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Bioavailability of biosolids- and consumer product-associated polybrominated diphenyl ether (PBDE) flame retardants to terrestrial invertebrates

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Bioavailability of Biosolids- and Consumer Product-Associated Polybrominated Diphenyl Ether (PBDE) Flame Retardants to Terrestrial Invertebrates

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Michael O. Gaylor

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APPROVAL SHEET

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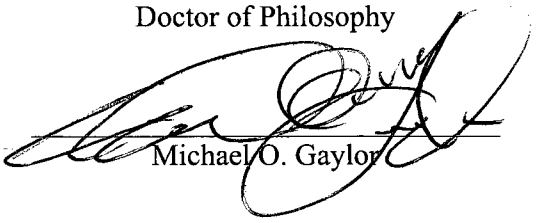

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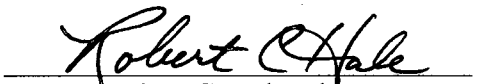

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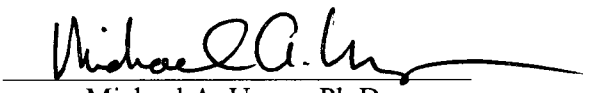

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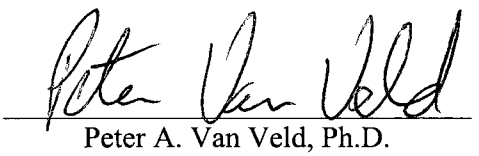

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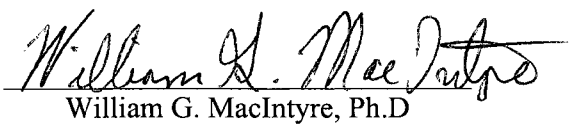

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It is surely cliché by now to profess that mere words are insufficient to convey the abyssal depths of my gratitude to those many in my life who have both facilitated and tolerated my sinuous sojourn in pursuit of this goal. A wise man once said that "...no man is an island..." For my own part, this could not be more accurate sentiment. So many have participated in, and sacrificed for, my attainment of this quite formidable and very special goal.

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ABSTRACT

Polybrominated diphenyl ether (PBDE) flame retardants have been widely used to flame retard consumer products, including polyurethane foam (PUF), thermoplastics and other polymers in home and office furnishings, vehicles, and electronics. Penta-BDE is used primarily in PUF. Deca-BDE is common in thermoplastics and textile back-coatings. Octa-BDE is a lesser-used product for wire coatings and thermoplastics. PBDEs are structurally similar to the persistent, bioaccumulative and toxic PCBs. PCB usage was accordingly discontinued in the mid-1970s. In contrast, PBDEs have continued to be intentionally added to consumer products at percent (by weight) levels. As PBDEs are not chemically reacted to polymers, they may migrate from products over time. Polymers themselves may deteriorate due to weathering and dispersion of the resulting fragments may increase exposure. Releases have resulted in the global distribution of PBDEs. Presently, ingestion of contaminated indoor dust is believed to be a major exposure route for humans. Transmission and bioavailability of PBDEs in the environment is poorly characterized. Despite most PBDEs being used in, released to and remaining in the terrestrial environment, less is known about their accumulation and fate in ecologically important resident invertebrates (e.g. crickets, spiders etc.) than in aquatic organisms or avian species. Some of these invertebrates frequent human habitations or are known to directly consume textiles and polymers. Exposure may also occur when organisms come into contact with discarded PBDE-treated products or other PBDE-containing materials.

Polymer products are manufactured in massive quantities. These in turn are delivered to consumers throughout the world, even to remote locations such as the Polar Regions. PBDE-laden particles may be transported long distances via wind, precipitation and biota and be deposited in soils, wastewater streams and ultimately sewage sludge. PBDEs have been detected at particularly high levels in North American sewage sludges. The pattern of the lower brominated congeners (i.e. 2 to 6 bromines) in sludge is strikingly similar to the Penta-BDE mixture used to flame retarded PUF. This supports the view that releases of and from PUF directly to sludge may play a major role in the observed PBDE burdens. About 60% of all sludge produced in the US is now disposed of via land application. Taken together, product- and sludge-derived PBDE emissions to the environment are enormous. Yet, our understanding of PBDE dispersal pathways and attendant bioavailability from soils receiving PBDE-containing materials such as biosolids is woefully incomplete. The primary objectives of this research were therefore to evaluate PBDE bioavailability to ecologically distinct soil invertebrates exposed to Penta-BDE-treated consumer PUF products and biosolid products with incurred PBDEs.

In laboratory bioassays, earthworms (*Eisenia fetida*) bioaccumulated ∑PBDEs (47+99+100+183) up to 11,000 μg/kg lipid after 28 days from a mixture of artificial soil and anaerobically-digested sludge biosolid (ADB). Earthworms also bioaccumulated ∑PBDEs (47+99+100+153+154+183) up to 13,500 and 838,000 μg/kg lipid after 28 d from a mixture of artificial soil and composted sludge biosolid (CB) and Penta-BDE-spiked artificial soil (SAS), respectively. No previous lab studies on bioaccumulation of PBDEs from sludge or sludge-amended soils have been published. Two publications, done by the same research group on the same sites in Sweden, do exist documenting

incidental levels of PBDEs in worms collected from historically sludge-amended agricultural fields. In the current research, biota-soil accumulation factors (BSAFs) were appreciably higher (dependent upon dose) than those reported in these Swedish field studies. PBDE usage in Sweden has been much less intense than in North America. Increasing BSAFs with decreasing biosolids-amended soil burdens may indicate that PBDE constituents within these biosolid products may impact earthworms at relatively low levels in soil.

Dispersal of Penta-BDE-containing PUF particles from indoor dust, deteriorating discarded polymer products, and from sewage sludge intentionally applied to land, contributes to environmental burdens. Hence, PBDE uptake by (and bioavailability to) soil biota was explored by dispersing Penta-BDE-containing PUF particles (< 75 μm) in artificial soil containing *E. fetida*. PUF particle dilution in the soil was approximately 1:2000. Earthworms ingested this mixture and accumulated ∑PBDEs (47+85+99+100+153+154) up to 3,740,000 μg/kg lipid after 28 days with no observed evidence of toxicity. Biota-soil accumulation factors (BSAFs) ranged from 4 to 33. These results indicate that PBDEs present in commercially used PUF products may be bioavailable to worms and their presence in the environment may result in the accumulation of appreciable burdens in such soil invertebrates. These may then be transferred to other sectors of the terrestrial food web.

The potential for a terrestrial arthropod, the house cricket (*Acheta domesticus*) to take up PBDEs directly from consumer PUF was also evaluated in a laboratory bioassay. These insects frequent indoor spaces and discarded materials and hence may have increased access (and thus exposure) to PBDE-treated polymers. Cricket nymphs were

reared in proximity to a commercially manufactured Penta-BDE-treated PUF. They accumulated ∑PBDEs (47+85+99+100+153+154) up to 14,200 μg/kg lipid after 28 days. Non-depurated crickets ingested ∑PBDE burdens up to 80,600 μg/kg lipid. Owing to the high PBDE content of the PUF (9% by weight) and the fact that much of it likely remained within the polymer matrix, cricket/PUF bioaccumulation factors (BAFs) were on the order of 10^{-4} to 10^{-3} for all PBDE congeners.

To evaluate real-world biosolid-associated PBDE bioavailability in the soil-associated terrestrial environment, a food web bioaccumulation study was conducted within a Mid-Atlantic US agricultural soil ecosystem receiving long-term (>20 years) sludge amendments. A managed pastoral ecosystem receiving only animal manure was examined for comparison. The reference site was formerly forested. PBDEs were below quantitation limits (BQL) in all samples collected from the former site. At the sludge-applied site, $\sum PBDE_{3\text{-}8}$ and $\sum PBDE_{9\text{-}10}$ in soils were 17,600±2330 μg/kg TOC (263±34 dw) and 7500±2770 μg/kg TOC (86±32 μg/kg dw), respectively. PBDEs were BQL in the dominant sludge-amended site vegetation, gamagrass (*Tripsacum dactyloides*). In contrast, PBDE bioaccumulation by native earthworms and soil arthropods was observed. $\sum PBDE_{3\text{-}8}$ and $\sum PBDE_{9\text{-}10}$ burdens in earthworms were 10,300±2670 μg/kg and 6490±4120 μg/kg (lipid), respectively. Highest arthropod $\sum PBDE_{3\text{-}8}$ burdens were 3000±227 μg/kg lipid in woodlice. However, $\sum PBDE_{9\text{-}10}$ was BQL in these organisms. BDE-209 burdens were detected at 86,500 μg/kg lipid in the single millipede composite obtained. High levels were also detected in one of three June beetle larvae composites. While sample contamination in the lab is possible, no PBDEs were quantifiable in any of the samples from the non-sludge amended site or in field and lab blanks. In addition to

earthworms, $\sum PBDE_{9\text{-}10}$ was also quantifiable in ground beetles (mean 5820±3843 µg/kg lipid) and in one of three cricket composites (1440 µg/kg lipid) from the sludge-amended field. BDE47/99 ratio patterns suggested differential uptake/elimination in dissimilar soil arthropods. Patterns of those with most intimate contact and reliance on the soil (e.g. earthworms) most closely reflected the soil/biosolid/commercial Penta-BDE fingerprint. PBDEs were BQL in the herbivorous grasshopper, in contrast to the closely related cricket, an omnivorous scavenger. Surprisingly, PBDEs were largely BQL in predaceous wolf spiders regardless of size or trophic level. Penta-BDE constituent biota-soil accumulation factors (BSAFs) ranged from 0.006 (crickets) to 1.2 (earthworms), while BDE-209 BSAFs ranged from 0.07 (earthworms) to 10.5 (millipedes). Lipid and TOC normalized PBDE burdens were strongly correlated for earthworms and ground beetles, perhaps indicative of attainment of steady state accumulation. In general, PBDE burdens decreased in the invertebrates with trophic level at the sludge-amended field. The pattern of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes in the taxa sampled suggests different trophic interactions at the non-sludge and sludge-applied fields. However, as only two sites were surveyed, a more exhaustive data set is needed in order to draw more definitive conclusions.

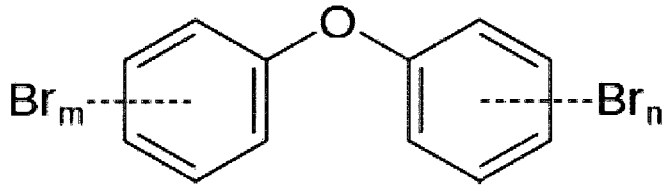
Results of these studies provide unequivocal evidence that PBDEs accumulate in soils where biosolids are applied. They also demonstrate that PUF- and biosolid-associated PBDEs are bioavailable and are accumulated to varying degrees by ecologically diverse soil-associated invertebrates. As such, they may become available for uptake by higher order terrestrial consumers, including reptiles, mammals and birds.

Bioavailability of Biosolids- and Consumer Product-Associated Polybrominated Diphenyl Ether (PBDE) Flame Retardants to Terrestrial Invertebrates

GENERAL INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are part of a large class of brominated flame retardant (BFR) chemicals that include tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD) and the polybrominated biphenyls (PBBs). PBDEs are incorporated in consumer products, often at percent by weight levels, to suppress the spread of fire and mitigate loss of human life. PBDEs are chemically similar to PBBs and polychlorinated biphenyls (PCBs), but differ slightly by the presence of an ether linkage between the two phenyl rings. PBDEs are also related to the polychlorinated diphenyl ethers (PCDEs), used historically as plasticizers, biocides and flame retardants. (Fig. 1).

Figure 1. General structure of PBDEs, where m + n = 1 to 10.



As with PBBs, PCBs and PCDEs, several industrial PBDE formulations exist. They differ primarily in their degree of bromine substitution. These include Deca-, Octa- and Penta-BDE, which are composed on average of diphenyl ether congeners substituted with ten, eight and five bromine atoms, respectively. The Deca-BDE mixture consists of a fully substituted diphenyl ether congener (BDE-209) with trace amounts of nona- and

octa-substituted congeners. The Octa-BDE mixture contains predominantly octa- and hepta-substituted congeners (~70-80%), while the Penta-BDE mixture contains primarily tetra- and penta-substituted congeners (WHO, 1994; La Guardia et al., 2006). Constituents of the latter mixture are widely regarded as the most bioaccumulative.

Like PBB and PCB formulations, 209 theoretical congeners are possible within the mixture. However, due to the influence of the electronegative oxygen linkage, yield of more diverse congeners is typically low. This accounts for the prevalence within most environmental samples of only a modest number of congeners. For example, two congeners dominate the Penta-BDE mixture (by weight), i.e. BDE-47 (2,2',4,4'-tetrabromodiphenyl ether; 38-43%) and BDE-99 (2,2',4,4',5-pentabromodiphenyl ether; 45-49%). The bulk of the remainder of Penta-BDE is composed of BDE-100 (2,2',4,4',6-pentabromodiphenyl ether; 8-13%), BDE-85 (2,2,3,4,4'-pentabromodiphenyl ether; 2-3%), BDE-154 (2,2',4,4',5,5'-hexabromodiphenyl ether; 3-5%) and BDE-153 (2,2',4,4',5,5'-hexabromodiphenyl ether; ~5%) (La Guardia et al., 2006).

PBBs and PCBs were banned in the US in the 1970s due to deleterious impacts to human, wildlife and ecological health. Though PBDE flame retardants serve a critical role in society (i.e. that of protecting life and property), their inclusion in myriad consumer products at substantial levels has resulted in their widespread dissemination in the global environment. Like their PCB and PBB analogs, PBDEs are hydrophobic and lipid soluble, making their release into the environment similarly problematic. Mounting scientific evidence of their potential to disrupt biological and ecological systems has resulted in a total ban (Europe) and an agreement between industry and the US EPA to cease manufacturing of the Penta- and Octa-BDE commercial formulations, effective

December 2004. A similar moratorium on production of Deca-BDE has been announced in the US, effective at the end of 2012, as well as other restrictions in selected US states and Europe (Vonderheide et al., 2008). Pre-existing stocks can be used after those dates. More importantly, their presence at percent levels in a myriad of long-lived consumer products will contribute substantially to global emissions well into the future (Alaee et al., 2003; Hale et al., 2003).

Based upon 2001 production data, the largest global market demand exists for the Deca (56,100 MT; 83.3%), followed by Penta (7,500 MT; 11.1%) and Octa (3,790 MT; 5.6%) formulations (BSEF, 2003). While viewed as a global problem, over 90% of Penta-BDE usage resided in North America in recent years (Hale et al, 2003). Table 1 summarizes world market demand by region. Congener composition and bromine substitution, as well as the physical properties of receiving products (e.g. polymers), determine into which materials these mixtures are incorporated. For instance, Octa- and Deca-BDE are used primarily in rigid thermoplastic polymers, such as computer and television display casings, appliance housings and wire and cable insulation (Alaee et al., 2003; La Guardia et al., 2006). Another Deca-BDE usage has been in textile-backcoatings. Penta-BDE has been used almost exclusively in flexible polyurethane foam (PUF) applications (e.g. furniture and vehicle cushioning, carpet underlayment etc.). PBDEs are generally added at levels ranging from 5-30% by weight (Hale et al., 2002, 2003; Alaee et al., 2003; La Guardia et al., 2006). As additive flame retardants, they are incorporated into the polymer matrix during formulation, but are not chemically bound. This permits some release from the polymer over time (Hutzinger and Thoma,

1987; Hale et al., 2003). In contrast, BFRs such as TBBPA are chemically reacted with the base polymer and are thus less susceptible to migration.

Table 1. The 2001 estimated world market demand for PBDE formulations (metric tons) (BSEF, 2003).

Formulation	North America	Europe	Asia	Rest of World	Total
Penta-BDE	7,100	150	150	100	7,500
Octa-BDE	1,500	610	1,500	180	3,790
Deca-BDE	24,500	7,600	23,000	1,050	56,100

PBDE physical properties vary with degree of bromination and substitution pattern. They are generally environmentally stable hydrophobic organic compounds (HOCs), characterized by low water solubilities and vapor pressures and high octanol-water partitioning coefficients (Kow). Consequently, PBDEs tend to partition strongly to the organic fraction of soils and sediments, and to preferentially partition into the lipid reserves of organisms upon release into the environment. This accentuates environmental persistence, bioaccumulation and potential for tropic transfer. Log Kow estimates for Octa- and Deca-BDE constituents range from about 7.9-9.9, while Penta-BDE constituent estimates are in the range of 5.8-7.8 (WHO, 1994). Log Kow estimates for Penta-BDE constituents indicate stronger bioaccumulation potential. Indeed, the bioaccumulative potential of tetra- to hexa-brominated congeners is now well established. For example, the highest tissue burdens of these congeners to date (47,900 μg/kg lipid) were reported in Virginia freshwater fishes (Hale et al., 2001). In that study, Penta-BDE constituents were detected in 89% of fish samples collected (n=332; 33 species). An extensive body of literature has documented the disposition of PBDEs within aquatic ecosystems.

However, few studies have examined PBDE bioaccumulation within terrestrial ecosystems. Deca-BDE constituents (e.g. BDE 209) were once believed to be essentially non-bioavailable. However, reports of accumulation of higher brominated constituents in wildlife, especially in terrestrial biota (Chen et al., 2008; Voerspools et al., 2007; Potter et al., 2009) and humans (Sjodin et al, 1999; Zota et al., 2008; Wu et al., 2007; Stapleton et al., 2008) are increasing. The pathway(s) for their introduction into terrestrial wildlife remains uncertain.

The potential for all PBDE constituents to disrupt endocrine systems is well established (Vonderheide et al., 2008). PBDEs (and their hydroxylated metabolites) are structurally similar to some thyroid hormones (e.g. T4) and are thus able to competitively bind with thyroid hormone receptors, such as the transport protein transthyretin (Vonderheide et al., 2008). Neurobehavioral disruption (i.e. learning and memory) (Ericksson et al., 2001) and fetal toxicity in rats and rabbits exposed to Penta- and Octa-BDEs have been reported (Darnerud, 2003). Deca-BDE can also induce morphological changes in thyroid and organ systems in exposed adults (Darnerud, 2003). Neonatal exposure to BDE-209 has also been reported, indicating permeability of the placental barrier (Riu et al., 2008). PBDEs may interfere with sexual development as well (Lilienthal et al., 2006). Though no definitive causal link with cancer has been established, PBDE serum levels have been correlated with increased incidences of non-Hodgkin's lymphoma (Vonderheide et al., 2008). Moreover, PBDEs are believed to act antagonistically and/or synergistically with other organic pollutants (e.g. PCBs) to enhance toxicity (Eriksson et al., 2006)

Despite considerable progress elucidating PBDE disposition in the environment, PBDE release pathways remain enigmatic. As PBDEs are present in many consumer products at percent by weight levels, multiple release pathways exist. These include release during: initial BFR synthesis, incorporation into plastics and formation of consumer products, and losses during product usage and after disposal (Hale et al., 2003). PBDEs may migrate from products or the product structure itself may deteriorate and fragment, carrying with it the associated BFR burden (Allen et al., 2008; Webster et al., 2009). Further fragmentation produces small, easily transported fragments with large surface areas. This, in turn, enhances the release of additives from the polymer remnant and particle size reduction increases the likelihood of organismal ingestion (Browne et al., 2008; Teuten et al, 2009). One pathway for PBDE release is land application of sewage sludge-derived biosolids containing incurred PBDEs. Historically, sewage sludge has been land-filled, incinerated or ocean-dumped. However, associated costs, dwindling landfill capacity and negative public reactions have rendered these disposal methods unattractive. Alternatively, sludge can be further treated to reduce pathogen loadings and then applied to agricultural and public lands as a soil amendment. The term *biosolid* is a euphemism for this stabilized sludge. About 8×10^6 dry tons of biosolids are generated in the US annually, more than half of which is now land-applied (US EPA, 2006). With more stringent wastewater treatment regulations and burgeoning human populations, the extent of sludge production and land disposal is likely to escalate (Harrison et al., 2006).

Biosolids are rich in nutrients and organic matter and thus facilitate the growth of plants. Biosolids are frequently provided by generating wastewater treatment facilities

to farmers and applied to agricultural fields at no cost, establishing an urban-rural export pathway. This reduces the farmer's fertilizer purchasing and application costs. In addition, the pH of many agricultural soils is low. Thus, the liming of biosolids to reduce pathogen content is also attractive, as it saves the farmer the costs of lime application. However, biosolids also contain complex mixtures of potentially toxic organic pollutants (Beck et al., 1996; Kinney et al., 2006), including PBDEs (Hale et al., 2001; Harrison et al., 2006; Eljarrat et al., 2008; Andrade et al., 2010; Xia et al., 2010). Their presence and potential for toxicological effects has spurred significant debate. In the US, biosolids are currently regulated for only nine metals and their pathogen content (US EPA, 1999; National Academy of Sciences, 2002).

Subsequent to the EPA risk assessment, wherein persistent organic pollutants (POPs) were assumed to be either absent or present at low and decreasing levels, high concentrations (1100-2300 μg/kg dw) of Penta-BDE constituents were detected in biosolids collected from across the US (Hale et al., 2001). Interestingly, a recent survey of selected pollutants in sludge from selected US wastewater treatment facilities (n=74) detected mono- to deca-PBDEs in virtually all samples, ranging in concentrations from 1.8 to 17,000 μg/kg dw (US EPA TNSSS, 2009). PBDEs have also been recently reported in US biosolids and biosolids-amended soils by others (Xia et al., 2010; Andrade et al., 2010). PBDEs have been detected in sludge and sludge-amended soils from several other countries as well, although generally at levels about 10- to 1000-fold lower than found in US sludges (Hale et al., 2003, 2008). The disparity is likely due to disproportionate usage of the Penta-BDE formulation in the US. The discovery of consistently high levels of PBDEs in biosolids and their intentional reintroduction to the

environment suggest that land application of sludge-derived biosolids may be an important conduit for redistribution of PBDEs.

