sediment: unresolved complex? *Environ. Sci. Technol.* **37**, 5197–5203 (2003).
54. Muijs, B. & Jonker, M. T. A closer look at bioaccumulation of petroleum hydrocarbon mixtures in aquatic worms. *Environ. Toxicol. Chem.* **29**, 1943–1949 (2010).
55. Muijs, B. & Jonker, M. T. O. Assessing the bioavailability of complex petroleum hydrocarbon mixtures in sediments. *Environ. Sci. Technol.* **45**, 3554–3561 (2011).
56. Fernandez, L. A. *Polyethylene Passive Samplers for Measuring Hydrophobic Organic Chemical Concentrations in Sediment Porewaters and their Use in Predicting Bioaccumulation in Soft-Shell Clams (*Mya arenaria*) from Sites Near Boston, MA*. PhD thesis, MIT (2010).
57. Perron, M. M., Burgess, R. M., Suuberg, E. M., Cantwell, M. G. & Pennell, K. G. Performance of passive samplers for monitoring estuarine water column concentrations: 1. Contaminants of concern. *Environ. Toxicol. Chem.* **32**, 2182–2189 (2013).
58. Oen, A. M. P. et al. In situ measurement of PCB pore water concentration profiles in activated carbon-amended sediment using passive samplers. *Environ. Sci. Technol.* **45**, 4053–4059 (2011).
59. Sanders, J. P., Andrade, N. A. & Ghosh, U. Evaluation of passive sampling polymers and nonequilibrium adjustment methods in a multiyear surveillance of sediment porewater PCBs. *Environ. Toxicol. Chem.* **37**, 2487–2495 (2018).
60. Nelson, D. W. & Sommers, L. E. *Total Organic Carbon and Organic Matter. 2nd edn, 539–579* (ASA and SSSA, 1982).
61. Karickhoff, S. W., Brown, D. S. & Scott, T. A. Sorption of hydrophobic pollutants on natural sediments. *Water Res* **13**, 241–248 (1979).
62. Endo, S., Grathwohl, P., Haderlein, S. B. & Schmidt, T. C. LFERs for soil organic carbon - water distribution coefficients (K OC) at environmentally relevant sorbate concentrations. *Environ. Sci. Technol.* **43**, 3094–3100 (2009).
63. Luthy, R. G. et al. Sequestration of hydrophobic organic contaminants by geosorbents. *Environ. Sci. Technol.* **31**, 3341–3347 (1997).
64. Alexander, M. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ. Sci. Technol.* **34**, 4259–4265 (2000).
65. Cornelissen, G. et al. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* **39**, 6881–6895 (2005).
66. Smedes, F. & Booij, K. Guidelines for Passive Sampling of Hydrophobic Contaminants in Water Using Silicone Rubber Samplers. Report No. 52, ICES Techniques in Marine Environmental Sciences (International Council for the Exploration of the Sea, 2012).
67. Jalalizadeh, M. & Ghosh, U. Analysis of measurement errors in passive sampling of porewater PCB concentrations under static and periodically vibrated conditions. *Environ. Sci. Technol.* **51**, 7018–7027 (2017).
68. Joyce, A. S. & Burgess, R. M. Using performance reference compounds to compare mass transfer calibration methodologies in passive samplers deployed in the water column. *Environ. Toxicol. Chem.* **37**, 2089–2097 (2018).
69. Lampert, D. J., Thomas, C. & Reible, D. D. Internal and external transport significance for predicting contaminant uptake rates in passive samplers. *Chemosphere* **119**, 910–916 (2015).
70. Jonker, M. T. O., Van Der Heijden, S. A., Kotte, M. & Smedes, F. Quantifying the effects of temperature and salinity on partitioning of hydrophobic organic chemicals to silicone rubber passive samplers. *Environ. Sci. Technol.* **49**, 6791–6799 (2015).
71. Jonker, M. T. O., Van Der Heijden, S. A., Kreitinger, J. P. & Hawthorne, S. B. Predicting PAH bioaccumulation and toxicity in earthworms exposed to manufactured gas plant soils with solid-phase microextraction. *Environ. Sci. Technol.* **41**, 7472–7478 (2007).

