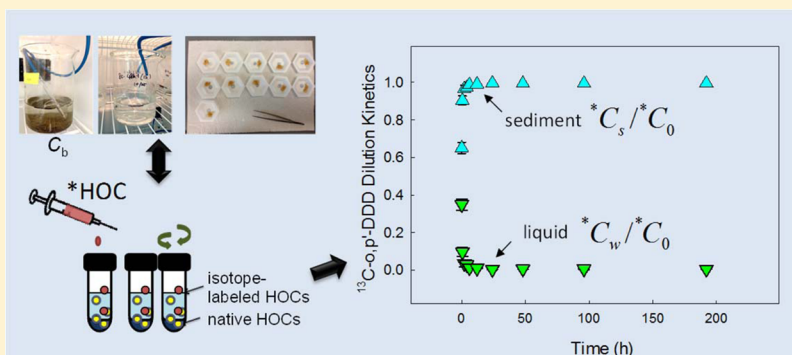


# Use of Isotope Dilution Method To Predict Bioavailability of Organic Pollutants in Historically Contaminated Sediments

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**S** Supporting Information



**ABSTRACT:** Many cases of severe environmental contamination arise from historical episodes, where recalcitrant contaminants have resided in the environment for a prolonged time, leading to potentially decreased bioavailability. Use of bioavailable concentrations over bulk chemical levels improves risk assessment and may play a critical role in determining the need for remediation or assessing the effectiveness of risk mitigation operations. In this study, we applied the principle of isotope dilution to quantify bioaccessibility of legacy contaminants DDT and PCBs in marine sediments from a Superfund site. After addition of  $^{13}\text{C}$  or deuterated analogues to a sediment sample, the isotope dilution reached a steady state within 24 h of mixing. At the steady state, the accessible fraction ( $E$ ) derived by the isotope dilution method (IDM) ranged from 0.28 to 0.89 and was substantially smaller than 1 for most compounds, indicating reduced availability of the extensively aged residues. A strong linear relationship ( $R^2 = 0.86$ ) was found between  $E$  and the sum of rapid ( $F_r$ ) and slow ( $F_s$ ) desorption fractions determined by sequential Tenax desorption. The IDM-derived accessible concentration ( $C_e$ ) was further shown to correlate closely with tissue residue in the marine benthic polychaete *Neanthes arenaceodentata* exposed in the same sediments. As shown in this study, the IDM approach involves only a few simple steps and may be readily adopted in laboratories equipped with mass spectrometers. This novel method is expected to be especially useful for historically contaminated sediments or soils, for which contaminant bioavailability may have changed significantly due to aging and other sequestration processes.

## INTRODUCTION

Because of their high affinity for solid particles, hydrophobic organic contaminants (HOCs) preferentially deposit in soil or sediment in the environment, where they may cause direct ecological effects or become secondary contamination sources. As shown in recent studies, HOCs in the soil or sediment are distributed in heterogeneous regions of organic matter.<sup>1–3</sup> Sequestration of HOCs in the “glassy” region of organic matter may lead to irreversible sorption or reduced bioaccessibility.<sup>1,3,4</sup> Therefore, use of the bulk chemical concentration may not convey the actual risk because the total concentration could substantially overexpress bioavailability.<sup>5</sup> Thus, predicting bioavailability that quantifies the actual amount of HOCs available for bioaccumulation or eliciting toxicity is essential for improving risk assessment.<sup>6</sup>

Changes in bioavailability may be especially pronounced for recalcitrant HOCs, e.g., dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), or polyaromatic

hydrocarbon (PAHs), from historical contamination episodes, because aging and other sequestration processes are known to decrease contaminant bioavailability.<sup>3,7,8</sup> Methods such as mild solvent extraction and sequential desorption have been previously tested for measuring HOC bioaccessibility. In mild extraction, a variety of “soft” solvents (e.g., *n*-butanol, methanol, ethyl acetate) are used to estimate the readily extractable fraction as an approximation of the bioavailable concentration.<sup>9–11</sup> However, in such applications, the measured value invariably depends on the strength of the selected solvent.<sup>6</sup> Sequential desorption employing sorbents such as Tenax and cyclodextrin has also been used in many studies, where the polymeric sorbent traps the desorbed HOC to facilitate

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desorption measurement.<sup>12–14</sup> However, the sequential desorption approach is extremely time-consuming, and laborious.<sup>12</sup> As an alternative, a single-point Tenax desorption has been tested,<sup>12,15</sup> but studies comparing the single- and serial desorption schemes often showed under- or overestimations.<sup>12,16</sup> Therefore, there is a great impetus to identify a simple and readily adoptable method for predicting bioaccessibility of HOCs in soil or sediment.