Striking similarities between PBDE congener patterns in biosolids and those found in PUF—the dominant product application for the Penta-BDE formulation—are compelling. PUF products are ubiquitous in human habitations and personal spaces and human contact with these products is frequent and intimate. About 5.5 x 10^8 kg of PUF is produced annually for the manufacture of upholstered furniture cushioning in the US (Alliance for Flexible Polyurethane Foam, 2010). Another 2.3 x 10^8 kg is employed in carpet underlayment and flexible PUF products, including extensive use in automotive products (Polyurethane Foam Association, 2010; Alcock et al., 2003). After use, PUF products are discarded in landfills or directly into the environment (Loudon and Mathews County, VA Supervisors, personal communication) where constituent PUF can degrade and release additive PBDEs (Osako et al., 2004; Odusanya et al., 2009). Some PUF is recycled into remanufactured products as well, such as carpet underlayment, from which PBDEs may also migrate. Such repackaging increases product-associated PBDE longevity within the anthroposphere. PUF is vulnerable to photodegradation and subsequent disintegration of its cellular structure (Dementev, 1999) and disintegration of treated PUF into minute particles has been observed after only four weeks exposure to ambient outdoor summer conditions (Hale 2001). PUF fragments retain the bulk of their original PBDE composition, may be transported to wastewater streams via runoff and aeolian processes and ultimately contribute to sludge burdens (Hale et al., 2001, 2002). Given the high levels of Penta-BDE used in PUF, only small amounts would be required

to contaminate large amounts of sludge with PBDEs at the mg/kg level. Reduction in fragment size will increase polymer surface area and possibly additive release.

Despite the proliferation of land application practices, few scientific assessments of the fate of PBDEs in sludge-amended ecosystems have been performed. Ecologically important soil-dwelling organisms, such as earthworms and arthropods, may be especially vulnerable to PBDE exposure. Taken together, these taxa represent substantial macroinvertebrate biomass in temperate soil and agricultural ecosystems (Dangerfield, 1990; Bouché, 1992; Coleman et al., 2004; El Titi, 2003). Both groups contribute significantly to soil conditioning and nutrient cycling and serve as critical food items for myriad consumers, such as reptiles, birds and mammals (Dangerfield, 1990; Bouché, 1992; McIntyre, 2000; Coleman et al., 2004) and in some cases humans (McIntyre, 2000). Owing to their intimate associations with soil, these organisms may function as biological indicators and integrators of terrestrial PBDE contamination (Walton, 1989; Matscheko et al., 2002; Sellström et al., 2005). Yet, surprisingly, the potential for earthworms to bioaccumulate sludge-associated PBDEs has received scant attention. Moreover, there appear to be no published reports to date investigating sludge-derived PBDE bioaccumulation in soil arthropods. Thus, there exists an urgent need for studies of sludge-associated PBDE bioaccumulation in soil ecosystems receiving sludge amendments. More data on the bioavailability of contaminants, such as PBDEs, from land-applied sludges derived from different treatment processes are also crucial to assess the risks of land application more comprehensively.

Another PBDE release/exposure pathway that has received scarce attention until quite recently is that of direct exposure to current use and derelict polymer products

containing high PBDE levels. There is growing awareness of the potential for consumer plastics to be significant sources of organic pollutants to the global environment (Teuten et al., 2007, 2009). Recent studies also confirm the importance of household dust as an important exposure route for PBDEs derived from proximate consumer products within homes, vehicles and the workplace (Allen et al., 2008; Frederiksen et al., 2009; Webster et al., 2009). PBDE releases from manufacturing sites are obvious potential exposure routes. However, owing to the high additive PBDE content of consumer PUF and the relative fragility of PUF, finished products may also contribute appreciably to global emissions.

To illustrate, high PBDE burdens were recently reported in sewage sludge from a major US Antarctic research facility (Hale et al., 2008). Penta-BDE concentrations were similar to those from mainland wastewater treatment plants serving major US cities. PBDEs are not manufactured in Antarctica. Congener profiles also matched commercial PBDE mixtures, rather than signatures expected to be generated by long-range transport and associated weathering processes. Hence, imported Penta-BDE-treated polymer products were posited as the likely ultimate source. It has also been estimated that about two to three times as much of the Penta-BDE constituents are imported into Europe via finished polymer products than are actually produced there (Prevedouros et al., 2004). In a similar study, total burdens of a major Penta-BDE constituent, BDE-47, available for distribution from consumer PUF goods to US and UK environments were estimated to be approximately 2600 and 520 metric tons, respectively (Alcock et al., 2003). These studies highlight the importance of the export of PBDE-containing commercial products to the rapid global dispersal of these POPs.

Modeling efforts based on physical-chemical properties further suggest that the bulk of PBDE-treated consumer products and PBDEs contained therein will be discharged to the terrestrial environment and remain there for long periods (Palm et al., 2002). Yet, a recent literature search revealed only one published report of PBDE uptake from a PBDE-treated product by a terrestrial arthropod (Hale et al. 2002). However, recent studies of the importance of spiders in transferring aquatic-derived PCBs (Walters et al., 2009) and mercury (Cristol et al., 2008) burdens to terrestrial-feeding birds have highlighted the relevance of land-based transport pathways. Recent findings of unexpectedly high PBDE burdens, especially of the "non-available," more brominated congeners, in terrestrial feeding birds of prey (Lindberg et al., 2004; Potter et al., 2009; Chen and Hale, 2010) further highlight the importance of terrestrial exposure routes.

Insects have cohabitated with humans throughout recorded human history (McIntyre, 2000) and are long known to consume anthropogenic materials such as textiles (Pruthi, 1938; Cheema, 1963). Yet, a literature search revealed only two published reports concerning PBDEs and terrestrial insects (Hale et al., 2002; Wu et al., 2009). It is noteworthy that the main focus of both studies related to PBDE uptake by amphibians and only considered insects tangentially. Clearly, more studies are needed to more critically evaluate the role of insects as PBDE receptors and vectors of their transport to and within terrestrial food webs.

To address the dearth of studies in these important areas, a series of lab- and field-based studies were conducted to evaluate the potential for PBDE accumulation from biosolid and commercial flame-retarded PUF products by representative soil invertebrates. Earthworms were exposed to model soils amended with: anaerobically

digested (Class B) and composted (Class "EQ") sewage sludge biosolids, PUF fragments, and solvent-delivered Penta-BDE. Evaluation of non-filled polymer passive sampling devices (PSDs) for estimating the bioavailable fraction of PBDEs of biosolids and a biosolids-amended soil was also undertaken. BFR-treated PUF, in-use or after its disposal, represents a massive store of PBDEs that may be bioaccessible. Some insects are known to directly consume polymers and textiles. This is a potential pathway for BFR entry and dissemination in terrestrial food webs. To evaluate this, house crickets were provided access to commercial PUF, coincident with food and water *ad libitum*, and resultant PBDE assimilation measured. Finally, PBDE accumulation in components of a real-world agroecosystem receiving long-term sludge amendments, including soil invertebrates, was evaluated relative to a reference site receiving only animal manure inputs. To further investigate trophic interactions, carbon and nitrogen isotope ratios in these ecosystems were also considered.

These studies addressed multiple hypotheses: 1) PBDE bioavailability to earthworms will differ in soils amended with biosolids generated by different wastewater sludge stabilization processes; 2) Solvent-borne PBDEs in soil will be the most bioavailable to earthworms, followed by sludge-associated and finally PUF-associated PBDEs; 3) PBDE uptake into PSDs from biosolids-amended soil will be proportional to, but slower than, uptake by earthworms; 4) Crickets provided access to Penta-BDE-treated PUF will ingest PUF fragments and accumulate PBDEs therein; 5) PBDEs will be enriched in soils receiving historical sludge amendments relative to a reference site receiving only animal manure inputs; 6) PBDEs in biosolids-amended field soil will be accumulated by soil invertebrates as a function of their ecological relationships.

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Chapter 1

Polybrominated Diphenyl Ether (PBDE) Accumulation in Earthworms After Exposure to Sewage Sludge, Polyurethane Foam Microparticles and Neat Chemical-Amended Soils

Abstract

Polybrominated diphenyl ethers (PBDEs) may enter soils or sewage sludge via fragmentation of, or migration from, polymer products. Land application of stabilized sewage sludge, also known as biosolids, is increasingly practiced to reduce attendant disposal costs and recycle agriculturally important nutrients. PBDEs in polymers are generally assumed to be largely biologically inaccessible. While PBDEs are persistent and bioaccumulative, no controlled experiments have examined their uptake from polymers or biosolids by soil-dwelling organisms. We exposed earthworms (*Eisenia fetida*) to composted biosolids (CB), anaerobically digested biosolids (ADB), polyurethane foam microparticles or solvent-delivered Penta-BDE (SAS) dispersed in an artificial soil. Worms in soils receiving the two biosolid classes accumulated comparable ∑PBDE (47+100+99+154+153+183) concentrations after 28 d. Worm concentrations were substantial in all doses, which overlapped reported PBDE burdens in the field. In the highest biosolid/soil doses (ADB: 169 and CB: 196 μg/kg dw) worm concentrations were 11,000 and 13,500 μg/kg lipid, respectively. Worms exposed at the lowest SAS dose (676 μg/kg dw) accumulated 64,300 μg/kg lipid. For individual PBDE congeners, biota-soil accumulation factors (BSAFs) ranged from 5 to 20 and were greatest for BDE 47. BSAFs were generally greater from lower dose biosolids-amended soils and within a factor of two of those for solvent-delivered PBDEs. BSAFs decreased with increasing congener log Kow. Uptake patterns in passive sampling devices (PSDs) inserted into the ADB-amended soil paralleled those in worms, although concentrations in the PSDs were much lower. Worms exposed to PUF-amended soil (∑Penta-BDE=83.2 mg/kg dw) exhibited ∑PBDE whole body burdens up to 3740 mg/kg lipid with no observable

toxicity. This high burden is likely related to residual PUF in the gut even after depuration. Apparent BSAFs ranged from 5 to 33 and were as much as two-fold higher than for worms exposed to SAS treatments of 5.6 mg/kg dw. Results demonstrate that worms can readily accumulate PBDEs from biosolids- and PUF-amended soils at levels comparable to those amended with solvent-delivered PBDEs. These burdens, in turn, would be available for subsequent transfer into terrestrial food webs.

Introduction

The toxicological implications of plastics discarded in the environment have generally focused on their potential to physically impair (i.e. entangle or block) the digestive tracts of aquatic wildlife, such as waterfowl (Ryan et al., 1988). Discarded plastics may also sorb in-situ hydrophobic environmental contaminants (Teuten et al., 2007). Hence, interest in the toxicological importance of these plastics following ingestion by wildlife, as well as the potential of polymers to ameliorate contaminated sites, have grown (Browne et al., 2008; Teuten et al., 2007; Teuten et al., 2009). Concentrations sorbed to plastics are typically in the μg/kg range. In contrast, chemicals intentionally added to polymers (e.g. plasticizers, stabilizers, antimicrobials and pigments) are often present at percent levels. However, it was long assumed that these additives were securely sequestered within the polymers and, hence, posed little toxicological threat. Recent studies indicate that additives may be released from plastics (Teuten et al., 2009) and consumer polymer products (Allen et al., 2008; Webster et al., 2009), thus calling into question the validity of this presumption.

Polybrominated diphenyl ethers (PBDEs) are one additive class of particular concern as they are persistent, bioaccumulative and toxic (PBT) chemicals. PBDEs have been used extensively to flame retard plastics common in the home, workplace and in vehicles. Of the three commercial PBDE formulations, Penta-BDE is the most environmentally mobile, toxic and bioaccumulative. It has been used for decades to flame retard polyurethane foam (PUF) consumer products. To meet stringent flame retardancy standards, consumer PUF typically contains about 10% by weight Penta-BDE (Hale et al., 2002). Consumption has been particularly intense in North America (Renner, 2000; Hale et al., 2002, 2006). Penta-BDE manufacture was discontinued in the US in late 2004, as a result of concerns over its accumulation in the environment and human tissues.

In plastics manufacturing PBDEs are incorporated within, but are not reacted with the polymer. Thus, some can escape over time. Plastics also may weather and lose structural integrity during product use or after disposal (Dementev, 1999; Teuten et al 2009; Osako et al., 2004; Odusanya et al., 2009). Recent publications document mg/kg PBDE burdens in indoor dust (Webster et al., 2009; Frederiksen et al., 2009) and establish a link between PBDE-treated consumer products and PBDE burdens in dust (Allen et al., 2008). Polymer breakdown may enhance additive release, as well as potential for ingestion of fragments by biota (Browne et al., 2008; Teuten et al 2009). Minute plastic fragments carry much of their original PBDE burden and congener signature. They also are easily transported via runoff and aeolian processes (Hale et al., 2001, 2002). Polymer microparticulates have even been found to translocate into the circulatory system of ingesting organisms (Browne et al., 2008). Hence, ingestion of

fragments, especially considering their intentional additive burdens, could represent an important exposure route for some organisms.

Most chemicals in commerce eventually enter wastewater treatment systems. Persistent, hydrophobic chemicals therein will concentrate in sewage sludge. Published reports describing organic pollutants in US biosolids have increased in recent years (Hale et al., 2001; Kinney et al., 2006; Harrison et al., 2006; Eljarrat et al., 2008; Andrade et al., 2010). Sewage sludges may be stabilized by anaerobic or aerobic digestion, composting, drying or liming and are then euphemistically termed *biosolids*. Due to their nutrient and organic matter content, such sludges are viewed as agricultural resources. However, the risks of organic pollutants in biosolids require further investigation. Given continued improvements in wastewater treatment technologies and burgeoning human populations, the quantity of biosolids and the scope and magnitude of land application are increasing (Harrison et al., 1999). About 8×10^6 dry tons of sludge is generated in the US annually, more than half of which is land-applied (US EPA, 2006). The US EPA National Sewage Sludge Survey (US EPA, 2009) reported a mean Penta-BDE concentration ($\sum$BDE 47, 99, 100, 153 and 154) of 2030 μg/kg (dw) in US biosolids from 74 publicly owned wastewater treatment plants. Yet, biosolids products remain unregulated for PBDEs. Assuming this material was land applied at one-time application rates of 20-60 MT/ha (Matscheko et al., 2002; Sellström et al., 2005), a mixing depth of 20 cm and a bulk soil density of 1.4 g/cm^3, a single sludge application would result in soil burdens of about 44 μg/kg dw. Indeed, Andrade et al. (2010) recently reported mean total PBDE concentrations in biosolids-treated agricultural soils from the US Mid-Atlantic region of 53 μg/kg dw. In that study, biosolids-applied fields had received historical applications

in the range of 9.4-74 MT/ha. Repeated, application of PBDE-contaminated sludges to soils may result in higher soil burdens (see Chapter 3). Eventually these may pose risks to ecologically critical soil biota, such as earthworms (Hale et al., 2001; Matscheko et al., 2002; Sellström et al., 2005).

Earthworms are abundant in many temperate soil ecosystems. Therein, they modify soil texture, moisture, nutrient and oxygen content via ingestion (Beck et al., 1996; Bouché, 1992; Coleman et al., 2004). Indeed, biomass densities of lumbricid earthworms in temperate soils often exceed that of all other invertebrate soil fauna combined (Bouché, 1992; Coleman et al., 2004). Earthworms are also preferred prey for myriad species of invertebrates, birds and mammals (Beck et al., 1996; Coleman et al., 2004). Thus, their exposure to contaminated soil may contribute to body burdens in organisms further up the food web (Beck et al., 1996; Matscheko et al., 2002; Sellström et al., 2005).

Due to the lack of controlled studies of the uptake and bioavailability of PBDEs, we exposed earthworms in the laboratory to solvent-delivered Penta-BDE, Penta-BDE-treated PUF fragments and sewage sludges (Class B and exceptional quality grades with incurred Penta-BDE constituents) dispersed in soil. Owing to the greater ecotoxicological potential of the Penta-BDE constituents, their substantial usage in PUF-containing consumer goods, and the vulnerability of PUF to deteriorate, fragment and enter soil and wastewater, our primary study objective was evaluation of Penta-BDE constituent bioavailability to earthworms. We also examined the utility of simple, polyethylene passive sampling devices (PSDs) to sample (coincident with earthworms) the fraction of PBDEs available for uptake from artificial soil amended with

anaerobically digested biosolid (ADB). We hypothesized that solvent-borne PBDEs in soil would be the most bioavailable to earthworms, followed by sludge- and finally PUF-associated PBDEs. Due to lack of soil ingestion, uptake of PBDEs from PSDs in ADB-amended soil was expected to be slower than in earthworms.

Experimental

Earthworms. A stock culture of red worms (*Eisenia fetida;* Worm World, Avella, PA) was established several weeks prior to exposure experiments to acclimate them to ambient laboratory conditions. To prepare earthworm bedding, alternating layers of sand and potting soil (~2.5 cm layers) were added to 20 L plastic buckets. To the top stratum was added approximately 25 cm of hydrated sphagnum peat moss. Drainage holes (~ 0.5 cm) were provided. Stock earthworms were then transferred to the buckets and acclimated for one week. Worms were fed every 3-4 d by covering the bedding surface with ~ 20 g dw of Worm Chow® (Purina, Saint Louis, MO). Feeding behavior was monitored. PBDEs were below detection in stock earthworms, bedding and food.

Biosolids. Two biosolids generated by Virginia (USA) publicly owned treatment works (POTW) were obtained in Summer 2002: 1) An "exceptional quality" (EQ) composted biosolid (CB) consisting of a mixture of dewatered sludge composted with recycled paper products, woodchips and yard waste; 2) An anaerobically digested Class B biosolid (ADB). Biosolids were sieved to < 2000 μm, homogenized and stored in covered 20 L plastic buckets at 4°C prior to use. ∑PBDE (47+99+100+153+154+183) burdens determined in the ADB (n=3) and CB (n=3) were 5560±440 and 1130±79 and μg/kg dw, respectively (Table S1).

Model soil. Artificial soil (AS) for use as a diluent for biosolid, PUF, solvent-spiking and control treatments. It was initially prepared by combining sand, kaolinite, peat moss and dolomite (69:15:15:1 w/w) and hydrating with deionized water to 45% (w/w) (ASTM, 1997). Batches of AS were sub-sampled to determine total organic carbon (TOC) content using an Exeter CHN Model 440 CE Elemental Analyzer (North Chelmsford, MA). The TOC of AS was 5.2±0.9% (n=3), 5.9±0.8% (n=3), 5.1±1.2% (n=3) and 4.8±0.8% (n=3) dw for batches intended for biosolids and PUF diluent and solvent-spiked and control AS, respectively. PBDEs were below detection in all soil components.

Biosolids substrate preparation. In preliminary testing, earthworms were only able to tolerate a 25% (w/w) mixture of the CB and 9% (w/w) mixture of the ADB before acute distress was observed (i.e. burrowing avoidance and then 100% mortality). Nontoxic dilutions of 6, 12 and 25% CB- and 3, 6 and 9% ADB-amended soils were thus subsequently prepared for use as low, medium and high dose treatments, respectively. These loadings approximated agronomic field application rates of 23, 47 and 70 MT/ha, respectively, comparable to the 10-70 MT/ha rate reported for Swedish agricultural soils (Sellström et al., 2002; Matscheko et al., 2005) and US agricultural soils (Andrade et al., 2010). Soil mixtures (~ 800 g) were transferred to pre-cleaned glass jars and equilibrated to laboratory conditions prior to exposure. Only artificial soil was used in solvent-delivered Penta-BDE (SAS) and unspiked (control) AS treatments. Control treatments (n=3) were run for each substrate tested.

For the SAS, nominal PBDE spiking levels were 10%, 50% and 100% of ∑PBDE levels previously determined in the 100% ADB. SAS was prepared by adding the

commercial Penta mixture DE-71 (Chemtura Corporation, West Lafayette IN), dissolved in 1 ml acetone to aliquots (~ 500 g) of sand. After spiking, sand was submerged in diethyl ether and agitated on a shaker table overnight to distribute the PBDEs. Control sand was manipulated identically, without the Penta-BDE spike. Residual ether was evaporated in a fume hood overnight. The sand was then combined with the other soil constituents in 20 L pre-cleaned plastic buckets and homogenized. Soils were then stored at 4°C for four weeks to equilibrate the mixture prior to exposure. ∑PBDE levels were 680±40 (n=3), 2900±200 (n=3) and 5600±330 (n=3) for low, medium and high dose SAS treatments, respectively (Table S1). PBDEs were below detection in unspiked AS controls.

PUF-amended soil substrate preparation. Commercial PUF was purchased locally and PBDE content therein determined (∑PBDEs=47+100+99+85+154+153=8.7±1.4% w/w; n=3). To generate microparticles, the PUF was frozen in liquid nitrogen, fragmented and sieved to < 75 μm. While *E. fetida* can ingest particles up to 2000 μm (Neuhauser et al., 1980), we selected the smaller size, as these are likely more common in the environment and more readily ingested by (and bioavailable to) the worms. PUF particles were dispersed in AS at 1:2000 (w/w) by mixing for 24 h on a rolling mixer. PBDE composition and concentration of the PUF-amended soil was determined prior to earthworm exposure. The soil was stored in the dark at ambient room temperature (~ 26°C) for one month prior to exposure to permit equilibration. After confirming soil homogeneity and quantifying PBDE levels (83.3±8 mg/kg dw), the PUF-amended soil substrate was hydrated to approximately 45% (w/w) and disbursed into glass jars (~ 600 g wet) and allowed to equilibrate under ambient laboratory conditions for one week prior

to exposure. For this PUF bioassay, the highest dose SAS (above) was used for quantitative comparison of relative PBDE uptake.

