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A stable isotope dilution method for measuring bioavailability of organic contaminants

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Abstract

Methods for determining bioavailability of organic contaminants suffer various operational limitations. We explored the use of stable isotope labeled references in developing an isotope dilution method (IDM) to measure the exchangeable pool (E) of pyrene and bifenthrin as an approximation of their bioavailability in sediments. The exchange of deuterated bifenthrin or pyrene with its native counterpart was completed within 48 h. The derived E was 38–82% for pyrene and 28–59% for bifenthrin. Regression between E and the sum of rapid and slow desorption fractions obtained from sequential desorption showed a slope close to 1.0. The ability of IDM to predict bioavailability was further shown from a strong relationship ($r^2 > 0.93$) between E and bioaccumulation into *Chironomus tentans*. Given the abundance of stable isotope labeled references and their relatively easy analysis, the IDM has the potential to become a readily adoptable tool for estimating organic contaminants bioaccessibility in various matrices.

Keywords

Isotope dilution method; Bioavailability; Pyrene; Bifenthrin

1. Introduction

Bioavailability of hydrophobic organic contaminants (HOCs) in environmental matrices (e.g., sediments, soils, water) has been a subject of many studies over the last two decades. Conceptually, in environmental matrices such as sediment or soil, a chemical must be in the freely dissolved form to be bioavailable, because only free molecules can cross a cell membrane and cause a biological response (Ehlers and Luthy, 2003; Semple et al., 2004). This is true even if the sediment or soil is ingested, as desorption (into gut fluid) must still precede biouptake (Weston and Maruya, 2002; Lu et al., 2003). As an organism moves through or ingests sediment or soil, it is immediately exposed to the freely dissolved concentration (C_{free}). As biouptake continues, C_{free} is depleted and then replenished via desorption of HOCs from the reversibly sorbed pool. Therefore, bioavailability is reflected in both C_{free} and what is available via desorption, with the latter often termed bioaccessibility (Ehlers and Luthy, 2003; Semple et al., 2004; Reichenberg and Mayer,

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Appendix A. Supplementary data: Additional details on sediment extraction protocol, Tenax desorption kinetics and relationship between E values and F_{rap} and $F_{\text{rap}} + F_{\text{slow}}$ of pyrene and bifenthrin are available in the online version at <http://dx.doi.org/10.1016/j.envpol.2013.01.036>.

2006). The C_{free} of HOCs in a sediment or soil can be determined using passive samplers such as solid phase microextraction (SPME) (Mayer et al., 2000; Bondarenko and Gan, 2009). However, there is ample ambiguity in the measurement of bioaccessibility (Reichenberg and Mayer, 2006). Bioaccessibility is usually measured using partial extraction methods with moderately polar solvents (Kelsey et al., 1997) or sequential desorption with sorbents (e.g., Tenax, cyclodextrin) (Reid et al., 2000; Cornelissen et al., 2001). The measured bioaccessibility invariably depends on the extraction or desorption conditions, e.g., solvent type, agitation speed, solid-to-solution ratio and desorption duration (Reichenberg and Mayer, 2006). Therefore, current methods for measuring bioaccessibility suffer operational limitations and can rarely be used across different chemicals or matrices (Chung and Alexander, 1998). An ideal method for bioaccessibility determination should be able to accurately and reproducibly quantify the reversibly sorbed fraction of HOCs.

In this study, we extended the isotopic dilution theory to the use of stable isotope labeled reference compounds to determine the bioavailability of HOCs in solid matrices. The isotope dilution method (IDM) has been well developed using radioactive isotopes, especially for heavy metals in understanding their distribution between the labile and fixed pools (Hamon et al., 2002b; Lombi et al., 2003; Donner et al., 2010). The use of isotope dilution on organic contaminants, however, has not been attempted, with a few exceptions where radioactive ^{14}C -labeling was used to characterize sorption of moderately hydrophobic compounds (e.g., pesticides) in soils (Celis and Koskinen, 1999a,b) sediments (Williams and Kookana, 2010) or HOC in organic matter (Sander and Pignatello, 2005). To date IDM has yet to be used for predicting environmental bioavailability of organic contaminants. The novel use of stable isotope labeling takes advantages of the increasing abundance of deuterium, ^{13}C or ^{18}O labeled compounds, as well as the availability of mass spectrometry (MS) detectors in most laboratories. Unlike radioactive isotope labeling, stable isotope labeled compounds are less expensive and safer to use, adding to the overall applicability.

1.1. Principles of the isotope dilution method (IDM)

The IDM has been developed from the use of radioactive isotopes for heavy metals (Hamon et al, 2002a,b, 2008; Lombi et al., 2003; Donner et al, 2010). A chemical within a solid (e.g., sediment, soil)–water system is distributed among different phases (Fig. 1) (Williams and Kookana, 2010), and the phase distribution may be expressed as (Hamon et al, 2002a):

$$C_{\text{total}} = (D \times C_w) + C_e + C_n \quad (1)$$

where C_{total} ($\mu\text{g g}^{-1}$) is the total concentration in the binary system, C_w ($\mu\text{g L}^{-1}$) is the concentration in the solution phase, C_e ($\mu\text{g g}^{-1}$) is the exchangeable portion of the sorbed concentration, C_n ($\mu\text{g g}^{-1}$) is the non-exchangeable portion, and D is the dilution factor that equals to the ratio of the solution volume to the solid mass. The sum of $(D \times C_w)$ and C_e is defined as the exchangeable pool E . The principle of IDM is based on the premise that, when a small amount of an isotopic analogue is introduced into a sediment-water (or soil–water) system, it will exchange isotropically with the native HOC in the exchangeable fractions (Celis and Koskinen, 1999a; Hamon et al, 2002a):

$$C_w^*/C_e^* = C_w/C_e \quad (2)$$

Where C_w^* ($\mu\text{g L}^{-1}$) is the concentration of the labeled analogue in the solution and C_e^* ($\mu\text{g g}^{-1}$) is the exchangeable portion of the labeled analogue. The total concentration of the labeled analogue introduced into the system, C^* ($\mu\text{g g}^{-1}$), is given by:

$$C^* = (D \times C_w^*) + C_e^* \quad (3)$$

Combining Eqs. (2) and (3), C_e may be written as:

$$C_e = (C_w / C_w^*) \times (C^* - C_w^* \times D) \quad (4)$$

Based on Eq. (4), E may be written as (Hamon et al., 2002a):

$$E = (D \times C_w) + C_e = (C_w / C_w^*) \times C^* \quad (5)$$

Therefore, after phase separation (e.g., centrifugation, filtration), analysis of the solution phase using GC-MS or LC-MS would give both C_w and C_w^* , which, when coupled with the predetermined spiking concentration of the labeled analogue C^* , will allow the estimation of E . The principle of IDM is based on the assumption that the added isotope labeled analogue behaves the same as its non-labeled counterpart in the sample, a condition that should be easily met if the isotope labeled analogue is the same compound as the analyte of interest.

2. Material and methods

2.1. Chemicals and sediments

Two compounds, bifenthrin as a representative compound from the pyrethroid insecticide family and pyrene, a member of polycyclic aromatic hydrocarbons (PAHs), were selected as the model HOCs in this method development study. Non-labeled standards of bifenthrin (99.0% purity) and pyrene (99.0% purity) were purchased from Chem. Service (West Chester, PA). Deuterated bifenthrin (99% purity) was purchased from Toronto Research Chemicals (North York, Ontario, Canada) and deuterated pyrene (98.9% purity) was supplied by C/D/N Isotopes (Pointe-Claire, Quebec, Canada). 4,5,9,10- ^{14}C -Pyrene (95% radiochemical purity; 50 mCi mmol $^{-1}$) from Sigma-Aldrich (St Louis, MO) and phenyl-ring- ^{14}C bifenthrin (95% radiochemical purity; 61 mCi mmol $^{-1}$) supplied by FMC Agricultural Products (Philadelphia, PA) were used in the bioaccumulation test. These chemicals were dissolved in acetone separately as stock solutions. All other solvents and chemicals used were of GC grade.

Five freshwater sediments were used: Jordan Lake Reservoir sediment (JL, Chatham County, NC), Greasy Creek sediment (GC, Benton County, OR), San Diego Creek sediment (SD, Irvine, CA), Leaf Lake sediment (LL, El Dorado County, CA) and Glen Charlie Pond sediment (GP, Wareham, MA). These sediments were selected based on their differing OC contents (0.08, 0.33, 0.92, 1.24 and 4.13% OC, increasing in the order of JL, GC, SD, LL and GP) and wide geographic locations. The sediments were wet sieved through a 2-mm mesh and stored at 5 °C before use. The background levels of bifenthrin (0–3 ng g $^{-1}$) and pyrene (0–13 ng g $^{-1}$) in the sediments were far below the spiked concentration (1.0 µg g $^{-1}$).

2.2. Method development experiments

The method development included separate experiments to determine the time to reach an isotope dilution steady state, the effect of dosing rates of labeled compounds, and the potential interference of the co-presence of dissolved organic matter (DOM). In the kinetics experiment, sediments were first spiked with bifenthrin and pyrene at a nominal concentration of 1 µg g $^{-1}$ (dry weight) by adding the chemicals in 0.5 mL of acetone to a small amount of silica sand and then mixing with 300 g (dry weight) of sediment in a 1.9-L wide-mouth glass jar. Water was then added to adjust the water-to-sediment ratio to about

1:1 (w/w). The spiked sediments were mixed at 15 rpm on a shaker at room temperature for 7 d to achieve a homogeneous distribution of the chemicals in the sediments. After this time the sediment was allowed to settle down and the overlying water was removed. To check if the spiked chemicals were homogeneously distributed in the sediment, aliquots (2 g dry weight) of sediments were taken and extracted as described in Delgado-Moreno et al. (2010) to derive the total sediment concentration C_{total} . Recoveries for the sediment extraction method were 87–110% and 99–111% for pyrene and bifenthrin, respectively (six replicates were used for the recovery test). The relative standard deviations of C_{total} from replicates and among sediments were 0.1–4.6% for pyrene and 1.7–4.3% for bifenthrin, suggesting uniform distribution of these chemicals in the spiked sediments.

Aliquots (2 g, dry weight) of the homogenized sediments were placed in glass centrifuge tubes and 20 mL sodium azide (200 mg L⁻¹) solution in water was added to suppress microbial activity. The samples were equilibrated by mixing on the shaker for 24 h, following which 10 µL of a solution containing the deuterated standards at 2 mg L⁻¹ was introduced. The amount of deuterated standards accounted for 1% of the total bifenthrin or pyrene amount. The samples were then returned to the shaker and mixed at 15 rpm. After different time intervals, i.e., 0.5, 1, 3, 6, 9, 16, 24, 48, 96 and 144 h, triplicate samples were removed and centrifuged at 670 g for 25 min. To determine C_w and C_w^* , the supernatant (18 mL) was extracted with 10 mL of hexane, followed by freezing the mixture in a conventional freezer (–18 °C) for 2 h to recover the hexane phase. The hexane extract was concentrated to 1.0 mL under nitrogen. Recoveries for the extraction method were 103 ± 10% and 114 ± 7% for pyrene and bifenthrin, respectively. For each time interval, E value was calculated using Eq. (5).

In the second experiment, the dosing rate of the isotope labeled standards was varied from 1 to 20% of that for the non-labeled HOCs, and E was measured after 48 h of mixing. Only JL and SD sediments were used, and the other conditions were kept the same except for the added amount of the deuterated standards.

Previous studies showed that centrifugation does not completely eliminate DOM from the supernatant, and that HOCs may sorb significantly to DOM in the aqueous phase (Delgado-Moreno et al., 2010; Wang et al., 2011). From Eq. (5), if a fraction of HOCs sorbed to DOM is not exchangeable, the inclusion of DOM in the analysis of C_w by solvent extraction may lead to biases in E measurement. A previously developed solid phase microextraction (SPME) method (Bondarenko and Gan, 2009) capable of selectively detecting C_{free} in the presence of DOM was used to evaluate this potential artifact. The supernatant samples from centrifugation were simultaneously analyzed using the above hexane extraction method and SPME. The SPME sampling and analysis were carried out using a 7-µm polydimethylsiloxane (PDMS)-coated fiber (Supelco, Bellefonte, PA) mounted on a Combi-Pal automated sampler (CTC Analytics, Zwingen, Switzerland). The sampling duration was 40 min, and after sample immersion, the fiber was injected into the GC inlet and analyzed under the same chromatographic conditions as for the liquid samples.

2.3. Method validation experiments

Multiple lines of evidence were used to demonstrate the capability of the IDM method for bioavailability prediction. Two method validation experiments were carried out. The IDM method was first compared to sequential Tenax extraction that has been frequently used for estimating bioaccessibility of HOCs (Cornelissen et al., 2001, 1997; Cui et al., 2010). Aliquots (2 g, dry weight) of the above sediments were simultaneously subjected to sequential desorption using a published Tenax extraction method (Cui et al., 2010). The sediment sample in 50 mL Teflon centrifuge tube was mixed with 20 mL sodium azide

solution (200 mg L⁻¹) and 0.05 g Tenax beads (Sigma–Aldrich, St. Louise, MO) on a horizontal shaker at 100 rpm at room temperature for 1 h, after which the Tenax beads were separated from the sediment phase by centrifugation at 1900 g for 20 min and filtration. Tenax beads retained on the filter paper were rinsed with deionized water. The same sediment sample was subjected to the same desorption step sequentially, using clean Tenax at each step. The incremental desorption intervals were 1, 2, 6, 10, 24, 498, 96, 144, 192, 240, and 312 h. Tenax beads from each desorption interval were extracted with 5 mL acetone:hexane (1:1, v/v) by sonication and an aliquot of the final extract was analyzed by GC–MS.

The desorption kinetics up to 312 h were used to construct the desorption curve by fitting the data to a triphasic model (Cornelissen et al., 1997):

$$S_t/S_0 = F_{\text{rap}} \left(\exp^{-k_{\text{rap}} \times t} \right) + F_s \left(\exp^{-k_s \times t} \right) + F_{\text{vs}} \left(\exp^{-k_{\text{vs}} \times t} \right) \quad (6)$$

where S_t and S_0 are the HOC concentration in the sediment at time t and time zero (before desorption started), respectively. The F_{rap} , F_s and F_{vs} are the HOC fractions in the rapid, slow and very slow desorption pools, respectively, and k_{rap} , k_s and k_{vs} are the corresponding desorption constant.

The freshwater invertebrate *Chironomus tentans* was used for bioaccumulation assays as a direct measurement of bioavailability. The organisms (Aquatic Bio-systems, Fort Collins, CO) were cultivated in the laboratory for several months before use (Cui et al., 2010). The sediments (60 g, dry weight) were spiked with 1.1×10^4 dpm ¹⁴C-pyrene or 1.8×10^4 dpm ¹⁴C-bifenthrin and with the non-labeled compound following the method described above to get an initial concentration of 1 µg g⁻¹. Water was used to adjust the water-to-sediment ratio to about 1:1 (w/w). The spiked sediments were capped and mixed at 15 rpm on a shaker at room temperature for 7 d to achieve a homogeneous distribution of the chemicals in the sediments. After equilibration, 10 g (dry weight) of the ¹⁴C-spiked sediment were transferred to a 75 mL aluminum foil container (Fisher Scientific, Pittsburgh, PA) and added with 30 mL of reconstituted hard water (Cui et al., 2010). After the sediment was allowed to settle overnight, 10 organisms with an average weight of 1.2 ± 0.3 mg were introduced to each test container. Four replicates were used for each sediment type. The organisms were harvested after 24 h of exposure by filtering the sediment sample through a 1 mm sieve. Previous studies showed that the uptake and elimination of phenanthrene or permethrin by *C. tentans* reached a steady state within 24 h (Cui et al., 2010; Hunter et al., 2008). Upon harvest, *C. tentans* were rinsed in deionized water and placed in new vessels containing clean sand and hard water for gut cleaning for 24 h. The gut-cleaned *C. tentans* were then placed on a piece of pre-weighed paper tissue to air dry for 24 h, and the dried samples were weighed again to obtain the dry mass of the exposed organisms. About 0.05 g (dry weight) of the sediment was simultaneously transferred onto an aluminum weighing dish and air dried. The dry *C. tentans* and sediment samples were combusted at 900 °C for 2 and 4 min, respectively, on an R.J. Harvey OX-500 Biological Oxidizer (R.J. Harvey, Hillsdale, NJ) and the evolved ¹⁴CO₂ was trapped in 15 mL of Carbon-14 cocktail (R.J. Harvey), followed by measurement of the radioactivity on a Beckman LS 5000TD liquid scintillation counter (LSC) (Fullerton, CA) to derive the body residue (BR , ng g⁻¹ dry weight) in *C. tentans* and sediment concentration C_s .

2.4. Chemical analysis

Analysis of non-labelled and stable isotope labelled pyrene and bifenthrin was carried out on a Varian 3800 gas chromatography (GC) (Varian Instruments, Sunnyvale, CA) coupled with

a Varian 1200 triple-quadrupole mass spectrometer (MS/MS). Separation was achieved on a Factor Four-5 MS (Varian) capillary column (30 m $\times$ 0.25 mm i.d.) with 5% diphenyl-95% dimethylsiloxane liquid phase (0.25 μ m film thickness). A 1.0- μ L aliquot of the final sample was injected at 260 $^{\circ}$ C in the splitless mode at a constant flow of 1 mL min⁻¹. Helium (99.999%) was used as the carrier gas in the pressure-pulse mode (45 psi for 0.8 min). The oven temperature was programmed to start at 80 $^{\circ}$ C, increase at 20 $^{\circ}$ C min⁻¹ to 300 $^{\circ}$ C and hold for 7 min. The MS/MS electron ionization source was 70 eV (EI), and the transfer line, manifold, and ionization source temperatures were 300, 40, and 170 $^{\circ}$ C, respectively. Argon (99.9999%) was used as the collision gas, with a resolution of quadrupoles equal to 1.2 and 2 for Q1 and Q3, respectively. Precursor ions for pyrene, pyrene-d₁₀, bifenthrin and bifenthrin-d₁₀ were 202, 212, 181 and 186, respectively. Product ions were 199, 182, 165 and 170 for pyrene, pyrene-d₁₀, bifenthrin and bifenthrin-d₁₀, respectively. The collision energy was 40 V for pyrene and pyrene-d₁₀ and 20 V for bifenthrin and bifenthrin-d₁₀.

2.5. Statistical analysis

STATGRAPHICS Centurion XVI, version 16.1.11 (Statistical Graphics, Princeton, NJ) was used for the one-way ANOVA analysis and for comparison of regression lines.

