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Assessing Bioavailability of DDT and Metabolites in Marine Sediments using Solid-Phase Microextraction with Performance Reference Compounds

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Abstract

Solid-phase microextraction (SPME) has often been used to estimate the freely dissolved concentration (C_{free}) of organic contaminants in sediments. A significant limitation in the application of SPME for C_{free} measurement is the requirement for attaining equilibrium partition, which is often difficult for strongly hydrophobic compounds such as DDT. A method was developed using SPME with stable isotope-labeled analogues as performance reference compounds (PRCs) to measure C_{free} of DDT and metabolites (DDTs) in marine sediments. Six ^{13}C -labeled or deuterated PRCs were impregnated into polydimethylsiloxane (PDMS) fiber before use. Desorption of PRCs from PDMS fibers and absorption of DDTs from sediment were isotropic in a range of sediments evaluated ex situ under well-mixed conditions. When applied to a historically contaminated marine sediment from a Superfund site, the PRC-SPME method yielded C_{free} values identical to those found by using a conventional equilibrium SPME approach (Eq-SPME), whereas the time for mixing was reduced from 9 d to only 9 h. The PRC-SPME method was further evaluated against bioaccumulation of DDTs by *Neanthes arenaceodentata* in the contaminated sediment with or without amendment of activated carbon or sand. Strong correlations were consistently found between the derived equilibrium concentrations on the fiber and lipid-normalized tissue residues for DDTs in the worms. Results from the present study clearly demonstrated the feasibility of coupling PRCs with SPME sampling to greatly shorten sampling time, thus affording much improved flexibility in the use of SPME for bioavailability evaluation. *Environ Toxicol Chem* 2013;32:1946–1953.

Keywords

Solid-phase microextraction (SPME); Bioavailability; Performance reference compound; Passive sampling; DDT

Introduction

Many hydrophobic organic chemicals (HOCs), such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, and DDT, have high affinity for sediment particles and are preferentially deposited into the bed sediment [1,2]. The freely dissolved concentration (C_{free}) of HOCs is increasingly regarded as a more relevant measurement over the bulk

concentration for predicting exposure to contaminated sediments [3]. The bioavailability of HOCs indicated in C_{free} is also an important parameter for evaluating the effectiveness of sediment remediation practices (e.g., activated carbon treatment) and for determining the endpoint of remediation treatments [4,5]. Consequently, a number of investigations have been conducted in recent years to develop and validate methods for C_{free} determination.

Passive samplers for detecting C_{free} that operate on the principle of passive equilibrium sampling [6–9] include the use of a sorbent, such as solid-phase microextraction (SPME) fiber [7,8,10–12], polyethylene membrane [6,13,14], or poly-oxymethylene film [9,15], among others. The SPME fiber has received widespread applications because of its flexibility and compatibility for both in situ and ex situ deployment [16–20]. In SPME sampling, a segment of fiber coated with a polymer (e.g., polydimethylsiloxane [PDMS]) is placed in the sediment, and HOCs partition into the nonpolar polymer under the chemical activity gradient until an equilibrium is reached [21]. At equilibrium, C_{free} in the sediment may be derived from the concentration on the fiber (C_f^e) through the use of a fiber–water partition coefficient (K_{FW})

$$C_{\text{free}} = \frac{C_f^e}{K_{\text{FW}}} \quad (1)$$

An essential requirement for equilibrium passive sampling is the attainment of equilibrium between the sampler and the sampled matrix [7]. For strongly hydrophobic compounds, however, a long time (often on the scale of weeks or months) may be needed, which greatly diminishes the practical value of these samplers [7,8]. One approach to circumvent this requirement, as shown for polyethylene devices [13,22], is to preload the passive sampler with a performance reference compound (PRC). In such applications, the desorption rate constant (k_{des}) of the preloaded PRC is used to approximate the absorption rate constant (k_{abs}) of the target analyte, a condition that should be readily met if the PRC is an isotope-labeled analogue. The value of k_{des} may be easily determined from the dosed amount of PRC (q_0) and the amount remaining on the sampler after time $t(q)$

$$q = q_0 \times e^{-k_{\text{des}} t} \quad (2)$$

The derived k_{des} may then be used to predict the analyte concentration in the sampler at equilibrium (C_f^e) [23]

$$C_f^e = \frac{C_{f(t)}}{1 - e^{-k_{\text{abs}} t}} \quad (3)$$

where $C_{f(t)}$ is the concentration of the target analyte in the sampler at time t . The PRC calibration allows a passive sampler to be used before equilibrium is reached, thus enabling the use of short or flexible sampling time. The calibration method has been successfully used on polyethylene devices to derive C_{free} [13,24,25] and also on SPME for in-fiber standardization in analysis [26]. However, so far it has yet to be integrated with SPME for measuring C_{free} .