Earthworm bioaccumulation bioassays. Jars were randomized as to treatment and location. Ten adult worms were placed on top of the soil and allowed to burrow. Jars were covered with perforated aluminum foil to reduce water loss, while permitting gas exchange and preventing worm escape. PBDE spiked and non-spiked controls were run simultaneously and manipulated identically. Worms were not depurated or rinsed of stock bedding material prior to exposure, as preliminary trials indicated that worms handled in this way would not burrow into the substrate. Trials also revealed that the originally prescribed organic matter content of ASTM artificial soil (i.e. 10% w/w peat moss) appeared to provide inadequate nutrition, resulting in worm burrowing avoidance and mortality. Increasing the peat moss content to 15% w/w obviated this. No PBDEs were detectable in stock bedding or food. Exposures were conducted at 30±3°C, relative humidity of 51±5% and a 12:12 light:dark photoperiod. Environmental conditions were monitored hourly using a programmable data logger. The experiment was monitored daily for mortality. Worms were fed twice weekly with 20 g per jar Worm Chow® (Nestlé Purina; St. Louis, MO). To evaluate PBDE uptake, worms were removed from substrates at 14 and 28 d, rinsed with deionized water and gut contents 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-exposed worms were removed from soil treatments at 7, 14 and 28 d intervals.

Passive sampling devices (PSDs). PSDs consisted of 86 μm thick lay-flat low-density polyethylene tubing (Brentwood Plastics, Saint Louis, MO). Prior to use, tubing was cut

into 9 cm^2 sheets, weighed and pre-extracted with 100% DCM. After air-drying, PSDs were wrapped in solvent-rinsed aluminum foil, sealed in plastic bags and frozen at -10°C until use. PSD masses used were consistent (1.5±0.02 g; n=15). PSDs were placed into the ADB-amended soils and non-spiked AS control soils coincident with worms. PSDs were completely buried, but did not obstruct earthworm movement. PSDs were removed at 14 and 28 d intervals along with exposed worms, rinsed with deionized water, wiped dry with a KimWipe® and stored at -10°C until analysis.

PBDE analysis. For soil/worms, analytical methodologies followed that of Chen et al., (2008) (see Supporting Information). PSDs were spiked with PCB 204 and extracted with methylene chloride overnight on a shaker table. Extracts were solvent exchanged to hexane and cleaned up directly on 2 gm silica gel SPE columns. PBDEs therein were determined as above.

Statistical analysis. 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, unless otherwise indicated. BSAFs were computed as the ratio of lipid-normalized whole body tissue PBDE burdens to TOC-normalized substrate PBDE concentrations. Relative uptake ratios (worm:soil burdens, dw basis) were also calculated for comparison with published uptake data. PSD accumulation factors (PAFs) were computed as the ratio of PSD (dw basis) to TOC-normalized substrate PBDE burdens. One-way Analysis of Variance (ANOVA) was used to determine significant dose and exposure duration differences in tissue PBDE burdens, BSAFs and PAFs, as well as congener uptake ratios and total lipid content. Pair-wise comparisons were made using Tukey post-hoc Honestly Significantly Different (HSD) testing. A two-sample t-

test was used when the data permitted comparison of only a single variable (i.e. due to levels < QL). Correlations between PBDE tissue, substrate and PSD burdens, earthworm BSAFs (and PSD PAFs) and literature-derived partitioning parameters were evaluated using multiple least squares linear regression. All data were normally distributed, as determined by the Kolmogorov–Smirnov test.

Results and Discussion

PBDE levels and trends: biosolids-exposed worms. Little research has examined the potential for PBDE bioaccumulation from biosolids. Here, ∑PBDEs in earthworms (lw) exposed to ADB-amended soil were five-fold higher ($p<0.05$) than those in the soil (TOC basis), confirming substantial PBDE availability (Fig. 1). Mean Penta-BDE levels in the ADB-amended soils were: 72 (low), 105 (med) and 169 (high) μg/kg dw (Table S1). We observed ∑Penta-BDE constituents of 260 μg/kg dw at a Mid-Atlantic field site receiving repeated biosolids applications over a 20 year period (see Chapter 3). Andrade et al. (2010) reported mean ∑PBDE (47+99+209) burdens of 53.0 μg/kg dw, while Xia et al. (2010) detected 650 μg/kg dw (BDE 47+99+100+153+154) in US surface soils receiving multiple biosolids applications. Mean ∑PBDE (47+99+100+153+154+183) in ADB-exposed earthworms reached 11,000 μg/kg lipid in the high dose treatment after 28 d (Fig. 1; Table S2). Worm PBDE concentrations were similar between doses and exposure times, suggesting steady state uptake by 14 d (Table S2). Congener burdens decreased in the following order: BDE 47 > 99 > 100 > 183. BDE 28, 85, 153 and 154 were below QL in worms and BDE 183 was below QL in all ADB soil treatments. Burdens of the most hydrophobic congener, BDE 183, were significantly higher in 28 d

medium and high doses, but not the low dose, compared to the same doses in 14 d treatments.

Our results are in agreement with trends reported by Sellström et al. (2005) for earthworms collected from Swedish agricultural fields receiving biosolids. Worms there were a mixture of *Lumbricus, Apporectodea* and *Allolobophora spp.* (see Matscheko et al., 2002). Their sludge-amended field sites generally exhibited soil Penta-BDE burdens 10- to 1000-fold lower (dw) than our ADB-amended treatments, reflective of the lower of Penta-BDE usage in Europe compared to North America (Hale et al, 2001; Law et al., 2006). However, one Swedish location exhibited high soil burdens (∑Penta-BDEs=1300 µg/kg dw), reputedly related to discharges from a textile manufacturer (Sellström et al., 2005).

To evaluate relative uptake into tissues and potential biotransformation, BDE 47/99 and 100/99 ratios were calculated for earthworms and substrate. These were similar with time and with ratios in soil, suggesting minimal differential biotransformation (Table S3). This is consistent with other earthworm/soil studies of PBDEs (e.g. Liang et al., 2010) and analogous POP (i.e. PCBs, PAHs, PBBs and chlorobenzenes) uptake (Blankenship et al., 2005; Kraus et al., 2005; Belfroid et al., 1995b). Mean BDE 47/99 and 100/99 ratios in 28 d high dose tissues were 2.1 and 0.2, respectively, while corresponding ADB-amended soil substrate ratios were 1.5 and 0.2, respectively (Table S3). Corresponding ratios of 0.79 and 0.27 were calculated for the commercial Penta-BDE product DE-71 based on data from La Guardia et al. (2006). Sellström et al. (2005) reported a soil 47/99 ratio of 0.76 at their most contaminated site. Our tissue BDE 47/99 ratios are two to three-fold higher than those derived from tissue

(lipid) PBDE burden data reported by Sellström et al. (2005) for field-exposed, depurated earthworms of various species. They are about twice (dw basis) those derived from a lab study by Liang et al (2010) involving *E. fetida* exposed to PBDE-spiked natural soil. However, our BDE 100/99 ratios are comparable to those derived from these two studies. The 47/99 ratio discrepancies may be due to decreased bioavailability of BDE 99 resulting from higher peat levels in our model soil. Hofman et al. (2008) reported lower bioavailability of phenanthrene to the oligochaete *Enchytraeus albidus* from artificial soil containing peat compared to a natural soil. Greater elimination of BDE-99 may also be a factor.

Liang et al., (2010) reported that uptake and elimination rate constants for BDE 47 after 28 d of exposure to spiked natural soil were higher than for BDE 99 and 100 in *E. fetida*. In contrast, elimination rate constants for BDE 99 were three times higher than BDE 47 in the aquatic worm, *Lumbriculus variegatus*, exposed to Penta-spiked artificial sediment or 100% of the same CB product evaluated here (Ciparis and Hale, 2005). Though lipid contents were not determined in the *L. variegatus* study, PBDE ratios derived from their reported tissue/substrate burdens were comparable to ours. Such different results highlight the importance of species, substrate and exposure mode/route in quantifying PBDE bioaccumulation by oligochaetes.

Biosolids that are composted differ from AD sludges in that they generally receive non-sludge related amendments of organic matter, followed by lengthy intervals of oxidation. In our CB-exposed earthworms, $\sum$PBDE burdens reached 13,500 μg/kg lw in the highest dose treatment after 28 d. These burdens are about a factor of four higher ($p<0.05$) than those measured in the CB soil substrate (TOC basis), but were not

statistically different from the ADB worm results (Fig. 1). In contrast to ADB-exposed worms, ∑PBDE burdens in CB-exposed worms were significantly ($p<0.05$) higher in the 28 d compared to 14 d high dose treatments (Table S2). Likewise, BDE 47, 99 and 100 worm burdens in our CB treatments were significantly greater in the 28 d high compared to the low dose. (Table S2). This may indicate that steady state was not yet reached. However, modeling efforts by Debruyn and Gobas (2006) suggested that lab-exposed earthworms should attain dietary steady state during 28 d soil exposures.

Extended contact time between soils and contaminants may dramatically reduce bioavailability (Alexander, 2000). Hence, studies employing chemicals added via solvent addition immediately before testing may result in higher BSAFs than those using field-collected matrices. In addition, sorbents such as carbon black (Brändli et al., 2008), or plastics (Teuten et al., 2007, 2009; Haukås et al., 2010), may reduce bioavailability of contaminants from soils and sediments. In both our ADB- and CB-exposure scenarios the PBDEs were present in the biosolids for substantial periods prior to worm exposure. In addition, at least some of the PBDEs likely originally entered the waste stream via losses from in-service or discarded PUF products. Recent studies have reported significant correlations between PBDE burdens in household dust and consumer products and forensic microscopy techniques have revealed a heterogeneous distribution of PBDEs in dust suggesting the presence of PBDE-containing plastic fragments (Allen et al., 2008; Webster et al., 2009). Though the extent of physical dissociation of the PBDEs from PUF fragments in sewage sludge is unknown, some likely remained therein and affected their bioavailability.

Mean TOC was high and comparable in all soil treatments we tested, i.e. 8-14, 7-

12 and 7-11% for ADB- and CB-amended soil and SAS, respectively. For comparison, Sellström et al. (2005) reported TOC (as loss on ignition) of 2 to 7% in sludge-amended field soils, while Liang et al. (2010) reported TOC of 1.7% for field-collected soils. The sources of the TOC in our study differed, as likely did relative lipophilicity. The organic matter in the SAS was wholly peat-derived. In the ADB- and CB-amended substrates, sludge organic matter supplemented the peat present in the model soil. The CB also contained contributions from paper, wood and yard waste. Significant differences in organic matter composition of sewage sludge have been reported (Smernik et al., 2003). Though comparable in the ADB- and CB-amended soil treatments, mean TOC content was significantly higher in the parent ADB (~25%) than in the CB (~9%) ($p<0.01$). These compositional differences may contribute to the disparities in the PBDE uptake rates.

For 28 d CB-exposed worms, mean tissue BDE 47/99 and BDE 100/99 ratios were 1.8 and 0.3, respectively, and were not significantly different with dose or time (Table S3). Mean high dose substrate ratios were 1.4 and 0.3, respectively, and were also not significantly different with time and dose. As seen in ADB treatments, BDE 47/99 ratios were generally higher than those derived from biosolid-exposed worms in the field by Sellström et al. (2005), spiked soil-exposed worms in the lab by Liang et al. (2010) and in DE-71 (La Guardia et al., 2006). When considered along with data presented in Figure 1, these results suggest that bioaccumulation of incurred PBDEs from soils amended with dissimilar biosolids occur via similar mechanism(s). Xia et al. (2010) recently reported median Penta-BDE burdens to be significantly lower in composted compared to anaerobically- and aerobically-digested biosolids. This could relate to

simple dilution of the sludge with non-PBDE containing materials such as yard wastes. In that study, PBDE congener patterns were similar in these biosolid types. We found congener patterns in parent ADB and CB used here to be similar as well.

PBDE uptake by earthworms from SAS treatments was also examined. Our low dose was similar to PBDE levels in field applied soils reported by Xia et al (2010). Our medium and high SAS doses were within the range of those observed in actual biosolids, but exceeded PBDE concentrations anticipated in sludge-applied agricultural soils. However, such high doses permitted evaluation of bioaccumulation of minor constituents (i.e. BDEs 85, 153, 154) that may be below typical quantitation limits in real world biosolid-amended soils. We were also interested in further exploring spiking dosage effects on BSAFs, as has been recently reported for *E. andrei* (Nyholm, 2009). However, subsequent quantitative comparisons of PBDE uptake from ADB- and CB-amended soil substrates are made only to the more environmentally relevant low dose (676 μg/kg dw) SAS.

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. The 28 d tissue ∑PBDE burdens were substantial, reaching a mean of 837,000 μg/kg lw in the highest dose treatment (5630 μg/kg dw). Mean worm burdens in 28 d low (676 μg/kg dw) and medium (2900 μg/kg dw) soil dose treatments were 64,300 and 345,000 μg/kg lw, respectively (Table S2). No acute toxicity was apparent at these levels. Asamoah (2005) reported comparable tissue lipid normalized burdens for *E. fetida* exposed to PBDE-spiked AS. However, direct comparison of our results is difficult as Asamoah only quantified BDE 85, 100 and 153 and no treatment replication was reported. Similarly,

Nyholm (2005) observed dose-dependent BSAFs (discussed below) for *E. fetida* exposed to PBDE-spiked SAS. Tissue burdens were not reported, again making direct comparisons difficult. However, spiking levels of 10, 100 and 10,000 μg/kg dw of BDEs 47, 99 and 153 were reported. Neither study has been published to date. Dose-dependent dietary accumulation of other hydrophobic organics (e.g. hexabromobenzene, PCB-153 and octachloronaphthalene) from artificial soil by *E. andrei* has also been reported (Belfroid et al., 1995b). In our 28 d low dose SAS-exposed worms, mean BDE 47/99 and BDE 100/99 ratios were 2.6 and 0.9, respectively. Ratios in treatment soils were 0.9 and 0.2, respectively, comparable to those in DE-71 (La Guardia et al, 2006). With the exception of the substrate BDE 100/99 ratio, these are significantly higher ($p<0.05$) than both congener ratios calculated for all tissue/substrate pairs evaluated. This was not observed in 14 d treatments (Table S3). BDE 47 is more environmentally mobile (e.g. more volatile and water-soluble) than BDE 99. It also appears to be more persistent than BDE 99 in some organisms. In a review of global PBDE burdens, Hites (2004) reported that BDE 47 was the dominant congener in wildlife and humans. Stapleton et al. (2004) reported debromination of BDE 99 to BDE 47 in lab-exposed carp. These factors may contribute to the enhanced BDE 47 burdens in earthworms.

PUF-exposed worms. Despite the role of polymer products as primary PBDE sources, and their percent levels therein, the possibility of direct biological uptake of these additives from polymers has received minimal attention. In an EU risk assessment (2001), considerable PUF-derived worm burdens were predicted from QSARs and pore water burdens. In our study, earthworms (*E. fetida)* accumulated substantial PBDE burdens after contact with soil containing PUF microparticles (<75 μm). Despite a 2000-

fold dilution of PUF fragments in soil, mean ∑Penta-BDE levels in PUF-amended soil were high, i.e. 83.2 mg/kg dw (Table S4).

PBDE uptake from PUF microparticle-amended soil and SAS generally increased with time for all congeners. ∑PBDE burdens in worms from the PUF-amended soil (83.3 mg/kg dw) ranged from 1890 mg/kg lipid (111 mg/kg dw) after 7 d to 3740 mg/kg lw (202 mg/kg dw) after 28 d. Burdens in worms exposed to SAS (5.6 mg/kg dw) ranged from 514 mg/kg lw (55 mg/kg dw) ∑PBDEs to 837 mg/kg lw (70 mg/kg dw) ∑PBDEs after 14 and 28 d, respectively (Table S4). The PBDE concentrations in SAS-exposed worms here were identical to the high dose used in conjunction with the biosolids exposures previously described. Unfortunately, 7 d soil treatment replicates were lost during sample processing. Nonetheless, the positive trajectories of the PBDE uptake indicate that the PBDE congeners had likely not reached steady state by 28 d. This is counter to the rapid time to steady state predicted by Debruyn and Gobas (2008) using a bioenergetic modeling approach and the experimental results of Liang et al. (2010) for uptake of solvent-spiked PBDEs from natural soil by *E. fetida*.

BSAFs. Depending upon soil properties, higher soil pollutant concentrations should result in higher earthworm tissue burdens (Belfroid et al., 1995; Sellström et al., 2005). This was generally the case in our study. ShuZhen et al. (2010) also reported similar results for *E. fetida* exposed to increasing soil doses of solvent-spiked Penta-BDE. However, we observed relatively higher burdens of BDE 100 in earthworms exposed to both PUF-amended soil and SAS. The ratio of 28 d worm tissue-to-soil ∑Penta-BDE burdens (dw basis) derived from the Liang et al. (2010) uptake study was about 48. Those derived from the ShuZhen et al. (2010) study were 20, 30, 31 and 7 for soil doses

of 10, 50, 100 and 500 μg/g ∑Penta-BDE (dw basis), respectively. Our apparent uptake ratios were lower, i.e. 2 and 13 (dw basis) for the PUF- amended soil and SAS treatments, respectively. In our and the Liang et al. (2010) studies, relative uptake from SAS and PBDE-spiked natural soil (on a dw basis) increased with decreasing concentrations (Fig. S1). In contrast, ShuZhen et al. (2010) observed increased relative uptake with increasing soil dose in all but their highest dose treatment (500 μg/g dw) (Fig. S1). Nyholm (2009) reported trends similar to those observed in our study in *E. fetida* exposed to PBDE-spiked artificial soil and hypothesized that this was a toxic response. Similarly, Blankenship et al. (2005), in a comparative study of PCB bioaccumulation at a Superfund site (soil ∑PCBs=6500 μg/kg wet weight (ww)) and a "clean" reference site (soil ∑PCBs=9 μg/kg ww), calculated BSAFs for depurated earthworms 5-fold higher at the reference site than calculated for the contaminated site. In a study of aging effects on PAH bioavailability, Chung and Alexander (1999) observed reduced phenanthrene assimilation (%) by *E. fetida* at increasing soil concentrations. Pyrene and PCB-77 BSAFs calculated for benthic invertebrates were also reported to decrease with increasing sediment concentration (Millward et al., 2001; Leppänen et al., 2003). In the Millward et al. (2001) study, lower BSAFs were attributed to decreased pore water (and/or gut fluid) solubility at higher sediment burdens, while Leppänen et al., (2003) attributed reduced BSAFs to compound-particle disequilibrium and reduced ingestion with increasing sediment concentration.

Worm uptake of the major Penta-BDE constituents (BDE-47, 99, 100) increased steadily with time in both our PUF-amended soil and SAS substrates. BDE-47 uptake from the PUF-amended soil mixture was not significantly different from BDE 99 at any

time point, but was significantly different with time from the SAS. While soil PBDE levels were 15-fold higher, mean 28 d ∑PBDE tissue burdens (lipid basis) in worms from the PUF-amended soil were only 4-fold higher than from the SAS (Table S4). This may indicate reduced PBDE bioavailability from the PUF. Tissue congener patterns resembled those of the Penta-BDE formulation, DE-71, and the PUF used in the exposures (Fig. 2). Even though homogenized, the presence of PUF microparticles and biosolids in soil would result in a heterogeneous "peppered" distribution of PBDEs similar to that reported by bromine in house dust reported by Webster et al (2009). Due to the physico-chemical sequestration of PBDEs within its structure, we anticipated greatly reduced relative uptake of PBDEs from the PUF compared to the SAS treatments. Instead, worms in the PUF-amended soil exhibited appreciably higher burdens. This may be due to inclusion of biochemically unassimilated PUF microparticles (and associated PBDEs) within the worm's digestive tract, despite a 24-hr depuration period. Ryan and Jackson (1987) reported a half-life of plastic pellets in the digestive tract of seabirds in excess of one year. Efforts to verify the presence of PUF microparticles in the worm gut using a dissection microscope were unsuccessful. More study is needed to evaluate the disposition of PUF microparticles, as well as fate of associated contaminants, within the worm gut.

Earthworm congener-specific BSAFs varied with substrate type, 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 ($0.001<p<0.05$) (Fig. 3; Table S5). Given the behavioral changes and mortality observed in earthworms exposed

to higher biosolids doses in preliminary testing, this pattern might be reflective of a reduction in assimilation due to acute toxicity. In contrast, the general BSAF trend in SAS-exposed worms was towards increasing (or steady) values with time and dose (low to high). This suggests that the reduction in BSAF with increasing dosage observed for the sludge treatments may not be due to the PBDEs, but rather other constituents or matrix interactions.

These SAS results contrast with those of Nyholm (2009) who observed decreasing BSAFs with increasing Penta-BDE spiking levels in SAS-exposed *E. andrei*. On the whole, our BSAFs for low and medium dose biosolids-exposed worms were comparable to those of SAS-exposed worms (Table S5). The Sellström et al. (2005) study of PBDE residues in earthworms collected from historically biosolids-amended soils in Sweden reported mean BSAFs of 5, 4.2, 4.6, 2.5 and 2.3 for BDEs 47, 99, 100, 153 and 154, respectively. Results from these field sites were generally consistent with those reported for the aquatic oligochaete, *Lumbriculus variegatus,* exposed to PBDE-spiked lake sediment (Leppänen and Kukkonen, 2004) and CB with incurred PBDEs (Ciparis and Hale, 2005). These BSAFs also agree with those reported for *E. fetida* in PBDE-spiked soil by Liang et al. (2010). Our high dose BSAFs calculated for ADB- and CB-exposed worms are comparable to those reports. However, our low and medium dose BSAFs for these biosolids-amended soils are considerably higher. Likewise, most of our BSAFs for BDE-47, 100 and 99 in SAS-exposed worms are higher (Fig. 3 and 4; Table S5 and S6). These results contrast with Hofman et al. (2008) who reported significantly reduced PAH bioavailability from solvent-spiked artificial compared to natural soil. Nyholm (2009) exposed *E. fetida* to artificial soil spiked with BDE-47, 99

and 153 at equal doses, at three levels (i.e. 10,000, 100 and 10 μg/kg dw). Maximum mean BSAFs for these congeners were 12, 9 and 4, respectively. Steady state was assumed by 28 d. In our study, maximum mean BSAFs for earthworms in the PUF-amended soil were about 3-fold higher (Fig. 4, Table S6). Maximum mean BSAFs for these congeners in our SAS treatments were about 17, 21 and 4, respectively (Fig. 4, Table S6). In earthworms exposed to artificial soils amended with sludges, BSAFs generally tracked those calculated for solvent spiked soil-exposed worms at comparable soil concentrations by Nyholm (2009).

