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Polybrominated Diphenyl Ether (PBDE) Accumulation by Earthworms (*Eisenia fetida*) Exposed to Biosolids-, Polyurethane Foam Microparticle-, and Penta-BDE-Amended Soils

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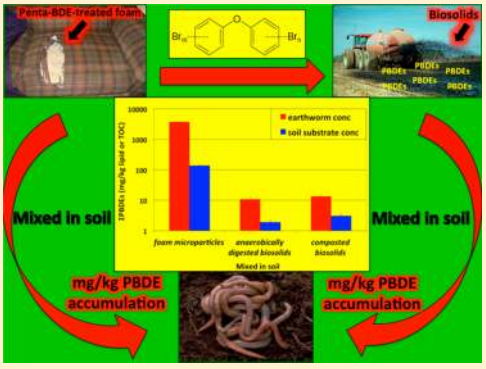
Supporting Information

ABSTRACT: Polybrominated diphenyl ether (PBDE) flame retardants have been used in consumer polymers at up to percent levels. While long viewed as biologically inaccessible therein, PBDEs may become bioaccessible following volatilization or polymer deterioration. PBDEs may then enter soils via polymer fragmentation or following land application of sewage sludge-derived biosolids. Studies of direct PBDE uptake from these materials by soil organisms are scarce. We thus exposed earthworms (*Eisenia fetida*) to artificial soil amended with a Class B anaerobically digested biosolid (ADB), an exceptional quality composted biosolid (CB), PBDE-containing polyurethane foam (PUF) microparticles, and Penta-BDE-spiked artificial soil (SAS). Worms accumulated mg/kg (lipid) ∑Penta-PBDE burdens from all substrates. Biota-soil accumulation factors (BSAFs) for worms exposed to ADB- and CB-amended soils were comparable after 28 d. BSAFs generally decreased with increasing congener K_{OW} and substrate dosage. Biosolids-associated PBDE bioavailability was lower than spiked PBDEs. BSAFs for worms exposed to PUF microparticles ranged from 3.9 to 33.4, with ∑Penta-PBDE tissue burdens reaching 3740 mg/kg lipid. Congener accumulation patterns were similar in worms and polyethylene passive sampling devices immersed in ADB-amended soil coincident with exposed worms. However, passive sampler accumulation factors were lower than BSAFs. Our results demonstrate that PBDEs may accumulate in organisms ingesting soils containing biosolids or waste plastics. Such organisms may then transfer their burdens to predators or translocate them from the site of application/disposal.



INTRODUCTION

In 2011 alone, 33 million tons of plastics entered the United States municipal solid waste stream.[1] Ecotoxicological concerns related to discarded plastics in the environment have historically focused on their potential to physically entangle aquatic wildlife or interfere with digestive processes.[2] But, discarded plastics also effectively sorb hydrophobic organic contaminants (HOCs) in situ,[3] and thus, interest in the impacts of sorbed contaminants following plastics ingestion by organisms has grown.[3−5] However, plastics-sorbed HOC levels are typically in the low μg/kg range, and while some studies have highlighted the comparatively substantial levels of additives (e.g., brominated flame retardants (BFRs)) in consumer materials such as baby products,[6] little research has evaluated actual environmental exposure and uptake following contact with additive-containing consumer polymers.[7] Indeed, it has long been assumed that such additives are largely sequestered within the polymers and thus inaccessible to biota. However, studies have increasingly documented high levels of BFRs in consumer products and in media downstream of their usage (e.g., indoor dust, sewage sludges, furniture cushioning, etc.).[7−13]

One group of BFRs, the polybrominated diphenyl ethers (PBDEs), has been used extensively in textile coatings, thermoplastics, and polyurethane foam (PUF), and of the three commercial PBDE formulations available, the Penta-BDE mixture has been used for decades primarily to treat PUF and is the most environmentally mobile, bioaccumulative, and toxic. To meet stringent United States flame retardancy standards, Penta-BDE has been added to PUF products at up to 10−30% w/w levels. Thus, > 90% of historical Penta-BDE demand has been in North America.[11,12] Penta-BDE manufacture was discontinued in the United States in late 2004 in response to escalating environmental and human health concerns. However, Penta-BDE constituents persist in myriad current-use and discarded PUF-based consumer products and continue to be reported in indoor air and dust, sewage sludges, soils, sediments, biota, etc.

PBDEs are incorporated within, but are not chemically bound to, consumer polymers. Thus, despite low volatilities and water solubilities, some may escape from products over time. Treated plastics also weather and lose structural integrity during use and especially after disposal,[4,14] with disintegrating

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microplastic particles retaining the bulk of their original additive burdens. Such particles can then easily enter soil and wastewater streams via runoff and aeolian processes.[10−12] Polymer degradation further enhances additive release and also facilitates subsequent microparticle ingestion and physiological absorption by biota.[4,5,7,14] Hence, ingestion of polymer microparticles containing percent levels of PBDE additives may represent an important but underappreciated exposure route for some organisms.[7] Indeed, ingestion of indoor dust is now viewed as a major human exposure route for PBDEs.[15]

Regardless of source, persistent HOCs sorb to organic-rich particulates and are concentrated in sewage sludge during wastewater treatment. Sewage sludges may subsequently be stabilized by anaerobic or aerobic digestion, composting, drying, or liming and are then euphemistically termed *biosolids*. Because of their high nutrient and organic matter content, such sludges have been promoted as soil amendments. However, biosolids also contain complex mixtures of organic pollutants,[10,16,17] and attendant exposure and toxicological risks require further investigation. About 8×10^6 tons of sewage sludge is generated in the United States per year, more than half of which is now land-applied.[18] PBDEs incurred in sludges intended for land application are of particular concern as they are persistent, bioaccumulative, toxic, and intentionally widely dispersed in the environment. The U.S. Environmental Protection Agency National Sewage Sludge Survey[19] has reported a mean Penta-BDE concentration (∑Penta-BDE 47, 99, 100, 153, and 154) of 2030 μg/kg (dw) in United States biosolids collected from 74 publicly owned wastewater treatment plants. Assuming this material was land-applied at one-time application rates in the range of 2.3−60 tonnes per hectare[20,21] and assuming a mixing depth of 7.6 cm[22] and a bulk soil density of 1.4 g/cm^3, a single sludge application would result in theoretical soil burdens of 4.4−115 μg/kg dw. Indeed, Andrade et al.[22] reported mean total PBDE concentrations (∑PBDE 47, 99, 209) of 53 μg/kg dw in agricultural soils from the United States Mid-Atlantic region receiving repeat biosolids applications in the range of 9.4−74.2 tonnes per hectare. Hale et al.[23] also found that soil PBDE burdens increased linearly with application rates in agricultural soils from the United States Midwest region receiving repeat biosolids applications, while Gorgy et al.[24] reported order-of-magnitude increases in soil PBDE burdens in response to a single biosolids application. Results from these studies are compelling and confirm that application of PBDE-contaminated sludges to soils results in higher soil burdens. Such elevated soil burdens may in turn impact ecologically critical soil biota, such as earthworms. Earthworms are abundant in many soil ecosystems, and their biomass in temperate soils often exceeds those of other soil invertebrate fauna.[25] Their reworking and ingestion of soil modifies its texture, moisture, nutrient, and oxygen content, and earthworms are also food sources for myriad species. Thus, their accumulation of soil contaminants contributes to body burdens in organisms throughout terrestrial food webs.[26]

Controlled studies of uptake and bioavailability to soil organisms of PBDEs present in environmentally relevant forms, such as sewage sludge-derived biosolids and consumer plastics, are scant and assessments of dust-associated PBDE bioavailability have yielded confounding results.[27,28] Moreover, evaluating PBDE mobility in complex soil and sediment matrices is challenging, as organic and particulate matter content and composition can vary widely.[29,30] Within polymer microparticulate and biosolid materials dispersed in soils, some fraction of PBDEs will be strongly retained by the polymer and sewage sludge matrices, respectively, which in turn will impact their bioavailability. Thus, to further evaluate the role of the matrix in PBDE bioavailability to soil organisms, we exposed earthworms to Penta-BDE-spiked artificial soil (SAS) and Penta-BDE-containing PUF microparticles dispersed in artificial soil (PUF-AS), as well as artificial soil (AS) amended with anaerobically digested biosolid (ADB) and exceptional quality composted biosolid (CB) with naturally incurred PBDEs. Finally, we examined the utility of simple polyethylene passive sampling devices (PSDs) to sample coincident with earthworms the fraction of PBDEs available for uptake from ADB-amended soil.