The objective of the present study was to develop and validate the PRC-SPME method for measuring C_{free} of HOCs in sediment. The legacy chlorinated insecticide DDT and its metabolites (DDTs) were used as the model HOCs. The PRC-SPME method was compared

with the conventional equilibrium method (Eq-SPME) under mixing conditions and further evaluated against bioaccumulation of DDTs by the marine polychaete *Neanthes arenaceodentata* in a historically contaminated marine sediment.

Materials and Methods

Chemicals and SPME fiber

The following chemicals were purchased from AccuStandard: 1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)ethane (*p,p'*-DDT), 1,1,1-trichloro-2,4-bis-(*o*-chlorophenyl)ethane (*o,p'*-DDT), 1,1-dichloro-2,2-bis-(chlorophenyl)ethane (*p,p'*-DDD), 1,1-dichloro-2,2-bis-(chlorophenyl)ethylene (*p,p'*-DDE), 1,1-dichloro-2, 4-bis-(chlorophenyl)ethylene (*o,p'*-DDE), 1,1-dichloro-2,4-bis-(chlorophenyl)ethane (*o,p'*-DDD), surrogate standards PCB-67 and PCB-191, and internal standards PCB-30 and PCB-82. The PRCs were composed of ^{13}C -labeled (^{13}C -*o,p'*-DDD and ^{13}C -*o,p'*-DDE) and deuterated compounds (*p,p'*-DDT- d_8 , *o,p'*-DDT- d_8 , *p,p'*-DDD- d_8 , and *p,p'*-DDE- d_8), purchased from Cambridge Isotope Laboratories or C/D/N Isotopes. Solvents and other chemicals were of analytical grade. Deionized water with electrical resistivity of $18.2 \text{ M}\Omega \cdot \text{cm}$ was prepared using a Barnstead E-pure system.

Thin fibers with a 430- μm glass core and 35- μm PDMS coating (Polymicro Technologies) were precleaned with ethyl acetate via Soxhlet extraction for 72 h. The cleaned fiber was cut into 1-cm-long pieces before use.

Sediments

Three marine sediments (0–15 cm) were collected from different locations and used in the present study. To further expand the range of sediment characteristics, some of these sediments were also amended with activated carbon or sand at different ratios. One of the sediments was taken from the ocean floor along the Palos Verdes Shelf (a Superfund site) off the coast of Los Angeles, California, USA, with known contamination of DDT and PCBs [27]; this sediment is termed PV8C in the present study. Two relatively clean sediments were collected from Newport Bay, California, USA (33.65°N, 117.87°W; NB), and New Fields in Port Gamble, Washington, USA (NF), and were used for preparation of spiked sediments. All collected sediments were sieved through a 2-mm mesh and stored at 4 °C before use.

The procedure used for sediment spiking followed the US Environmental Protection Agency's guidelines [28]. Briefly, 10 g sand was uniformly spread on the bottom of 1.5-L glass jar and spiked with 1.0 mL of acetone solution containing a mixture of DDT compounds. The spiked sand was allowed to dry in a fume hood, followed by the addition of 1.0 kg (dry wt equivalent) of wet sediment. The glass jar was sealed and rotated at 80 rpm for 1 wk at room temperature. To create activated carbon- or sand-amended sediments, aliquots of the spiked sediment or PV8C sediment were mixed with activated carbon (Calgon Carbon; <0.15 mm) or sand (<0.15 mm) at a rate of 1, 2, or 5% (w/w). The amended sediment samples were mixed for another week to achieve uniformity. The organic carbon contents of all sediments were determined on a Flash EA 1112 series N/C Analyzer (Thermo Electron) and are shown in the Supplemental Data, Table S1.

Measurement of fiber-seawater partition coefficient (K_{FW})

The K_{FW} values of DDTs were essential in deriving C_{free} from the equilibrium fiber concentration C_f^e . A procedure similar to that of Yang et al. was used to estimate K_{FW} [29]. Briefly, a 1-cm PDMS fiber was attached to a cotton thread and suspended in 110 mL high-purity water containing 0.27 $\mu\text{g/L}$ DDTs, 35‰ NaCl, and 0.2% NaN_3 in a 125-mL glass

vial. The containers were continuously agitated on a stirring plate at 700 rpm (10.9 g force) at room temperature. Triplicate samples were removed after 3,6,9,12,19, and 25 d of mixing, and the fiber and artificial salt water were separately analyzed for the levels of DDTs.