We recently explored the use of isotope dilution for predicting bioavailability of HOCs and evaluated the method using bifenthrin and pyrene as model contaminants in laboratory spiked freshwater sediments.<sup>17</sup> The principle of isotope exchange was previously used for measuring metal bioavailability.<sup>18–23</sup> However, sorption of metals to soil or sediment is known to differ mechanistically from that of organic molecules, as metal ions are adsorbed to specific charged sites.<sup>24,25</sup> In this study, we extended the isotope dilution method (IDM) to sediments historically contaminated with recalcitrant HOCs. Specifically, we evaluated the validity and performance of IDM for marine sediments from a Superfund site contaminated with DDT and PCBs from many decades ago, and demonstrated the predictability of IDM for contaminant bioavailability in extensively aged matrices.

## MATERIALS AND METHODS

**Chemicals and Sediments.** Seven legacy HOCs, including four DDT derivatives (*o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, and *p,p'*-DDD, denoted as DDXs) and three PCB congeners (PCB 52, PCB 70 and PCB 153), were considered in this study. Standards of PCB 67 and PCB 191 were used as external (recovery) surrogates and PCB 30 and PCB 82 as internal standards. Stable isotope labeled HOCs (denoted as \*HOCs herein) including <sup>13</sup>C-*o,p'*-DDD, <sup>13</sup>C-*o,p'*-DDE, <sup>13</sup>C-PCB 52, and <sup>13</sup>C-PCB 153 and deuterated compounds *p,p'*-DDD-*d*<sub>8</sub>, *p,p'*-DDE-*d*<sub>4</sub>, and PCB 70-*d*<sub>3</sub> were used in the IDM method development and evaluation. Sources and purities of these chemicals may be found in the Supporting Information (SI).

Marine sediments (0–15 cm) historically contaminated with DDT and PCBs were collected at two sites (denoted as PV6C and PV8C) of the Palos Verdes Shelf (PVS) Superfund site off the coast of Los Angeles, CA. Both sediments were sieved through a 2 mm mesh and stored at 4 °C before use. Contamination of the PVS ocean floor was due to many decades of discharge of wastewater by the Los Angeles County Sanitation District and disposal of wastewater containing DDT by the Montrose Chemical Company, once the largest DDT manufacturer in North America, from 1950s to 1970s. As a result, the PVS Superfund site encompasses about 44 km<sup>2</sup> of sediment that is heavily contaminated with DDXs and PCBs. In some areas, levels of DDXs were as high as 200 mg/kg.<sup>26,27</sup> Monitoring showed that DDXs and PCBs at PVS have the potential to accumulate into marine benthic invertebrates and fish, posing human health risks via dietary exposure. The PV8C sediment was collected from the hot spot near the outfalls, whereas the PV6C sediment was relatively less contaminated.<sup>27</sup> Sediment properties (texture and organic carbon content) were analyzed at the University of California Davis Analytical Laboratory using standard methods (Davis, CA).<sup>28</sup> The measured organic carbon (OC) contents of PV6C and PV8C were 2.34% and 3.54%, respectively. The sand, silt, and clay contents were 36%, 51%, and 13% for PV6C and 48%, 39%, and 13% for the PV8C sediment, respectively.

**Characterization of HOC Phase Distribution.** A batch equilibration experiment was carried out to determine the time for each native HOC to reach sorption equilibrium and the apparent sediment–water partition coefficient *K*<sub>d</sub>. Briefly, 2 g of the homogenized PV8C sediment (dry weight basis) was placed in a 50 mL glass centrifuge tube, and 20 mL of 32 ‰ artificial seawater containing 200 mg/L sodium azide was added. The tubes were sealed with aluminum foil lined caps and mixed at 120 rpm on a horizontal shaker. After 0.5, 1, 2, 4, 6, 12, 24, 48, or 96 h of mixing, triplicate samples were removed and centrifuged at 670g for 30 min for phase separation. A similar protocol was used for the PV6C sediment, except that the samples were retrieved after 12, 24, and 48 h of mixing.

After centrifugation, the supernatant was collected and extracted using a liquid–liquid extraction (LLE) method. The details of the LLE method are given in SI. The final extracts were reconstituted in 200 μL of hexane. After addition of internal standards, an aliquot of the final extract was analyzed on a Varian 3800 GC equipped with a Varian 1200 triple quadrupole mass spectrometer (GC–MS/MS) (Varian, Sunnyvale, CA). Details of the instrumental analysis are provided in SI. Sediment samples were freeze-dried and then extracted following a modified EPA method 3620C. The details of sediment extraction and method performance may be found in SI. The final extracts were reconstituted in 1 mL of hexane and analyzed by GC–MS/MS.