EXPERIMENTAL SECTION

Earthworms and Biosolids. A stock culture of earthworms (*Eisenia fetida*; Worm World, Avella, PA) was established several weeks prior to exposure experiments to acclimate them to ambient laboratory conditions (See Supporting Information for worm rearing details). PBDEs were below the quantitation limit (QL) in all stock worms, bedding, and food (Tables S1QC and S2QC, Supporting Information). Two biosolids were obtained from publicly owned treatment works (POTW): (1) a Class B anaerobically digested biosolid (ADB) and (2) an exceptional quality composted biosolid (CB) consisting of dewatered sludge composted with recycled paper products and yard waste. Biosolids were sieved to <2000 μm, homogenized, and stored at 4 °C prior to use. ∑Penta-PBDE (47 + 99 + 100 + 153 + 154 + 183) burdens determined in 100% CB and ADB were 1130 ± 79 and 5560 ± 440 μg/kg dw, respectively (Table S1, Supporting Information).

Soil Substrate Preparation. Artificial soil (AS) was generated for use as exposure and control substrates by combining sand, kaolinite, peat moss, and dolomite (69:15:15:1 w/w) and hydrating with deionized water to 45% (w/w).[31] Batches of AS were subsampled to determine total organic carbon (TOC) content (Exeter CHN Model 440 CE Elemental Analyzer; North Chelmsford, MA). AS TOC content was 5.2 ± 0.90%, 5.9 ± 0.80%, 5.1 ± 1.2%, and 4.8 ± 0.80% dw for batches used in diluting biosolids and PUF microparticles and the corresponding SAS and control substrates, respectively. PBDEs were below QL in all soils (Table S1QC and S2QC, Supporting Information). Worms tolerated a maximum of only 9% (w/w) of the ADB and a 25% (w/w) mixture of the CB before burrowing avoidance and 100% mortality was observed. Thus, AS dilutions of 3%, 6%, and 9% ADB and 6, 12, and 25% CB were prepared for use as *low*, *medium*, and *high* dose treatments, respectively. Dilutions approximated agronomic field applications of 23, 47, and 70 tonnes per hectare, respectively, comparable to the 10−70 tonnes per hectare range reported for Swedish[20,21] and United States agricultural[22] soils. Soil mixtures (~800 g) were transferred to precleaned glass jars and equilibrated to laboratory conditions prior to exposure. AS controls (N = 3) were run simultaneously for each substrate tested.

For the corresponding SAS exposures, nominal PBDE spiking levels were 10%, 50%, and 100% of ∑Penta-PBDE levels in the 100% ADB (Table S1, Supporting Information). The Penta-BDE mixture, DE-71 (Chemtura Corporation, West Lafayette IN), was dissolved in acetone and added to sand, which in turn was combined with the other soil constituents. AS control sand was manipulated identically but without the Penta-BDE spike. Soils were stored at 4 °C for four weeks prior

to exposure. Final mean ∑Penta-PBDE levels were 676, 2910, and 5630 μg/kg dw for *low*, *medium*, and *high* dose SAS treatments, respectively (Table S1, Supporting Information). PBDEs were below QL in all AS controls (Table S1QC and S2QC, Supporting Information).

Commercial PUF was obtained and determined to contain 8.7 ± 1.4% dw ∑Penta-PBDEs (47 + 100 + 99 + 85 + 154 + 153). To generate ingestible microparticles, PUF was frozen in liquid nitrogen and fragmented to <75 μm (Supporting Information). PUF microparticles were dispersed in AS (1:2000 w/w) by mixing for 24 h on a rolling mixer. ∑PBDEs in the PUF-amended soil (PUF-AS) were determined prior to earthworm exposure (83 mg/kg dw). PUF-AS was stored at 4 °C for one month prior to exposure. The soil was then hydrated to approximately 45% (w/w), disbursed into glass jars (~600 g wet) and equilibrated under ambient laboratory conditions for 7 d prior to exposure.

Bioaccumulation Bioassays. Jars were randomized as to treatment and location. Ten adult worms were placed on the soil surface and allowed to burrow. Jars were covered with perforated aluminum foil to reduce water loss and prevent worm escape. The corresponding SAS and nonspiked AS control exposures were run simultaneously and manipulated identically alongside both biosolids- and PUF-amended soil bioassays. Worms were not depurated or rinsed of stock bedding prior to exposure, as preliminary trials indicated that worms thus handled would not burrow. Trials also revealed that the ASTM-prescribed organic matter content of the AS provided inadequate nutrition, resulting in worm burrowing avoidance and mortality. Increasing the peat moss content to 15% w/w obviated this. No PBDEs were detected in stock bedding or food. Exposures were conducted over a 12:12 light:dark photoperiod at 30 ± 3 °C and 51 ± 5% relative humidity. Environmental conditions were monitored hourly using a data logger. Treatments were monitored daily for worm mortality, and worms were fed twice weekly with 20 g per jar Worm Chow (Nestlé Purina; St. Louis, MO). Biosolids- and corresponding SAS-exposed worms were removed from substrates at 14 and 28 d and rinsed with deionized water, and gut contents were depurated for 24 h on moistened KimWipes. Worms were rinsed again with deionized water, patted dry with KimWipes, and frozen at −10 °C until analysis. PUF-AS- and corresponding SAS-exposed worms were removed from substrates at 7, 14, and 28 d.

Passive Sampling Devices (PSDs). PSDs were constructed from 86 μm thick lay-flat low-density polyethylene film (Brentwood Plastics, Saint Louis, MO). Prior to use, stock film was cut into 9 cm^2 sheets, weighed, and pre-extracted with methylene chloride (DCM). After air-drying, PSDs were wrapped in solvent-rinsed aluminum foil, sealed in plastic bags, and frozen at −10 °C until use. PSDs (1.5 ± 0.020 g; n = 15) were placed into the ADB-amended and AS control soils coincident with worms so as not to obstruct worm movement. PSDs were removed at 14 and 28 d intervals, rinsed with deionized water, wiped dry with a KimWipe, and stored at −10 °C until analysis.

Chemical and Statistical Analysis. All materials (except PSDs) were analyzed according to previously published methods[32] (see Supporting Information for more details). PSDs were spiked with PCB 204 and extracted with DCM overnight on a shaker table. Extracts were solvent exchanged to hexane and purified on 2 g silica SPE columns. PBDEs were determined using gas chromatography−mass spectrometry (GC-MS) (see Supporting Information for details). Statistical evaluations were performed using StatPlus:Mac (AnalystSoft Inc.; Vancouver, BC, Canada). Significance was determined at the α = 0.05 level using two-tailed testing (see Supporting Information for details).

RESULTS AND DISCUSSION

Biosolids-Exposed Earthworm Burdens. Despite widespread application of biosolids to land, minimal research has examined the bioaccumulation of PBDEs from amended soils, especially under controlled conditions. Here, ∑Penta-PBDE (lipid weight (lw)) burdens in worms exposed to ADB-amended soil for 28 d were 5-fold higher (p < 0.05) than those in the substrate, confirming substantial bioavailability (Figure 1). Exposure levels were environmentally realistic.[22,23,33] Mean

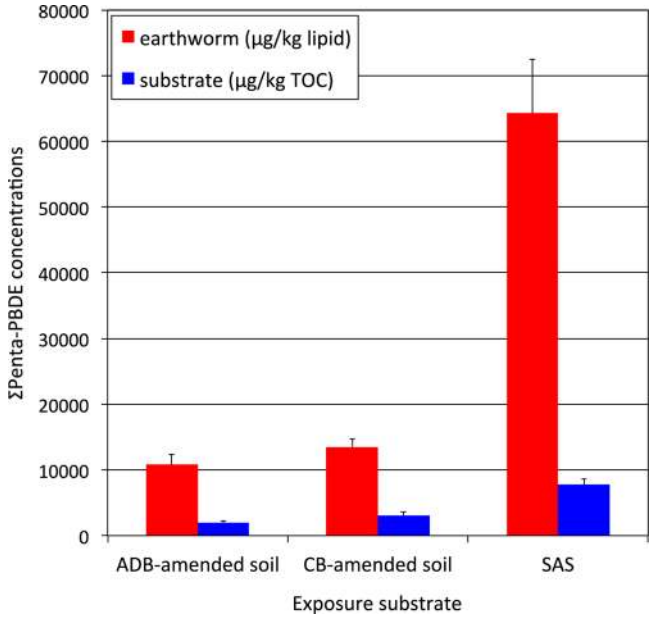

Figure 1. Mean 28 d concentrations of ∑Penta-BDE constituents in earthworms (μg/kg lipid) versus those in *high* dose ADB- and CB-amended soil substrates and the corresponding *low* dose SAS substrate (μg/kg TOC). Error bars represent standard deviations from the means (N = 3).

∑Penta-PBDE burdens in ADB-exposed worms reached 11,000 μg/kg lw in the *high* dose treatment (169 μg/kg) after 28 d, with apparent steady state uptake achieved by 14 d (Figure 1; Figure S1A, Supporting Information). However, a dose−response accumulation pattern was observed in CB-exposed worms after 28 d (Figure S1B, Supporting Information). Tissue burdens generally decreased in the following order: BDE 47 > 99 > 100 > 85 > 154 > 153. BDE 85, 153, 154, and 183 were below QL in all biosolids-exposed worms (and most soil treatments). Our findings are generally consistent with trends reported by Sellström et al.[21] for worms collected from Swedish agricultural fields receiving biosolids. There, worms were a mixture of *Lumbricus*, *Apporectodea*, and *Allolobophora* spp.[20] Their sludge-amended field sites exhibited soil Penta-BDE burdens about 10- to 1000-fold lower (dw) than our ADB-amended treatments, reflective of the much lower Penta-BDE usage in Europe.[10,12] However, one Swedish site showed high soil ∑Penta-BDE burdens (1300

μg/kg dw), putatively related to discharges from a textile manufacturer.[21]

To evaluate relative uptake into tissues and potential biotransformation, BDE 47/99 and 100/99 ratios were calculated for worms and corresponding substrates. Congener ratios were generally comparable with time and substrate, suggesting minimal differential uptake or biotransformation (Table S2, Supporting Information), consistent with other studies of PBDE[21,34,35] and PCB[36] uptake by worms. For ADB-exposed worms, mean BDE 47/99 and 100/99 ratios in the 28 d *high* dose treatments were 1.8 and 0.3, respectively, while corresponding ADB substrate ratios were 1.5 and 0.3, respectively. Ratios of 0.79 and 0.27 were calculated for the commercial Penta-BDE product DE-71 using data from La Guardia et al.[37] For comparison, we calculated a soil BDE 47/99 ratio of 0.76 for the most contaminated soil reported in the Sellström et al. study.[21] Our tissue BDE 47/99 ratios were about 2-fold higher (dw basis) than those calculated from a lab study by Liang et al.[34] wherein *E. fetida* were exposed to PBDE-spiked natural soil. Our higher BDE 47/99 ratios may be due in part to decreased bioavailability of BDE 99 resulting from higher peat levels in our soil. Hofman et al.[38] reported lower bioavailability of phenanthrene to the oligochaete *Enchytraeus albidus* from peat-based artificial soil compared to natural soil. Greater elimination of BDE-99 may also be a factor. Liang et al.[34] reported that uptake and elimination rate constants for BDE 47 were higher than for BDE 99 and 100 in *E. fetida* after 28 d. In contrast, BDE 99 elimination rate constants were three times higher than for BDE 47 in *Lumbriculus variegatus* exposed to Penta-BDE-spiked artificial sediment and the 100% CB product evaluated here.[39] Such disparate results are intriguing and highlight the importance of species, substrate, and exposure mode in quantifying PBDE bioaccumulation by oligochaetes.

CB-exposed worms incurred ∑Penta-PBDE burdens of 13,500 μg/kg lw in the *high* dose treatment after 28 d (Figure 1; Figure S1B, Supporting Information). These burdens are about 4-fold higher ($p < 0.05$) than those measured in the CB-amended soil substrate itself but were not statistically different from ADB-exposed worm burdens. Composted biosolids are typically produced by mixing sludge with wood chips and yard waste and incubating the resulting mixture for weeks under aerobic conditions. Unlike ADB-exposed worms, ∑Penta-PBDE burdens in CB-exposed worms were significantly higher ($p < 0.05$) in 28 d compared to 14 d *high* dose treatments (Figure S1A and B, Supporting Information). Likewise, BDE 47, 99, and 100 burdens in CB-exposed worms were significantly higher ($p < 0.05$) in the 28 d *high* versus *low* dose (Figure S1B, Supporting Information). This may indicate delayed steady state uptake in CB-exposed worms. Extended contact time between soils and contaminants may reduce bioavailability. Hence, experiments wherein chemicals are added via solvent addition immediately before organism exposure may generate higher BSAFs than those using aged or field-collected matrices.[34,40] The presence of plastic microparticles may further reduce POP bioavailability in soils and sediments.[3,4] In both our ADB- and CB-amended soil exposures, incurred PBDEs were present in the biosolids for substantial periods prior to exposure. Also, at least some of the PBDEs therein likely entered the waste stream via fragmentation of current-use or discarded PUF products, as revealed in previous X-ray fluorescence and energy dispersive spectrometry studies of PBDE distributions in indoor dust.[8,9]

Mean TOC contents were similar in all substrates examined (e.g., 8−14%, 7−12%, and 7−11% for the ADB- and CB-amended soils and corresponding SAS, respectively). These are high compared to those of Sellström et al.[21] and Liang et al.[34] who reported 2−7% TOC in sludge-amended field soils and 1.7% TOC for field-collected soils, respectively. Our SAS organic matter was wholly peat-derived and ADB- and CB-amended soil organic matter was supplemented with peat. Significant differences in organic matter composition of sewage sludges have been reported.[41] Though comparable in our ADB- and CB-amended substrates, mean TOC content was higher in the pure ADB (25%) than in the pure CB (9%). Such differences may also reflect compositional variation that contributed to our observed PBDE uptake disparities. For 28 d CB-exposed worms, mean tissue BDE 47/99 and BDE 100/99 ratios were also 1.8 and 0.3, respectively. Ratios were not significantly different with soil dose or exposure duration (Table S2, Supporting Information). In the *high* dose substrate, the mean ratios were 1.4 and 0.3, respectively, and did not differ with time or dose. PBDE congener patterns were also similar in ADB- and CB-amended substrates, consistent with previous findings in sludge-applied United States agricultural soils.[33]

SAS-Exposed Earthworm Burdens. Our *low* dose SAS levels (676 μg/kg dw) were comparable to ∑Penta-PBDE levels reported by Xia et al.[33] (658 μg/kg dw) for natural soils receiving biosolids but about 3.5 to 14 times higher than those used in our biosolids exposures. Our *medium* (2910 μg/kg) and *high* (5630 μg/kg) SAS doses were within the range of those observed in pure biosolids but exceeded PBDE burdens expected in sludge-applied agricultural soils. Such higher doses permitted evaluation of uptake of minor constituents (i.e., PBDEs 85, 153, 154) typically below QL in biosolids-amended field soils. They also permitted exploration of spiking dosage effects on BSAFs. However, quantitative comparisons of PBDE uptake from ADB- and CB-amended substrates are only made here to the more environmentally relevant *low* dose SAS substrate.

A significant ($p < 0.05$) dose-dependent increase in uptake of all PBDE congeners was observed for SAS-exposed worms in 14 and 28 d treatments (Figure S1C and D, Supporting Information). The 28 d tissue ∑Penta-PBDE burdens were substantial, reaching 840,000 μg/kg lw in the *high* dose treatment. Mean worm burdens in 28 d *low* and *medium* dose treatments were 64,300 and 345,000 μg/kg lw, respectively (Figure S1C and D, Supporting Information). No acute toxicity was observed. In our 28 d *low* dose SAS-exposed worms, mean BDE 47/99 and BDE 100/99 ratios were 2.6 and 0.6 compared to 0.9 and 0.2, respectively, in the soil substrate (Table S2, Supporting Information).

Foam Microparticle-Exposed Earthworm Burdens. Despite the role of polymer products as PBDE repositories and the percent PBDE levels therein, direct transfer of these additives from polymers to the environment (or to resident biota) has received minimal attention. However, uptake of PBDEs following ingestion of PUF by house crickets has been reported.[7] Here, *E. fetida* accumulated substantial PBDE concentrations after exposure to soil containing microparticles derived from a commercial PUF product. Despite a 2000-fold dilution of PUF microparticles in AS, mean substrate ∑Penta-PBDE levels reached 83 mg/kg dw (Table S3, Supporting Information).

PBDE uptake from PUF-AS and the corresponding SAS increased with time for all congeners (Figure S2A and B,

Supporting Information). BDE-47 uptake from the PUF-AS mixture was not significantly different from BDE 99 uptake at any time point, but it was significantly different with time from the SAS. Similar trends were also observed for the lesser contributing congeners and tissue congener patterns resembled those of the commercial DE-71 mixture, the PUF and PUF-AS substrate (Figure 2; Figure S2B, Supporting Information).

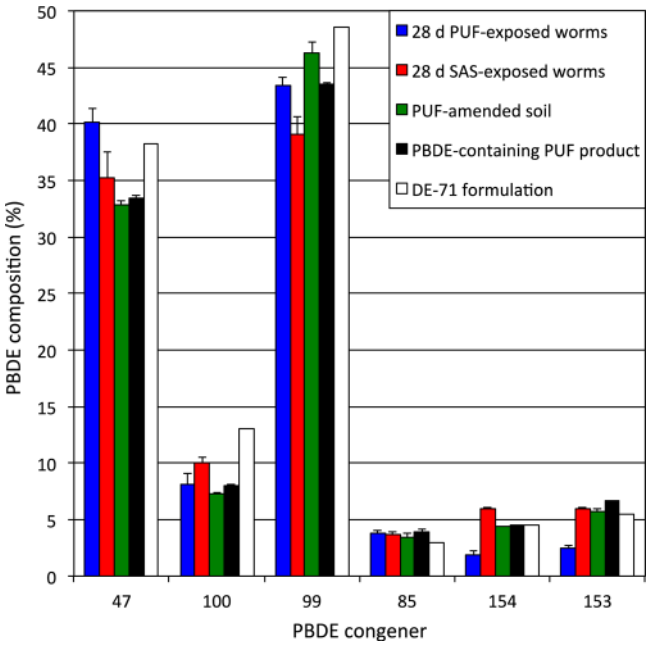

Figure 2. Penta-BDE congener composition in worms exposed to the PUF-AS and SAS substrates for 28 d. Data are compared to PBDE congener composition in the PUF-AS, in the PBDE-containing PUF product, and in the Penta-BDE formulation, DE-71. DE-71 composition data were taken from ref 37. Error bars represent standard deviations from the means (N = 3).

∑Penta-PBDE burdens in worms exposed to PUF-AS ranged from 1890 mg/kg lw (111 mg/kg dw) after 7 d to 3740 mg/kg lw (202 mg/kg dw) after 28 d. ∑Penta-PBDE burdens in worms exposed to the corresponding high dose SAS (5.6 mg/kg dw) ranged from 510 mg/kg lw (55 mg/kg dw) to 840 mg/kg lw (70 mg/kg dw) after 14 and 28 d, respectively (Table S3, Supporting Information). While PUF-AS ∑Penta-PBDE levels were about 15-fold higher than those of the SAS, mean 28 d ∑Penta-PBDE tissue burdens (lw) in PUF-AS-exposed worms were only about 4.5-fold higher than those of SAS-exposed worms. This may reflect reduced PBDE bioavailability from ingested PUF microparticles due to reduced fugacities of polymer-associated additives.

PBDE tissue burdens in the SAS-exposed worms were not significantly different from those of the corresponding SAS-exposed worms examined alongside our biosolids exposures. Unfortunately, our 7 d SAS substrate replicates were lost during sample processing. Nonetheless, the positive trajectories of PBDE uptake from the SAS indicate that the PBDE accumulation had likely not reached steady state by 28 d (Figure S2A and B, Supporting Information). This contrasts with experimental results of previous studies of spiked PBDE uptake by *E. fetida*.[34,40] The presence of PUF microparticles and biosolids in AS likely yield a more heterogeneous distribution of PBDEs consistent with that of elemental bromine reported in house dust.[9] Due to the physicochemical sequestration of PBDEs within the polymer, we anticipated and observed reduced relative uptake of PBDEs from the PUF-AS compared to the SAS substrates, as well as congener signatures similar to the PUF and the commercial Penta-BDE mixture (Figure 2). Nonetheless, PUF-AS-exposed worms accumulated appreciable burdens and thus calculated BSAFs were substantial (Table S5, Supporting Information). This may be due to some retention of PUF microparticles in the gut, despite depuration. Efforts to verify the presence of PUF microparticles in the worm gut by light microscopy were unrevealing. However, Browne et al.[5] reported that microplastic particles were able to cross the gut wall and into the hemolymph of exposed mussels. More study is needed to evaluate the physical fate of PUF microparticles and associated additives in vivo.

Depending upon soil properties, higher soil pollutant concentrations should result in higher worm tissue burdens.[20,21] This was the case for our SAS-exposed worms. Zhu et al.[35] reported similar results for *E. fetida* exposed to increasing doses of solvent-spiked Penta-BDE. To better compare our data to previous lab studies, we calculated 28 d worm tissue-to-soil ∑Penta-PBDE uptake ratios (dw basis) from our data and those reported by Liang et al.[34] and Zhu et al.[35] Uptake ratios were about 49 in the former study and about 20, 30, 31, and 7 for soil doses of 10, 50, 100, and 500 μg/g (dw basis), respectively, in the latter study. Our 28 d ratios were lower, i.e., 2 and 13 for the PUF-AS and SAS treatments, respectively (Figure S3, Supporting Information). Interestingly, relative PBDE uptake from our PUF-AS and SAS substrates decreased with increasing soil concentrations, while Zhu et al.[35] observed increased relative uptake with increasing soil dose in all but their highest dose treatment.