72. Lambert, M. K., Friedman, C., Luey, P. & Lohmann, R. Role of black carbon in the sorption of polychlorinated dibenzo-p-dioxins and dibenzofurans at the diamond alkali Superfund site, Newark Bay, New Jersey. *Environ. Sci. Technol.* **45**, 4331–4338 (2011).
73. Witt, G. et al. Passive equilibrium sampler for in situ measurements of freely dissolved concentrations of hydrophobic organic chemicals in sediments. *Environ. Sci. Technol.* **47**, 7830–7839 (2013).
74. Choi, Y., Wu, Y., Luthy, R. G. & Kang, S. Non-equilibrium passive sampling of hydrophobic organic contaminants in sediment pore-water: PCB exchange kinetics. *J. Hazard. Mater.* **318**, 579–586 (2016).
75. Booij, K., Sleiderink, H. M. & Smedes, F. Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. *Environ. Toxicol. Chem.* **17**, 1236–1245 (1998).
76. Choi, Y., Cho, Y. M. & Luthy, R. G. Polyethylene-water partitioning coefficients for parent- and alkylated-polycyclic aromatic hydrocarbons and polychlorinated biphenyls. *Environ. Sci. Technol.* **47**, 6943–6950 (2013).
77. Reitsma, P. J., Adelman, D. & Lohmann, R. Challenges of using polyethylene passive samplers to determine dissolved concentrations of parent and alkylated PAHs under cold and saline conditions. *Environ. Sci. Technol.* **47**, 10429–10437 (2013).
78. Smedes, F., Geertsma, R. W., Van Der Zande, T. & Booij, K. Polymer-water partition coefficients of hydrophobic compounds for passive sampling: application of cosolvent models for validation. *Environ. Sci. Tech* **43**, 7047–7054 (2009).
79. Smedes, F. SSP silicone–, lipid– and SPMD–water partition coefficients of seventy hydrophobic organic contaminants and evaluation of the water concentration calculator for SPMD. *Chemosphere* **223**, 748–757 (2019).
80. Booij, K., Smedes, F. & Allan, I. J. Guidelines for determining polymer-water and polymer-polymer partition coefficients of organic compounds. Report No. 61, ICES. Techniques in Marine Environmental Sciences (International Council for the Exploration of the Sea, 2017).
81. Fernandez, L. A., Harvey, C. F. & Gschwend, P. M. Using performance reference compounds in polyethylene passive samplers to deduce sediment porewater concentrations for numerous target chemicals. *Environ. Sci. Technol.* **43**, 8888–8894 (2009).
82. Shen, X. & Reible, D. An analytical model for the fate and transport of performance reference compounds and target compounds around cylindrical passive samplers. *Chemosphere* **232**, 489–495 (2019).
83. Apell, J. N. & Gschwend, P. M. Validating the use of performance reference compounds in passive samplers to assess porewater concentrations in sediment beds. *Environ. Sci. Technol.* **48**, 10301–10307 (2014).
84. Thompson, J. M., Hsieh, C. H. & Luthy, R. G. Modeling uptake of hydrophobic organic contaminants into polyethylene passive samplers. *Environ. Sci. Technol.* **49**, 2270–2277 (2015).
85. Booij, K., Hoedemaker, J. R. & Bakker, J. F. Dissolved PCBs, PAHs, and HCB in pore waters and overlying waters of contaminated harbor sediments. *Environ. Sci. Technol.* **37**, 4213–4220 (2003).
86. Rusina, T. P., Smedes, F., Koblizkova, M. & Klanova, J. Calibration of silicone rubber passive samplers: experimental and modeled relations between sampling rate and compound properties. *Environ. Sci. Technol.* **44**, 362–367 (2010).
87. Smedes, F. Silicone–water partition coefficients determined by cosolvent method for chlorinated pesticides, musks, organo phosphates, phthalates and more. *Chemosphere* **210**, 662–671 (2018).
88. Booij, K., Smedes, F. & Van Weerlee, E. M. Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers. *Chemosphere* **46**, 1157–1161 (2002).

## Acknowledgements

M.T.O.J. acknowledges financial support from the European Chemical Industry Council's Long-range Research Initiative program (Cefic-LRI), under contracts ECO22 and ECO43. F.S. acknowledges support by the Czech Ministry of Education, Youth, and Sports (LM2018121) and the European Structural and Investment Funds, Operational Program Research, Development, and Education (CZ.02.1.01/0.0/0.0/16_013/0001761). R.L. acknowledges support from SERDP ER-2538. This publication represents U.S. Environmental Protection Agency ORD-033094.

## 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-tune several conceptual and methodological aspects. R.L. drafted the 'PRC' section in the 'Experimental design' section. R.M.B., U.G., P.M.G., S.E.H., M.J.L., K.A.M., and D.R. contributed to improving the manuscript by providing comments and edits.

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41596-020-0311-y.

**Correspondence and requests for materials** should be addressed to M.T.O.J.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 22 September 2019; Accepted: 10 February 2020;
Published online: 20 April 2020

**Related links**
**Key reference using this protocol**
Jonker, M. T. O. et al. *Environ. Sci. Technol.* **52**, 3574–3582 (2018): https://doi.org/10.1021/acs.est.7b05752

nature research

Corresponding author(s): M.T.O. Jonker

Last updated by author(s): Jan 17, 2020

# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|---|---|
| ☒ | ☐ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒ | ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☒ | ☐ The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☒ | ☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | No data were collected during this study. |
| Data analysis | No data were analysed during this study. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

No datasets were generated or analysed during the current study

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Study description | This study concerns the writing of a standard protocol. No actual samples were investigated and no data were analysed. |
| Research sample | No actual samples were investigated during this study. |
| Sampling strategy | No actual samples were taken during this study; hence, there was no sampling strategy. |
| Data collection | No data were collected during this study. |
| Timing and spatial scale | No data were collected during this study. |
| Data exclusions | No data were collected during this study.; hence, no data were excluded |
| Reproducibility | No data were collected during this study; hence, reproducibility was not tested. |
| Randomization | No actual samples were investigated in this study; hence, allocation into groups is not applicable. |
| Blinding | No data acquisition was performed during this study; hence, blinding is not applicable. |

Did the study involve field work? ☐ Yes ☒ No

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |