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Bioaccumulation of Highly Hydrophobic Organohalogen Flame Retardants from Sediments: Application of Toxicokinetics and Passive Sampling Techniques

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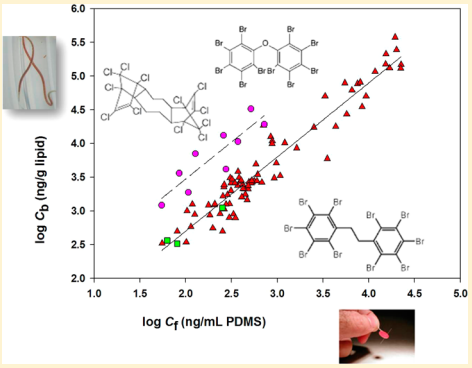
Supporting Information

ABSTRACT: Highly hydrophobic organohalogen flame retardants (HHOFRs) are found ubiquitously in the environment; therefore, a better understanding of their bioavailability is needed. In the current study, bioaccumulation testing using the oligochaete, *Lumbriculus variegatus*, and passive sampling (solid-phase microextraction (SPME)) were performed to study the bioaccumulation potential of HHOFRs, including decabromodiphenyl ether (*deca*-BDE), decabromodiphenyl ethane (DBDPE), and dechlorane plus (DP), in laboratory-spiked and field-collected sediments. The HHOFRs were bioavailable to *L. variegatus* even though their biota-sediment accumulation factors were low (0.016 ± 0.002 to 0.48 ± 0.082 g organic carbon/g lipid, *syn*-DP > *anti*-DP > *deca*-BDE > DBDPE). Hydrophobicity and stereoisomerism affected HHOFR bioavailability. Meanwhile, HHOFR concentrations on the SPME fibers (C_f) correlated with those in biota (C_b), suggesting the potential application of SPME in bioavailability prediction for those compounds. The log C_f to log C_b correlation for *deca*-BDE and DP had a greater intercept than that for polychlorinated biphenyls (data obtained from the literature) although the slopes were similar, while data for DBDPE fell on the regression line for PCBs, implying some uncertainty in application of SPMEs across chemical classes. The increasing sorptive ability of proteins for HHOFRs in comparison to the less-brominated BDEs suggested that protein-binding should be considered when estimating bioaccumulation potential of HHOFRs in benthic invertebrates.



INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) have become ubiquitous in the environment due to their extensive use as flame retardants.[1] The widespread presence and potential toxicity of the less-brominated BDEs has led to their restriction in the early 2000s, and the production of *deca*-BDE has also been restricted, due to the possibility of forming less-brominated BDEs through debromination.[2] These restrictive regulations on the manufacture and use of PBDEs promoted a growing demand for their replacements, such as decabromodiphenyl ethane (DBDPE) and dechlorane plus (DP).[3−5] Unfortunately, an alarming increase in DBDPE and DP sediment concentrations has been recently documented, raising concern about their bioaccumulation potential.[3−6]

Both *deca*-BDE and its replacements (DBDPE and DP) are highly hydrophobic organohalogen flame retardants (HHOFRs) with octanol−water partition coefficients (log K_{ow}) greater than 9 (Table S1 and Figure S1 in the Supporting Information, SI). Although these HHOFRs were once considered to not be bioavailable,[7] the concurrent detection of these compounds in fish and sediment revealed their bioaccumulation potential.[8−10] Benthic organisms directly contact sediment and are the main food source for many fish species; therefore, understanding the bioavailability of HHOFRs to benthic organisms is vital to understand their transfer pathways from the sediment to the aquatic food web.

Bioaccumulation testing using the freshwater oligochaete, *Lumbriculus variegatus*, was a conventional way to evaluate the bioavailability of sediment-associated contaminants.[11] Alternatively, passive sampling methods (PSMs), e.g. solid phase microextraction (SPME), have been developed to quantify the freely dissolved chemical concentrations in sediment porewater (C_{free}).[12,13] Since C_{free} determines the effective concentration for diffusive uptake and partitioning, it is a preferable metric for bioavailability prediction compared to bulk sediment concen-

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Table 1. Highly Hydrophobic Organohalogen Flame Retardant (HHOFR) Concentrations in Two Laboratory-Spiked Sediments at High and Low Levels and a Field-Collected Sediment (C_s, μg/g Organic Carbon (OC)) and in *Lumbriculus variegatus* (C_b, μg/g Lipid) at Steady State[b]