Earthworm Biota-Soil Accumulation Factors (BSAFs). BSAFs varied with substrate, dose, and exposure duration. In 28 d ADB-exposed worms, BSAFs for BDEs 47, 99, and 100, decreased significantly with increasing dose (*low* to *high*; $0.0001 < p < 0.01$). A similar dose-dependent response was observed in CB-exposed worms ($0.001 < p < 0.05$) (Figure 3; Table S4, Supporting Information). Given the profound behavioral changes and mortality observed in worms exposed to higher biosolids doses during preliminary testing, this trend may reflect a reduction in assimilation due to behavior modification or toxicity. In contrast, BSAF trends in SAS-exposed worms were generally toward increasing values with time and dose (Table S4, Supporting Information). These trends suggest influences by other matrix constituents.

Our SAS uptake results are interesting in both corroborating and contrasting those of Nyholm et al.[40] for SAS-exposed *E. fetida*. There, BSAFs increased with spiking dosage to a point and then decreased significantly at their highest dosage. On the whole, our BSAFs for 28 d *low* and *medium* dose biosolids-exposed worms were comparable to those of our SAS-exposed worms (Table S4, Supporting Information). For comparison, BSAFs calculated for worms collected from Swedish biosolids-amended fields were 5, 4.2, 4.6, 2.5, and 2.3 for BDEs 47, 99, 100, 153, and 154, respectively.[21] These are generally consistent with those reported for *L. variegatus* exposed to PBDE-spiked lake sediments[42] and to our 100% CB,[39] as well as those reported for *E. fetida* in PBDE-spiked natural soil.[34] BSAFs calculated for our *high* dose ADB- and CB-exposed worms are comparable to these reports. However, our *low* and *medium* dose BSAFs for these substrates are considerably higher. Likewise, most of our BSAFs for BDE-47, 100, and 99 in SAS-

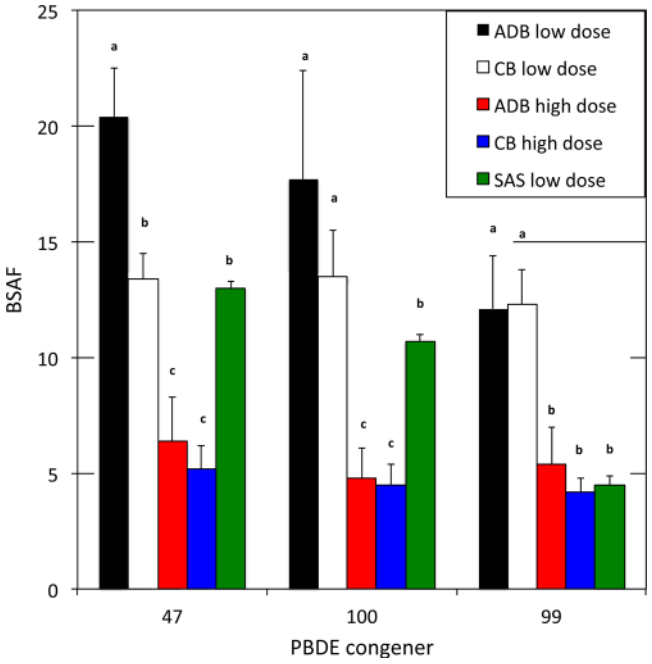

Figure 3. Mean 28 d earthworm BSAFs for BDE 47, 100, and 99. *Low* and *high* dose ADB- and CB-amended soil substrate and corresponding *low* dose SAS substrate exposure results are presented. For each congener, bars with different letters are statistically different (ANOVA; $p < 0.05$). Error bars represent standard deviations from the means ($N = 3$).

exposed worms were higher (Figure 3; Figure S4, Tables S4 and 5, Supporting Information).

These results contrast with Hofman et al.[38] who reported significantly reduced PAH bioavailability from solvent-spiked artificial compared to natural soil. Nyholm et al.[40] exposed *E. fetida* to artificial soil spiked with BDE-47, 99, and 153 at 10, 100, and 10,000 μg/kg dw and reported maximum mean BSAFs for these congeners of about 11, 9, and 4, respectively. Here, maximum mean BSAFs for worms exposed to the same PUF-associated PBDEs were about 3-fold higher (Figure S4, Table S5, Supporting Information), while those calculated for our SAS-exposed worms were about 17, 21, and 4, respectively. Our ADB- and CB-exposed worm BSAFs generally tracked those calculated for solvent spiked soil-exposed worms at comparable soil concentrations by Nyholm et al.[40] Interestingly, Blankenship et al.,[36] in a comparative study of PCB bioaccumulation at a Superfund site (soil $\sum$PCBs = 6500 μg/kg ww) and a reference site (soil $\sum$PCBs = 9 μg/kg ww), calculated about 5-fold higher BSAFs for worms at their reference site than for worms at their contaminated site. Decreasing BSAFs with increasing concentration has also been reported for other HOCs. For example, Millward et al.[43] reported a lower BSAF with increasing pyrene sediment concentration for exposed aquatic oligochaetes (*Limnodrilus hoffmeisteri*) and attributed this to decreased pore water and/or gut fluid solubility of the higher sediment burdens. Similar trends were observed for *L. variegatus* exposed to spiked PCB-77 and were attributed to compound-particle disequilibrium.[44]

At steady state, HOC partitioning from soil particles into organism lipids should increase with compound log K_{ow} up to a threshold value of about 7. Here, BSAFs decreased consistently with increasing log K_{ow} for worms exposed to all substrates (Figure 4A, B). For biosolids-exposed worms, slopes were

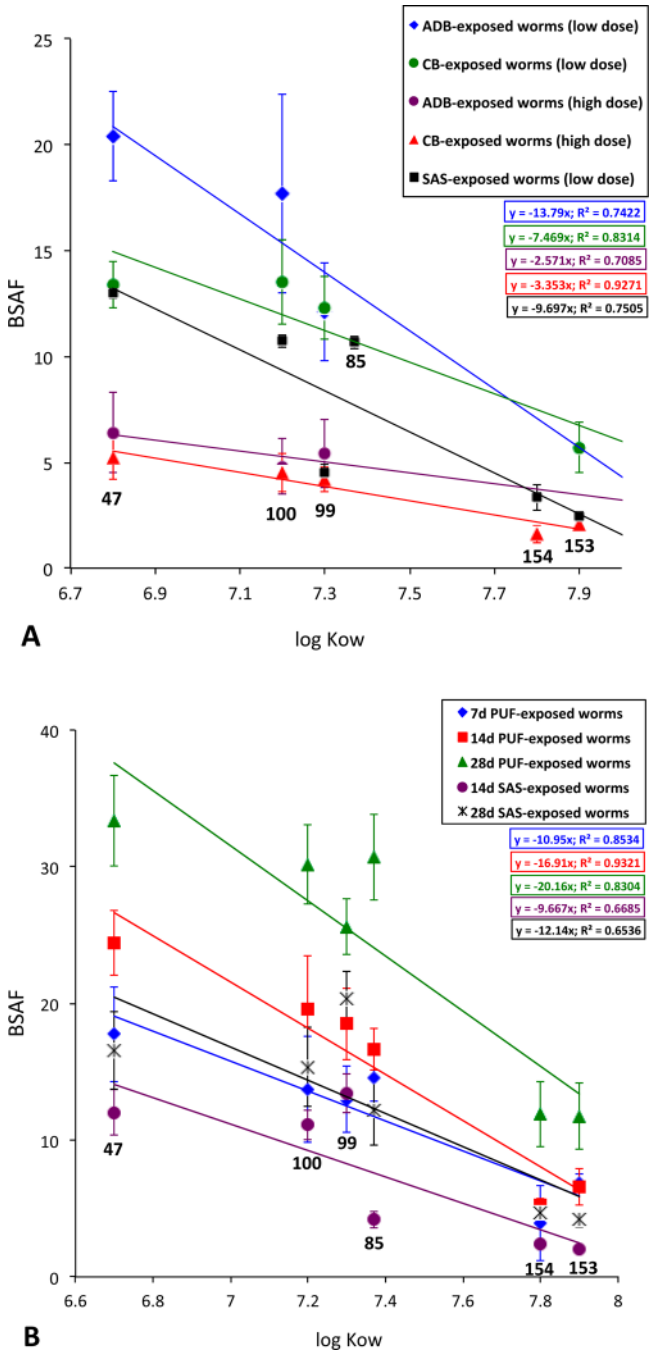

Figure 4. (A) Least squares regression analyses of mean 28 d Penta-BDE BSAFs for *low* and *high* dose ADB- and CB-exposed worms and corresponding *low*-dose SAS-exposed worms versus published log K_{ow} values. Log K_{ow} data were taken from ref 52. Congeners for which data are missing were < QL. Error bars represent standard deviations from the means ($N = 3$). (B) Least squares regression analyses of Penta-BDE BSAFs for PUF- and corresponding SAS-exposed worms versus published log K_{ow} values. Log K_{ow} data were taken from ref 52. Error bars represent standard deviations from the means ($N = 3$).