At steady state, partitioning of hydrophobic chemicals from soil into organismal lipids should be independent of log Kow (Di Toro et al., 1991; Belfroid et al., 1995c; Ma et al., 1998). However, we observed that BSAFs decreased linearly with increasing compound log Kow for worms exposed to biosolids-amended soils (Fig. 5). Slopes were significantly lower in high compared to low dose biosolid substrates, largely as a result of greater BSAFs for BDE-47, 99 and 100. Interestingly, although the total PBDE burden of the low dose SAS (676 μg/kg) exceeded those of the low and high dose biosolids its regression line was intermediate. This BSAF pattern may be a function of the TOC content of the soil substrates. We thus examined the relationship between tissue lipid burdens and log Kow and detected significant differences ($p<0.05$) in BDE-47 and 99 burdens between high and low dose CB-amended soil treatments (Fig. S2). Differences between these congener burdens were not significantly different ($p>0.05$) in ADB-amended soil treatments. Moreover, these parameters were generally more strongly correlated in CB- compared to ADB-exposed worms. The stronger correlations observed in CB-exposed worms might be indicative of a failure to reach steady state by 28 d. The

trend of decreasing BSAFs with log Kow observed in our study (Fig. 5 and 6) agree with those reported for mixed worm species collected from Swedish agricultural fields previously treated with biosolids (Sellström et al., 2005) and for *E. fetida* exposed to solvent-spiked soil in the lab (Liang et al., 2010), as well as analogous soil-associated POPs (Krauss et al., 2005; Belfroid et al., 1994; Jager et al., 2005). Previous studies have shown that uptake from soil of hydrophobic chemicals (log Kow > 5), such as the PBDEs studied here, by *E. andrei* occurs primarily via the gut (Jager et al., 2003; Belfroid et al., 1995a,c).

Condition of worms. Significantly higher ($p<0.05$) fresh weights after depuration were observed in the ADB-exposed worms relative to CB- and SAS-exposed and control worms (Table S7). Contreras-Ramos (2009) also reported fresh weight increases for *E. fetida* in sludge-amended soils compared to PAH-contaminated vermicompost. ShuZhen et al. (2010) observed significant growth inhibition (relative to non-exposed controls) in *E. fetida* exposed to solvent-delivered DE-71 at all exposure doses (100-1000 μg/g dw). There, soil treatments were also augmented with food (corn meal). Rapid worm growth dilution could contribute to an apparent decrease in tissue burdens with increasing biosolid doses. Though differences were not statistically significant ($p>0.11$), this may help to explain why ∑PBDE burdens in ADB- exposed worms appear proportionally lower by 28 d compared to CB-exposed worms. Interestingly, burdens in low dose ADB-exposed worms were higher in 14 d treatments compared to corresponding CB-amended soil-exposed worms ($p<0.05$; Table S2).

Lipid reserves are an essential energy store for insects and as such their status may indicate overall organism fitness (Arrese and Soulages, 2010). For worms exposed

to a given substrate, differences in percent lipid content (as another general indicator of worm health) with increasing dose were not observed (Table S7). Total lipids in both SAS-exposed and control worms were significantly lower ($p<0.05$) in 28 d than 14 d samplings for all treatment doses. This may reflect the lower nutrient value of the artificial soil alone relative to the biosolids-amended soil, despite feeding and augmentation of the ASTM-recommended 10% to 15% (w/w) peat moss. Total extractable lipid values for PUF-exposed worms (Table S8) were about half those of biosolids-amended soil- and SAS-exposed and control worms (Table S7). This may be indicative of a nutritional effect or other stress in worms ingesting PUF microparticles. This would potentially inflate lipid-adjusted tissue burdens in PUF-exposed worms. Nonetheless, PBDE uptake (on a dw basis) still increased steadily with time. For SAS- and biosolids-exposed worms, total lipid levels were consistent with those reported for *E. fetida* exposed to PBDE-spiked artificial soil (Asamoah, 2005; Nyholm, 2009) and *Lumbricus terrestris* exposed to field-contaminated soils with incurred PCBs and PAHs (Krauss et al., 2000). However, as noted, our soils contained enhanced peat levels. Total extractable lipids in worms exposed to the PUF-amended soil were not significantly different with time. Total lipids in worms exposed to SAS and non-spiked control AS were not significantly different ($p>0.05$).

PBDE accumulation by PSDs. Plastics adsorb lipophilic contaminants effectively. For example, Teuten et al (2007) reported 100-fold or greater equilibrium partition coefficients for phenanthrene and polyethylene than for marine sediments. Other researchers have examined the performance of various PSDs in soils for POPs, including Liang et al. (2010) who used Tenax for $\sum$Penta-BDEs; Awata et al. (2000) who used C_{18}

silica-filled polyethylene for OC pesticides and van der Wal et al. (2004) who used solid-phase micro-extraction (SPME) fibers for organochlorine POPs. Accordingly, we examined PSDs in the ADB-amended soil treatments coincident with worms to evaluate their biomimetic efficacy in regards to PBDE partitioning.

In our study, mean PSD ∑Penta-BDE concentrations ranged from 45 to 142 μg/kg, comparable to those of the respective soil substrate doses (72 to 169 μg/kg dw; Table S9). In regards to individual congeners, the PSD BDE 47 burdens exceeded those in the ADB soil treatments (dw basis; $p<0.05$) after 28 d (Fig. 7), but BDE 100 and 99 PSD concentrations were lower than soil levels (dw basis; $p<0.05$ and $p<0.01$, respectively) (Fig. 7). This may relate to hysteresis in their release and subsequent transfer from the soil to the PSD. BDE 153 and 154 were < QL in the PSDs buried in the ADB-amended soil, likely due to a combination of modest soil concentrations and uptake hysteresis. Strandberg et al. (1997) reported that triolein-filled SPMDs sampled only about 1% of the total burden of a suite of incurred organochlorine pollutants in household compost. As PSDs are stationary, localized depletion of available PBDEs may also occur.

In contrast, worms sojourn through the soil column, ingesting fresh material. While BDE-47, 99 and 100 were quantifiable in our PSDs, concentrations were about 1% of those in worms, but comparable those in soil. In support of the importance of feeding behavior, Jager et al. (2003) observed reduced uptake with increasing log Kow in worms (*E. andrei*) prevented from feeding in soil receiving solvent-spiked POPs. Leppanen and Kukkonen (2004) obtained similar results for aquatic oligochaetes in sediments receiving solvent-spiked PBDEs. Experiments in which PSDs were buried in 100% ADB also

indicated greater BDE 47 uptake (Fig. S3), despite higher BDE 99 ADB burdens (Table S1). Liang et al. (2010) reported greater uptake of BDE-47 and 99 relative to BDE-100 from Penta-BDE-spiked natural soil by *E. fetida*. In their study, tissue burdens of BDE-47 and 99 were similar (dw basis). They also reported that PBDE accumulation on Tenax followed similar patterns and equated this to its efficient sampling of the rapidly desorbing fraction from soil. In our study, PSD uptake of BDE 47, 100 and 99 from 100% ADB was statistically different for all but the t=3 d time point (i.e. 47>99>100; $p<0.05$). The different results observed here may be explained by differences in substrate burdens and in congener composition of the parent ADB. ∑Penta-BDE levels in the 100% ADB were about 56-fold higher (dw basis) than spiking levels examined by Liang et al. (2010). To compare PSD accumulation to that seen in worms, and better compare our results to those of Liang et al. (2010), we also evaluated the relationship between PSD and worm tissue burdens (lipid basis) using least squares regression. These quantities were well correlated after 14 d ($r^2 \geq 0.76$) and 28 d ($r^2 \geq 0.85$), regardless of treatment dose.

We also calculated PSD accumulation factors (PAFs), i.e. ratio of PSD levels (dw basis) to TOC-normalized soil PBDE levels. PAFs were much lower than BSAFs for worms, i.e. from 0.03 to 0.2 versus 4.8 to 20, respectively (Table S10). As observed in worms, PBDE concentrations in the PSDs generally increased with soil dose while PAFs generally decreased or were unchanged with dose. Calculated PAFs generally tracked those of medium and high dose worm BSAFs ($0.71<r^2<0.97$), whereas low dose PAFs were not as well correlated ($r^2=0.58$). PBDE partitioning into PSDs presumably occurs via passive partitioning between substrate TOC and the surface layer of the PSD polymer

phase. Regression analysis of PSD and ADB-amended soil PBDE congener burdens (TOC-normalized) revealed that the two quantities were well correlated in 14 d ($r^2 \geq 0.91$) and 28 d ($r^2 \geq 0.89$) treatments.

While previous lab studies have demonstrated the availability of PBDEs following their addition via solvent carrier to soil our results demonstrate that PBDEs are similarly bioavailable from soils amended with sewage sludge biosolids generated by different wastewater treatment processes. Class B biosolids (consistent with our ADB) is the most commonly land-applied in North America. "EQ" biosolids (e.g. our CB) are promoted as pathogen-free and containing fewer contaminants. EQ distribution to and use by the general public is unregulated and essentially untracked. Its recent distribution as "organic compost" has been controversial in some US cities, e.g. San Francisco (Washingtontimes.com, 2010). Data on PBDE burdens in worms collected from historically sludge-amended field sites are crucial. However, the extent of exposure is difficult to characterize. Nonetheless, BSAFs estimated from controlled exposures to well characterized biosolid-amended soils (dependent upon dose) in the lab were comparable to values reported for worms of several species obtained from biosolids-amended agricultural soils in Sweden (Matscheko et al., 2002; Sellström et al., 2005). This is important as *E. fetida* is not common in agricultural soils and species-specific behavior in soils could impact contaminant accumulation. For example, Kelsey et al. (2005) reported that DDE accumulation from natural soils by *E. andrei* was significantly higher compared to uptake by *Lumbricus terrestris* (anecic) and *A. caliginosa*. In contrast, however, van der Wal et al. (2004) and Jager et al. (2005) found that uptake of PCBs and organochlorine pesticides from natural soils by the epigeic *E. andrei* and

endogeic *Apporectodea caliginosa* was comparable. From our PUF bioassay results, it is clear that earthworms exposed to small amounts of PUF particles in soil are able to accumulate PBDEs therein to high levels with no apparent toxicity. The earthworm burdens may be readily transferred further into terrestrial food webs. Though Penta-BDE manufacture has ceased, emission from extant and relic PUF will likely contribute substantially to global emissions for decades to come.

Figures

Figure 1. Mean ∑Penta-related PBDEs in worms (μg/kg lipid) versus levels in high dose ADB- and CB-amended soils and low dose Penta-BDE spiked AS (μg/kg TOC) after 28 d. Worm concentrations exceeded those in soils in all non-control treatments ($p<0.01$; two-sample t-test, assuming unequal variance). Error bars represent standard deviations from the means.

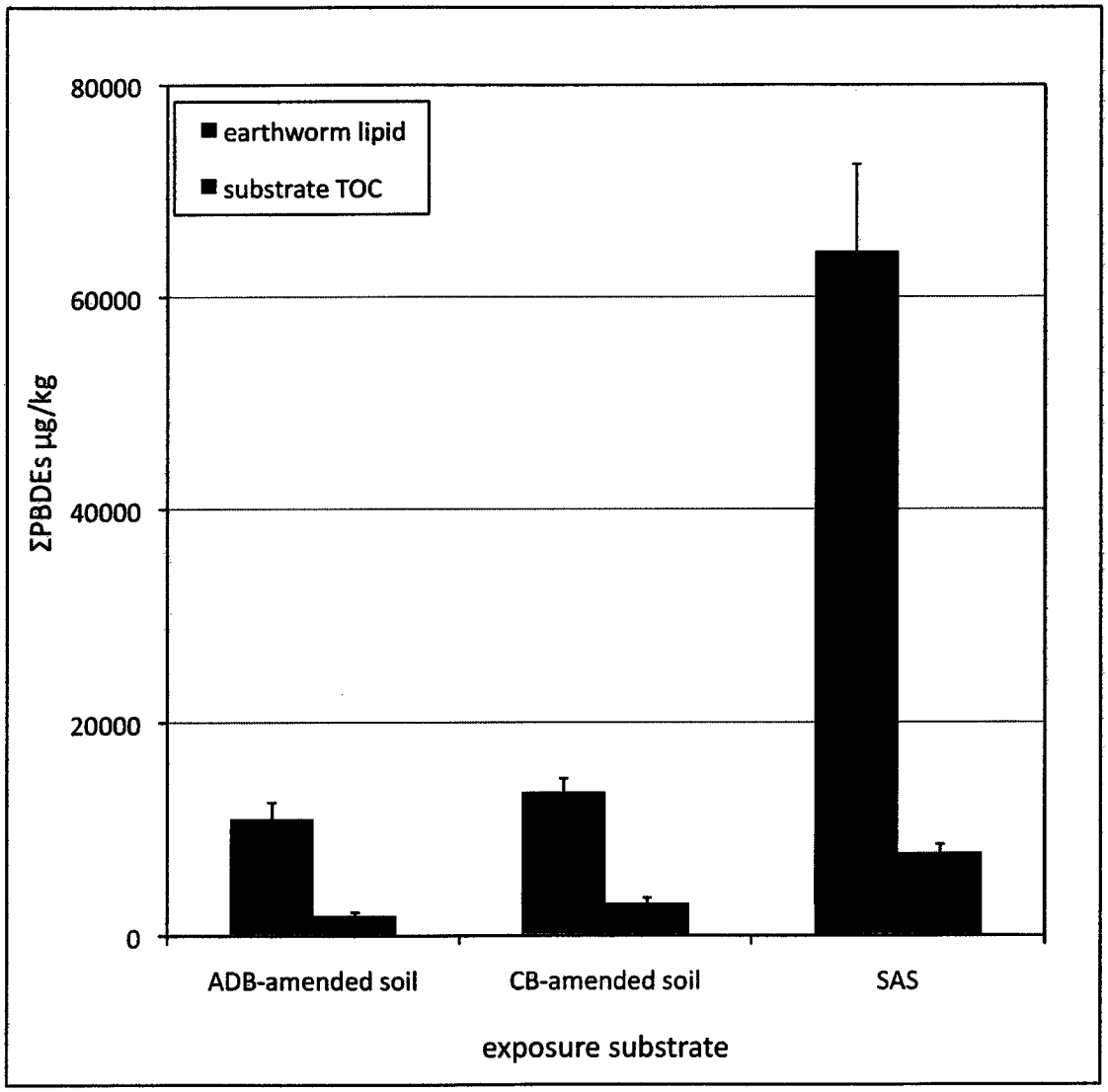


Figure 2. PBDE congener composition in worms exposed to soil with dispersed PUF microparticles and solvent-spiked Penta-BDE for 28 d (N=3); compared to PUF, PUF-amended soil and a commercial Penta-BDE formulation, DE-71. Error bars are standard deviations from the means.

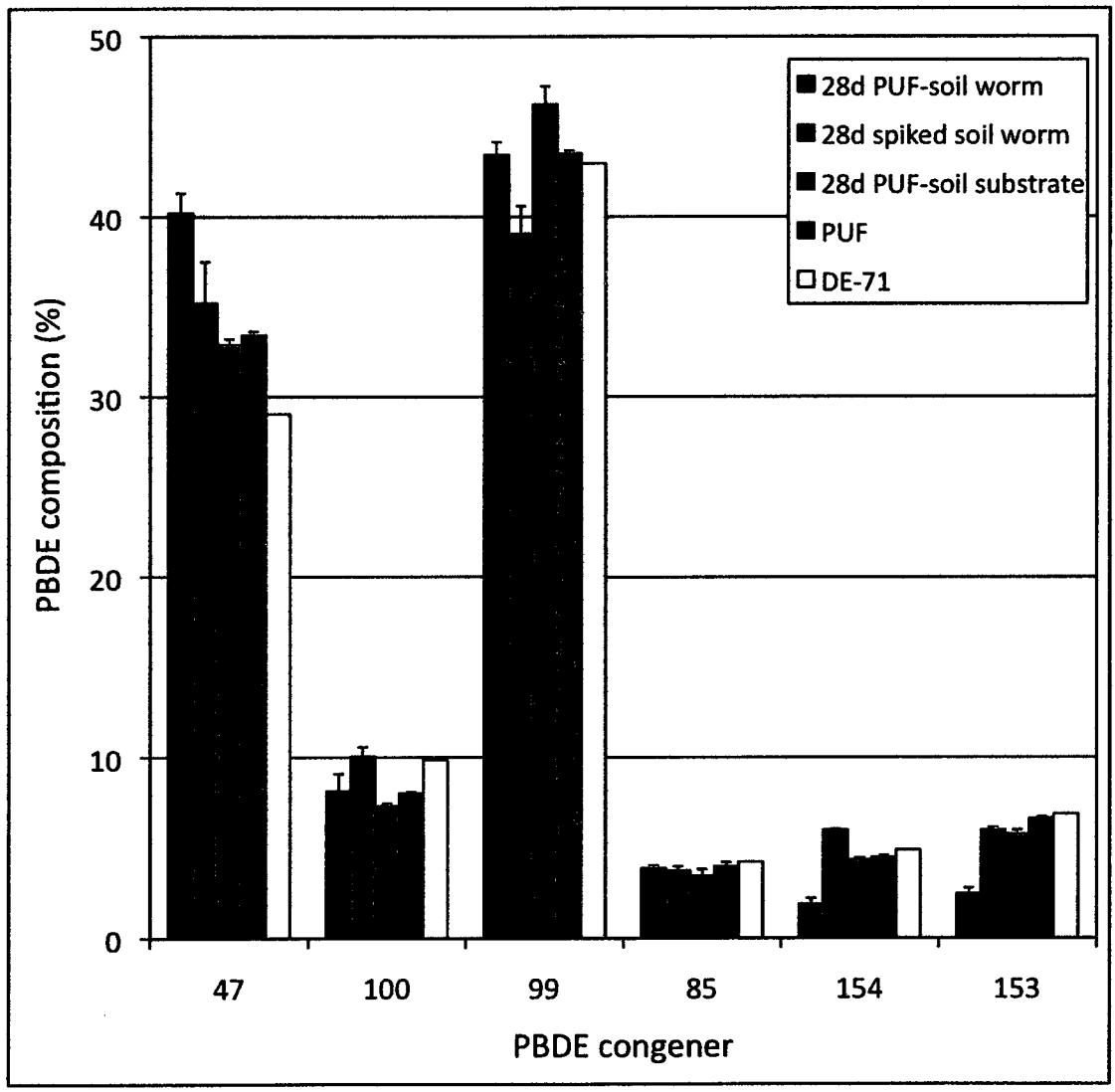


Figure 3. Mean worm BSAFs (lipid/TOC normalized basis; N=3) for BDE-47, 100 and 99. Low and high dose ADB and CB and low dose SAS soil exposure results on 28 d shown. Soil ∑PBDE concentrations in μg/kg dw (see legend). For each congener, bars with different letters are statistically different (ANOVA with Tukey HSD testing). Pair-wise comparisons: ADB low versus high doses (BDE 47 $p<0.001$; BDE 100, 99 $p<0.01$); CB low versus high doses (BDE-47, 100, 99 $p<0.01$). High dose BSAFs for all congeners in all biosolid treatments are significantly lower than low dose BSAFs ($0.001<p<0.05$). For the SAS substrate, means of all congener BSAFs are significantly different from each other, i.e. BDE 47>100>99 ($0.001<p<0.05$). Error bars represent standard deviations from the means.