**Development of Isotope Dilution Method (IDM).** In the use of IDM for bioaccessibility prediction, the fraction of native HOC reversibly sorbed may be inferred from the sorption of the externally added, isotope-labeled HOC counterpart (\*HOC). The principle of IDM is based on assumptions that \*HOCs added to the sediment distribute themselves just between the aqueous phase and the labile fraction after short-term mixing, and that the addition of \*HOCs does not modify equilibrium of the native HOCs. Under these assumptions, the distribution of native HOCs in sediment may be expressed as

$$C_T = (D \times C_w) + C_s^e + C_s^{ne} \quad (1)$$

where *C*<sub>T</sub> (μg/kg) is the total HOC concentration in sediment, *C*<sub>w</sub> (μg/L) is the HOC concentration in the solution phase, *C*<sub>s</sub><sup>e</sup> (μg/kg) is the accessible concentration of sediment-sorbed HOC, *C*<sub>s</sub><sup>ne</sup> (μg/kg) is the nonaccessible concentration of sediment-sorbed HOCs, and *D* (L/kg) is the dilution factor equaling the ratio of the solution volume to the solid mass. The *C*<sub>T</sub> may be characterized through an exhaustive extraction following the same method as described above. The overall accessible concentration *C*<sub>e</sub> (μg/kg) in sediment is

$$C_e = (D \times C_w) + C_s^e \quad (2)$$

When \*HOC is introduced into the sediment–water binary system, it will readily redistribute itself between the solution and labile phases, which are in dynamic equilibrium<sup>17,29</sup>

$$\frac{*C_w}{*C_s^e} = \frac{C_w}{C_s^e} \quad (3)$$

where \**C*<sub>w</sub> (μg/L) is the concentration of \*HOC in the solution and \**C*<sub>s</sub><sup>e</sup> (μg/kg) is the sorbed concentration of \*HOC. The total concentration of \*HOC in sediment (\**C*<sub>0</sub>, μg/kg) is

$$*C_0 = D \times *C_w + *C_s^e \quad (4)$$

Combining eqs 3 and 4,  $C_s^e$  may be given as

$$C_s^e = \frac{C_w}{*C_w} \times (*C_0 - D \times *C_w) \quad (5)$$

Combining eq 2 and 5, accessible concentration  $C_e$  may be given as

$$C_e = (D \times C_w) + C_s^e = \frac{C_w}{*C_w} \times *C_0 \quad (6)$$

Furthermore, the ratio of  $C_e$  over the total chemical concentration  $C_T$  gives the accessible fraction of the HOC,  $E$ :

$$E = \frac{C_e}{C_T} \quad (7)$$

A similar protocol to the above batch equilibration experiment was followed to evaluate partition kinetics of externally added  $*\text{HOCs}$  and to estimate  $C_e$  and  $E$  from simultaneous measurements of  $C_w$  and  $*C_w$ . Briefly, to a 2 g sediment and 20.0 mL of artificial seawater, 10  $\mu\text{L}$  of a working solution containing each stable isotope-labeled HOC at 5 mg/L was added. The sediment–water slurry was mixed at 120 rpm on a horizontal shaker. Triplicate samples were removed after 0, 0.5, 2, 4, 6, 12, 24, 48, 96, or 192 h for both PV8C and PV6C sediments. Samples were centrifuged at 670g for 30 min for phase separation, and the supernatant was collected. Both the sediment and supernatant were separately analyzed for levels of HOCs following the same methods as described above. Determination of  $*\text{HOCs}$  in both the solution and solid phases allowed construction of isotope dilution kinetics and estimation of the time for isotope tracers to reach dilution equilibrium. When  $*\text{HOCs}$  in the aqueous phase reached a steady state, the measured  $C_w$  and  $*C_w$ , along with  $*C_0$ , were used to calculate  $C_e$  and  $E$  of the native HOCs in the target sediment samples using eqs 6 and 7.

**Tenax-Aided Desorption Experiment.** To validate the performance of IDM, the same sediment samples were subjected to sequential desorption in which Tenax beads were used to recover the desorbed HOCs to facilitate measurement. Tenax-aided desorption has been previously used in a number of studies, and fit of the desorption curve to a three-phase model gives estimates of the relative distribution of sorbed-HOCs in the rapid, slow and very slow desorption pools.<sup>12–14</sup> Briefly, a 2-g aliquot of sediment was placed in a 50 mL polyethylene centrifuge tube. Tenax beads (0.15 g, Scientific Instrument Services, Ringoes, NJ) and 20 mL artificial seawater with 200 mg/L sodium azide were added. The tube was closed and mixed horizontally on a mechanical shaker. For PV6C, after 1, 2, 6, 12, 24, 48, 96, 144, 192, or 312 h of shaking, the sample with Tenax was centrifuged at 670g for 30 min. The Tenax beads were collected by passing the supernatant through a Whatman No. 41 filter paper (Whatman, Maidstone, UK). The trapped beads were rinsed thoroughly with the deionized water and air-dried before they were transferred to 20 mL glass vials and then mixed in 3 mL of acetone-hexane (1:1, v:v), followed by sonication for 5 min by a Fisher Sonic 550 ultrasonic processor. The extraction was repeated three times. The extracts were combined, concentrated under  $\text{N}_2$  to near dryness, and reconstituted in 1 mL of hexane. The desorption step was repeated sequentially for additional increments. A similar protocol was used for PV8C sediment. However, owing to its high levels of DDXs and

PCBs, the sequential desorption was continued up to 837 h to obtain the entire desorption kinetics.