HHOFR	sediment	C_s (μg/g OC)	C_b (μg/g lipid)	$k_s \times 10^3$ (g OC/g lipid/d)	k_e (1/d)	BSAF (g OC/g lipid)	t_{95} (d)
deca-BDE	high	162 ± 14.2	19.1 ± 3.77	11.6 ± 1.36	0.098 ± 0.013	0.12 ± 0.021	30
	low	52.8 ± 4.55	4.15 ± 0.76	10.4 ± 1.13	0.13 ± 0.016	0.079 ± 0.013	23
	field[a]	103 ± 8.84	10.6 ± 2.77	21.6 ± 3.53	0.21 ± 0.038	0.10 ± 0.025	14
DBDPE	high	61.9 ± 4.82	1.11 ± 0.21	2.39 ± 0.29	0.13 ± 0.018	0.018 ± 0.003	23
	low	21.0 ± 1.74	0.33 ± 0.058	2.12 ± 0.22	0.14 ± 0.016	0.016 ± 0.002	22
	field[a]	17.4 ± 1.45	0.36 ± 0.054	2.82 ± 0.23	0.14 ± 0.013	0.021 ± 0.003	22
anti-DP	high	83.1 ± 6.43	32.6 ± 4.93	37.3 ± 3.18	0.095 ± 0.009	0.39 ± 0.051	32
	low	24.3 ± 1.87	7.04 ± 1.17	26.6 ± 2.54	0.092 ± 0.010	0.29 ± 0.042	33
	field	8.80 ± 0.68	1.88 ± 0.39	37.4 ± 4.75	0.18 ± 0.025	0.21 ± 0.041	17
syn-DP	high	27.3 ± 1.85	13.1 ± 2.44	69.8 ± 7.97	0.15 ± 0.018	0.48 ± 0.082	21
	low	7.66 ± 0.65	3.62 ± 0.62	29.8 ± 2.77	0.063 ± 0.007	0.47 ± 0.071	48
	field	3.54 ± 0.39	1.21 ± 0.24	44.2 ± 4.90	0.13 ± 0.017	0.34 ± 0.058	23

[a]Data from Zhang et al.[26] [b]The uptake and elimination rate constants (k_s, g OC/g lipid/d and k_e, 1/d), biota-sediment accumulation factors (BSAFs, g OC/g lipid), and the time to reach steady state in the organisms (t_{95}, d) are also presented. Data are reported as mean ± standard deviation.

trations.[14−16] Previous studies demonstrated good correlations between SPME measurements and body residues, but these studies mainly targeted contaminants with log K_{ow} < 7, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).[14−18] On the other hand, the application of PSMs to contaminants with high hydrophobicity is a greater challenge, because these compounds have a strong affinity for sediment organic carbon (OC) and have extremely low C_{free} values. Recent studies extended the use of SPME to quantify C_{free} of less-brominated BDEs, which have log K_{ow} values of approximately 8.[19,20]

In the current study, bioaccumulation kinetic testing was performed with *L. variegatus* exposed to *deca*-BDE, DBDPE, and DP in laboratory-spiked and field-collected sediments. The impact of chemical hydrophobicity and stereoisomerism were investigated on HHOFR bioaccumulation. In addition, the potential of using SPME to predict the bioavailability of HHOFRs (log K_{ow} > 9) was assessed by correlating the chemical concentrations accumulated on the SPME fibers with the concentrations in the organisms using HHOFRs from the current study and PCBs and less-brominated BDEs obtained from the literature.[17−20]

MATERIALS AND METHODS

Chemicals, Sediments, and Organisms. Two laboratory-spiked sediments spiked with two concentrations of *deca*-BDE, DBDPE and DPs and a field-collected sediment from an electronic recycling site in South China (Figure S2 in the SI) were used in the current study. Detailed information on the chemicals and reagents used in the study, sediment collection and spiking procedures, measurements of the contents of OC and black carbon in the sediments, and methods for culturing the test species are presented in the SI.

Bioaccumulation Testing. Bioaccumulation potential of sediment-associated HHOFRs was estimated using the freshwater oligochaete *L. variegatus*. Worms were cultured in accordance with U.S. Environmental Protection Agency (USEPA) standard protocols[11] at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (GIGCAS).

Bioaccumulation tests were conducted in triplicate in beakers containing approximately 120 g of wet sediment and 300 mL of laboratory-made moderately hard reference water, which served as overlying water for the tests. After the sediment was allowed to settle overnight, 30 worms were randomly introduced into each beaker. The tests were performed at 23 ± 1 °C with a 16:8 light:dark photoperiod. The organisms were not fed throughout the tests, and approximately 150 mL of overlying water was changed twice daily using an automated water-delivery system. Water quality parameters including pH, temperature, conductivity, and dissolved oxygen were monitored daily, while ammonia concentrations were analyzed weekly.

Bioaccumulation testing included uptake and elimination phases. In the uptake phase, 30 worms per replicate were exposed to test sediment, and three replicates were sampled at predetermined time intervals. Sampling times during the uptake phase were 3, 7, 14, and 28 d for laboratory-spiked sediments and 1, 3, 5, 7, 14, and 28 d for field-collected sediment. At the end of uptake phase, *L. variegatus* from the remaining replicates were sieved from the sediment and transferred to clean sediment, and the elimination phase was initiated. The termination of the uptake phase (28 d) served as the beginning of the elimination phase. Elimination sampling times were 5, 14, and 28 d post uptake phase for the spiked sediments and 3, 7, and 21 d for the field-collected sediment, respectively. At each predetermined time-point, worms were sampled in triplicate by sieving them from the sediments, transferred to 300 mL of moderately hard reference water for gut-purging for 6 h, weighed, and frozen at −20 °C until analysis. Concurrently, bioaccumulation testing was also processed using control Conghua sediment, and sampling time-points were the same as the spiked sediments.

The HHOFR concentrations in the sediments were analyzed in triplicate before and after the 28-d uptake phase. After exposure, one worm per replicate was removed to quantify lipid content, and the remaining worms were used for HHOFR body residue analyses. The extraction, cleanup, and gas chromatography/mass spectrometry (GC/MS) analytical methods for sediment and tissue are detailed in the SI. Information on the quality assurance and quality control for the analyses is also presented in the SI.

Passive Sampling Measurements. The bioavailability of HHOFRs was also estimated using disposable SPME fibers with a coating of 10 μm of polydimethylsiloxane (PDMS) and a phase volume of 0.069 μL/cm (Fiberguide Industries, NJ, USA) following a previously developed method.[17] Briefly, the fibers, which were protected with stainless steel envelopes with

110 μm openings, were sequentially washed by sonication with methanol and distilled water three times each before use. The vials containing approximately 10 g of wet sediment and 30 cm of fibers were shaken at 120 rpm at 23 °C. Three replicate fibers were sampled at predetermined time intervals, and these sampling times were the same as those used in the bioaccumulation tests. After being removed from the sediment, fibers were washed with distilled water, dried, and extracted using sonication with 3 mL of a 50% dichloromethane in hexane solution for 5 min. The extraction was repeated twice, and the extracts were combined, cleaned with concentrated H_2SO_4, evaporated to 50 μL of hexane, and analyzed on GC/MS after the addition of the internal standard. Partition coefficients for the HHOFRs and PBDEs between the PDMS disk and protein (bovine serum albumin (BSA) as the representative protein) ($K_{PDMS/BSA}$) were also measured, and detailed methods are included in the SI.

Data Analysis. Biota-sediment accumulation factors (BSAFs, g OC/g lipid) were used to describe the bioaccumulation potential of HHOFRs and were calculated using a kinetic approach (eq 1)

$$\mathrm{BSAF} = \frac{k_s}{k_e} \tag{1}$$

where k_s (g OC/g lipid/d) and k_e (1/d) are the uptake and elimination rate constants, respectively. Since HHOFR concentrations in sediment did not change during the bioaccumulation tests and no obvious growth of the worms occurred as indicated by the similar worm weights per replicate before and after the testing, a first-order kinetic model was employed to estimate the rate constants (eq 2)[21]

$$\frac{dC_b}{dt} = k_s \times C_s - k_e \times C_b \tag{2}$$

where C_b is the lipid-normalized HHOFR concentration in the organism at time t (d), and C_s was the OC-normalized HHOFR concentration in sediment (Table 1).

The HHOFR concentrations on the SPME fibers at equilibrium (C_f) were calculated using eq 3, and C_{free} was calculated from C_f (eq 4)

$$C_t = C_f(1 - e^{-k \cdot t}) \tag{3}$$

$$C_{free} = \frac{C_f}{K_{fw}} \tag{4}$$

where C_t is the HHOFR fiber concentration (ng/mL PDMS) at time t (d), and k is the desorption rate constant (1/d). Since experimentally measured PDMS fiber-water partition coefficients (K_{fw}) were not available for the HHOFRs studied in the current study, K_{fw} values were calculated from K_{ow} values using the following regression equation: log K_{fw} = 0.665 log K_{ow} + 1.394 (Table S1). The equation was derived from a data set for PBDEs that was presented in the literature.[22−24] The uncertainty in the K_{fw} values caused by the variability noted in the K_{ow} values was quantified using a Monte Carlo simulation (Crystal Ball, Oracle, v11.1) after 20,000 simulations, and the results of that procedure are presented in Table S2 in the SI.