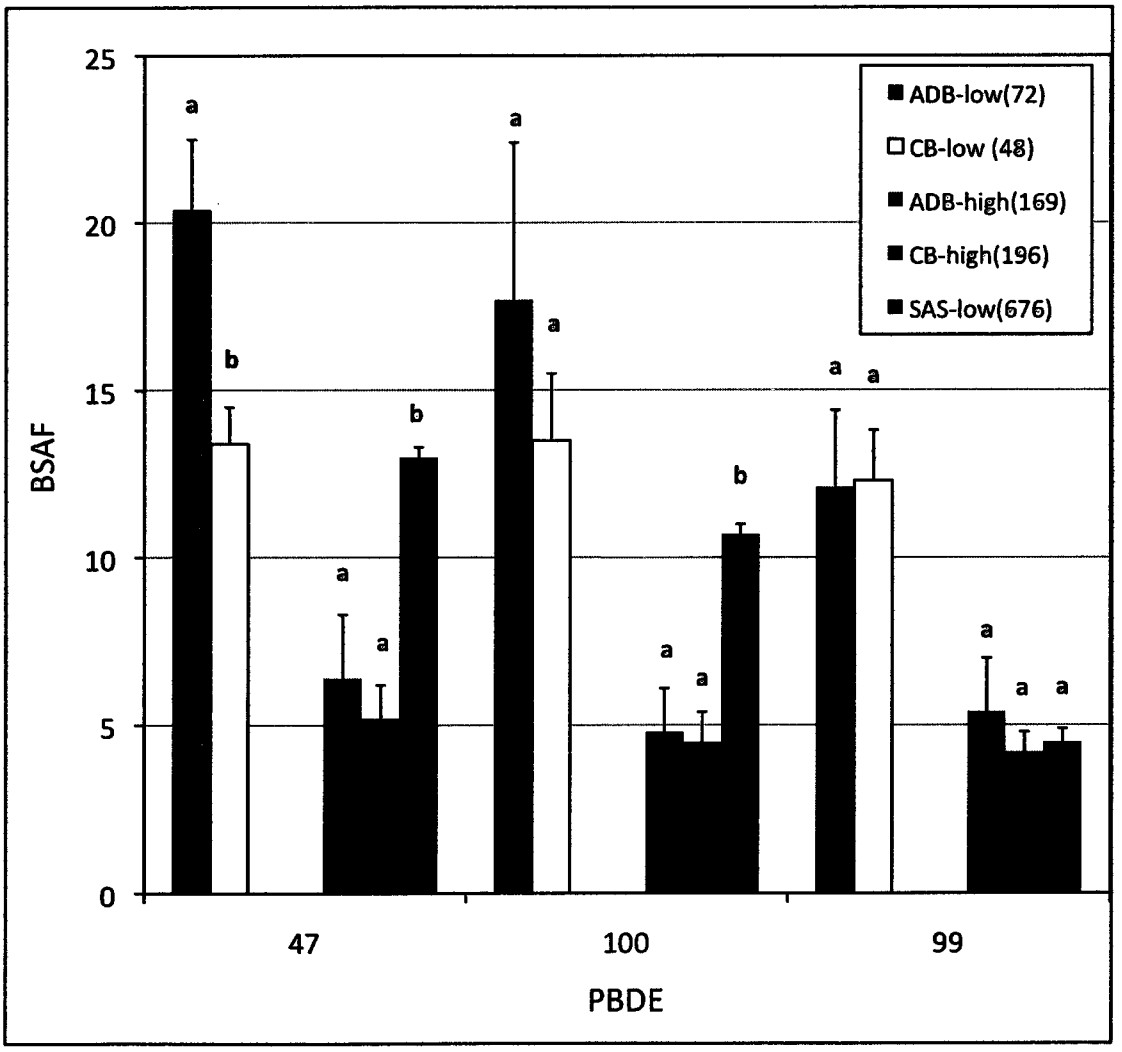
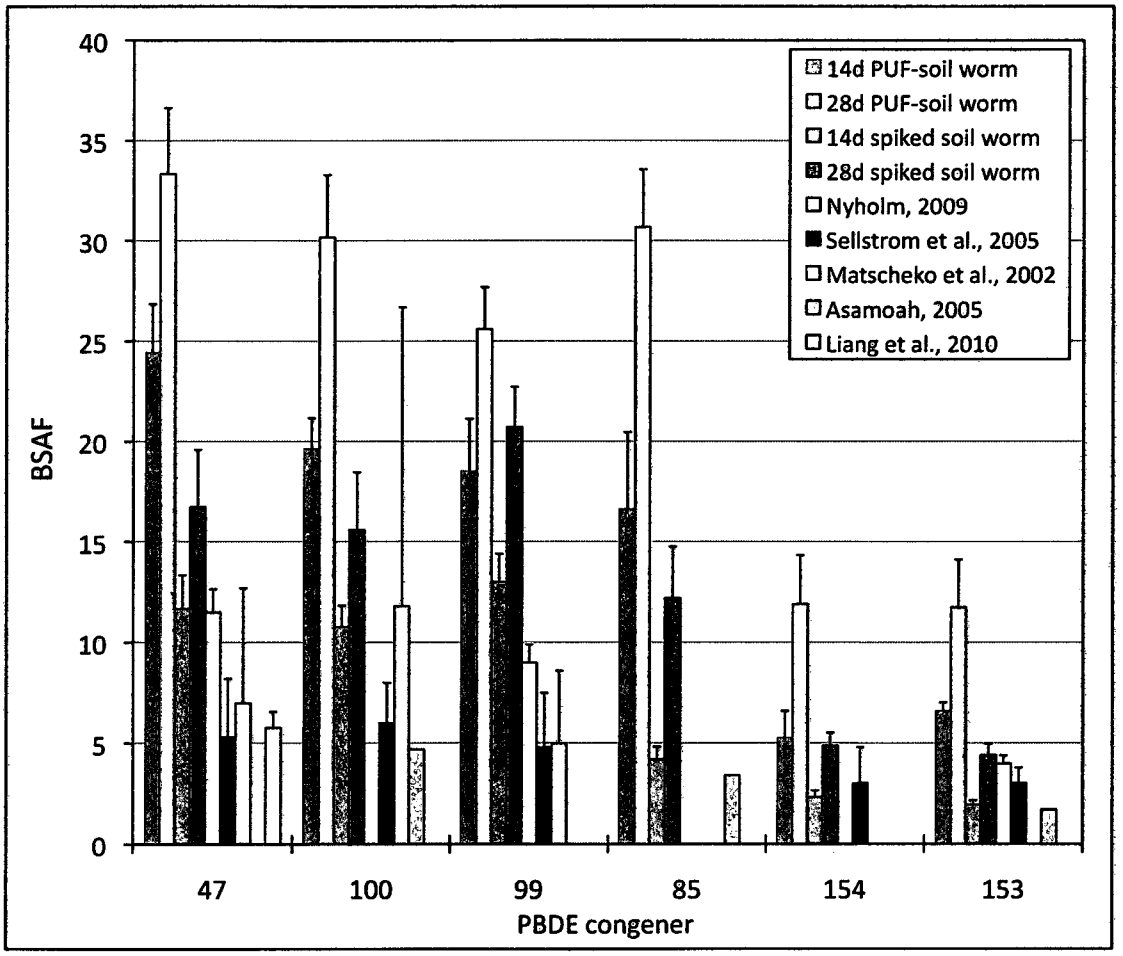


Figure 4. Comparison of mean PBDE BSAFs calculated for earthworms exposed to the PUF-amended soil mixture (83.3 mg/kg dw) and the solvent-spiked artificial soil (SAS; 5.6 mg/kg dw) here and those previously reported for earthworms exposed to PBDE-spiked artificial (Asamoah, 2005; Nyholm, 2009) and natural (Liang et al., 2010) soils and biosolids-amended field soils (Sellström et al., 2005; Matscheko et al., 2002).



Additional notes: For the Matscheko et al. (2002) data, we calculated means for their reported BSAFs for seven (BDE-47 and 99) and four (BDE 100) sludge-amended field sampling sites, respectively. Error bars are standard deviations from treatment means for this study and our meta-analysis of the Matscheko et al. (2002) means. Error bars for Liang et al. (2010) data are their reported standard deviations for BDE 47. In that study, means for all other congeners were reported as a range and are thus not plotted. Error bars are standard error of the mean for Sellström et al. (2005) data and one standard deviation from the mean for the Nyholm (2009) data. No replication was reported in the Asamoah (2005) study. Note that only highest dose (100 μg/kg dw) BDE-47 BSAF data were available for analysis from the Liang et al. (2010) study (all other means given as a range). Missing congeners were not reported for a given data set.

Figure 5. Mean 28 d Penta-BDE BSAFs (for low- and high-dose biosolids-exposed worms and low-dose Penta-BDE-spiked SAS-exposed worms) versus published log Kow values (Braekevelt et al., 2003). Congeners for which data are missing were below QL. Error bars represent standard deviations from the means (N=3).

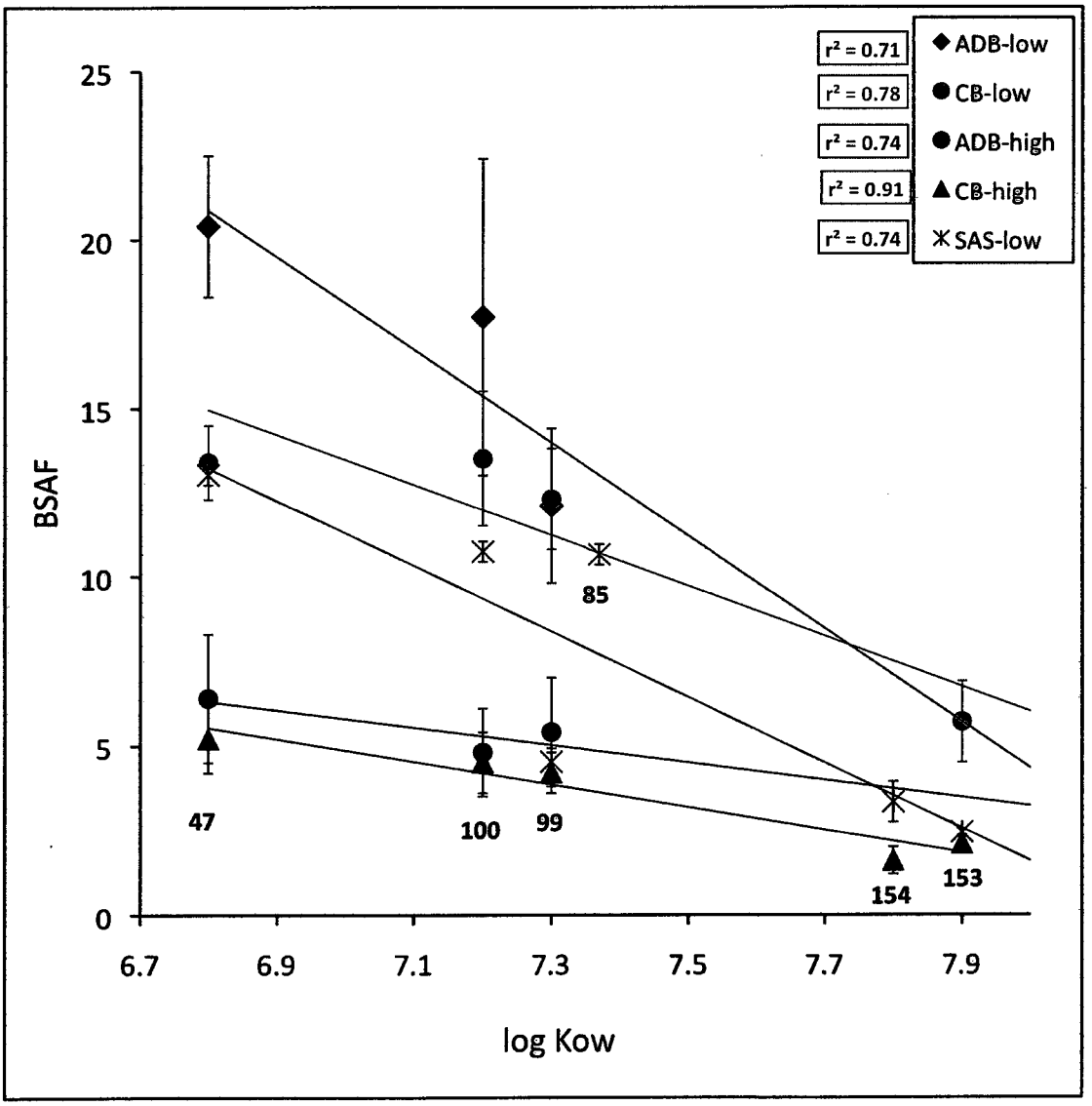


Figure 6. Least squares regression analysis of mean earthworm PBDE BSAFs for PUF microparticle and associated SAS exposures versus log Kow values (from Braekevelt et al., 2003). All correlations are statistically significant (PUF-amended soil $p<0.01$) or nearly significant (SAS $0.05>p<0.052$). Error bars are standard deviations from treatment means (N=3).

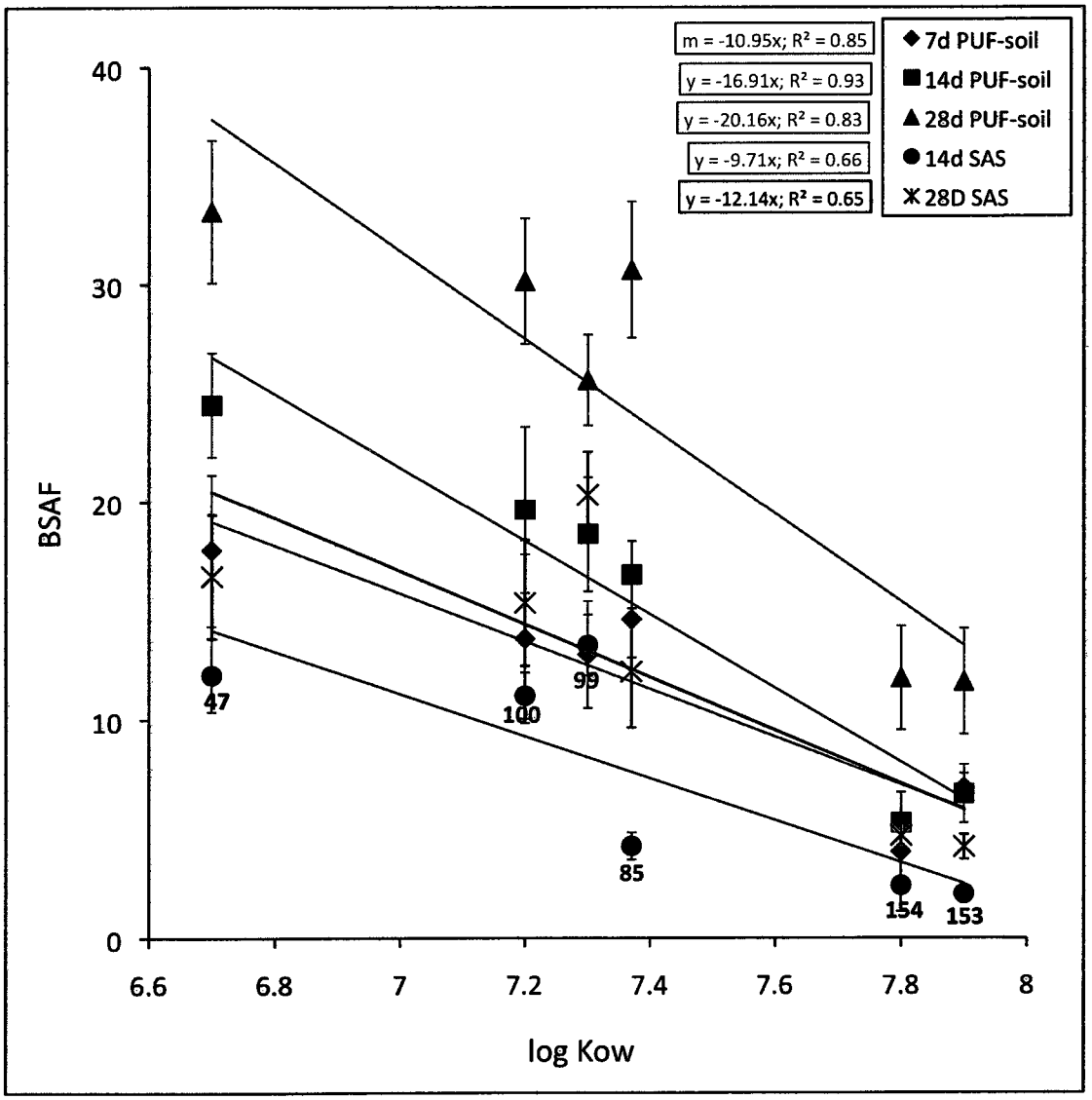
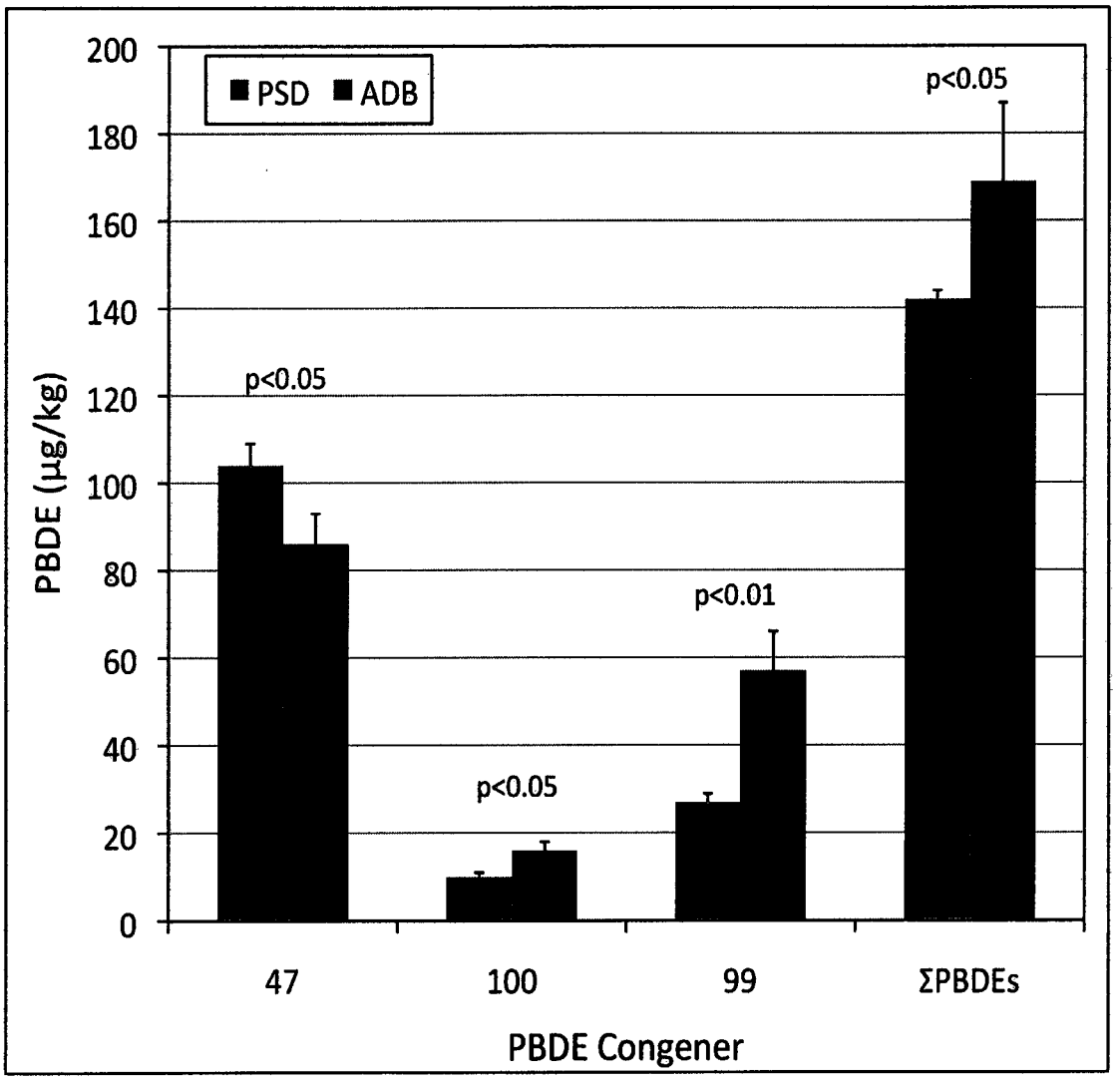


Figure 7. BDE-47, 99, 100 and ∑PBDEs concentrations in passive sampling devices (PSDs; µg/kg PSD) versus the corresponding ADB substrate (µg/kg dw) after 28 d. p values shown. Error bars represent standard deviations from the means (N=3).



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Chapter 2

Accumulation of Brominated Flame Retardants by Insects from Polyurethane Foam Derived from Household Furniture

Abstract

Polybrominated diphenyl ethers (PBDEs) are flame retardant additives in common at percent levels in polymer products pervasive in homes and the workplace. These PBDEs were long assumed sequestered within plastics. However, mg/kg burdens have recently been detected in indoor dust and are believed to arise from these consumer products. Most PBDEs enter and reside in terrestrial compartments, yet few investigations of their fate here have been conducted. Insects play a critical role in terrestrial ecosystems and several taxa are known to consume in-use and discarded textiles and polymers. Direct ingestion of even minute amounts of PUF containing PBDE additives may yield substantial body burdens in such organisms. Here, house crickets (*Acheta domesticus)* were allowed free access to PUF containing Penta-BDE at 8.7% (dry wt) in the laboratory. Crickets allowed to depurate after 28 d exposure exhibited body burdens of up to 13.4 mg/kg lipid ∑Penta-BDE. These burdens are 1000-fold higher than typically reported in humans. Non-depurated crickets contained up to 80.6 mg/kg lipid ∑Penta-BDE. Molted exoskeleton burdens contained up to 63.3 mg/kg lipid. Congener patterns of whole cricket and molts resembled those of PUF and the commercial Penta-BDE formulation, DE-71, indicative of minimal discrimination or biotransformation. Determination of precise cricket accumulation factors was hampered by difficulties in determining the exact extent of PUF ingestion. However, estimated accumulation factors were in the range of 10^{-4} to 10^{-3}. The magnitudes of the factors were likely low due to the limited bioavailability of the PBDEs contained within the polymer. Nonetheless, even these low transfer efficiencies resulted in substantial burdens in these insects due to the percent PBDE levels in such polymers. Once ingested even burdens not absorbed

across the gut wall may be dispersed geographically by the insect's movements or transferred within food webs as a result of predation.