The desorption kinetics up to 312 h for the PV6C sediment and up to 837 h for the PV8C sediment were used to construct the desorption curve and for estimating the rapid ( $F_r$ ), slow ( $F_s$ ), and very slow ( $F_{vs}$ ) desorption fractions by fitting data to a triphasic model<sup>13</sup> using SigmaPlot 11.0 (San Jose, CA)

$$\frac{S_t}{S_0} = F_r e^{-k_r t} + F_s e^{-k_s t} + F_{vs} e^{-k_{vs} t} \quad (8)$$

$$F_r + F_s + F_{vs} = 1 \quad (9)$$

where  $S_t$  and  $S_0$  ( $\mu\text{g/kg}$ ) are concentrations of HOCs in sediment after desorption time interval  $t$  (h) and before the initiation of desorption, respectively, and  $k_r$ ,  $k_s$ , and  $k_{vs}$  are the rate constants ( $\text{h}^{-1}$ ) for the rapid, slow, and very slow desorption fractions, respectively.

**Bioaccumulation Experiment.** The usefulness of IDM was further demonstrated by evaluating the correlation between  $C_e$  and uptake of HOCs into a marine deposit feeder *Neanthes arenaceodentata*. The selected marine polychaete is a predominant benthic invertebrate in the surface and subsurface macrofauna community in the PVS area and is an important food source for the bottom feeding flatfish California halibut (*Paralichthys californicus*).<sup>30</sup> The overall method for the bioaccumulation test was modified from the American Society for Testing and Materials (ASTM) sediment toxicity test with polychaetous annelids.<sup>31</sup> Briefly, 2–3 week old *N. arenaceodentata* worms (Aquatic Toxic Support, Bremerton, WA) were acclimated in aerated artificial seawater at room temperature ( $20 \pm 1$  °C) for 1 week and fed with dry algae. After the acclimation, 15 worms were transferred to a 1 L beaker containing 20 g (dry weight basis) of PV8C or PV6C sediment and 350 mL artificial seawater. The worms were exposed at 20 °C for 4 d with air bubbled into the overlying water. The water level, salinity, and ammonia contents were checked daily and kept constant throughout the exposure. A control group without exposure to the sediment was included. After 4-d of exposure, the worms were retrieved by sieving and depurated in clean artificial seawater (32‰) for 48 h. The analysis of the tissue samples for DDXs and PCBs and lipid content are given in SI.

**Quality Assurance and Quality Control.** Several practices were used to ensure the accuracy and reproducibility of sample analysis. First, the recoveries of target HOCs and surrogate standards spiked in the sediment and worm tissue samples were verified in method development (SI). Second, both external surrogates and internal standards were spiked in all samples to monitor the extraction efficiency and check for instrument drift in sensitivity (SI). In addition, a laboratory blank was included with every batch of nine samples to monitor for potential contamination from laboratory materials and solvents. Furthermore, a standard reference material, SRM 1947 Lake Michigan Fish Tissue, was purchased from the National Institute of Standards and Technology (NIST) and used for validating the analysis of tissue samples of *N. arenaceodentata* in this study. The calibration curve standards were prepared daily and were used only when the regression coefficient was greater than 0.999. The difference between different treatments was determined by one-way analysis of variance (ANOVA) and Tukey test, using SPSS 15.0 (SPSS, Chicago, IL).



## RESULTS AND DISCUSSION

**Phase Partition Kinetics.** The measurement of DDXs and PCBs in the sediment phase ( $C_s$ ) and in the aqueous phase ( $C_w$ ) over time allowed the characterization of phase distribution kinetics of the extensively aged HOCs in the marine sediments. Figures S1 and S2 (SI) display the relative phase distribution of HOCs in the PV8C and PV6C sediments at different time intervals. In the PV8C sediment, an apparent phase equilibrium was reached before 12 h of mixing, and the apparent  $K_d$  remained constant for up to 96 h. One-way ANOVA analysis showed that there was no difference among the distribution values beyond 12 h for PCBs ( $p = 0.163$ – $0.365$ ), DDEs ( $p = 0.227$ – $0.528$ ), or DDDs ( $p = 0.433$ – $0.911$ ). In the PV6C sediment (Figure S2, SI), it appears that *o,p'*-DDD and *p,p'*-DDD reached an apparent equilibrium within 12 h, while all PCBs and DDEs reached a steady state after 24 h. This observation was in agreement with other studies, where phase equilibrium was reached between sediment and liquid phases for DDT and its metabolites within 4 h.<sup>32</sup> Therefore, 24 h was considered as the time duration sufficient for achieving phase distribution equilibrium for the native HOCs in the historically contaminated sediments in this study.