Apparent steady state was defined as the state when chemical concentration in a phase reached 95% of the concentration at equilibrium and the time to reach apparent steady state (t_{95}) for HHOFRs in the worms and the fibers was calculated using k_e and k, respectively (eq 5).

$$t_{95} = \frac{-\ln(0.05)}{k_e \text{ or } k} = \frac{2.996}{k_e \text{ or } k} \tag{5}$$

Uptake and elimination estimates were performed by fitting the data using Scientist software (Micromath, Salt Lake City, UT, USA). Statistical differences among groups were compared by ANOVA, and Tukey's Honestly Significant Difference was introduced for further comparison. Statistical significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Bioaccumulation Tests. Control and field-collected sediments contained 2.75 ± 0.07% and 1.80 ± 0.13% of OC and 0.093 ± 0.003% and 0.103 ± 0.007% of black carbon, respectively. Sediment HHOFR concentrations before and after the bioaccumulation tests were not significantly different, thus average values were used (Table 1). In general, the difference in the HHOFR concentrations in the spiked and field-collected sediments was within a factor of 10, except for *syn*-DP in the high-level spiked sediment, which had 17.5 times greater concentration than that in the field-collected sediment. All of the HHOFR concentrations in the spiked sediments were within the range of previously reported values in South China, where as high as 7.3, 1.8, 5.4, and 2.1 μg/g dry weight (dw) of *deca*-BDE, DBDPE, *anti*-DP, and *syn*-DP have been detected, respectively.[8,25]

Dissolved oxygen (5.4 ± 0.4 mg/L), pH (7.6 ± 0.2), temperature (23.6 ± 0.8 °C), conductivity (316 ± 10 μs/cm), and ammonia (< 0.7 mg/L) were all within tolerance limits for *L. variegatus* throughout the bioaccumulation tests.[11] The worms in the spiked and field collected sediments behaved similarly to those in the controls. No overt avoidance of the sediment was observed, and the total worm weight per replicate changed little before and after the tests.[26] No significant reproduction of the worms was observed during the bioaccumulation testing.

As shown in Figure S3 in the SI, HHOFR residues in biota increased in the first 14-d of exposure and thereafter reached a plateau, resulting in similar body residues at 14- and 28-d. After the worms were transferred into control sediment, the HHOFRs were quickly eliminated from the worms, and k_e values ranged from 0.063 to 0.209 1/d (Table 1). Using the k_e estimates, t_{95} values were calculated using eq 5, and the results suggested that all of the HHOFRs approached steady state levels in the organisms within 33 d, with the exception of *syn*-DP in the low-level-spiked sediment (48 d, Table 1).

Recent advances in analytical techniques have facilitated the detection of trace levels of HHOFRs in biota, thus the bioavailability of these contaminants is now a potential issue[26,27] even though HHOFRs were once considered not to be bioavailable.[7,28] While HHOFRs bioaccumulated in *L. variegatus*, their bioavailability was relatively low with BSAF values ranging from 0.021 ± 0.003 to 0.481 ± 0.082 g OC/g lipid (Table 1). All of the BSAFs calculated in the current study were less than 1, which suggested that HHOFRs were more likely to bind to sediment OC than the lipids of the test organism based on the assumption that no significant biotransformation occurred in the worms and that the uptake and depuration kinetics were controlled by the same mechanism. Nevertheless, HHOFRs can still bioaccumulate in

Table 2. Highly Hydrophobic Organohalogen Flame Retardant (HHOFR) Concentrations on the Polydimethylsiloxane (PDMS) Fiber (C_f, ng/mL PDMS) at Equilibrium after Exposures to Two Laboratory-Spiked Sediments at High and Low Levels and a Field-Collected Sediment and the Respective Freely Dissolved Concentration in Sediment Porewater (C_{free})[c]

HHOFR	sediment	C_f (ng/mL PDMS)	C_{free}[a] (pg/L)	k (1/d)	t_{95} (d)	r^2
deca-BDE	high-spiked	725 ± 29	7.99 ± 0.32	0.166 ± 0.027	18	0.983
	low-spiked	277 ± 9	3.05 ± 0.10	0.321 ± 0.055	9	0.986
	field[b]	372 ± 24	4.10 ± 0.27	0.152 ± 0.027	20	0.981
DBDPE	high-spiked	253 ± 11	0.197 ± 0.008	0.221 ± 0.041	14	0.979
	low-spiked	81.3 ± 3.6	0.0634 ± 0.0028	0.333 ± 0.081	9	0.972
	field[b]	63.3 ± 3.6	0.0494 ± 0.0028	0.246 ± 0.052	12	0.979
anti-DP	high-spiked	516 ± 16	13.6 ± 0.4	0.219 ± 0.030	14	0.989
	low-spiked	128 ± 4	3.38 ± 0.11	0.326 ± 0.061	9	0.983
	field	108 ± 10	2.84 ± 0.27	0.150 ± 0.039	20	0.961
syn-DP	high-spiked	260 ± 8	6.85 ± 0.21	0.281 ± 0.042	11	0.988
	low-spiked	84.8 ± 3.2	2.24 ± 0.08	0.218 ± 0.038	14	0.981
	field	54.9 ± 3.7	1.45 ± 0.10	0.138 ± 0.024	22	0.982