3. Results and discussion

3.1. Isotope exchange kinetics

The exchange of a labeled reference with the pre-existing non-labeled compound was expected to progress and reach a steady state once the exchange with the exchangeable fractions is completed (Celis and Koskinen, 1999a; Sander and Pignatello, 2005). Thus, it is important to know the time needed for the exchange to reach the steady state. As shown in Fig. 2, the derived *E* values for pyrene or bifenthrin increased with mixing time initially, but reached a plateau after 48 h. The increase was relatively rapid, between 30 min and 24 h. Although still increasing, the exchange between 24 and 48 h was drastically slower (Fig. 2). No significant differences in *E* values were observed for time intervals longer than 48 h and those at 48 h (*P* values were 0.11–0.95 for pyrene and 0.26–0.66 for bifenthrin). Thus, 48 h appeared to be adequate for the completion of isotope exchange under the used conditions, and was selected as the mixing time in the subsequent experiments. Studies with metals have shown that after 2–3 d of mixing, the activity of the radioactive element in the solution remains almost constant for weeks (Young et al, 2000; Tongtavee et al, 2005; Oliver et al, 2006). Studies to determine the sorption hysteresis of several organic compounds using ¹⁴C-labeling also showed that isotopic exchange was completed in a relatively short time, e.g., 1 d for pesticides triadimefon and imidacloprid in soil (Celis and Koskinen, 1999a) and 4 d for naphthalene in kerogen organic matter (Sander and Pignatello, 2005). Our results suggest that the use of IDM allows the characterization of the labile pool of HOCs in sediments in a relatively short time frame as compared to other methods for assessing bioaccessibility that may take many days or even weeks (Cornelissen et al., 2001; Maruya et al., 2009).

For the different sediments, fractions of pyrene and bifenthrin in the labile pool, as denoted by *E* measured at the 48-h interval, were 38–82% and 28–59%, respectively (Table 1). Other studies using partial extraction methods to measure bioavailability of HOCs reported similar values for the bioavailable fraction of pyrene and bifenthrin in sediments. For example, You et al. (2009) found that the bioavailable fraction of bifenthrin measured by Tenax extraction was 39.5% and 18.0% for two sediments containing 1.3% and 7.9% of OC, respectively. Reid et al. (2000) reported that the extractable fraction of pyrene using cyclodextrin extraction was 35.4% in a soil containing 3.7% of OC. Cornelissen et al. (2001) found that the rapid desorption fraction of pyrene in three different sediments varied from 31 to 71%. In the same sediment, the labile pool was consistently larger for pyrene than that for

bifenthrin. This compound-specific difference may be attributed to the molecular differences (You et al., 2007) and the relatively lower hydrophobicity of pyrene ($\log K_{ow} = 5.2$ (Chiou et al., 1998)) as compared to that of bifenthrin ($\log K_{ow} = 6.4$ (Laskowski, 2002)). Previous studies have shown that more hydrophobic chemicals tend to have lower molecular diffusion rates and thus slower desorption from sediments (Reid et al., 2000; Chiou et al., 1998; Ruus et al., 2010). For different sediments, E values for pyrene or bifenthrin consistently decreased as the sediment OC content increased, suggesting that the presence of OC in sediment increased the irreversible sorption of HOCs and thus decreased their bio-availability (Chiou et al., 1998; Di Toro et al., 1991). However, the relationship between OC content and E values was not statistically significant for pyrene ($r^2 = 0.60$; $P = 0.13$) or bifenthrin ($r^2 = 0.65$; $P = 0.10$) when all sediments were pooled, suggesting that the quality of OC also exerted influences on the sorption of HOCs.

3.2. Applicability considerations

Presence of DOM in the solution phase may contribute to C_w in Eq. (5) and may thus affect the derived E value. The effect of DOM may be determined by the comparison of E values calculated using C_{free}/C_{free}^* as given by the SPME measurement (E_{SPME}) and C_w/C_w^* as given by the conventional solvent extraction method. If E_{SPME} is smaller than E , a fraction of the chemical sorbed to DOM may be assumed non-exchangeable. Fig. 3 shows the correlation between E_{SPME} and E values for pyrene (Fig. 3A) and bifenthrin (Fig. 3B). For the different sediments studied, the slope of correlation was close to 1 for both chemicals. Statistical analysis showed that the correlation had a slope equal to 1 ($P = 0.73$ and 0.79 for pyrene and bifenthrin, respectively). These results suggest that the presence of DOM in the aqueous phase did not affect the measurement of E . It is important to note that HOCs such as pyrene and bifenthrin partition overwhelmingly to the sediment solid phase (Gan et al., 2008). For example, at equilibrium, only <5% of pyrene or <4% of bifenthrin in the entire sediment-water system was in the aqueous phase. Thus, the influence of the non-exchangeable fraction of DOM-sorbed HOCs, if any, would be negligibly small as compared to the exchangeable pool of the entire system. Nevertheless, the feasibility of using C_w from centrifugation (or filtration) to derive E values makes IDM a simple method readily adoptable in the most laboratories.