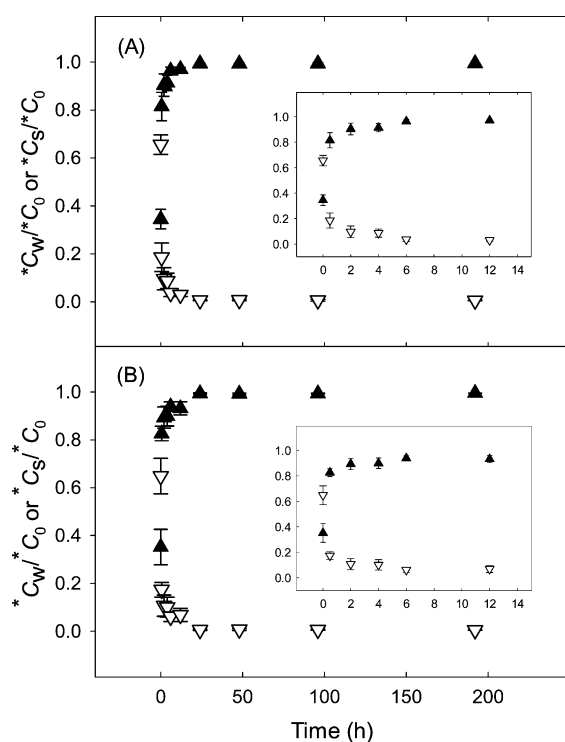
The time necessary for the externally added \*HOCs to reach phase distribution equilibrium was subsequently determined. Upon introduction to the sediment slurry, \*HOCs rapidly partitioned to the sediment phase and an apparent phase distribution equilibrium was reached after a short period of mixing. Figure 1 shows the changes of aqueous ( $*C_w/*C_0$ ) or sediment phase fraction ( $*C_s/*C_0$ ) of <sup>13</sup>C-PCB 52 and *p,p'*-DDE-*d*<sub>4</sub> as a function of mixing time. The kinetics of other \*HOCs may be found in the SI (Figure S3). The strong sorption of \*PCBs and \*DDXs to the sediment resulted in

rapid dilution of the added isotope tracers in the aqueous phase, as reflected from the rapid decreases in  $*C_w/*C_0$  (Figure 1). For example, after 2 h of mixing, 90.2% and 89.1% of the initially added <sup>13</sup>C-PCB 52 and *p,p'*-DDE-*d*<sub>4</sub> were found in the sediment phase for the PV8C sediment or the original concentration in the solution phase was diluted by 10.2 and 9.1 times, respectively. High percentages of dilution were also found for the other isotope-labeled HOCs (7.9–28.9 fold) in the PV8C sediment at 2 h (Figure S3, SI). Fast partitioning of \*HOCs was further observed for the PV6C sediment (Figure S4, SI). Dilution beyond the 2 h interval was much more gradual. From 24 to 192 h, the fractions of \*HOCs in both sediment and aqueous phases were essentially constant, suggesting that phase distribution of the isotope-labeled tracers reached an apparent equilibrium. One-way ANOVA analysis showed no significant change in the fraction of \*HOCs in the sediment or solution phases ( $p = 0.276$ – $0.890$  for \*PCBs,  $p = 0.536$ – $0.624$  for \*DDEs, and  $p = 0.388$ – $0.769$  for \*DDD) for the PV8C sediment between 24 and 192 h (Figures 1 and S3, SI). Similar results ( $p = 0.109$ – $0.878$ ) were also observed for the PV6C sediment (Figure S4, SI). At the steady state, less than 2% of the introduced \*HOCs remained in the aqueous phase for either PV8C or PV6C sediment.

The apparent  $K_d$  values calculated for native HOCs using the derived  $C_s$  and  $C_w$  (Figure S5, SI) showed no detectable influence from the addition of isotope analogues. The  $K_d$  values of each HOC obtained from the previous batch sorption experiment and the ones after the addition of isotope tracers were found to be essentially identical (Tables 1 and S2, SI)

**Table 1. Log  $K_d$  Values of PCBs and DDXs in PV8C Sediment before ( $N = 9$ ) and after ( $N = 9$ ) the Addition of Isotope-Labeled Analogues**

compd	log $K_d$ (before)	log $K_d$ (after)
PCB 52	3.70 ± 0.20	3.69 ± 0.21
PCB 70	3.74 ± 0.19	3.68 ± 0.22
PCB 153	3.50 ± 0.38	3.27 ± 0.18
<i>o,p'</i> -DDE	3.37 ± 0.13	3.66 ± 0.24
<i>p,p'</i> -DDE	3.37 ± 0.18	3.73 ± 0.31
<i>o,p'</i> -DDD	3.54 ± 0.25	3.86 ± 0.34
<i>p,p'</i> -DDD	4.07 ± 0.44	4.21 ± 0.52



**Figure 1.** Fractions of isotope tracers (\*HOC) in the sediment (solid triangle) and liquid phase (open triangle) in the PV8C sediment slurry as a function of mixing time: (A) <sup>13</sup>C-PCB 52; (B) *p,p'*-DDE-*d*<sub>4</sub>.