[a]The C_{free} was estimated by dividing the C_f by the partition coefficient for the PDMS fiber and water (K_{fw}), which is shown in Table S1 in the Supporting Information. [b]Data from Zhang et al.[26] [c]The elimination rates (k, 1/d), time to reach equilibrium (t_{95}, d), and r^2 for the model fitting are also represented. Data are reported as mean ± standard deviation.

benthic organisms at detectable levels due to their elevated sediment concentrations[3−6,8−10,25]and then possibly biomagnify throughout aquatic food webs.[8,29]

Factors Affecting HHOFR Bioaccumulation. Bioaccumulation potential was significantly different ($p < 0.05$) among the three target HHOFRs in the current study. Specifically, BSAF values followed the order of DBDPE < *deca*-BDE < DP, and this was the same order as their relative hydrophobicity and molecular weights (Tables 1 and S1). The chemical structures of *deca*-BDE and DBDPE were similar; however, the substitution of an −O− group in *deca*-BDE for $-CH_2-CH_2-$ in DBDPE made DBDPE less bioaccumulative in the oligochaete. As shown in Table 1, k_s values decreased in the same order as the BSAFs (DP > *deca*-BDE > DBDPE), whereas the k_e values were not significantly different for any of the HHOFRs. This suggested that the larger molecular size and hydrophobicity hindered the uptake of HHOFRs by the worms, leading to decreased bioaccumulation potential. A similar reduction in BSAF and k_s values was also noted for the less-brominated PBDEs with an increase in log K_{ow}.[26] The PCB concentrations in the field-collected sediment were also analyzed using bioaccumulation data obtained from the literature.[17,18] Interestingly, the PCB congeners did not follow the same trend with a reduction in BSAF values with increasing hydrophobicity, but instead the BSAF values for the PCBs remained relatively constant (1.68 ± 0.47) regardless of the log K_{ow} value used (Figure S4 in the SI).

Stereoisomerism affected the bioaccumulation of the DPs. As shown in Table 1, *syn*-DP had larger BSAF values than *anti*-DP, and the stereodifference was significant for DP isomers from the low-level-spiked and field-collected sediments ($p < 0.05$). The fraction of *anti*-DP (f_{anti}) in commercial DP products was approximately 0.75,[5] while for f_{anti} the values were 0.75, 0.76, and 0.71 in the high- and low-level-spiked and field-collected sediments, respectively. Nevertheless, f_{anti} was reduced to 0.71, 0.66, and 0.61 in *L. variegatus*, which were exposed to the respective sediments. The reduction in f_{anti} through bioaccumulation processes was consistent with previous field observations in South China, where f_{anti} was approximately 0.75 in sediment but 0.14 to 0.68 in aquatic species.[8] Tomy et al.[30] also noted distinct f_{anti} patterns in field-collected sediment and biota from Lake Ontario (0.51−0.76 in fish and 0.86 in sediment) and Lake Winnipeg (0.04−0.96 in biota and 0.61 in sediment).

Stereospecific uptake and species-specific biotransformation were proposed as the explanations for the change in f_{anti} during the bioaccumulation process, yet it was hard to elucidate the process with data using field-collected samples alone.[30] Instead, controlled experiments using laboratory-spiked sediment provided more information on the bioaccumulation kinetics. As shown in Table 1, *syn*-DP generally had larger k_s values than *anti*-DP, and this difference in k_s values for the DP isomers was significant for the high-level-spiked sediment ($p < 0.05$). At the same time, *syn*-DP had significantly smaller k_e values than *anti*-DP for the low-level-spiked and field-collected sediments. This suggested that stereospecific uptake and elimination processes may be occurring and could be the main reasons for the decline in f_{anti} in biota, since *syn*-DP had more accumulation (larger k_s) but less elimination (smaller k_e) than *anti*-DP in the organisms. In the high-level-spiked sediment, *syn*-DP had a significantly larger k_e than *anti*-DP. The ratio of uptake rates for the DP isomers ($k_{s,syn\text{-DP}}/k_{s,anti\text{-DP}}$) was 1.9 in the high-level-spiked sediment, while the ratios were 1.12 and 1.15 for the low-level-spiked and field-collected sediment, respectively. This suggested that the significantly faster uptake rate of *syn*-DP compared to *anti*-DP from the high-level-spiked sediment compared to the other two sediments might have accelerated the elimination of *syn*-DP.