Each fiber was transferred into a 350-μL glass insert (housed in a 2-mL gas chromatography vial) containing 200 μL hexane and sonicated in a water bath (FS110H; Fisher Scientific) for 20 min. Before instrumental analysis, PCB-30 and PCB-82 (in 2 μL hexane) were added to each sample as internal standards. The saltwater sample was spiked with surrogate standards (PCB-67 and PCB-191) and then transferred to a glass separatory funnel and extracted 3 times, each time with 20 mL of dichloromethane. The solvent extracts were combined, dried with anhydrous sodium sulfate, and concentrated to 1 to 2 mL on a vacuumed rotary evaporator at 40 °C. The concentrated extracts were redissolved in hexane and reduced to 0.5 mL under nitrogen. Internal standards (PCB-30 and PCB-82) in 5 μL hexane were added to each sample prior to instrumental analysis.

Fiber preloading and isotropy validation experiments

To preload PDMS fibers with PRCs, 38 clean fibers of 1 cm were placed in a 125-mL glass jar with a 50-mL acetone-water (1:4, v/v) solution containing all PRCs (20 μg/L for each compound) and shaken at 80 rpm for 24 h. The preloaded fibers were rinsed with deionized water before use. A subset of 5 preloaded fibers was randomly selected to determine the exact impregnated PRC amounts.

The first step in method development was to validate isotropic exchange between the isotope-labeled PRCs on the fiber and their nonlabeled counterparts (target analytes) in the sediment matrix, with the purpose of testing whether k_{des} equals k_{abs} . If k_{abs} equals k_{des} , the following relationship holds [26]

$$\frac{q}{q_0} + \frac{C_{f(t)}}{C_f^e} = 1 \quad (4)$$

The NB sediment, amended with activated carbon or sand at 0, 1, 2, or 5% (w/w), was used for the 4 DDT metabolites, and native NF sediment was used for *o,p'*-DDT and *p,p'*-DDT. Preliminary experiments showed that *o,p'*-DDT and *p,p'*-DDT were unstable in the NB sediment. Briefly, a preloaded fiber, an aliquot of 2.0 g (dry wt) spiked sediment, and 1.0 mL NaN_3 (2 mg/L) were placed in a 20-mL glass vial. All sample vials were sealed and mixed at 80 rpm on a horizontal shaker. After 3, 7, 12, 24, 36, 48, 72, 96, 144, 216, or 288 h of mixing, triplicate vials were sacrificed and the fibers were retrieved from the sediment slurry, rinsed with deionized water, gently wiped with a sheet of tissue paper to remove particles from the fiber, and subjected to solvent extraction as described in the section *Measurement of fiber-seawater partition coefficient*.

Bioaccumulation experiment

The developed PRC-SPME method was validated by comparison against the Eq-SPME method as well as tissue concentrations (C_{bio}) of DDTs in a marine polychaete exposed to the PV8C sediment. To expand the range of sediment characteristics and to alter bioavailability, activated carbon or sand was mixed with the PV8C sediment at 0.5, 2, or 5% (w/w). The overall protocol for the bioaccumulation test was similar to that of Lotofu et al. [30]. Briefly, *N. arenaceodentata* worms (2–3 wk old; Aquatic Toxic Support) were acclimated in 32‰ artificial seawater for 1 wk with feeding of dry algae (once) before exposure at 20 °C. For exposure, 10 worms were transferred to a 1-L beaker containing 20 g (dry wt) sediment and 350 mL 32‰ artificial seawater. The exposure experiment was

maintained at 20 °C. The overlying water was aerated via continuous bubbling, and the water level was maintained by periodically adding artificial seawater. At the end of a 4-d exposure, the worms were harvested by sieving the sediment slurry through a 100-mesh sieve. The relatively short exposure time was chosen through preliminary experiments to avoid potential toxicity effects to the test organism. The worms were allowed to stay in clean artificial seawater for 48 h for depuration, after which they were stored at –20 °C until chemical analysis.

Simultaneous to the bioaccumulation test, subsamples of PV8C sediments were equilibrated with PRC-preloaded fibers for 9 h, or clean PDMS fibers for 9 d (for Eq-SPME), under mixing conditions. At the end of mixing, the fibers were retrieved and analyzed as described above, from which C_f^e was directly measured for Eq-SPME, or calculated using Equation 4 for the PRC-SPME measurement. The C_f^e values were then used in Equation 1 to obtain C_{free} values.