significantly lower ($p < 0.05$) in *high* compared to *low* dose soil treatments. These trends are consistent with those reported for mixed worm species collected from Swedish agricultural fields receiving biosolids,[21] *E. fetida* exposed to solvent-spiked soil,[34] and *Lumbricus terrestris* exposed to PCB-contaminated natural soils.[45]

Lipids are essential energy stores, and their status may reflect overall organism fitness. Here, differences in worm total extractable lipids were not observed with increasing biosolids dose. However, total lipids in both SAS-exposed and control worms were significantly lower ($p < 0.05$) in 28 d than in 14 d treatments (Table S6, Supporting Information). This may reflect the lower nutrient value of the peat-amended AS relative to the biosolids-amended soil. Total lipids in PUF-AS-exposed worms were about half those in biosolids- and SAS-exposed and control worms and did not differ with time (Tables S6, S7, Supporting Information). This too may be indicative of nutritional or other physiological stress in worms ingesting PUF microparticles. Numerically, this would inflate lipid-normalized tissue burdens in PUF-AS-exposed worms. Nonetheless, PBDE uptake by PUF-AS-exposed worms still increased steadily with time.

Coincident PBDE Accumulation by PSDs. To evaluate their biomimetic potential to accumulate PBDEs, we examined PSDs immersed in ADB-amended soil substrates coincident with worms. Many plastics effectively sorb lipophilic contaminants. For example, Teuten et al.[3] reported 100-fold or greater equilibrium partition coefficients for phenanthrene binding to polyethylene particulates compared to natural marine sediments. Others have examined the potential of various PSD materials to sorb POPs from soils, including Tenax and PBDEs,[34] C_{18} silica-filled polyethylene and organochlorine pesticides,[46] and solid-phase microextraction (SPME) fibers and organochlorine POPs.[47] Here, mean PSD ∑Penta-PBDE concentrations ranged from 47 to 142 μg/kg dw, comparable to those of their respective substrate doses (Table S8, Supporting Information). PSD BDE 47 levels (dw basis) exceeded those in the corresponding substrate ($p < 0.05$) after 28 d, while BDE 100 and 99 PSD burdens were lower ($p < 0.05$ and $p < 0.01$, respectively) (Figure 5). The lower accumulation of the latter congeners may relate to hysteresis in their release and subsequent transfer from substrate to PSD. PSD uptake of BDE 153 and 154 was below QL, likely due to a combination of low substrate concentrations and uptake hysteresis. As PSDs are stationary, localized depletion of available PBDEs may also occur.

In contrast to PSDs, worms are mobile and continuously ingest soil amounts 5−30 times their body weight per day.[25] While BDE 47, 99, and 100 were quantifiable in our PSDs, burdens were about 1% of those in ADB-exposed worms. This is consistent with the findings of Strandberg et al.[48] who reported that triolein-filled SPMDs sampled only about 1% of organochlorine pollutants incurred in household compost. In support of the importance of feeding behavior, Jager et al.[49] observed reduced uptake of POPs in *E. andrei* prevented from ingesting POPs-spiked soil. Similar results have also been reported for *L. variegatus* exposed to PBDE-spiked sediments.[42] Experiments in which PSDs were immersed in 100% ADB for 28 d also indicated preferential accumulation (i.e., BDE 47 > 99 > 100), despite higher BDE 99 burdens in the 100% ADB (Figure S5, Table S1, Supporting Information). Liang et al.[34] reported greater uptake of BDE 47 and 99 relative to BDE 100 from Penta-BDE-spiked natural soil by *E. fetida*. They also reported that PBDE accumulation on Tenax followed similar patterns and equated this to its efficient sampling of the more rapidly desorbing soil fraction. Here, PSD uptake of congeners from 100% ADB was statistically different ($p < 0.05$) for all but the $t = 3$ d time point (i.e., BDE 47 > 99 > 100). ∑Penta-PBDE levels in our 100% ADB were about 56-fold higher (dw basis) than spiking levels examined by Liang et al.[34] Thus, to better compare PSD accumulation to that seen in our ADB-exposed worms and to the Liang et al.[34] data, we calculated PSD accumulation factors (PAFs) (Table S9, Supporting Information) and evaluated PSD and worm burdens (lw) using least-squares regression. These data were well correlated regardless of time and treatment dose (see Supporting Information for discussion).

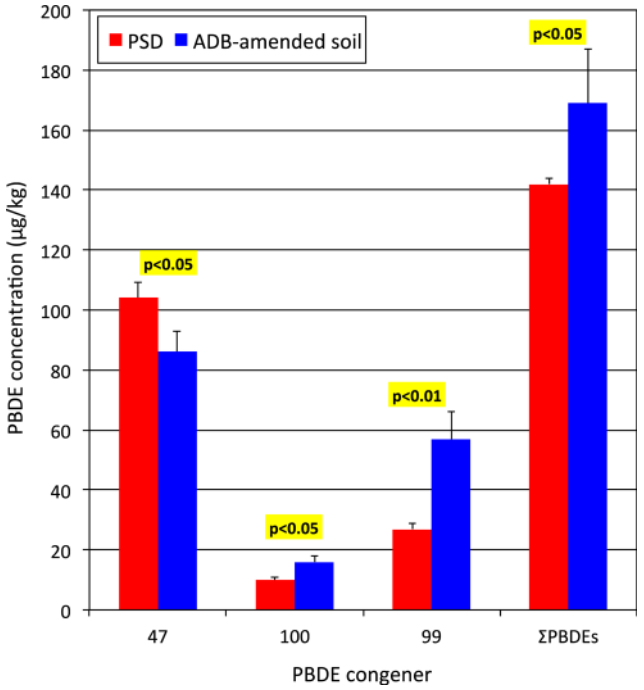

Figure 5. Mean 28 d BDE 47, 99, 100, and ∑PBDE concentrations (μg/kg) in polyethylene passive sampling devices (PSDs) relative to those in the corresponding *high* dose ADB-amended soil (μg/kg dw). Statistically significant concentration differences are indicated above each pair of data points. Error bars represent standard deviations from the means ($N = 3$).