Chemical concentration is another factor that can affect the bioaccumulation potential. Burkhard et al.[31] found an inverse correlation between BSAFs and PCB concentrations in sediment. The BSAFs for the HHOFRs used in the current study were not significantly different at the two spiked levels (Table 1), but it is difficult to draw any conclusion on this issue due to the limited data collected in the current study.

Furthermore, it has been well documented that sediment aging affects the bioaccumulation potential of contaminants.[32] For example, Klosterhaus and Baker[33] observed that the bioavailability of PBDE to *Nereis virens* was less when they were exposed to field-collected sediment than when exposed to aged spiked sediment. In the current study, the reduction in bioavailability of sediment-associated HHOFRs after prolonged aging times was obvious for DP, whose BSAFs were significantly smaller from field-collected sediment than those

from spiked sediments (Table 1). Conversely, the BSAFs for *deca*-BDE and DBDPE were similar from spiked and field-collected sediments, and this might be due to the extremely small amount of these compounds that was bioavailable within the sediment. This is consistent with the findings by Nyholm et al.[34] who estimated BSAFs for PBDEs from soil to earthworms and found that aging soil decreased the bioavailability of the less-brominated BDEs but did not affect the bioavailability of *deca*-BDE. It was difficult to draw any conclusion from the data collected in the current study on the influence of sediment aging on bioaccumulation potential of sediment-associated HHOFRs. Therefore, more research is needed in order to provide a quantitative evaluation.

Passive Sampling Measurements. The bioavailability of HHOFRs was also estimated using SPME fibers, and the HHOFRs were all detectable on the fibers. The C_f and k were determined using a first-order kinetic model (eqs 3 and 4) with r^2 values being fairly high ranging from 0.96 to 0.99 (Table 2 and Figure S5 in the SI). Although sediment concentrations were at mg/kg levels, only a small portion of the HHOFRs was freely dissolved in the pore water with C_{free} at pg/L levels (Tables 1 and 2). The low C_{free} values were consistent with the low BSAFs for the HHOFRs, further confirming their low bioavailability. Because of their extremely high hydrophobicity and low water solubility, it is problematic to measure K_{fw} for the HHOFRs, and experimentally measured K_{fw} values were not available.[24] Instead, K_{fw} values were calculated from K_{ow} values using the equation derived from the data sets for less-brominated BDEs[22,23] (Table S1 in the SI). Inevitable uncertainties, however, were introduced in estimating K_{fw} values and corresponding C_{free} with this extrapolation (Table S2 in the SI); therefore, an alternative method to predict C_b using SPME measurements was applied.

Alternatively, C_b was directly predicted from C_f by using the fibers as biomimetic samplers.[14−16] This approach was advantageous for estimating HHOFR bioavailability, because it circumvented the measurement of K_{fw}. Concentrations of *deca*-BDE, DPs, and DBDPE on the SPME fiber and in the organisms at steady state are shown in Figure S6 in the SI, and the PCB data from the literature[17,18] were also included for comparison. A good relationship was found between C_f and C_b for the PCBs ($\log C_b = (1.10 \pm 0.04) \log C_f + (0.50 \pm 0.11)$, $r^2 = 0.92$, $p < 0.05$, $n = 80$), suggesting C_f could be a good indicator for C_b. Interestingly, C_f and C_b values for *deca*-BDE and DPs were positively correlated ($\log C_b = (1.10 \pm 0.22) \log C_f + (1.28 \pm 0.52)$, $r^2 = 0.77$, $p < 0.05$, $n = 9$), with the same slope, but a greater intercept compared to that for the PCBs (Figure 1). A greater intercept suggests a stronger affinity of the chemicals for the organism assuming no significant degradation occurred in the system. Since both intercepts were positive, HHOFRs and PCBs appear to prefer the worm lipids over the PDMS fibers. The average ratio of C_b/C_f was 35 for *deca*-BDE and DPs, suggesting that the capacity of the worms to accumulate *deca*-BDE and DPs was about 35-fold higher than the PDMS, while the difference in binding capacities between the lipids and the PDMS for PCBs was only 7-fold higher (the average ratio of C_b/C_f was 7). A previous study[35] also reported that the C_f to C_b relationship established for PCB data underestimated C_b for BDE-47. Conversely, the DBDPE data fell on the PCB line (Figure 1), but the reasons for the coincidence are unknown. The results suggested some uncertainty in application of SPMEs across chemical classes, and additional uptake routes and protein-binding of the HHOFRs may be the reasons for the discrepancy in the C_b/C_f linearity.