Introduction

Increasingly, concerns are being raised about the accumulation of plastics in the environment, most recently illustrated by the appearance of "garbage patches" of immense sizes in oceanic gyres (Rios et al., 2007; Teuten et al., 2009). Greatest attention has centered on biological impacts due to physical entanglement or digestive blockages due to ingestion of plastics in aquatic environments (Ryan et al., 1988; Browne et al., 2008). However, most plastics are used, discarded and remain in the terrestrial compartment. Recently, the toxicological implications of the ingestion of polymers with sorbed hydrophobic contaminants have received increasing attention (Heudorf et al., 2007; Teuten et al., 2007; Teuten et al., 2009). Plastic surfaces may concentrate such contaminants from ambient air and water by orders of magnitude. Indeed, researchers make use of this phenomenon in the design of passive samplers for collection of airborne semi-volatile persistent organic pollutants (POPs) (Foday et al., 2004). Levels of sorbed POPs are typically orders of magnitude lower than those of chemicals intentionally used as additives in many polymer products. For example, polybrominated diphenyl ethers (PBDEs) have been widely added to flame retard consumer plastics. Of the three commercial PBDE mixtures, Penta-BDE is the most bioaccumulative and toxic. It is dominated by six congeners, with BDE 47 and 99 comprising about 86% of the formulation (La Guardia et al., 2006). While viewed by the scientific community as a

global pollutant of concern, historically over 90% of worldwide Penta-BDE usage has occurred in North America, with most of that in the US (Hale et al., 2003).

Penta-BDE was used primarily to flame retard polyurethane foam (PUF) products. About 5.5 x 10^8 kg of PUF is produced annually for the manufacture of upholstered furniture cushioning in the US (Alliance for Flexible Polyurethane Foam, 2010). Another 2.3 x 10^8 kg is used for carpet underlayment, in the automotive sector and in other applications (Polyurethane Foam Association, 2010; Alcock et al., 2003). To meet strict US flame retardancy standards manufacturers have added percent levels by weight of Penta-BDE to PUF intended for consumer products (Hale et al., 2002). PBDEs are incorporated in, but not chemically reacted with, the polymer. This increases the potential for additive escape over the product's lifetime. This contradicted a long held view that PBDEs were permanently sequestered in finished polymer products. The observation of mg/kg levels of PBDEs in indoor dust, and their increasing levels in humans and wildlife serves to undermine this assumption and underscore the need for a better understanding of the role of polymer products in exposure. In the face of increasing environmental and human health concerns, US Penta-BDE production ceased in December 2004.

While generally viewed as very long-lived in the environment, many plastics weather and disintegrate relatively rapidly (Dementev et al., 1999; Browne et al, 2008; Barnes et al, 2009; Saido et al., 2009). For example, upon environmental exposure, PUF has been observed to begin to crumble into small fragments in a few weeks, while retaining the bulk of its original PBDE composition (Hale et al., 2001). As particle size decreases, surface area increases and hence the potential for additive release and

dispersal. The importance of finished products rather than manufacturing as a source for PBDEs to the environment was highlighted in a study of samples obtained in and near an Antarctic research facility (Hale et al., 2008). No BFR or plastics manufacturing occurs in Antarctica. Nonetheless, substantial PBDE concentrations were discovered in indoor dust within facility buildings, sewage sludge from its new wastewater treatment plant and aquatic organisms collected nearby.

Legacy persistent organic pollutants (POPs), such as PCBs, historically were released primarily through industrial or heavy commercial processes. Human exposure to such POPs has since occurred predominantly via consumption of contaminated wildlife (i.e. fish), rather than direct contact with PCB-containing products (Harrad and Diamond, 2006). In contrast, the dominant pathway for human PBDE exposure appears to be ingestion of indoor dust arising from PBDE-treated polymer products in homes, vehicles and the workplace (Harrad and Diamond, 2006; Allen et al., 2008; Frederiksen et al., 2009; Webster et al., 2009). Some of the PBDE within such dust may still be contained within polymer fragments (Webster et al., 2009) and this may control its bioavailability. Owing to extensive usage in polymer products and their ubiquity within human living spaces, losses from in-use and discarded products will be important PBDE sources to the global environment well into the future (Frederiksen et al., 2009). The total reservoir of BDE 47 alone available for distribution from finished PUF products to the US and UK environments has been estimated to be about 2620 and 520 metric tons, respectively (Alcock et al., 2003)

Fugacity modeling indicates that the bulk of PBDE-treated polymer products and associated PBDEs will be discharged to, and remain in, the terrestrial environment (Palm

et al., 2002). In addition, insects (e.g. crickets, carpet beetles, silverfish, termites, ants and moth larvae) have long invaded human habitations causing serious damage to household items and structural elements. Nonetheless, most insect-related POP studies have focused on aquatic rather than terrestrial species. Recent studies of the role of spiders in transferring aquatic-derived mercury and PCB burdens to terrestrial-feeding birds have highlighted the importance of terrestrial transport pathways via lower organisms (Cristol et al, 2008; Walters et al., 2009). Findings of substantial burdens of highly brominated PBDEs in terrestrial feeding birds of prey (Chen and Hale, 2010) also support the need for further investigation of terrestrial exposure routes. Insects also constitute a significant portion of terrestrial ecosystem species and biomass. Terrestrial epigeic arthropods can reach densities of 10-20 g m^{-2}, depending upon biotope (Dangerfield, 1990). Yet we are aware of only two published reports concerning PBDEs and terrestrial insects (Hale et al., 2002; Wu et al., 2009). In both, determining PBDE burdens in insects were incidental aspects of larger amphibian bioaccumulation studies.

Bioaccumulation and toxicity of soil PCBs to the house cricket *A. domesticus* (Paine et al., 1993), as well as the utility of this species to serve as a bioindicator of POP contamination (Walton, 1989; Paine et al., 1993), has been demonstrated. For example, *A. domesticus* has been used as a model organism to assess PAH (He et al., 1998) and PCB (Li and McKee, 1992) toxicokinetics. Such studies demonstrate the potential for crickets to accumulate/eliminate POPs and transfer accumulated burdens within food webs. Yet, the extent to which such terrestrial organisms may assimilate PBDEs directly from such polymers has remained largely uninvestigated. To further evaluate the potential of insects to accumulate and eventually transport PBDEs within terrestrial food

webs, we evaluated accumulation from a commercial PUF product by a common insect, the house cricket.

Experimental

Cricket-PUF bioassay. The ASTM soil toxicity/bioaccumulation bioassay (ASTM E-1676, 1997) was used as a starting point to assess the accumulation and bioavailability of Penta-BDE contained in commercial PUF to house crickets (*A. domesticus)*. Briefly, 21-day nymphs were purchased (Fluker Farms; Port Allen, LA) and acclimated to laboratory conditions for 48 hours prior to exposure. Groups of nymphs (n=10) were randomly assigned to 2 L beakers (N=4 treatments and N=4 controls). Food and water (Fluker Farms Cricket Quencher© and Cricket Feed©) were provided *ad libitum* for the duration of exposure. PUF was obtained from a local upholstery shop. Small (0.5 g) cylinder-shaped cores (6 cm x 2 cm o.d.) were cut from this PUF. Cores and cardboard shelters were placed in the test beakers. Cores were weighed before and after exposure to estimate PUF ingestion. Crickets in control beakers were treated identically, except with no access to PUF. The bioassay was conducted at 26±3°C, relative humidity of 45±5%, with a 12:12 light:dark photoperiod. PUF-exposed and control crickets were collected at day 14 and 28 of the experiment. Crickets were depurated by removing the PUF from treatment beakers and allowing them to consume only fortified food and water *ad libitum* for 96 h after each exposure time. Cricket body lengths were measured.

Exoskeleton molts were collected as discovered and frozen until analysis. Those obtained from exposed and control treatments were small (< 0.1 g dw), necessitating pooling to allow PBDE quantitation (n=2 composites each for treatment and control

crickets). To conserve sample for PBDE analysis, lipid content determinations were, conducted on molts (n=3) collected from crickets of the same age cohort from the stock population. To estimate growth and minimize stress to test crickets, individuals (n=60) were selected at random from the stock culture at the start of the bioassay. These were considered "t=0" crickets and were not used in the subsequent exposure assay. Crickets and molts were rinsed with deionized water to remove any adhering material, lyophilized and homogenized prior to analysis.

PBDE analysis. The analytical methodology generally followed that of Chen et al. (2008). Briefly, freeze-dried samples were spiked with a surrogate standard (PCB 204) to monitor analyte recoveries and then subjected to enhanced solvent extraction (Dionex ASE 200; Sunnyvale, CA) using methylene chloride at 100°C and 1000 psi. The lowest concentration PBDE standard (16 ng/ml) yielding a S/N ratio of ≥ 3 was used to establish quantitation limits (QLs). Thus QLs varied by sample weight available for extraction: 23-81 µg/kg dw (213-870 µg/kg lipid) for whole cricket samples, 128 µg/kg dw (1280 µg/kg lipid) for molts and 81 µg/kg dw (133 µg/kg C) for PUF replicates. Total extractable lipids in solvent extracts were estimated by evaporating 10% of the total to constant weight. The remainder of the extracts were purified on an EnviroSep® size-exclusion column (Phenomenex; Torrance, CA) by elution with methylene chloride, followed by polarity-based purification on 2 g silica gel solid-phase extraction (SPE) columns (EnviroPrep®; Honeywell Burdick & Jackson; Muskegon, MI). Following solvent exchange to hexane and addition of an internal quantitation standard, (decachlorodiphenyl ether: DCDE; Ultra Scientific; Kingston, RI), PBDEs were separated on a gas chromatograph (GC; Varian 3400; Sugar Land, TX) equipped with a

15 m DB-5 column (J&W Scientific; Folsom, CA; 0.25 μm film, 0.32 mm ID) and Varian 8200 CX autosampler. The GC carrier gas was helium and injections were made in the splitless mode. Samples were analyzed with full scan electron ionization mass spectrometry (MS; Varian 4D ion-trap; Sugar Land, TX). To quantify PBDEs, the area of three major ions of each PBDE congener was summed relative that of the internal standard, DCDE, using an 8-point calibration curve. PBDE standards examined included the dominant congeners in the commercial Penta-BDE mixture, DE-71 (Chemtura Corporation; West Lafayette, IN), i.e. BDE 28, 47, 85, 99, 100, 153 and 154 (Cambridge Isotope Laboratories; Andover, MA). All PBDE data were corrected for PCB 204 recoveries (mean recoveries in crickets: 77±28%, n=20; molts: 83±12%, n=2; PUF: 91±16%, n=4). BDE-28 and 85 were detectable in all cricket samples, but were below QLs. PUF total organic carbon content (TOC) was determined using an Exeter CHN Model 440 CE Elemental Analyzer (North Chelmsford, MA).

QA/QC. Care was taken throughout the study to minimize sample contamination and analyte carryover among samples. Prior to exposure, crickets, food and housing materials were analyzed to verify that PBDEs were below detectable levels. Composite cricket samples and procedural blanks (ignited sodium sulfate) were also spiked with the PBDEs of interest and a surrogate standard (PCB 204) and analyzed in triplicate. Recoveries were excellent (∑PBDEs: 89±11%; PCB-204: 85±8%). Random subsamples (n=4) of the exposure PUF were collected and analyzed to ensure homogeneity of PBDE distribution within the PUF (discussed below). Instrumental QC included daily injections of standard solutions (at multiple dilutions) and solvent blanks. Multiple dilutions of quantitation/calibration and retention standards were run and quantified along with each

set (10-20) of samples analyzed. BDE 47 and 99 were detected in a few procedural blanks, but were below the QL in all instances.

Statistical analysis. Statistical evaluations were performed using StatPlus for Mac (AnalystSoft Inc.; Vancouver, BC, Canada). Significance was determined at the α=0.05 level using two-tailed testing. One-way Analysis of Variance (ANOVA) was used to determine significant differences in mean tissue PBDE burdens, bioaccumulation factors (BAFs) and total extractable lipids among treatments. BAFs were computed as the ratio of lipid-normalized whole body tissue PBDE burdens in depurated crickets to organic carbon-normalized PBDE concentrations in subsamples (n=4) of exposure PUF cores. One-way ANOVA was also used to determine significant differences in cricket body length and relative growth among treatments and compared to controls. A two-sample t-test (assuming unequal variance) was used to determine significant differences in mean tissue PBDE congener levels within treatment groups, as well as differences in apparent PUF mass ingested and mortality. Where ANOVA detected significant differences in means, pair-wise comparisons were made using a Tukey post-hoc Honestly Significantly Different (HSD) test. All data were normally distributed as determined using the Kolmogorov–Smirnov test.

Results and Discussion

PBDE bioaccumulation in PUF-exposed crickets. Despite provision of fortified food and water *ad libitum*, we observed that house crickets frequently browsed directly on the PBDE-treated PUF. These insects accumulated substantial levels of PBDEs, but displayed no obvious signs of distress. Total mortality among treatment crickets was not

significantly different ($p>0.05$) from controls. The mean ∑PBDE (BDE-47, 85, 99, 100, 153, 154) concentration in the PUF itself was 8.7±1.4% (n=4). This is in agreement with expected Penta-BDE levels reported for such products (Hale et al., 2002) and is orders of magnitude greater than levels typically reported in common environmental matrices such as indoor dust, soils, sediments or biota (Hale et al., 2003).

After a 96 h depuration period, mean whole cricket ∑PBDE burdens were high, i.e. 14.9±2.5 and 13.4±3.8 mg/kg lipid (1.6±0.3 and 1.4±0.3 mg/kg dw) in crickets exposed for 14 d and 28 d, respectively (Fig. 1; Table S1). PBDE burdens in all non-exposed control crickets were below the QL. In our study of earthworms exposed to PUF-amended soil (∑Penta-BDE=83.2 mg/kg dw), these oligochaetes exhibited ∑PBDE whole body burdens up to 3740 mg/kg lipid (see Chapter 1). By comparison, Wu et al. (2009) reported ∑Penta-BDE burdens of only 14.1 μg/kg ww in insect composites collected from an e-waste recycling facility in China. However, the dominant PBDE mixture used in electronics is Deca-BDE. In our crickets, differences in ∑Penta-BDE tissue concentrations at 14 and 28 days were not significantly different. Whole body ∑PBDE burdens in non-depurated crickets were 7.3±1.9 and 80.7±11.1 mg/kg lipid for 14- and 28 d treatments, respectively (Fig 1; Table S1). The much greater levels in the 28 d non-depurated crickets are believed due to retention of PUF particles in the gut. Hale et al. (2002) reported even higher mean ∑Penta-BDE burdens (257 mg/kg lipid) in a serendipitous analysis of non-depurated PUF-exposed crickets used as food for a lab-reared amphibian population. PUF levels there were 32% by weight, about 3-fold higher than those in our exposure cores. In that study, predator-prey contaminant transfer was

observed, i.e. frogs feeding on PUF-exposed crickets accumulated ∑Penta-BDE burdens of 209 mg/kg lipid.

Most published chemical exposure studies using terrestrial invertebrates have focused on mortality and reproductive impairment in targeted insect pests from intentionally engineered biocides. Assessments were generally conducted on short time scales (i.e. 24-72 hr) (see Burrow, 1989 for a review), as rapid time to death was a desired pesticide performance criterion. In contrast, research on POP bioaccumulation in non-target terrestrial invertebrates has been limited. However, a few recent studies have highlighted the important role of major terrestrial arthropods, i.e. *Coleoptera* and *Orthoptera* (Blankenship et al., 2005) and *Tetranathidae* and *Araneidae* (Walters et al., 2009), in transferring PCBs within terrestrial food webs. Earlier efforts (Davis et al., 1981; Watson et al., 1985) showed that soil arthropods (including *Orthoptera*) could accumulate μg/g PCB burdens (lipid basis) from field-contaminated soil. These studies suggest that soil arthropods may serve as indicator species of contamination.

PBDE congener signatures can provide valuable clues as to exposure sources and biotransformation processes (Ikonomou et. al, 2002). After 28 d, the PBDE congener patterns of whole cricket extracts and molted exoskeletons resembled that of the PBDE-treated PUF and the commercial Penta-BDE formulation, DE-71 (Fig. 2). This suggests minimal congener-specific discrimination or in-vivo PBDE biotransformation over the exposure period. However, a general trend towards decreasing BDE 47/99 and 100/99 congener ratios with time was observed. But, only the BDE 47/99 ratio was statistically different ($p<0.05$) with time (comparison of congener ratios in crickets, sub-sampled exposure PUF cores and the US commercial Penta-BDE formulation is provided in

Supporting Information Fig. S1). Interestingly, comparable congener patterns were also observed in our study of earthworms (*Eisenia fetida*) exposed to PUF-amended soil in the lab (see Chapter 1).

PUF mass losses from the exposure cores, as an estimate of apparent total PUF ingestion, were 7.3±2.1 and 5.7±4.2 mg in 14 d and 28 d depurated treatments, respectively. PUF mass losses from non-depurated treatments were 11.4±5.3 and 10.4±4.9 mg, respectively. Estimates were not significantly different, regardless of treatment. These values are in the range of 1.5 to 2.8% of total exposure PUF mass provided. Values denote the percent mass removed from the foam cores provided, not the amount ingested. Hence, in lieu of increases in the apparent mass of the remaining foam by fouling, the estimate is likely an upper bound.

It is uncertain why PUF ingestion was not higher in crickets exposed for a longer period. One possibility is that crickets found the PUF increasingly unpalatable, perhaps as a result of impacts on digestive processes due to PUF gut retention, and reduced their intake accordingly. This would also help to explain why tissue ∑PBDE burdens were generally unchanged with exposure time in depurated crickets. Apparent PUF ingestion was not proportional to the higher whole body burdens measured in 28 d non-depurated crickets. As crickets are messy feeders (Clifford and Woodring, 1990), this lack of apparent ingestion increase might also have been due to an augmentation in PUF core weight due to fouling or PUF fragments retained in the gut. No data on retention of plastics in the insect gut are available. However, greater gut retention time permits enhanced extraction of food constituents (Yang and Joern, 1994). Substantial gut retention times in other wildlife have been observed. For example, Ryan and Jackson

(1987) estimated a half-life of plastic particulates in the digestive tract of seabirds in excess of one year. After normalizing BAFs to the PUF mass loss from the exposure cores, calculated cricket/PUF apparent BAFs were on the order of 10^{-3} to 10^{-4} for depurated and non-depurated crickets (Table 1).

Little published data exist for dietary ingestion rates for crickets. Woodring et al. (1977) reported a mean daily feeding rate of 31.4 mg/d for late instar female *A. domesticus* larvae, while Clifford et al. (1990) reported 16-34 mg/d for similar age instars and adults. Though we did not quantify food ingestion directly, our estimates of apparent PUF mass ingested by foam-exposed crickets are more than 10-fold lower than theirs. Our estimates are also lower than those reported for *A. domesticus* exposed to PCB (Aroclor 1254)-spiked natural soil in a controlled 14 d toxicity bioassay (Paine et al., 1993). There, mean food consumption by crickets was consistent with that observed by Woodring et al. (1977) and Clifford et al. (1990) for non-exposed crickets and did not vary significantly (e.g. 364-476 mg) over a range of exposure concentrations (i.e. 0-2000 μg/g dw), despite significant mortality at the highest soil doses (e.g. 1000 and 2000 μg/g dw; 144-149 μg/g ww tissue burdens).

Humans spend about 80% of their lives indoors, in proximity to flame retarded polymer products. The observed cricket PBDE concentrations are about 1000-fold higher than those typically reported for human sera (Frederiksen et al., 2009). Our data suggest that insects frequenting homes or having access to discarded PUF products may ingest small amounts of polymers and that these may in turn transmit large amounts of PBDEs. Associated PBDE body burdens would then be available for transfer to predators, regardless of the assimilation efficiency within the prey organism. The low calculated

BAFs presented here are likely due to the limited assimilation of the PUF-associated PBDEs.

Crickets are proficient at shredding and masticating ingested food items, including man-made materials. Associated increases in surface area of plastics may enhance additive bioaccessibility. PBDEs are not chemically bound to PUF. In addition, PUF exhibits a large surface area, due to its open-cell structure. This contrasts with dense plastics such as high-impact polystyrene used in electronics casings. However, the high affinity of the PBDEs for the PUF, the viscosity of the polymer and the rate of diffusion to the polymer surface will limit transfer to air/water interstitial spaces in soils or gut fluids following PUF ingestion. POPs incurred in environmental solids such as sediments are typically assumed to be sorbed to complex pools of natural organic carbon accreted on particles and thereby less accessible to biota. Teuten et al (2007) reported phenanthrene K_d values of 19 and 135 for sandy and silty aquatic sediment, respectively, but a mean value of 38,100 for polyethylene powder. However, on an organic carbon basis, partition coefficient values (K_{oc}) were within a factor of five. Naturally occurring organic carbon also occurs at relatively low levels in soil and sediments (i.e. 2-5% w/w basis). In contrast, the organic carbon content of the PUF used in this study was 61±6% (n=4).

Mechanisms to explain PBDE desorption from PUF and subsequent tissue absorption once ingested are speculative. We are unaware of any studies examining ingestion/absorption of synthetic polymer additives by insects, although many are able to break down natural polymers such as cellulose. In terms of digestive physiology in *A. domesticus*, Teo and Woodring (1985) observed gut pH to vary from 4.1-7.7 depending

upon compartment and feeding regimen. PUF is resistant to degradation within this pH range *in vitro*. It is therefore unlikely that ingested PUF was chemically degraded appreciably by simple acid-catalyzed hydrolysis within the cricket gut. Lepom et al. (2010) found that PBDEs were quantitatively extracted from contaminated house dust using artificial digestive fluids. Wang et al. (1997) reported that synthesized poly(ester)urea-urethane PUF was biodegraded in an artificial digestive system via incubation with cholesterol esterase.

Saido et al. (2009) recently reported that polystyrene might be relatively rapidly degraded in the ocean, thereby releasing oligomers and potentially toxic plastic additives. In their study, a low temperature polyethylene glycol extraction procedure was used and resulting products compared to chemical constituents observed in the environment. However, release of xenobiotic chemicals from polymer particulates into gut fluids may not translate directly into increased accumulation into tissues. For example, in modeling organic chemical uptake from soil by earthworms, Jager et al. (2003) concluded that, though digestive fluids increase dissolved concentrations in the gut, they do not alter the fugacity gradient of the chemical. Commercial PUF used in upholstered furniture and automobile cushioning is commonly derived from polyether precursors (Beyler and Hirschler, 1995) and may be susceptible to degradation by a variety of microorganisms (e.g. Ghazali et al., 2005). Minute PUF fragments may also be able to cross the gut wall intact, thereby increasing PBDE uptake into tissues. For example, Browne et al. (2008) found that plastic microparticles (9.6 μm maximum particle size) ingested by filter-feeding mussels (*Mytilus edulis*) accumulated in the haemolymph and persisted there for up to 48 d.