Sediment and tissue extraction and analysis

Sediment samples (2 g dry wt) were mixed with 20 g anhydrous sodium sulfate in 250-mL glass centrifugation bottles, and the mixture was sonicated in 70 mL acetone–dichloromethane (1:1, v/v) for 30 min in a water bath. Before extraction, the recovery surrogate standards (PCB-67 and PCB-191) in 10 µL acetone were added to each sediment sample. The sediment mixture was centrifuged at 500 rpm (69.8 g force) for 15 min, after which the supernatant was collected and filtered through a sodium sulfate–filled funnel into a 250-mL glass flask. The same extraction step was repeated 2 additional times. The extracts were combined and then condensed to 1 to 2 mL on a vacuumed rotary evaporator at 40 °C. The concentrated extracts were redissolved in hexane and reduced to 1.0 mL. Extract purification was performed by eluting the sample extract through a solid-phase cartridge packed with 1 cm acidic silica, and the sample was eluted with 20 mL hexane–dichloromethane (1:1, v/v). The effluent was concentrated to 0.5 mL under nitrogen and spiked with the internal standards (PCB-30 and PCB-82 in 10 µL) prior to instrumental analysis.

Worm tissues were freeze-dried at –45 °C, and the dry mass was recorded. The sample was sonicated in 40 mL acetone–dichloromethane (1:1, v/v) for 30 min and the same extraction was repeated 3 times. The combined extract was concentrated, and one-fifth of the extract was removed for analysis of lipid content. The remaining sample was cleaned up as described above. Preliminary experiments showed that the recoveries of DDT compounds were 88 to 136% for sediment samples and 94 to 141% for tissue samples.

Analysis of DDT compounds was carried out on an Agilent 6890N gas chromatography system coupled with a 5973 mass selective detector, using electron ionization in the selective ion scanning mode. The samples (2 µL) were injected at 200 °C in the splitless mode. A DB-5 capillary column (60 mm × 0.25 mm inner diameter, 0.25 µm film thickness) was used for separation. The oven temperature was programmed from 80 °C (held for 1 min) to 210 °C at a rate of 10 °C/min and then ramped to 300 °C at 5 °C/min (held for 8 min). The temperatures of the mass spectrometry source, quadrupole mass spectrometry and AUX-2 were 230, 150, and 300 °C, respectively. The column flow of carrier gas (helium) was 1.0 mL/min. Under these conditions, the decomposition rate was less than 10% for both *o,p'*-DDT and *p,p'*-DDT.

Quality assurance and quality control

Laboratory blanks for fiber, saltwater, sediment, and tissue samples were included throughout the experiments. No target analyte was detected in the blank samples. The

recoveries of surrogate standards (PCB-67 and PCB-191) were $109 \pm 11\%$ and $93 \pm 9\%$ in salt water, $97 \pm 27\%$ and $90 \pm 17\%$ in sediment, and $86 \pm 3\%$ and $88 \pm 3\%$ in tissue samples, respectively. During instrumental analysis, a standard was injected after the analysis of every 10 samples for calibration of a standard curve.

Results and Discussion

PRC preloading and isotropy validation

The amounts of isotope-labeled DDT compounds on the fiber were analyzed from 9 fibers after the preloading procedure, and the relative standard deviation was found to be smaller than 4% (Supplemental Data, Table S2). Analysis of subsets of preloaded fibers throughout the study showed that the relative standard deviation always remained at $<5.8\%$ for all DDT compounds, except for *p,p'*-DDT-d₈, for which it was $<11\%$ (Supplemental Data, Table S2). These observations suggested that the simple preloading method was capable of producing accurate and reproducible PRC-impregnated fibers.

The isotropy between the analytes in sediment and their corresponding isotope-labeled analogues (as PRCs) on the fiber was tested in native and activated carbon- or sand-amended NB or NF sediments under mixing conditions. Absorption kinetics of DDT compounds and desorption kinetics of their corresponding PRCs were found to be symmetrical, as shown in Figure 1 for all DDT compounds in the native NB sediment (DDT metabolites) and NF sediment (*o,p'*-DDT and *p,p'*-DDT). The sum of ratios for absorption of nonlabeled DDTs (n/n_e) and those for desorption of the labeled PRCs (q/q_0) statistically equaled 1.0 throughout the mixing duration in all sediments (Figure 1). The k_{abs} (h^{-1}) and k_{des} (h^{-1}) were derived through regression analysis of the kinetic curves, with $r^2 = 0.92$ and $p < 0.01$ for all treatments (Supplemental Data, Table S3). No significant difference ($p > 0.05$) was found between k_{abs} and k_{des} for the same compound (Table 1 and Supplemental Data, Table S3). These results clearly suggested that absorption of nonlabeled DDTs from the sediment to the PDMS fiber was isotropic with desorption of the isotope-labeled DDT analogues (as PRCs) from the fiber into the sediment, validating the use of PRCs in SPME sampling.