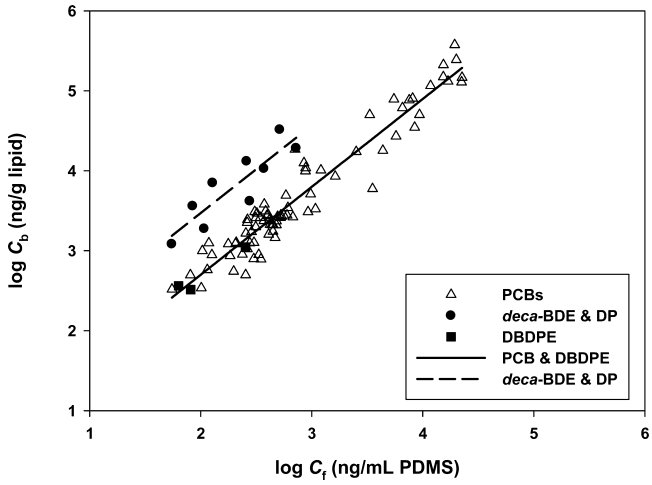

Figure 1. Logarithmic relationships between polychlorinated biphenyls (PCBs) concentrations and highly hydrophobic organohalogen flame retardants (HHOFRs) in *Lumbriculus variegatus* (C_b, ng/g lipid) and polydimethylsiloxane (PDMS) fibers (C_f, ng/mL PDMS) after sediment exposures. The PCB data were obtained from the literature,[17,18] while the HHOFR data were measured in the current study.

Additional Uptake Routes. The presence of additional uptake routes other than passive partitioning may enhance HHOFR accumulation in the worms. The C_{free} determines the effective concentration for diffusive uptake but fails to incorporate active processes within biota. Uniform k_e values implied the involvement of active kinetic processes in HHOFR bioaccumulation, thereby causing greater amounts of HHOFR to be accumulated in the worms than what would be expected from passive partitioning alone (C_f).[26,36] At the same time, k_e values for various PCB congeners in *L. variegatus* were also similar.[37] Therefore, enhanced bioaccumulation due to active kinetic processes would not cause the different intercepts noted in the C_f to C_b relationships between HHOFRs and PCBs. Furthermore, C_b may be overquantified if sediment particles were left in the worm after gut-purging, but no obvious sediment particles were found in the worm guts. In the current study, the accumulation of DBDPE in the worms was considerably lower and fell on the PCB line, also suggesting that the particles left in the worm's gut were not the reason for the greater than expected C_b concentrations for *deca*-BDE and DP. Thus, the presence of additional uptake routes was not likely the reason for the greater accumulation of *deca*-BDE and DP in organisms compared to the expectation from the C_f to C_b relationship for the PCB data set.

Protein-Binding of HHOFRs. The presence of additional binding phases in the worms other than lipids may have contributed to the elevated bioaccumulation of the HHOFRs. Lipids are the principal storage compartment for hydrophobic contaminants, and lipid-normalization is the traditional way to assess bioaccumulation potential.[11] The oligochaetes had a low f_{lip} of 1.2−1.3% (1.20 ± 0.13% for HHOFRs and 1.29% for PCBs[17,18]) but relatively higher $f_{protein}$ of 5.54 ± 0.26%. Thus, proteins may serve as an additional binding phase for contaminants in oligochaetes. DeBruyn and Gobas[38] found that the sorptive capacity of solid animal proteins was 1−10% of that of lipids. Serum albumin is the most abundant protein in

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the blood serum and serves as an important carrier for endogenous and exogenous chemicals in the blood.[23] Bovine serum albumin is frequently used as a complement for the growth medium of mammalian cells in biological assays, because it is a critical component in fetal bovine serum.[23] Endo et al.[23,24] demonstrated that binding of PBDEs to serum albumin played a role in both *in vivo* and *in vitro* assays with K_{BSAw} values being only one log unit less than K_{lipw}. Thus, BSA was used as the representative protein in the current study.

As reported by Endo et al.,[23,24] relationships between log K_{lipw} and log K_{ow} were similar for PCBs and PBDEs, but distinct relationships were noted for log K_{BSAw} versus log K_{ow} for PBDEs and other HOCs (PCBs, PAHs, chlorobenzenes, and benzene). A peak value of log K_{BSAw} occurred at a log K_{ow} of ~6 for the other HOCs, whereas log K_{BSAw} values for PBDEs continued to increase as log K_{ow} increased over 8.[23,24] Therefore, the different affinities of the other HOCs, i.e. PCBs, and PBDEs for proteins may explain greater amounts of HHOFRs accumulated in the worms compared to those estimated from the C_f to C_b relationship with lipid-normalized PCB data.