(paired  $t$  test,  $p = 0.209$ ). This finding suggested that addition of isotope tracers did not alter the phase distribution of the native HOCs, validating one of the prerequisites for IDM. This was expected as the tracers were isotope-labeled counterparts of the native HOCs and should have very similar physicochemical properties.<sup>33</sup>

**Accessibility of HOCs Estimated by Isotope Dilution Method (IDM).** When the isotope dilution reached an apparent equilibrium (e.g.,  $\geq 24$  h), the accessible concentration  $C_e$  was estimated using eq 6 with the derived  $C_w$ ,  $*C_w$ , and  $*C_0$  (predetermined). The  $C_e$  values of PCBs or DDXs were much higher in PV8C than those in PV6C (Table 2), which coincided with the overall higher contamination levels of HOCs at the PV8C location.<sup>27</sup> The overall  $C_e$  of DDXs and PCBs were estimated at 14052 and 223  $\mu\text{g}/\text{kg}$  for the PV8C sediments, respectively, which was consistent with the overall higher levels of DDXs in these sediments than PCBs.<sup>26,27</sup>

The relative accessibility  $E$  was calculated as the ratio of  $C_e$  over  $C_T$  for each HOC in the sediments (Table 2). The use of  $E$  eliminated the effect of different contamination levels, allowing

**Table 2.** Accessible Concentrations ( $C_e$ ,  $\mu\text{g/kg}$ ) and Fractions ( $E$ ) of PCBs and DDXs Determined by the Isotope Dilution Method in the PV8C and PV6C Sediments

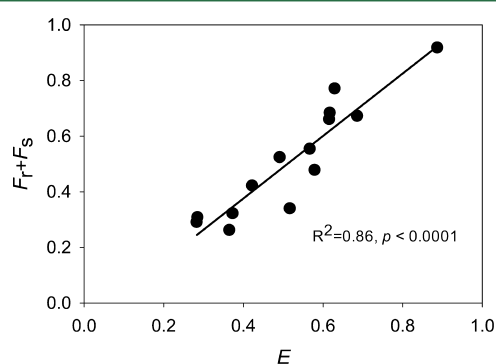
	PV8C		PV6C	
	$C_e$	$E$	$C_e$	$E$
PCB 52	$99.6 \pm 21.2$	$0.49 \pm 0.17$	$9.2 \pm 1.6$	$0.89 \pm 0.12$
PCB 70	$107.1 \pm 18.8$	$0.42 \pm 0.10$	$9.6 \pm 1.9$	$0.62 \pm 0.08$
PCB 153	$15.9 \pm 2.7$	$0.52 \pm 0.07$	$4.8 \pm 1.6$	$0.58 \pm 0.20$
<i>o,p'</i> -DDE	$1250.9 \pm 165.3$	$0.37 \pm 0.11$	$179.5 \pm 57.8$	$0.63 \pm 0.24$
<i>p,p'</i> -DDE	$9168.3 \pm 1380.4$	$0.37 \pm 0.13$	$1082.4 \pm 426.5$	$0.57 \pm 0.20$
<i>o,p'</i> -DDD	$528.6 \pm 153.3$	$0.29 \pm 0.09$	$13.6 \pm 1.6$	$0.62 \pm 0.08$
<i>p,p'</i> -DDD	$3104.3 \pm 1948.5$	$0.28 \pm 0.23$	$78.3 \pm 3.9$	$0.69 \pm 0.03$

more straightforward comparison of contaminant accessibility. The derived  $E$  values were all smaller than 1, with most  $E$  values <0.50 for the HOCs in the PV8C sediment (Table 2), clearly suggesting that a significant fraction of the native HOCs was in regions of sediment that were not accessible by the externally added \*HOCs. The derived  $E$  values of PCBs and DDXs in the PV8C sediment were consistently smaller than those in the PV6C sediment, which may be attributed to the higher OC content in the PV8C sediment (3.54%) as compared to that in the PV6C sediment (2.34%). For example, the averaged  $E$  values of DDEs and DDDs were 0.37 and 0.28, respectively, in the PV8C sediment, which were smaller than those in the PV6C sediment (0.60 for DDEs and 0.65 for DDDs). In the same sediment,  $E$  also varied among the different HOCs. For example, in the PV6C sediment,  $E$  values for PCB 52 (0.89), PCB 70 (0.62), and PCB 153 (0.58) displayed a decreasing trend as the chlorine substitution or  $K_{ow}$  increased.<sup>34</sup> In the PV8C sediment, a similar trend was not observed, and the  $E$  values for the different PCB congeners were statistically the same. This suggests that there were other factors (e.g., aging time) affecting the accessibility of HOCs. Contact time or aging has been often found to diminish the availability of PCBs in sediments.<sup>35–37</sup>