Another consideration in the applicability of IDM is the amount of isotope labeled compounds added to a sample. The amount of the labeled compounds added should not be so large to significantly perturb the equilibrium of the compound of interest (Hamon et al., 2008), or so small to hinder accurate measurement. The E values were derived from the same sediment samples with the deuterated standards added at 1–20% of the non-labeled compounds (Fig. 4), and were found statistically equivalent for both pyrene ($P = 0.33$ and 0.23 for SD and JL sediments, respectively) and bifenthrin ($P = 0.10$ and 0.12 for SD and JL sediments, respectively). Thus, varying the amount of deuterated standard added to the sediment suspension did not affect the derived E values. The absence of a significance influence may be attributed to the fact that IDM is based on the measurement of an isotope ratio (C_w/C_w^*), not individual concentrations. Sterckeman et al. (2009) also did not detect a significant spiking effect on the E value of Cd in soils using Cd isotope. The lack of a significant influence implies that IDM may be reliably used for field samples for which the concentration ranges of HOCs are not yet known.

3.3. Bioavailability predictability

Tenax extraction has been used in many studies as a biomimetic tool to estimate bioaccessibility of HOCs in soils or sediments (Cui et al., 2010; Cornelissen et al., 1997; You et al., 2007). The desorption kinetics of pyrene and bifenthrin measured using Tenax were fitted to Eq. (6) to derive F_{rap} , F_s , and F_{vs} (Fig. A.1 and Table A.1). The triphasic

desorption model accurately described the desorption kinetics for all treatments, with $r^2 = 0.98$ ($P < 0.001$). A significant linear relationship ($r^2 = 0.86$, $P < 0.0001$) was observed between the total amount of pyrene or bifenthrin extracted by Tenax (up to 312 h) and the E values (Fig. 5). The slope of the linear relationship was close to 1. It may thus be concluded that the IDM method provided a good measurement of the fraction potentially bio-available via desorption. Many studies showed good correlations between F_{rap} or F_{rap} and F_s with HOC bioaccumulation potentials (You et al., 2007, 2011). The correlation of E values with F_{rap} or the sum of F_{rap} and F_s for pyrene and bifenthrin in all sediments was evaluated (Fig. A.2A). The derived E values of pyrene and bifenthrin were about twice as large as F_{rap} (Fig. A.2A). However, a strong positive relationship was found between E values and the sum of F_{rap} and F_s (Fig. A.2B) and the slope of the linear relationship was close to 1 (Fig. A.2B). These results suggest that the very slow desorption pool as estimated by Tenax extraction did not participate in the exchange with the isotope labeled HOCs, and would likely be unavailable for bioaccumulation by benthic organisms (Tang et al., 1999; Lamoureux and Brownawell, 1999). It must be noted that Tenax-aided sequential desorption is much more time consuming and laborious as compared with the single-step measurement by the IDM method.

The bioaccumulation of ^{14}C -bifenthrin or ^{14}C -pyrene was measured by exposing *C. tentans* to the same sediments, and evaluated against the derived E values. Survival of *C. tentans* was 90–100% in the exposure vessels. A strong linear relationship was found between E values and BR in *C. tentans* for pyrene ($r^2 = 0.93$, $P < 0.0001$) as well as bifenthrin ($r^2 = 0.88$, $P < 0.0001$) on the logarithmic scales (Fig. 6). Regression analysis further showed a close linear correlation between biota sediment accumulation factor ($BSAF$) and E values for both pyrene ($r^2 = 0.93$, $P < 0.001$) and bifenthrin ($r^2 = 0.94$, $P < 0.001$) when the data were pooled for all sediments (data not shown). The correlation was much more improved than that between $BSAF$ and $1/K_d$ for pyrene ($r^2 = 0.42$, $P = 0.01$) or bifenthrin ($r^2 = 0.82$, $P < 0.001$) (Fig. A.3). The close dependence of BR or $BSAF$ on E clearly validated that IDM can be used as a surrogate method for assessing the bioavailability of pyrene and bifenthrin in sediments and that E values may be used to predict the bioaccumulation potential of HOCs. It is worth noting that the sediments in this study were laboratory-spiked and thus underwent the same incubation prior to the measurement of E or BR . It is anticipated that more pronounced improvements over other conventional approaches may be achieved by using IDM if field-contaminated sediments are used, because factors such as aging are known to cause variations in bioavailability.