The desorbed fraction of PRCs varied with the specific DDT compounds as well as the sediment type (Supplemental Data, Figures S1 and S2). In general, within 9 h of mixing, 21 to 90% of desorption occurred for different PRC compounds in all sediments. In the present study, 9 h was selected as the time duration for subsequent applications of the PRC-SPME method. As suggested for polyethylene film-based samplers, a desorption fraction of 20 to 80% of PRCs was desirable, as desorption less than 20% may result in increased analytical uncertainties (Equation 4) [31], whereas desorption greater than 80% may lead to diminished analytical sensitivity. For Eq-SPME, as evident from Figure 1, a much longer time was needed to attain the equilibrium state for the different DDT compounds. For example, an apparent equilibrium was not reached until 4 d of mixing for *o,p'*-DDE and *p,p'*-DDE in NB sediment. For *o,p'*-DDE and *p,p'*-DDE, the time to reach an apparent equilibrium state was 6 d. In the present study, 9 d was therefore selected for the evaluation of Eq-SPME for the purpose of method validation.

In a previous study using the polyethylene film sampler [31], 28 d of equilibration was selected for a range of PCB congeners under mixing (resuspension) conditions to coincide with the duration used for organism exposure. Desorption of all PRCs exceeded 15%, and a good linear relationship ($r^2 = 0.877$) was observed between the estimated equilibrium concentrations of PCBs in the PE strips and lipid-normalized tissue concentration in *Nereis virens* [31]. In Fernandez et al. [12], the deployment time for PE samplers was determined to be the time after which the desorbed fraction of PRCs was greater than the given

measurement uncertainty (20%) in analysis. When a mixture of compounds is targeted for analysis, the minimum sampling time would depend on the PRC compound that desorbs the slowest. In the present study, 9 h was chosen as the sampling time when the desorbed fraction of ^{13}C -*o,p'*-DDE and *p,p'*-DDE-d₈ from the fiber were close to 20% (Supplemental Data, Table S4). It should be noted that the sampling time may be influenced by other conditions such as mixing speed and polymer coating thickness. A shorter sampling time may be achieved with a higher mixing speed or thinner polymer coating. Conversely, longer sampling time may be necessary under static conditions.

Comparison between PRC-SPME and Eq-SPME

The K_{FW} values were determined for individual DDT compounds. As shown in Supplemental Data, Figure S3, all 6 DDT compounds appeared to have reached an apparent equilibrium after 6 d of mixing. Therefore, data from all sampling points after 6 d were used to calculate K_{FW} values of DDTs (Table 2). The K_{FW} values were generally similar to those reported by Zeng et al. for 100- μm -thick PDMS fiber [32], except for *o,p'*-DDD, *p,p'*-DDD, and *p,p'*-DDT. The K_{FW} values of *o,p'*-DDD, *p,p'*-DDD, and *p,p'*-DDT were 0.4 log units to 0.5 log units smaller than those in Zeng et al. [32]. The differences may be attributed to the source or dimension of the PDMS fibers used in the different studies. Yang et al. [33] also observed that the partition coefficient of ^{14}C -labeled *p,p'*-DDE (5.29 ± 006) between the 35- μm -thick PDMS fiber (same as in the present study) and freshwater was smaller than that between the Supelco 30- or 100- μm PDMS fiber and freshwater (6.66 ± 0.11 or 6.12) found in other studies [8,34].