Previous lab studies have demonstrated the bioavailability of PBDEs from solvent-spiked soils. However, our results show that PBDEs are similarly bioavailable from soils amended with biosolids generated by widely used and distinct wastewater treatment processes. Class B biosolids (our ADB) are the most commonly land-applied type in North America and composted biosolids (our CB) are promoted as pathogen-free with minimal contaminant burdens. Further, their distribution to (and use by) the general public is unregulated and untracked. Some data on PBDE burdens in worms collected from historically sludge-amended fields are available. However, associated exposure mechanisms may be confounded by uncontrolled factors. BSAFs derived from our controlled exposures of worms to well-characterized biosolids-amended soils (dependent upon dose) are comparable to values reported for worms of several species obtained from biosolids-amended agricultural soils in Sweden.[20,21] This is important, as *E. fetida* is not common in agricultural soils and species-specific behavior in soils could influence contaminant accumulation. For example, Kelsey et al.[50] reported that DDE accumulation from natural soils by *E. andrei* (epigeic species) was significantly higher than uptake by *L. terrestris* (anecic species) and *A. caliginosa* (endogeic species). In contrast, van der Wal et al.[47] and Jager et al.[51] found that uptake of PCBs and organo-

chlorine pesticides from natural soils by *E. andrei* and *A. caliginosa* were comparable. From our PUF bioassay results, it is also clear that worms in contact with soils containing PUF microparticles (such as have been observed in indoor dust) can accumulate substantial amounts of PBDEs. These may be subsequently transferred throughout terrestrial food webs and translocated to different soil strata and/or locales via organismal movement. Hence, we believe that more rigorous evaluation of the direct transfer of additives from consumer polymers to the environment and resident biota is merited.

■ ASSOCIATED CONTENT

Supporting Information

Additional details on analytical methodology, quality control/quality assurance (QA/QC), supporting experimental, results and discussion, and references, as well as supporting Tables S1QC−S2QC, Tables S1−S9, and Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ REFERENCES

(1) Municipal Solid Waste (MSW) in the United States: Facts and Figures, U.S. Environmental Protectin Agency, 2013. http://www.epa.gov/osw/nonhaz/municipal/msw99.htm (accessed August 18, 2013).

(2) Ryan, P. G.; Connell, A. D.; Gardener, B. D. Plastic ingestion and PCBs in seabirds: Is there a relationship? *Mar. Pollut. Bull.* **1988**, *19*, 174−176.

(3) Teuten, E. L.; Rowland, S. J.; Galloway, T. S.; Thompson, R. C. Potential for plastics to transport hydrophobic contaminants. *Environ. Sci. Technol.* **2007**, *41*, 7759−7764.

(4) Teuten, E. L.; et al. Transport and release of chemicals from plastics to the environment and to wildlife. *Phil. Trans. R. Soc., B* **2009**, *364*, 2027−2045.

(5) Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol.* **2008**, *42*, 5026−5031.

(6) Stapleton, H. M.; Klosterhous, S.; Keller, A.; Ferguson, P. L.; van Bergen, S.; Cooper, E.; Webster, T. F.; Blum, A. Identification of flame retardants in polyurethane foam collected from baby products. *Environ. Sci. Technol.* **2011**, *45*, 5323−5331.

(7) Gaylor, M. O.; Harvey, E.; Hale, R. C. House Crickets can accumulate polybrominated diphenyl ethers (PBDEs) directly from polyurethane foam common in consumer products. *Chemosphere* **2012**, *86*, 500−505.

(8) Allen, J. G.; McClean, M. D.; Stapleton, H. M.; Webster, T. F. Linking PBDEs in house dust to consumer products using x-ray fluorescence. *Environ. Sci. Technol.* **2008**, *42*, 4222−4228.

(9) Webster, T. F.; Harrad, S.; Millette, J. R.; Holbrook, R. D.; Davis, J. M.; Stapleton, H. M.; Allen, J. G.; McClean, M. D.; Ibarra, C.; Abdallah, M. A.; Covaci, A. Identifying transfer mechanisms and sources of decabromodiphenyl ether (BDE 209) in indoor environments using environmental forensic microscopy. *Environ. Sci. Technol.* **2009**, *43*, 3067−3072.

(10) Hale, R. C.; La Guardia, M. J.; Harvey, E.; Gaylor, M. O.; Mainor, T. M.; Duff, W. H. Persistent pollutants in land-applied sludge. *Nature* **2001**, *412*, 140−141.

(11) Hale, R. C.; La Guardia, M. J.; Harvey, E.; Mainor, T. M. Potential role of fire retardant-treated polyurethane foam as a source of brominated diphenyl ethers to the US environment. *Chemosphere* **2002**, *46*, 729−735.

(12) Hale, R. C.; La Guardia, M. J.; Harvey, E.; Gaylor, M. O.; Mainor, T. M. Brominated flame retardant concentrations and trends in abiotic media. *Chemosphere* **2006**, *64*, 181−186.

(13) Hale, R. C.; Kim, S. L.; Harvey, E.; La Guardia, M. J.; Mainor, T. M.; Bush, E. O.; Jacobs, M. J. Antarctic research bases: local sources of polybrominated diphenyl ether (PBDE) flame retardants. *Environ. Sci. Technol.* **2008**, *42*, 1452−1457.

(14) Andrady, A. L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596−1605.

(15) Johnson, P. I.; Stapleton, H. M.; Sjodin, A.; Meeker, J. D. Relationships between polybrominated diphenyl ether concentrations in house dust and serum. *Environ. Sci. Technol.* **2010**, *44*, 5627−5632.

(16) Kinney, C. A.; Furlong, E. T.; Zaugg, S. D.; Burkhardt, M. R.; Werner, S. L.; Cahill, J. D.; Jorgensen, G. R. Survey of organic wastewater contaminants in biosolids destined for land application. *Environ. Sci. Technol.* **2006**, *40*, 7207−7215.

(17) Davis, E. F.; Klosterhous, S.; Stapleton, H. M. Measurement of flame retardants and triclosan in municipal sewage sludge and biosolids. *Environ. Int.* **2012**, *40*, 1−7.

(18) *Emerging Technologies for Biosolid Management*; EPA 832-R-06-005; U.S. Environmental Protection Agency, Office of Water: Washington, DC, 2006.

(19) *Targeted National Sewage Sludge Survey Sampling and Analysis Technical Report*; EPA-822-R-08-016; U.S. Environmental Protection Agency, Office of Water: Washington, DC, 2009. http://www.epa.gov/waterscience/biosolids/tnsss-stat.pdf (accessed August 18, 2013).

(20) Matscheko, N.; Tysklind, M.; De Wit, C.; Bergek, S.; Andersson, R.; Sellström, U. Application of sewage sludge to arable land-soil concentrations of polybrominated diphenyl ethers and polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls, and their accumulation in earthworms. *Environ. Toxicol. Chem.* **2002**, *21*, 2515−2525.

(21) Sellström, U.; de Wit, C. A.; Lundgren, N.; Tysklind, M. Effect of sewage-sludge application on concentrations of higher brominated diphenyl ethers in soils and earthworms. *Environ. Sci. Technol.* **2005**, *39*, 9064−9070.

(22) Andrade, N. A.; McConnell, L. L.; Torrents, A.; Ramirez, M. Persistence of polybrominated diphenyl ethers in agricultural soils after biosolids applications. *J. Agric. Food Chem.* **2010**, *58*, 3077−3084.

(23) Hale, R. C.; La Guardia, M. J.; Harvey, E.; Chen, D.; Mainor, T. M.; Luellen, D. R. Polybrominated diphenyl ethers in U.S. sewage sludges and biosolids: temporal and geographical trends and uptake by corn following land application. *Environ. Sci. Technol.* **2012**, *46*, 2055−2063.

(24) Gorgy, T.; Li, L. Y.; Grace, J. R.; Ikonomou, M. G. Migration of polybrominated diphenyl ethers in biosolids-amended soil. *Environ. Pollut.* **2013**, *172*, 124−130.

(25) Coleman, D. C., Crossley, D. A., Hendrix, P. F. *Fundamentals of Soil Ecology*; Academic Press: New York, 2004.