4. Conclusions

Although only two compounds were examined in this study, the same method should be equally applicable to other HOCs for which isotope labeled references are available. Considering that the measurement of E only involves a simple mixing and centrifugation step, the proposed IDM method should be easily implementable as a simple and inexpensive technique for measuring bioavailability of sediment or soil-borne HOCs. The requirement of only rudimentary devices (i.e., shaker, centrifuge) also allows the handling of multiple samples, increasing sample throughput. It must be noted that many laboratories nowadays are already using stable isotope standards in other contexts (e.g., recovery surrogates, internal standards). Therefore, adoption of IDM may only require simple changes to existing methods or practices. These features together make IDM an ideal tool to be incorporated in the risk assessment of HOC-contaminated sediments or soils. Future studies may explore the various application scenarios for IDM, such as evaluation of remediation effectiveness or endpoints, understanding the influence of contact time (i.e., aging) or organic carbon properties on bioavailability, and testing if IDM may be used across different organism species or on biological matrices (e.g., serum).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Diagram of the isotope exchangeable and non-exchangeable pools of a hydrophobic organic contaminant in sediment (adapted from Williams and Kookana, 2010).

Fig. 2. Effect of mixing time on the isotope exchangeable pool (E) for A) pyrene and B) bifenthrin. The E values are expressed as a percentage of the total chemical concentration in the sediment. Error bars are standard errors of triplicate samples.

Fig. 3. Comparison of isotope exchangeable pool (E) for A) pyrene and B) bifenthrin determined from C_{free}/C_{free}^* measured by SPME (E_{SPME}) and C_w/C_w^* measured by solvent extraction (E). The E values are expressed as a percentage of the total chemical concentration in the sediment. The line represents the 1:1 ratio.

Fig. 4. Isotope exchangeable pool (E) for A) pyrene and B) bifenthrin measured after addition of the deuterated reference at different percentages of the non-labeled chemical for Jordan Lake (JL) and San Diego Creek (SD) sediments. Horizontal line is the average of all measurements and error bars are the standard error of triplicate samples.

Fig. 5. Relationship between E values and total amount of chemical extracted by Tenax expressed as percentage of the amount of chemical in the sediment before desorption.

Fig. 6.

Correlation between isotope exchangeable pool (E , ng g^{-1}) and body residue (BR , ng g^{-1}) of bifenthrin and pyrene in *Chironomus tentans* exposed in contaminated sediments. Solid line represents regression line for pyrene ($\log BR = 3.18 \times \log E - 7.00$; $r^2 = 0.93$, $P < 0.0001$) and dashed line represents regression line for bifenthrin ($\log BR = 2.41 \times \log E - 4.63$; $r^2 = 0.88$, $P < 0.0001$).

Table 1

Isotope exchangeable pool (*E*) of pyrene and bifenthrin in different sediments expressed as a percentage of the total concentration of the chemical in the sediment.

	JL ^a	GC	SD	LL	GP
Pyrene	81.3 ± 2.5	73.4 ± 2.1	50.5 ± 4.6	46.8 ± 2.9	38.0 ± 1.0
Bifenthrin	57.2 ± 0.7	44.9 ± 1.5	41.6 ± 2.2	39.5 ± 1.8	30.4 ± 0.6

^aJL: Jordan Lake sediment; GC: Greasy Creek Pond sediment; SD: San Diego Creek sediment; LL: Leaf Lake sediment; GP: Glen Charlie Pond sediment.