The performance of the PRC-SPME method was first compared against the conventional Eq-SPME method in the bioaccumulation experiment. The C_{free} values were derived by dividing the measured (Eq-SPME) or calculated (PRC-SPME) fiber concentration at equilibrium C_f^e by K_{FW} . The C_{free} values of DDTs derived from the 2 methods were analyzed statistically using an independent sample *t* test. All *p* values were greater than 0.05, except for *p,p'*-DDD in the PV8C sediment (*p* = 0.041). Therefore, in general, C_{free} values of the same DDT compound obtained with the 2 methods were statistically identical in all sediments (Table 3). For example, in the native PV8C sediment, the C_{free} of *p,p'*-DDE was 57 ± 8.1 ng/L for the Eq-SPME method, whereas it was 71 ± 16 ng/L for the PRC-SPME measurement. Likewise, the C_{free} values for *o,p'*-DDE, *o,p'*-DDD, and *p,p'*-DDD were 16 ± 1.5 ng/L, 8.7 ± 0.16 ng/L, and 108 ± 21 ng/L when measured using Eq-SPME, and the corresponding values derived from PRC-SPME were 31 ± 11 ng/L, 8.8 ± 0.68 ng/L, and 70 ± 6.0 ng/L. These results clearly indicated that the PRC-SPME method was similarly accurate and reproducible in predicting C_{free} compared with the conventional Eq-SPME method.

Correlation between porewater concentration and short-term uptake in *N. arenaceodentata*

To evaluate the performance of the developed PRC-SPME method in estimating the bioavailability of HOCs in sediment, the marine polychaete *N. arenaceodentata* was exposed to PV8C sediments in a 4-d exposure test. The observed tissue residue (C_{bio}) was correlated with C_{free} derived from either PRC-SPME or Eq-SPME. The marine polychaete *N. arenaceodentata* was known to be present at the Palos Verdes Shelf Superfund site through routine surveys and is considered a critical component of the food web for native fish species such as the flatfish California halibut (*Paralichthys californicus*) [35]. In the PV8C sediment, all 6 DDT compounds were detected, with the levels of *p,p'*-DDE and *p,p'*-DDD being extremely high, ranging up to 19 and 16 $\mu\text{g/g}$ (dry wt; Supplemental Data, Table S5), respectively. The levels of DDTs in the PV8C sediment were comparable to those measured yearly by the Sanitation District of Los Angeles County (unpublished data). The source of DDT contamination at the Palos Verdes Shelf site was the result of many decades

of discharge of municipal wastewater and DDT-containing wastewater at the ocean outfall [27]. It is estimated that a total of 40 km² of the ocean floor, with a sediment layer from 5 to 60 cm in thickness, still contains DDTs and PCBs, with the location where the PV8C sample was taken as the hot spot [36].

Accumulation of DDTs into *N. arenaceodentata* was observed after the 4-d exposure. Figure 2 shows the correlations between lipid content-normalized C_{bio} of *p,p'*-DDE and *p,p'*-DDD, and the equilibrium fiber concentration C_f^e independently derived by PRC-SPME or Eq-SPME. Strong significant linear relationships are seen between C_f^e and C_{bio} for both *p,p'*-DDE ($r^2 = 0.84$) and *p,p'*-DDD ($r^2 = 0.83$ and 0.80) for both SPME methods. The slopes of the linearized relationships were statistically identical between the PRC-SPME method and the Eq-SPME method for *p,p'*-DDE (1.31 ± 0.23 vs 1.03 ± 0.18) and *p,p'*-DDD (1.51 ± 0.27 vs 1.17 ± 0.23). These results demonstrated that the PRC-SPME method was a suitable alternative to Eq-SPME. In addition, both methods equally predicted the decreasing trend in bioaccumulation of *p,p'*-DDE and *p,p'*-DDD by *N. arenaceodentata* with increasing activated carbon amendment rates in the PV8C sediment. The inhibitory effect of carbonaceous materials such as activated carbon on the bioaccumulation of HOCs has been frequently observed and is the basis for the use of activated carbon in sediment remediation [4,5]. For example, Tomaszewski et al. observed 83% reduction in the aqueous concentration of DDTs in sediment 1 mo after amendment of activated carbon at 3.2% [37]. In the present study, bioaccumulation of DDTs in *N. arenaceodentata* decreased by 88% with 5% activated carbon amendment. In contrast, amending the sediment with sand up to 5% had a negligible effect on bioaccumulation. The inhibition of the activated carbon amendment on bioaccumulation or C_{free} of DDTs was attributable to the changes in the overall sediment organic carbon content (Supplemental Data, Table S1) and also to the fact that activated carbon was an exceptionally strong adsorbent for DDTs. In contrast, addition of sand up to 5% did not result in any notable changes in C_{free} . As shown in Figure 2, addition of 5% sand (organic carbon content 3.06%) did not cause a significant change in the overall sediment organic carbon content compared with the native PV8C sediment (organic carbon content 3.08%). It must be noted that capping of a contaminated sediment bed with sand or gravel can provide a physical barrier under field conditions, allowing natural attenuation to take place while limiting exposure to some organisms [38].