(26) Vermeulen, F.; Covaci, A.; D'Havé, H.; Van den Brink, N. W.; Blust, R.; De Coen, W.; Bervoets, L. Accumulation of background levels of persistent organochlorine and organobromine pollutants through the soil-earthworm-hedgehog food chain. *Environ. Int.* **2010**, 721−727.

(27) Lepom, P., Berndt, M., Duffek, A., Warmbrunn-Suckrow, E. Oral Bioaccessibility of PBDEs in Dust Using an in Vitro Gastrointestinal Model. BFR2010: Fifth International Symposium on Brominated Flame Retardants, April 7−9, 2010, Kyoto Japan. http://www.bfr2010.com/abstract-download/2010/90145.pdf (accessed August 18, 2013).
(28) Huwe, J. K.; Hakk, H.; Smith, D. J.; Diliberto, J. J.; Richardson, V.; Stapleton, H. M.; Birnbaum, L. S. Comparative absorption and bioaccumulation of polybrominated diphenyl ethers following ingestion via dust and oil in male rats. *Environ. Sci. Technol.* **2008**, *42*, 2694−2700.
(29) Mattila, T. J.; Verta, M. Modeling the importance of biota and black carbon as vectors of polybrominated diphenyl ethers (PBDEs) in the Baltic Sea ecosystem. *Environ. Sci. Technol.* **2008**, *42*, 4831−4836.
(30) Ter Laak, T. L.; Van Eijkeren, J. C. H.; Busser, F. J. M.; Van Leeuwen, H. P.; Hermens, J. L. M. Facilitated transport of polychlorinated biphenyls and polybrominated diphenyl ethers by dissolved organic matter. *Environ. Sci. Technol.* **2008**, *43*, 1379−1385.
(31) *Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm Eisenia fetida*; ASTM E 1676-97; Committee E-47 on Biological Effects and Environmental Fate, ASTM: West Conshohocken, PA, 1997.
(32) Chen, D.; La Guardia, M. J.; Harvey, E.; Amaral, M.; Wohlfort, K.; Hale, R. C. Polybrominated diphenyl ethers in Peregrine Falcon (*Falco peregrinus*) eggs from the northeastern US. *Environ. Sci. Technol.* **2008**, *42*, 7594−7600.
(33) Xia, K.; Hundal, L. S.; Kumar, K.; Armbrust, K.; Cox, A. E.; Granato, T. C. Triclocarban, triclosan, polybrominated diphenyl ethers and 4-nonylphenol in biosolids and in soil receiving 33-year biosolids application. *Environ. Toxicol. Chem.* **2010**, *29*, 597−605.
(34) Liang, X.; Zhu, S.; Chen, P.; Zhu, L. Bioaccumulation and bioavailability of polybrominated diphenyl ethers (PBDEs) in soil. *Environ. Pollut.* **2010**, *158*, 2387−2392.
(35) Zhu, S.; Liu, M.; Tian, S.; Zhu, L. Bioaccumulation and single and joint toxicities of penta-BDE and cadmium to earthworms (*Eisenia fetida*) exposed to spiked soils. *Sci. China Chem.* **2010**, *53*, 1025−1032.
(36) Blankenship, A. L.; Zwiernik, M. J.; Coady, K. E.; et al. Differential accumulation of polychlorinated biphenyl congeners in the terrestrial food web of the Kalamazoo River superfund site, Michigan. *Environ. Sci. Technol.* **2005**, *39*, 5954−5963.
(37) La Guardia, M. J.; Hale, R. C.; Harvey, E. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used Penta-, Octa-, and Deca-PBDEs technical flame-retardant mixtures. *Environ. Sci. Technol.* **2006**, *40*, 6247−6254.
(38) Hofman, J.; Rhodes, A.; Semple, K. T. Fate and behaviour of phenanthrene in the natural and artificial soils. *Environ. Pollut.* **2008**, *152*, 468−475.
(39) Ciparis, S.; Hale, R. C. Bioavailability of polybrominated diphenyl ether flame retardants in biosolids and spiked sediment to the aquatic oligochaete (*Lumbriculus variegatus*). *Environ. Toxicol. Chem.* **2005**, *24*, 916−925.
(40) Nyholm, J. R.; Asamoah, R. K.; van der Wal, L.; Danielsson, C.; Andersson, P. L. Accumulation of polybrominated diphenyl ethers, hexabromobenzene, and 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane in earthworm (*Eisenia fetida*): Effects of soil type and aging. *Environ. Sci. Technol.* **2010**, *44*, 9189−9194.
(41) Smernik, R. J.; Oliver, I. W.; Merrington, G. Characterization of sewage sludge organic matter content using solid-state carbon-13 nuclear magnetic resonance spectroscopy. *J. Environ. Qual.* **2003**, *32*, 1516−1522.
(42) Leppänen, M. T.; Kukkonen, J. V. K. Toxicokinetics of sediment-associated polybrominated diphenyl ethers (flame retardants) in benthic invertebrates (*Lumbriculus variegatus*: Oligochaeta). *Environ. Toxicol. Chem.* **2004**, *23*, 166−172.
(43) Millward, R. N.; Fleeger, J. W.; Reible, D. D.; Keteles, K. A.; Cunningham, B. P.; Zhang, Li. Pyrene bioaccumulation, effects of pyrene exposure on particle-size selection and fecal pyrene content in the oligochaete, *Limnodrilus hoffmeisteri* (Tubificidae, Oligochaeta). *Environ. Toxicol. Chem.* **2001**, *20*, 1359−1366.
(44) Sormunen, A. J.; Leppänen, M. T.; Kukkonen, J. V. K. Influence of sediment ingestion and exposure concentration on the bioavailable fraction of sediment-associated tetrachlorobiphenyl in oligochaetes. *Environ. Toxicol. Chem.* **2008**, *27*, 854−863.
(45) Krauss, M.; Wilcke, W.; Zech, W. Availability of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) to earthworms in urban soils. *Environ. Sci. Technol.* **2000**, *34*, 4335−4340.
(46) Awata, H.; Cobb, G. P.; Anderson, T. A. A chemical test for determining biological availability of aged chemicals in soil. *Int. J. Environ. Anal. Chem.* **2000**, *78*, 41−49.
(47) van der Wal, L.; Jager, T.; Fleuren, R. H. L. J.; Barendregt, A.; Sinnige, T. L.; Van Gestel, C. A. M.; Hermens, J. L. M. Solid-phase microextraction to predict bioavailability and accumulation of organic micropollutants in terrestrial organisms after exposure to field-contaminated soil. *Environ. Sci. Technol.* **2004**, *38*, 4842−4848.
(48) Strandberg, B.; Wågman, N.; Bergqvist, P.; Haglund, P.; Rappe, C. Semipermeable membrane devices as passive samplers to determine organochlorine pollutants in compost. *Environ. Sci. Technol.* **1997**, *31*, 2960−2965.
(49) Jager, T.; Fleuren, R. H. L. J.; Hogendoorn, E. A.; De Korte, G. Elucidating routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta). *Environ. Sci. Technol.* **2003**, *37*, 3399−3404.
(50) Kelsey, J. W.; Colino, A.; White, J. C. Effects of species differences, pollutant concentration and residence time in soil on the bioaccumulation of 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene by three earthworm species. *Environ. Toxicol. Chem.* **2005**, *24*, 703−708.
(51) Jager, T.; van der Wal, L.; Fleuren, R. H. L. J.; Barendregt, A.; Hermens, J. L. M. Bioaccumulation of organic chemicals in contaminated soils: Evaluation of bioassays with earthworms. *Environ. Sci. Technol.* **2005**, *39*, 293−298.
(52) Braekevelt, E.; Tittlemier, S. A.; Tomy, G. T. Direct measurement of octanol−water partition coefficients of some environmentally relevant brominated diphenyl ether congeners. *Chemosphere* **2003**, *51*, 563−567.