Passive diffusion across the waxy cuticle may be an additional pathway by which soil arthropods are exposed to lipophilic chemicals. Though the mechanism was not identified, Paine et al. (1993) observed uptake of PCBs by house crickets suspended in cages just above field-contaminated soils (mean soil burdens=1150 μg/g dw). There, crickets accumulated tissue whole body burdens of 1.6 μg/g ww after 3 d exposure. In the absence of direct contact with the soil, the authors hypothesized that cricket burdens were derived primarily from gas phase transfer. A similar phenomenon has been observed for sorption of PBDEs to plant surfaces (e.g. Huang et al., 2010).

To better understand the potential of the cricket cuticle to accumulate PUF-associated PBDEs, burdens in molted exoskeletons were also examined. Molt ΣPenta-BDE burdens (63±13 mg/kg lipid) were 4-fold higher than burdens in whole depurated crickets, but lower than those measured in 28 d non-depurated crickets (Table S1). With the exception of BDE-100, molt congener patterns were comparable to whole body crickets, PUF and DE-71 (Fig. 2). For comparison of accumulation, we calculated cricket molt/PUF BAFs as the ratio of lipid-normalized molt PBDE burdens to TOC-normalized PBDE concentrations in apparent PUF ingested. After log-transformation, these values were poorly correlated with published log Kow values (r^2=0.35; $p>0.24$). Molt PBDE burdens (lipid basis) were, however, strongly correlated (r^2=0.99; $p<0.0002$) with PBDE concentrations (TOC basis) in the apparent PUF ingested (data not shown).

Some limited outgassing of additive PBDEs from finished polymer products occurs over time. Indeed, Wilford et al. (2003) examined vapor phase desorption from PBDE-treated PUF by passing air through PBDE-treated PUF plugs and then trapping the resulting volatilized PBDEs. The original congener pattern in the PBDE-treated PUF

(i.e. BDE-99 > 47 > 100 > 153 > 154) differed from that of the sampled air (i.e. BDE-47 >> 99 > 100 ~ 28 > 49 ~ 17), indicating preferential stripping of the more volatile congeners. Prevedouros et al. (2004) predicted similar preferential outgassing of BDE 47 from in-use consumer products. Such preferential release has also been invoked as one explanation for congener patterns observed in some indoor dust (Hale et al., 2006). Regardless of accumulation pathway, shedding of contaminated molts will further redistribute PBDE burdens within terrestrial ecosystems via direct soil deposition or ingestion by other organisms (Ghouri and McFarlane, 1958). Crickets molt 8-10 times over the course of their life cycle (Clifford and Woodring, 1990) and a myriad of organisms prey on soil-dwelling arthropods, including a variety of both terrestrial and aquatic birds (Walters et al., 2009; Dauwe et al., 2009).

PUF-exposed cricket growth. Evaluation of PUF/PBDE toxicity was not a major objective of our study. However, data on relative growth and lipid content of exposed crickets among treatments and relative to controls are informative. As expected when exposing nymphs, cricket size in our exposures increased significantly with time in depurated and non-depurated exposure treatments ($p<0.00001$). Growth did not differ ($p>0.05$) among depurated and non-depurated treatments regardless of time. Growth rates (relative to t=0 crickets) of PUF-exposed crickets did not differ significantly from controls ($p>0.05$), regardless of depuration, indicating that ingestion of PUF/PBDEs did not inhibit growth (Fig. S2). Mean growth rates for depurated treatments were 0.03±0.008 (14 d) and 0.03±0.005 (28 d) cm/d while those for non-depurated treatments were 0.02±0.003 and 0.02±0.004 cm/d. For control treatments, mean growth rates were 0.02±0.004 (14 d) and 0.03±0.005 (28 d) cm/d. On a percent basis, relative growth was

35.1% (14 d) and 52.9% (28 d) for depurated and 25.5% and 40.6% for non-depurated crickets. Relative growth of control crickets was 29.6% (14 d) and 47.9% (28 d). On a mass basis, our estimates are consistent with those reported for 21 d-old *A. domesticus* nymphs exposed to PCB (Aroclor 1254)-spiked natural soil for 14 d under controlled conditions (Paine et al., 1993). In that study, growth was comparable for crickets exposed to non-spiked soil substrates as well (Burrows, 1989). Their relative growth for crickets reared on a non-soil substrate for 14 d was 27.4%, consistent with our results. In contrast, relative growth for their crickets reared on a non-contaminated soil was 51.7%.

While exposure to PBDEs via ingestion of PUF by crickets did not appear to impair relative growth in our study, ShuZhen et al. (2010) observed significant inhibition (relative to non-exposed controls) in earthworms exposed to a range of solvent-delivered DE-71 soil doses (100 to 1000 μg/kg dw). Similarly, Wollenberger et al. (2005) found that Penta-BDE constituents inhibited larval development in the marine copepod (*Acartia tonsa*) at only 1-13 μg/L. These constituents were found to be 100- to 800-fold more toxic than other BFRs examined (e.g. tetrabromobisphenol A). Tissue total extractable lipids (as a general indicator of growth and organism health) were not significantly different among our exposed depurated and non-depurated and control crickets either (Table 2).

These results indicate that terrestrial insects may consume PUF present in many in-use and derelict consumer products and accumulate appreciable PBDE burdens. Insects may therefore be underappreciated vectors of PBDE transfer from consumer products into terrestrial food webs. Future risk assessments should therefore be more inclusive of their role.

Figures

Figure 1. Mean tissue PBDE burdens (mg/kg lipid) for depurated and non-depurated crickets (N=4 pools per treatment; 10 crickets per pool) after 14- and 28-d exposure to PBDE-treated PUF. For a given PBDE congener, treatments with a different lowercase letter are significantly different (BDE-47: $p<0.01$; BDE-100: $p<0.05$; BDE-99: $0.0001<p<0.05$; BDE-154: $0.01<p<0.05$; BDE-153: $0.001<p<0.05$). Error bars represent standard deviations from the means.

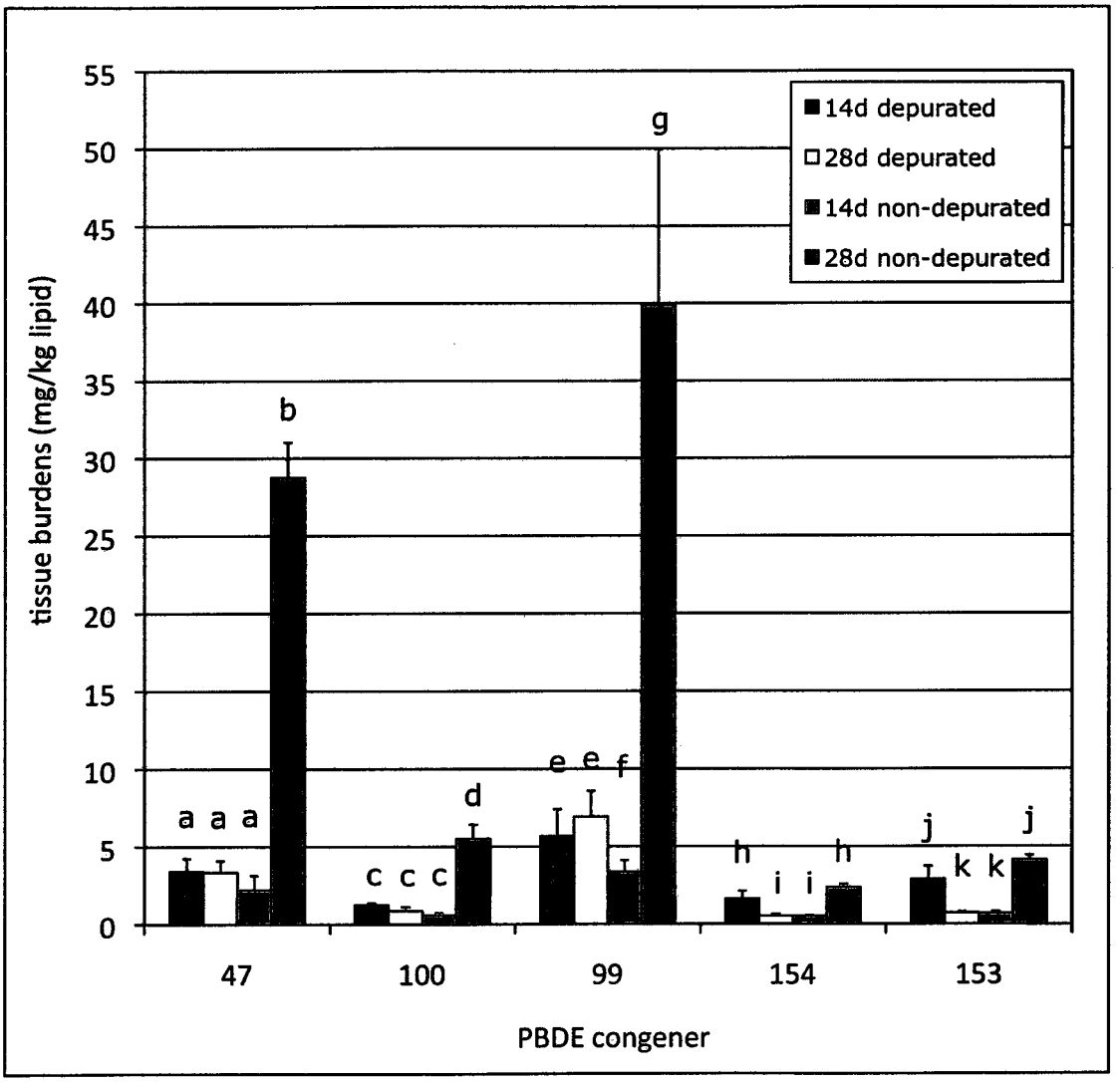

Figure 2. Contribution of major Penta-BDE constituent congeners in 28d: depurated and non-depurated cricket treatments (N=4 pools per treatment; n=10 crickets per pool), molts from PUF-exposed crickets (N=2), commercial PUF (n=4) and DE-71 (data from La Guardia et al., 2006). Error bars represent standard deviations from the means for cricket tissues and PUF. For molts, error bars represent the range of the measurements.

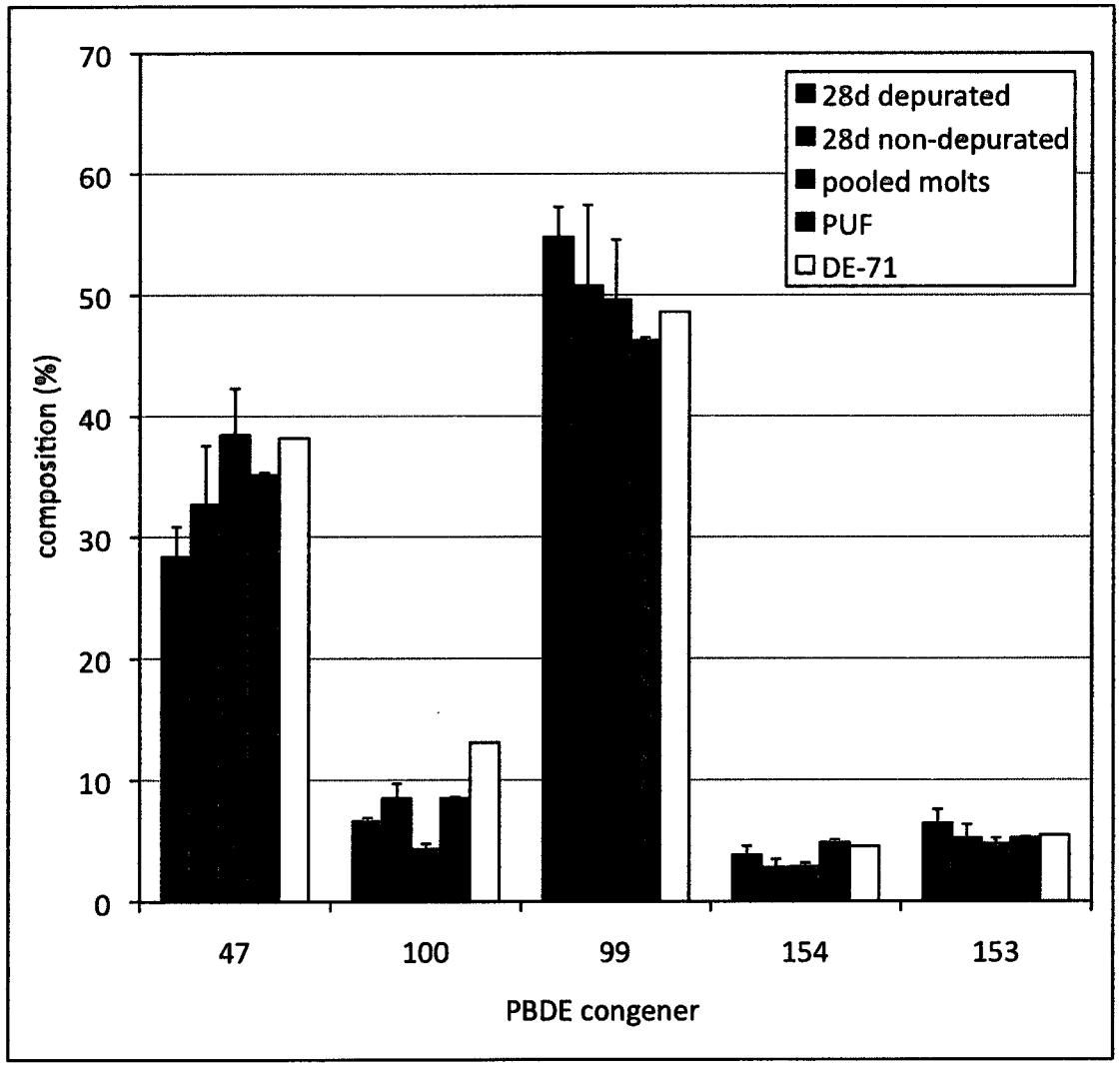

Table 1. Calculated mean PBDE cricket/PUF bioaccumulation factors (BAFs) ($x10^{-4}$) for depurated and non-depurated crickets exposed to PBDE-treated PUF. BAFs were computed as the ratio of lipid-normalized tissue PBDE burdens to TOC-normalized PUF burdens. Values are normalized for apparent PUF mass ingested. %RSD=percent relative standard deviations from treatment means (N=4).

	depurated cricket-PUF BAFs				non-depurated cricket-PUF BAFs			
PBDE	14d($x10^{-4}$)	%RSD	28d($x10^{-4}$)	%RSD	14d($x10^{-4}$)	%RSD	28d($x10^{-4}$)	%RSD
47	5.3	23.6	4.1	22.3	4.0	25.6	34.6	8.1
100	8.0	10.4	4.3	27.1	4.1	15.9	27.3	17.1
99	6.7	30.2	6.4	23.7	4.2	20.1	36.6	25.9
154	17.1	29.1	4.2	27.7	4.2	12.6	19.2	7.6
153	24.3	19.3	4.7	4.8	5.8	8.3	25.7	6.5
ΣPBDE	7.6	16.9	5.3	27.8	4.1	19.6	31.9	14.4

Table 2. Mean (±S.D.) total extractable lipid (%) in depurated (N=4) and non-depurated (N=4) crickets, cricket molts (N=3) and non-exposed control crickets (N=3 for both exposure periods). For lipid determinations, cricket molts (N=3) were collected as discovered from crickets of the same age cohort as the stock population. Differences are not statistically different ($p>0.05$), regardless of treatment (ANOVA).

cricket total extractable lipid (%)				
treat	depurated	non-depurated	molt	control
14d	11.1±0.7	10.8±1.6	10.3±0.7	10.6±1.3
28d	10.8±1.5	10.9±0.9	9.5±0.8	10.2±1.1

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Chapter 3

Accumulation of Polybrominated Diphenyl Ether (PBDE) Flame Retardants in an Agricultural Soil Ecosystem Receiving Long-Term Sewage Sludge Amendments

Abstract

While land application of sewage sludge is widely practiced, little research has examined uptake of organic pollutants therein by soil-associated invertebrates. PBDE burdens in such organisms and grasses in an agroecosystem receiving long-term (>20 yr) sludge amendments were evaluated versus a field receiving no biosolids. No PBDEs were detected in samples from the latter, but surficial soil in the former contained 10,300±2700 μg/kg of ∑Penta-BDE (BDE 28+47+99+100+153+154) on a total organic carbon (TOC) basis. PBDEs were below quantitation limits in grasses in the sludged field. ∑Penta-BDE concentrations were highest in invertebrates with greatest soil contact , rather than with increasing trophic position. Earthworms contained ∑Penta-BDE at 17,600±2300 μg/kg lipid weight (lw). Maximum ∑Penta-BDE burdens in detritivorous woodlice were lower than worms, 3000±230 μg/kg lw. PBDEs were below quantitation in all but one wolf spider sample. Worm and soil BDE-209 burdens were 6500±4100 μg/kg lw and 7500±2800 μg/kg TOC, respectively. BDE-209 occurred in fewer taxa, but levels as high as 86,500 μg/kg lw were detected in millipedes. PBDE congener patterns suggest differential uptake/elimination in dissimilar soil invertebrates. Penta-BDE constituent congener biota-soil accumulation factors (BSAFs) ranged from 0.006 (crickets) to 1.2 (earthworms), while BDE-209 BSAFs ranged from 0.07 (earthworms) to 10.5 (millipedes). Tissue lw and soil TOC normalized PBDE burdens were strongly correlated for earthworms. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures were poorly correlated with PBDE bioaccumulation. However, samples from the sludge-applied site were more enriched in $\delta^{15}N$ relative to the non-applied site.

Introduction

Polybrominated diphenyl ethers (PBDEs) have been used worldwide to flame retard consumer products in order to minimize loss of life and property. As a result they have become globally dispersed. Production of the Penta-BDE formulation has recently been largely phased out due to the persistence and bioaccumulation potentials of its constituents. Historically, the dominant PBDE product in use has been Deca-BDE. It was widely assumed that Deca-BDE exhibited negligible bioaccumulation potential (Hardy, 2002; Ross et al., 2009). However, substantial levels have been detected in some terrestrial-feeding birds of prey (Lindberg et al., 2004; Chen and Hale, 2010). Deca-BDE's major constituent, BDE 209, also appears subject to dehalogenation to more bioaccumulative PBDE congeners in the environment (La Guardia et al., 2007). Hence, Deca-BDE is also being phased out. Despite these regulatory actions, PBDEs are persistent in the environment, so residues are building up in soils and sediments and may present hazards for decades. Unlike legacy pollutants such as PCBs, released primarily through industrial activities, PBDEs were intentionally incorporated at percent levels into high-volume consumer products, such as furniture cushioning and carpet underlayment (World Health Organization, 1994; Hale et al., 2002). These high PBDE concentrations and the abundance of treated items in the indoor environment have enhanced the potential for human exposure.

Once believed safely sequestered in polymers, PBDEs may migrate from plastics and coated textiles throughout their life. This may be a primary pathway for human exposure in the indoor environment (Frederiksen et al., 2009). Upon release, PBDEs accumulate in biota, soils, sediments and sewage sludges. PBDEs thus pose risks to both

human and ecological health. At present, our understanding of PBDE emission and exposure pathways remains incomplete. One route is land application of stabilized wastewater treatment sludges, also known as biosolids.

About 8 x 10^6 metric tons (dry weight) of sewage sludge is generated in the US annually, more than half of which is applied to agricultural and other soil ecosystems (US EPA, 2006). Burgeoning human populations and increasingly stringent wastewater effluent standards may escalate sludge production and subsequent disposal issues. Though offering numerous soil enrichment benefits, sludge also contains a complex mixture of inorganic and organic pollutants. In the past, attention was focused on metals due to their toxicity and persistence. Organic pollutants were presumed to be degradable or below levels of concern. In the US, organic pollutants incurred in biosolids destined for land application are currently unregulated. Recently, concern has risen over such chemicals, including PBDEs, and published reports on US biosolids and biosolids-amended soils have increased (e.g. Hale et al., 2001; Kinney et al., 2006; US EPA TNSSS, 2009).

The disproportionately large demand for the Penta-BDE product in North America has resulted in mg/kg sludge burdens here, 10-fold or greater than elsewhere in the world (Hale et al., 2001). Levels and patterns of the Penta-BDE constituents (i.e. BDE-47, 100, 99, 154, 153) in sludge samples are quite consistent across the US (Hale et al., 2001, US EPA TNSSS, 2009). Substantial burdens have also been detected in indoor dust and the extent of these has been correlated with the prevalence and usage of flame-retarded products (Allen et al, 2008; Webster et al., 2009). These patterns, and the discovery of similar concentrations in sludge and indoor dust from a geographically

isolated US Antarctic research station (Hale et al., 2008), suggest that much of the Penta-BDE burden in sludge arises from releases from polymer products, i.e. polyurethane foam (PUF), rather than from industrial sources.

Significant gaps remain in our understanding of their fate in soils receiving sludge and the risks to associated biota. To date, only two field studies have addressed exposure of ecologically critical soil organisms, in both cases earthworms, to sludge-associated PBDEs (Matscheko et al., 2002; Sellström et al., 2005). These studies were performed by the same research group on the same sites in Sweden, where PBDE usage has been much lower than in the US. No published studies of PBDE accumulation in ecologically significant sludge-exposed soil arthropods were located. Accordingly, we investigated PBDE bioaccumulation in an agroecosystem receiving long-term (> 20 yr) sludge amendments. For comparison, we evaluated PBDE burdens within a soil ecosystem receiving only animal manure amendments.