**Validation of IDM by Sequential Tenax Desorption and Bioaccumulation Assays.** The sequential Tenax-aided desorption was applied to the same PV8C and PV6C sediments to evaluate HOC accessibility by fitting the desorption curves (Figure S6, SI) to the triphasic model to derive  $F_r$ ,  $F_s$ , and  $F_{vs}$ . The regression fit to the model was excellent for all sediment-HOC treatments (Table S3, SI). The estimated rapid fraction ( $F_r$ ) was consistently smaller in the PV8C sediment than that in the PV6C sediment for the same compound. For example, the averaged  $F_r$  values were 0.15, 0.08, and 0.10 for PCBs, DDEs, and DDDs in the PV8C sediment, which were smaller than those in the PV6C sediment (0.30, 0.30, and 0.33, respectively). However,  $F_r$  of HOCs was only about a half of  $E$  given by IDM for the same HOC. For example, in the PV8C sediment, the  $E$  and  $F_r$  of PCB 52 were 0.49 and 0.22, respectively. Similar differences were observed in a previous study where spiked sediments (pyrene and bifenthrin) were considered.<sup>17</sup> Studies showed that in addition to  $F_r$ , the slow desorption fraction  $F_s$  also contributed to bioavailability.<sup>38–41</sup> For instance, when the rapid desorption fraction was removed, PAHs in the slow desorption compartment migrated to the rapid desorption pool over time.<sup>41</sup> When benthic deposit feeders were exposed to sediments with the rapid desorption fraction removed, there was still accumulation of PCBs and PAHs.<sup>38</sup> The biodegradation of PAHs by bacteria also exceeded the measured  $F_r$  in sediments.<sup>40</sup> These observations together suggest that bio-

accessibility of sediment-borne HOCs may encompass both  $F_r$  and  $F_s$  as estimated from desorption kinetics.

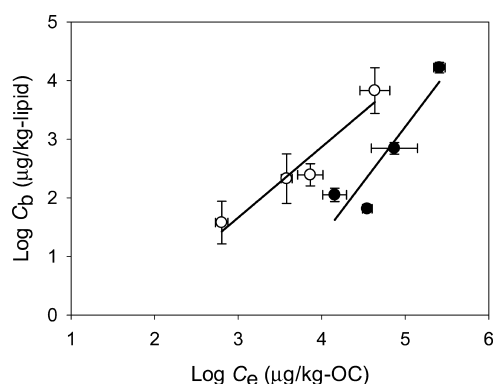
A good linear relationship ( $R^2 = 0.86$ ,  $p < 0.01$ ) was observed between the  $E$  values and the sum of  $F_r$  and  $F_s$  (Figure 2). The

**Figure 2.** Linear correlation between the accessibility ( $E$ ) given by the isotope dilution method and the sum of rapid and slow desorption fractions ( $F_r + F_s$ ) derived by Tenax desorption (slope =  $1.12 \pm 0.13$ ,  $R^2 = 0.86$ ,  $p < 0.0001$ ).

linear slope ( $1.12 \pm 0.13$ ) was close to 1, implying that the accessibility predicted by IDM was close to the sum of  $F_r$  and  $F_s$  estimated from desorption kinetics and likely a better indicator for bioavailability than  $F_r$ . The concentrations of PCB 153 in the PV8C and PV6C sediments were relatively low (26.3 and 11.4  $\mu\text{g/kg}$ ), resulting in a large variance in the estimated  $F_r$ ,  $F_s$ , and  $F_{vs}$  values for this PCB congener. For example,  $F_r$ ,  $F_s$ , and  $F_{vs}$  of PCB 153 in the PV6C sediment were  $0.10 \pm 0.33$ ,  $0.38 \pm 3.42$ , and  $0.52 \pm 3.74$ , respectively. If the data points for PCB 153 were excluded from the correlation, the linear relationship further improved substantially ( $R^2 = 0.93$ ,  $p < 0.01$ ).

The performance of IDM was further evaluated by comparing the measured  $C_e$  of HOCs with 4 d accumulation of HOCs into the marine polychaete *N. arenaceodentata*. A previous study showed that the uptake of HOCs by the polychaete reached a steady state after 4 d of exposure.<sup>42</sup> After 4 d of exposure, tissue residue ( $C_b$ ) of HOCs was analyzed and expressed on both dry weight (dw) and lipid weight (lw) bases. Owing to the relatively low levels of PCBs in the sediment, only DDEs and DDDs were reliably detected in the tissue samples. For example,  $C_b$  of DDEs was  $1595.0 \pm 326.8 \mu\text{g/kg}$  (dw) for the PV6C sediment exposure and  $3282.0 \pm 192.9 \mu\text{g/kg}$  (dw) for the PV8C sediment. The values for DDDs were  $42.1 \pm 11.7$  and  $161.2 \pm 20.6 \mu\text{g/kg}$  (dw), respectively. Strong linear relationships were found between  $C_e$  ( $\mu\text{g/kg}$ ) normalized by OC content and  $C_b$  ( $\mu\text{g/kg}$ ) normalized by lipid content for DDEs and DDDs in the PV8C ( $R^2 = 0.84$ ) and PV6C ( $R^2 =$