Conclusions

Results from the present study showed that PDMS fiber may be coupled with the use of PRCs to circumvent the requirement for equilibrium that usually requires a long time to attain. As shown for DDT compounds in marine sediments, the use of isotope-labeled analogues such as PRCs shortened the sampling time from 9 d to only 9 h under mixing conditions. The C_{free} estimated by the PRC-SPME method was consistently similar to that of the conventional SPME method in a range of sediments. Furthermore, C_{free} values of DDT and metabolites were closely correlated with their residues in a marine polychaete exposed to the same sediment conditions. The short and flexible sampling time is expected to greatly improve the feasibility of SPME sampling for various scenarios. For field deployment of SPME samplers, shorter or flexible sampler placement time would increase the applicability while offering more instantaneous measurements reflecting real-time contaminant distribution. The PRC-SPME method may be used in assessing changes in bioavailability of sediment-borne HOCs during remediation operations. Although not tested in the present study, the same approach should be equally applicable for other HOCs or other sediment types. In contrast, if SPME samplers are imbedded in the sediment statically, such as under field conditions, more time is likely needed to achieve substantial desorption

(e.g., >20%) of the preloaded PRCs. Although this time is expected to be significantly shorter than that for Eq-SPME, research is needed to quantify this time duration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Absorption kinetics of DDT and its metabolites and desorption kinetics of corresponding isotope-labeled analogs (as performance reference compounds) on the polydimethylsiloxane (PDMS) fiber in unamended Newport Bay (**a–d**) or New Fields sediment under mixing conditions (**e–f**). q_0 = initial amount of performance reference compounds (PRC) on the fiber; q = amount of PRC remaining on the fiber at time t ; n and n_e = amounts of target compound on the fiber at time t and at equilibrium; Sum = $q/q_0 + n/n_e$; p,p' -DDT = 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane; o,p' -DDT = 1,1,1-trichloro-2,4-bis-(*o*-chlorophenyl)ethane; p,p' -DDD = 1,1-dichloro-2,2-bis-(chlorophenyl)ethane; p,p' -DDE =

1,1-dichloro-2,2-bis-(chlorophenyl)ethylene; *o,p'*-DDE = 1,1-dichloro-2,4-bis-(chlorophenyl)ethylene; *o,p'*-DDD = 1,1-dichloro-2,4-bis-(chlorophenyl)ethane.

Figure 2. Correlations between the equilibrium concentrations (C_e) of 1,1-dichloro-2,2-bis-(chlorophenyl)ethylene (p,p' -DDE; **a,b**) and 1-dichloro-2,2-bis-(chlorophenyl)ethane (p,p' -DDD; **c,d**) on the disposable fiber estimated by performance reference compound-calibrated solid-phase microextraction (PRC-SPME) and from SPME with the equilibrium method (Eq-SPME) and tissue concentrations (C_{bio}) in the *Neanthes arenaceodentata* exposed to Palos Verdes Shelf (PV8C) sediment amended without and with 0.5, 2, and 5% sand (S) or activated carbon (AC) for 4 d. The sampling times of PRC-SPME and Eq-SPME were 9 h and 9 d, respectively.

Table 1
Rate constants (h^{-1}) of absorption (k_{abs}) and desorption (k_{des}) of DDTs and performance reference compounds (PRCs) on polydimethylsiloxane (PDMS) fiber in marine sediments under the mixing conditions^a

Compound	k_{abs}	PRC	k_{des}	p^d
<i>o,p'</i> -DDD ^b	0.29 ± 0.08 (n = 5)	¹³ C- <i>o,p'</i> -DDD	0.27 ± 0.06 (n = 6)	0.49
<i>o,p'</i> -DDE ^b	0.058 ± 0.01 (n = 5)	¹³ C- <i>o,p'</i> -DDE	0.053 ± 0.009 (n = 6)	0.18
<i>p,p'</i> -DDD ^b	0.32 ± 0.06 (n = 5)	<i>p,p'</i> -DDD-d ₈	0.28 ± 0.03 (n = 6)	0.40
<i>p,p'</i> -DDE ^b	0.048 ± 0.01 (n = 5)	<i>p,p'</i> -DDE-d ₈	0.049 ± 0.006 (n = 6)	0.96
<i>o,p'</i> -DDT ^c	0.034 ± 0.001 (n = 1)	<i>o,p'</i> -DDT-d ₈	0.036 ± 0.003 (n = 1)	
<i>p,p'</i> -DDT ^c	0.048 ± 0.003 (n = 1)	<i>p,p'</i> -DDT-d ₈	0.055 ± 0.004 (n = 1)	

^aThe data are shown as mean $\pm$ standard deviation; n = number of sediments. See Figure 1 caption for definition of compound abbreviations.