To evaluate the contribution of protein sorption to HHOFR bioaccumulation, we attempted to determine the K_{BSAw} and K_{lipw} for HHOFRs by measuring the K_{fw}, $K_{PDMS/lip}$, and $K_{PDMS/BSA}$ values (eqs S1 and S2), but most tests failed with the exception of the $K_{PDMS/BSA}$ measurements. Experimental variation was caused by the extremely high hydrophobicity of the target chemicals, which made the K_{fw} and $K_{PDMS/lip}$ and subsequently K_{BSAw} and K_{lipw} estimations unsuccessful. The relationship between the experimentally measured log $K_{PDMS/BSA}$ and the log K_{ow} is presented in Figure 2, and results

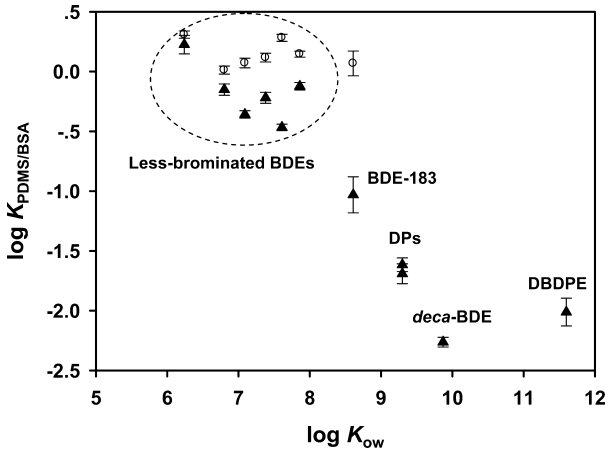

Figure 2. Logarithmic relationship between octanol−water partition coefficients (log K_{ow}) and the measured polydimethylsiloxane (PDMS)-bovine serum albumin (BSA) partition coefficients (log $K_{PDMS/BSA}$) of the less-brominated polybrominated diphenyl ethers (PBDEs) (BDE-28, -47, -99, -100, -153, -154), BDE-183, and highly hydrophobic organohalogen flame retardants (*deca*-BDE, DPs, and DBDPE). The values determined in the current study (filled triangles) and in Endo et al.[23] (open circles) were both included in the figure.

showed that the $K_{PDMS/BSA}$ values for the HHOFRs were 1−2 orders of magnitude smaller than for the less-brominated BDEs. This suggested that the difference in sorptive capacity between proteins and PDMS was larger for the HHOFRs than the less-brominated BDEs, which had consistent $K_{PDMS/BSA}$ values. Endo et al.[23] also reported that PBDEs (BDE-183 with a log K_{ow} 8.61[39] was the most hydrophobic chemical to have data) had relatively uniform $K_{PDMS/BSA}$ values across K_{ow} values. The stronger sorption capacity of HHOFRs to BSA than the less hydrophobic compounds may have enhanced their bioaccumulation in the worms.

The likely contribution of additional sorptive phases for HHOFRs besides lipids has also previously been reported. Wan et al.[9] found the distribution of *deca*-BDE in Chinese sturgeon was different for less-brominated BDEs and was not controlled by f_{lip} alone, suggesting the presence of additional binding phases. Therefore, the greater bioaccumulation noted for *deca*-BDE and DP compared to the estimation from the PCB-based C_f to C_b relationship may be explained by the additional protein-binding process, which was ignored by the conventional calculation with lipid-normalization. Nevertheless, it is unknown if proteins played a role in the bioaccumulation of the most hydrophobic compound, DBDPE. More studies are needed to better understand the chemical binding processes to lipids and proteins as well as the role of the binding phases on the bioaccumulation of hydrophobic contaminants.

Overall, the current study found that the extremely hydrophobic *deca*-BDE, DBDPE, and DP were bioavailable to deposit-feeding organisms. Bioaccumulation potential of these HHOFRs decreased with increasing hydrophobicity. Specifically for DPs, stereoisomerism also affected the uptake process and subsequently the bioavailability. The low bioavailability of HHOFRs to *L. variegatus* was confirmed by their low concentrations in SPME fibers. To our knowledge, this is the first report to link chemical concentrations in biota with PSM measurements for sediment-associated contaminants with log K_{ow} > 9. The C_f to C_b relationship for *deca*-BDE and DPs had a significantly greater intercept than that for PCBs (data obtained from the literature[17,18]), indicating that biota concentrations of *deca*-BDE and DPs would be significantly underestimated using the C_f to C_b relationship derived from PCB data alone. Therefore, applications of PSMs in bioavailability prediction across chemical classes should be done with caution. The distinct contribution of protein-binding fractions to total body residues for *deca*-BDE and DPs and PCBs was the likely reason for the different intercepts, and the protein-binding of HHOFRs should not be ignored in bioaccumulation potential estimation for deposit-feeding invertebrates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional text describing sample preparation and instrumental analyses. Figures showing chemical structures, sampling sites, kinetic curves for the worms and fibers, and the relationships between the BSAF values and chemical hydrophobicity are shown in Figures S1 to S6, and chemical properties and the analysis of uncertainty in estimating K_{fw} values were presented in Tables S1 and S2. 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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