0.94) sediments (Figure 3). The slopes for the linear relationships were  $1.88 \pm 0.57$  and  $1.20 \pm 0.22$  for PV8C



**Figure 3.** Linear correlation between the lipid content-normalized tissue residues of DDXs ( $\log C_b$ ,  $\mu\text{g/kg-lipid}$ ) in *N. arenaceodentata* and the organic carbon-normalized accessible HOC concentrations ( $\log C_e$ ,  $\mu\text{g/kg-OC}$ ) in both the PV8C (solid circle) and PV6C sediments (open circle).

and PV6C sediments, respectively. Although more studies may be required to test other HOCs or organisms, the accessible concentration  $C_e$  derived by IDM was clearly predictive of the bioaccessibility of DDT derivatives in the historically contaminated marine sediments to the benthic polychaete *N. arenaceodentata*.

**Considerations on Use of IDM for Predicting Bioaccessibility.** As demonstrated in this study, the use of IDM to derive  $C_e$  or  $E$  involves only a few simple steps, i.e., addition of isotope-labeled analogues to a sediment sample, mixing the sample for a relatively short duration (e.g., 24 h), and analyzing the aqueous concentrations of both labeled and nonlabeled (native) HOCs using a MS-equipped chromatographic system. Standards labeled with  $^{13}\text{C}$  or deuterium are widely available, especially for legacy contaminants. At present, MS-equipped instruments are also found in most research and commercial laboratories. In fact, many laboratories already use stable-isotope labeled standards as recovery surrogates or internal standards in routine analysis of environmental samples. Therefore, the measurement of  $C_e$  or  $E$  may require only minor modifications to existing procedures.

In this study, the PV6C and PV8C sediments contained very different levels of DDXs and PCBs. The isotope-labeled analogues were added at a fixed rate (i.e.,  $25 \mu\text{g/kg}$ ) to the sediment samples, regardless of the bulk sediment concentrations of the individual HOCs. When expressed as the ratio to the native HOC level,  $*C_0/C_T$  varied from 0.09% to 82.4% for the PV8C sediment and from 1.3% to 301% for the PV6C sediment. Therefore, results of this study suggested that it is not necessary to control the ratio of  $*C_0/C_T$  in the use of IDM. This offers the advantage that the concentrations of native HOCs in a contaminated sample need not to be known beforehand and that a single rate of isotope-labeled tracers may be used irrespective of the levels of native HOCs. This lends a great versatility to the method because field-contaminated samples often contain a mix of HOCs over a wide range of concentrations.

Another important consideration in the use of IDM is the time for the externally introduced isotope tracers to reach dilution equilibrium. In the present study, 24 h was selected on the basis of the observed isotope dilution kinetics. There was

no significant difference in the time to equilibrium among the different HOCs or between different sediments. As shown in this study, isotope dilution did not change appreciably during 24–192 h for both sediments and all HOCs considered. Therefore, after dilution equilibrium is reached, the specific time interval used for mixing is not critical, which adds flexibility to the method. Moreover, a mixing interval of 24 h is relatively short and convenient, contributing to a high sample throughput and hence feasibility.

The PV Shelf Superfund site represents a vast contaminated area where a multitude of factors could have affected the concentration profiles of DDXs and PCBs at a given location, including effluent discharge history, distance to the outfalls, topography of the ocean floor, and sediment deposition, burial, and resuspension caused by ocean currents. The two locations considered in this study, PV8C and PV6C, were about 2 km apart, with PV8C close to the outfalls. As shown in this study, contaminant accessibility as estimated by IDM ranged from 0.28 to 0.89 and was substantially smaller than 1 for most HOCs, suggesting that aging and other factors may have significantly decreased the bioavailability of HOCs at this Superfund site. Thus, the use of bioavailability-relevant measurements over bulk sediment levels may provide improved risk assessment that is valuable in decision making, in, e.g., designation of specific areas for remediation. In addition, findings from the current study also offered a preliminary comparison between locations at the PV Shelf site. The relative accessibility of DDXs and PCBs in the PV8C sediment was consistently smaller than in the PV6C sediment. The underlying mechanisms for changes in contaminant accessibility merit further research. Although this study only considered marine sediments and a select group of HOCs, the principle of IDM should be similarly applicable to other solid matrices (e.g., soil) and contaminants and should be further explored.

## ■ ASSOCIATED CONTENT

### Supporting Information

Additional details on description of methods and results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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