^bMeasurements in amended sediments from Newport Bay, California, USA.

^cMeasurements in unamended sediments from New Fields in Port Gamble, Washington, USA.

^dPaired samples *t* test were performed between the k_{abs} s and k_{des} s of *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD and their corresponding PRCs with SPSS 13.0.

Table 2
Partition coefficient (K_{FW}) of DDT and its metabolites between the 35- μm disposable polydimethylsiloxane (PDMS) fiber and 35‰ seawater

Compound ^a	$\log K_{OW}$ ^b	$\log K_{FW}$ ^c	$\log K_{FW}$
		35 μm	100 μm
<i>o,p'</i> -DDD	6.22	4.96 ± 0.04	$5.36^d, 5.20^d$
<i>o,p'</i> -DDE	6.96	5.75 ± 0.03	$5.96^d, 5.66^d$
<i>p,p'</i> -DDD	6.22	4.82 ± 0.03	5.20 ± 0.03^e
<i>p,p'</i> -DDE	6.96	5.86 ± 0.03	5.74 ± 0.07^e
<i>o,p'</i> -DDT	6.91	5.47 ± 0.05	$5.71^d, 5.69^d$
<i>p,p'</i> -DDT	6.91	5.12 ± 0.10	5.63 ± 0.10^e

^aData were acquired from De Bruijn et al. [39].

^bData were acquired from De Bruijn et al. [39].

^cThe values were obtained from the present study.

^dThe values were obtained from Difilippo et al. [40].

^eData were obtained from Zeng et al.[32].

Data were obtained from Zeng et al.[32].

Table 3
Comparison of the freely dissolved concentrations (ng/L) of DDTs from Eq-SPME and PRC-SPME in PV8C sediments (amended without and with 0.5, 2, and 5% sand or activated carbon

	<i>o,p'</i> -DDD			<i>o,p'</i> -DDE			<i>p,p'</i> -DDD			<i>p,p'</i> -DDE		
	Eq-SPME	PRC-SPME		Eq-SPME	PRC-SPME		Eq-SPME	PRC-SPME		Eq-SPME	PRC-SPME	
PV8C	8.7 ± 0.16	8.8 ± 0.68		16 ± 1.5	31 ± 11		108 ± 21	70 ± 6.0		57 ± 8.1	71 ± 16	
PV8C + 0.5% sand	9.6 ± 0.26	8.7 ± 0.04		17 ± 0.63	25 ± 3.3		74 ± 22	71 ± 2.6		58 ± 5.1	69 ± 11	
PV8C + 2% sand	9.0 ± 0.38	8.3 ± 0.37		17 ± 0.11	24 ± 2.6		65 ± 20	63 ± 3.3		61 ± 0.87	70 ± 8.3	
PV8C + 5% sand	8.0 ± 0.06	8.6 ± 0.73		16 ± 0.09	22 ± 0.42		65 ± 11	72 ± 1.9		58 ± 0.30	58 ± 0.14	
PV8C + 0.5% AC	6.4 ± 0.21	5.2 ± 0.35		13 ± 0.46	16 ± 5.6		45 ± 7.8	38 ± 2.1		49 ± 1.7	51 ± 20	
PV8C + 2% AC	1.5 ± 0.08	1.2 ± 0.41		4.5 ± 0.01	3.5 ± 0.1		23 ± 12	7.1 ± 0.85		18 ± 0.85	13 ± 0.32	
PV8C + 5% AC	0.27 ± 0.07	0.2 ± 0.02		0.93 ± 0.09	0.74 ± 0.11		3.0 ± 0.95	1.4 ± 0.02		4.2 ± 0.28	2.9 ± 0.5	

Eq-SPME = solid-phase microextraction with equilibrium method; PRC-SPME = reference compound-calibrated solid-phase microextraction; PV8C = Palos Verdes Shelf, California, USA; *o,p'*-DDD = 1,1-dichloro-2,4-bis-(chlorophenyl)ethane; *o,p'*-DDE = 1,1-dichloro-2,4-bis-(chlorophenyl)ethylene; *p,p'*-DDD = 1,1-dichloro-2,2-bis-(chlorophenyl)ethane; *p,p'*-DDE = 1,1-dichloro-2,2-bis-(chlorophenyl)ethylene; AC = active carbon.