Experimental

Study sites. The sludge-amended study area was an untilled hayfield (Mid-Atlantic region, USA). The dominant vegetation was eastern gamagrass (*Tripsacum dactyloides*), a forage species native to the area, related to corn (*Zea mays*). Precise estimates of sludge tonnage applied were unavailable. However, landowners confirmed sludge application to the field at agronomic nitrogen rates in alternate years since the mid-1980s. The field (~60,000 m^2) last received an application in Summer 2008. A comparable non-sludge-applied field (~6000 m^2) located about 160 km south of the sludge-amended site was selected for comparison. It had been cleared of trees a few years prior to sampling

and since had received periodic animal manure inputs. The dominant vegetation currently on-site was orchard grass (*Dactylis glomerata L.*).

Paired soil-earthworm and vegetation sampling. Paired soil and earthworm samples were collected from randomly selected locations by excavating the upper 15 cm of surface soil and hand sorting worms and soils. Worms (~10 g) and collocated soil (~1 kg) were pooled to yield paired earthworm-soil composite samples. Earthworms in composite samples were mixtures of *Lumbricus terrestris and L. rubellus, Apporectodea caliginosa, Apporectodea. spp. and Allolobophora spp.* Gamagrass (sludge-applied field) and orchard grass (reference site) vegetative shoots were collected just above the root crown within each sampling quadrat. All samples were stored on ice for transport back to the laboratory. There, earthworms were rinsed with deionized water, depurated on moistened KimWipes® for 24 hr and rinsed again with deionized water. Soil was sieved to < 2000 μm and stored at 4°C until analysis.

Soil arthropod sampling. Arthropods were collected at or near the soil surface and included wolf spiders (*Araneae; Lycosidae; Hogna, spp., Schizocosa spp.* and *Rabidosa spp.*), millipedes (*Polydesmida:Paradoxosomatidae*), woodlice (*Isopoda:Porcellionidae*), grasshoppers (*Orthoptera:Acrididae*), field crickets (*Orthoptera:Gryllidae*), ground beetles (*Coleoptera:Carabidae*) and firefly larvae (*Coleoptera:Lampyridae*), June beetles (*Coleoptera:Scarabidae*) and June beetle larvae (*Phyllophaga spp.*). Web spider samples were also collected and pooled and consisted of mixtures of garden spiders (*Argiope spp.*), crab spiders (*Thomisidae*), black widows (*Theridiidae*) and other small orb-weavers (e.g. *Mangora spp.)*. Arthropods were allowed to purge their gut contents overnight in the collection containers and then stored at -10°C until analysis. Individual

wolf spiders were weighed, and sorted into pools based upon fresh weight (i.e. <0.1g, 0.1-0.2g, 0.2-0.3g, 0.3-0.4g, >0.4 g; n=347 sludge-amended site; n=245 reference site). Samples were cleaned of obvious soil and vegetation and then identified to at least Family.

Chemical analysis. Similar to Chen et al. (2008), lyophilized samples were spiked with a surrogate standard (PCB 204) to monitor recoveries and then subjected to enhanced solvent extraction (Dionex ASE 200; Sunnyvale, CA) with methylene chloride. Total extractable lipids in samples were estimated by evaporating 10% of the extract to constant weight. Remaining worm, soil and vegetation extracts were purified on an EnviroSep® size-exclusion column (Phenomenex; Torrance, CA) by elution with methylene chloride, followed by polarity-based purification on 2 g silica gel solid-phase extraction (SPE) columns (EnviroPrep®; Honeywell Burdick & Jackson; Muskegon, MI). Eluent was solvent exchanged to hexane, cleaned up on 2 gm silica gel SPE columns and then analyzed for PBDEs (28, 47, 99, 100, 153, 154 and 183) as previously described. Due to their small size, arthropod ASE extracts were purified using silica gel SPE only. Following addition of an internal quantitation standard, decachlorodiphenyl ether (DCDE) (Ultra Scientific; Kingston, RI), PBDEs were separated on a Varian 3400 GC equipped with a Varian 4D ion-trap MS (Sugar Land, TX). The GC was equipped with a 60 m DB-5 column (J&W Scientific; Folsom, CA; 0.25 μm film, 0.32 mm ID) and Varian 8200 CX autosampler. Analytes were detected in the full scan electron ionization (EI) mode. To quantify PBDEs, the three major ions of each PBDE congener were summed relative to the area of the internal standard using an 8-point calibration curve. The GC carrier gas was helium and injections were made in the splitless mode. Initial

GC column temperature was held at 75°C for 2 min, then programmed to 320°C at 4°C/min and held for 15 min. As this GC-MS system is limited to a mass range limit of 650 amu, analysis of BDE-206, 207, 208 and 209 were conducted according to La Guardia et al. (2006). Briefly, extract constituents were separated on an Agilent 6890N GC (Agilent Tech., Palo Alto, CA) coupled to a JMS-GC Mate II MS (JEOL, Peabody, MA). The GC was equipped with a 30 m DB-5HT capillary column (0.25 mm i.d. x 0.1 μm; J&W Scientific). Analytes were detected in the full scan electron capture negative chemical ionization (ECNI) mode. QA/QC and stable isotope analysis procedures are provided in Supporting Information.

Statistical analysis. Statistical evaluations were performed using StatPlus:Mac (AnalystSoft Inc.; Vancouver, BC, Canada). Statistical significance was determined at the α=0.05 level using two-tailed testing, unless otherwise indicated. BSAFs were computed as the ratio of lw-normalized whole body tissue PBDE burdens to TOC-normalized substrate PBDE concentration. One-way Analysis of Variance (ANOVA) with Tukey's honestly significant difference (HSD) post-hoc testing was used to determine significant differences among mean tissue PBDE burdens, BSAFs and congener uptake ratios. PBDE burdens < QL were not considered in calculating means. Correlations between PBDE tissue and soil burdens, earthworm BSAFs and literature-derived parameters were evaluated using least squares linear regression. Principle components analysis (PCA) (XLStat; Addinsoft, New York, USA) was used to identify patterns in PBDE burdens normalized to percent of $\sum$Penta-BDE. Congeners < QL were assigned a value of half the QL. Normality of data was determined using the Kolomogorov–Smirnov test. Relationships between stable isotope and PBDE burdens

and BSAFs were evaluated using least squares linear regression analysis. Differences in stable isotope ratios among taxa and between sites were evaluated using One-way ANOVA with Tukey's HSD.

Results and Discussion

PBDE levels and trends in soil and plants. PBDEs are hydrophobic and concentrate onto solids during wastewater treatment. Sludge application to land will transfer associated burdens to soils and PBDE persistence will result in their accumulation over time. However, to date, few studies have examined this phenomenon and fewer still have considered PBDE uptake in exposed terrestrial organisms. Mean Penta-BDE and deca-BDE concentrations in the US EPA Targeted National Sewage Sludge Survey (US EPA TNSSS, 2009) were 2030 and 2180 μg/kg (dw), respectively, in sludge collected from 74 publicly owned treatment works (POTWs) throughout the US in 2006-2007. At our sludge-amended site, all congeners sought (BDE 28, 47, 99, 100, 153, 154, 183, 206, 207, 208 and 209) were detectable in soils, but BDE-28 and 183 were <QL. In contrast, all targeted congeners were < QL at the unsludged reference site. Mean BDE 209 burdens in the sludge-applied soil (7510 μg/kg TOC; 86 μg/kg dw) were 2- to 3-fold lower than the sum of tri- to octa-brominated congeners ($\sum PBDE_{3\text{-}8}$; 17,600 μg/kg TOC; 260 μg/kg dw). Andrade et al. (2010) and Xia et al. (2010) reported ∑PBDE burdens in US sludge-amended soils of 53 μg/kg dw (BDE 47+99+209) and 650 μg/kg dw (BDE 47+99+100+153+154), respectively. Consistent with our observations, BDE-183 was < QL in most biosolid and biosolids-amended soil samples examined in the Andrade et al.

(2010) study. For comparison, soils receiving only atmospheric inputs of ∑Penta-BDE burdens are typically in the low (e.g. 1-12) μg/kg range (Hale et al., 2006).

The ratio of ∑Penta-BDE to BDE 209 in sludge and soils receiving sludges may decrease in the future as use and release of Penta-BDE diminishes. Hale et al. (2001) observed higher ∑Penta-BDE than BDE 209 concentrations in US biosolids collected prior to the 2004 discontinuation of US Penta-BDE production. Comparable mean Penta-BDE and BDE 209 burdens were found in biosolids in the US EPA TNSSS, but some analytical problems were encountered with BDE 209 (US EPA TNSSS, 2009). In contrast, Andrade et al. (2010) observed that BDE 209 levels exceeded those of ∑Penta-BDE by several-fold (dw basis) in Mid-Atlantic biosolids collected between 2005 and 2008. Conversely, BDE 47+99 burdens were found to be higher than BDE 209 burdens in biosolids-applied soils.

In our study, soil $\sum PBDE_{3-8}$ burdens across the sludge-applied site were consistent (RSD=13%, N=10), suggesting uniform distribution. Mean $\sum PBDE_{3-8}$ soil burdens (TOC basis) in our sludge-amended agricultural field were similar to that reported for the most heavily sludge-amended Swedish field (19,900 μg/kg; normalized to a loss on ignition basis) reported by Sellström et al. (2005). Soil burdens at other sludge-amended sites in the Swedish study were appreciably lower. In general, PBDE burdens in US sludge-amended soils are higher than counterparts in other countries due to greater North American PBDE usage. The anomalously high Swedish site had received sludge from a wastewater facility serving textile plants. Sellström et al. (2005) reported this sludge-amended soil contained the less brominated congeners in the order: BDE 99>47>>153~154. This is consistent with their abundance in commercial Penta-BDE

mixtures (La Guardia et al, 2006). Congener patterns in our sludge-amended soil were BDE 47~99>>100>154>153, with BDE 154 burdens about 4-fold higher than BDE 153 (Table 1). BDE 154 and PBB 153 were fully separated on the 60 m GC column, hence the contribution of the latter could be excluded. In our soils, no indication of BDE 209 debromination, as evidenced by elevated levels of nona-brominated congeners relative to their presence in commercial Deca-BDE, was observed (BDE-206, 207 and 208 detectable in all soils, but below QL).

Both humans and wildlife consume plants, hence the question of PBDE accumulation in vegetation is critical. Uptake of hydrophobic contaminants by vegetation through the root system has been previously reported to be limited, although partitioning from air to leaf surfaces may be significant for some compounds (Simonich and Hites 1995). In the gamagrass shoots examined here, PBDEs were below QL (TOC normalized QL 16-31 μg/kg with TOC of 40.1 to 42.7%). These findings are consistent with those of Xia et al. (2010) who reported PBDEs below detection in corn tissues from sludge-amended soil plots. Corn (*Zea mays*) is closely related to gamagrass. Results of both studies contrast with findings by Huang et al. (2010). They exposed a variety of plants to soil spiked with solvent-borne BDE 209 (3613 μg/kg dw) and observed shoots to contain up to 490 μg/kg dw. Mueller et al (2006) spiked planted and unplanted soil with the Penta-BDE mixture, DE-71, at 75 μg/kg dw. They reported PBDE recoveries of <10% from aged soil with solvent extraction, except in experiments where mixed species plantings (e.g. radishes and zucchinis) occurred. In soils with mixed plantings, they observed greater recovery of BDE 47 than BDE 99 or 100. However, uptake of PBDEs in the plants was low, i.e. < 5 μg/kg dw in roots and shoots.

PBDE levels and trends in earthworms. Earthworm $\sum PBDE_{3-8}$ concentrations were significantly higher than in all arthropods ($p<0.001$). Blankenship et al. (2005) noted comparable differences in PCB burdens between worms and soil arthropods at a field-contaminated site. With the exception of BDE 28, targeted Penta-BDE constituents were detectable (S/N > 3) in virtually all biota samples. But only BDE 47 and 99 were consistently above QL in most samples (see Supporting Information for QA/QC details). Mean earthworm $\sum PBDE_{3-8}$ burdens were 10,300 μg/kg lw and, like soil burdens, were quite uniform throughout the sampling area (n=10; RSD=26%). In these worms, no significant differences were detected between BDE 47 and 99 levels, but BDE 100, 153 and 154 were significantly lower than both, with the latter two congeners significantly lower than BDE 100 ($p<0.05$). Worm burdens generally tracked those of the collocated soil, similar to PCB burdens in worms from field-contaminated soil (Blankenship et al., 2005).

In worms, ingestion of PBDEs from soil is a more important route of exposure than dermal contact (Jager et al., 2003; Sellstrom et al., 2005). Uptake generally decreases as soil organic matter/PBDE binding and compound log Kow increases. Extended contaminant/soil contact times may further reduce bioavailability, as residues migrate to less accessible locations within the matrix (Alexander, 2000). Likewise, PBDEs remaining within polymer remnants may exhibit low bioavailability, but levels within these may be at percent level (see Chapters 1 & 2). Hence, given the same soil composite concentration, one might expect lower biological uptake from biosolids exposures than from lab exposures using solvent-delivered contaminant doses. Differences may also exist between species, dependent upon ecology (Alexander 2000).

However, we observed relatively similar uptake in earthworms exposed to composted and anaerobically digested biosolids and solvent-amended soils in a series of lab experiments (see Chapter 1). Anaerobically digested sludges are by far the most commonly land-applied. In our field study, mean BDE-209 burdens in worms were 6500 (±4120) μg/kg lw, consistent with worm burdens (5200 μg/kg lw) at the most heavily contaminated sludge-applied site in the Swedish study by Sellstrom et al. (2005). However, at the latter site, reported soil burdens of BDE-209 (33,600 μg/kg; loss on ignition basis) were about four-fold higher than mean soil burdens measured here.

Mean BSAFs for Penta-BDEs reported by Sellström et al. (2005) were 5, 4.2, 4.6, 2.5 and 2.3 for BDE-47, 99, 100, 153 and 154, respectively. BSAFs generally decreased with increasing log Kow, consistent with a greater resistance to mass transfer and hence lower bioavailability. Though our earthworm BSAFs were lower (Table 2), we observed the same decreasing trend with log Kow (obtained from Braekevelt et al., 2003) (Fig. 1). We observed comparable decreasing trends with log Kow in our lab study of PBDE uptake from biosolids-amended soils by worms (see Chapter 1). However, there, BSAFs were 5- to 20-fold higher than our field-exposed worms and 2- to 4-fold higher than field-exposed worms in the Sellström et al. (2005) study. In our lab study (Chapter 1), worm BSAFs generally decreased with increasing biosolids dose as well. BSAFs for Penta-BDE constituents in the range of 0.9-5.8 have also been reported for *E. fetida* exposed to Penta-BDE-spiked natural soil (Liang et al., 2010). Nyholm (2009) reported lower BSAFs for higher brominated PBDEs in the lab study. Sellström et al. (2005) also reported lower BSAFs for these congeners, with that for BDE-209 being 0.3. This is about 4-fold higher than corresponding BSAFs calculated in our field study (Table 2).

Nyholm (2009) also observed significant decreases in BSAFs with increasing concentrations of all congeners (including BDE-183 and 209). Similarly, Blankenship et al. (2005), in a comparative study of PCB bioaccumulation at a Superfund site (soil ∑PCBs=6500 μg/kg wet weight (ww)) and a "clean" reference site (soil ∑PCBs=9 μg/kg ww), calculated BSAFs for plants and soil arthropods two orders of magnitude higher at the reference compared to contaminated site. Interestingly, BSAFs for depurated earthworms at their reference site were 5-fold higher than at the contaminated site, while those for non-depurated worms were about 30% higher. Increased atmospheric deposition at the reference site and, more generally, concentration-dependent uptake from soil was posited to account for this.

PBDE levels and trends in soil-associated arthropods. A literature search revealed only two studies that considered PBDE accumulation in terrestrial insects (Hale et al., 2002; Wu et al., 2009). However, in both, PBDE uptake by insects was incidental to the primary focus. The Hale et al. (2002) study described serendipitous findings regarding burdens in crickets in contact with Penta-BDE-treated polyurethane foam in the lab that were fed to frogs. Mean burdens in frogs and crickets were 209,000 and 257,000 μg/kg lw, respectively. Congener profiles in the foam, crickets and frogs were similar. Wu et al., (2009) reported PBDE levels in terrestrial (mixed species) insect composites as part of a frog monitoring effort near a Chinese electronics waste recycling site. Deca-BDE, not Penta-BDE, is typically used in electronics. Nonetheless, congeners associated with the Penta-BDE mixture dominated in most of the frog and insect composites examined. The mean BDE 47/99 ratio derived from the composite insect burdens reported in the Wu et al. (2009) study was 0.83. This is consistent with that in the DE-71 mixture (0.79; La

Guardia et al., 2006), but is lower than nearly all of the soil dwelling invertebrates in our study (e.g. 1.1-4.3) except our cricket samples (0.19-0.37). Soil burdens were not reported in the Wu et al. (2009) study.

Principal components analysis (PCA) was used to visualize Penta-BDE congener contributions in the sample types collected in our study (Fig. 2). A biplot of scores and loadings showed that PC1 and PC2 explained 88.8% of the variation in the data set. Congener patterns for crickets, woodlice, June beetles and soil are well separated in the PCA plot, while ground beetles, June beetle larvae and earthworms are more closely associated. Results indicate "weathering" of Penta-BDE constituents in moving from soil into the invertebrate food web, similar to that observed for PCB congeners accumulated in a soil food web by Blankenship et al. (2005). The sludge-amended soil was enriched in BDE 154 and 153, with congener patterns resembling that of the DE-71. Woodlice, June beetle (adults and larvae), firefly larvae and millipedes were generally more enriched in BDE-47, perhaps indicative of its increased bioavailability. Overlap of congener pattern variance among worms, June beetle larvae and ground beetles may be indicative of the latter's preference for soft-bodied prey. While BDE 47 typically dominates in wildlife, it was near the QL in crickets. Instead BDE 99 dominated, in excess of its proportion in soil or biosolids. This may indicate biotransformation in this species.

Except for crickets and ground beetles, BDE 47 burdens were significantly ($p<0.05$) higher than the other Penta-BDE constituents in all other arthropods we analyzed. This pattern differed from the earthworms and soil, wherein BDE 99 was substantial. This is also the case with the commercial Penta-BDE product, DE-71, and

biosolids. For example, the range of BDE 47 burdens in June beetles and firefly larvae was about 2- to 3-fold higher than BDE 99 burdens (Table 1). BDE-153 and 154 were above the QL only in ground beetles and worm tissues. June beetle larvae concentrations were comparable to those in the millipede composite, but were about one-third those of adult June beetles and firefly larvae. June beetle larvae burdens were significantly lower than ground beetles ($p<0.05$; paired t-test). In crickets, BDE 47 levels were the lowest among the Penta-BDE congeners and their $\sum PBDE_{3\text{-}8}$ burdens were lower than in all taxa except the spiders. BDE 100 was quantifiable only in soil, earthworms, woodlice and ground beetles and levels were not statistically different between soil and worms or between woodlice and ground beetles. BDE 100 burdens in both arthropods were about 20% of those measured in worms and soil.

Though detectable in several sample types (i.e. soil, worms, millipedes, June beetle larvae), the major Octa-BDE constituent (BDE-183) was too low to quantify in any of them. The nona-brominated congeners present in the commercial Deca-BDE mixture (BDE-206, 207 and 208) were also detectable in several of these sample types, but were only quantifiable in the single millipede composite. There, congener burdens were 8520, 9260 and 3990 µg/kg lw for BDE-206, 207 and 208, respectively. The major Deca-BDE constituent (BDE-209) was quantifiable only in soil (n=6), worm (n=6), woodlice (n=1), millipedes (n=1), crickets (n=1), ground beetles (n=3) and one (0.1-0.2 g) wolf spider replicate. Descriptive statistics were thus calculable only for soil, worms and ground beetles. ∑Deca-BDE burdens (206+207+208+209) ranged from 1440 to 86,500 µg/kg lw in crickets and millipedes, respectively. BDE-209 burdens were not as uniform (i.e. higher RSDs) as observed for Penta-BDE constituents.

Among arthropods, woodlice (n=3 at the sludge-amended site) exhibited the highest $\sum PBDE_{3\text{-}8}$ burdens, i.e. 3000±227 µg/kg lw. But, BDE 209 levels were below QL. Quantitation limits (3210-4290 µg/kg lw) were high, but comparable to those derived for earthworms. The BDE 47/99 ratio in woodlice exceeded 4, compared to a mean of 1.1 in earthworms and 1.0 in collocated soil (Table 2). The lower relative BDE 99 burden (Fig. 3; Table 1) may be due to the isopod's preference for ingesting decaying organic matter, rather than bulk soil. Alternatively, it may reflect more rapid elimination of BDE 99. Higher woodlice burdens are consistent with the findings of Hopkin and Martin (1982) who observed higher metals accumulation in woodlice than all other terrestrial organisms examined. This was believed due to the especially large surface area and absorption capacity of the isopod hepatopancreas (Hames and Hopkin, 1989). Stroomberg et al. (2003) also reported that woodlice are able to accumulate PAHs from contaminated soil, but then rapidly biotransform them.

Millipedes, like woodlice, prefer to consume decaying vegetation. The $\sum PBDE_{3\text{-}8}$ in the millipede composite sample (n=1) was lower than in woodlice or earthworms, i.e. 589 µg/kg lw. The BDE 47/99 ratio was intermediate (2.0) between that of the soil/earthworms and woodlice. BDE 209 was detected at 64,700 µg/kg lw in the millipede composite. While no BDE 209 was detected in lab and field blanks or in samples from the unsludged site (N=39), this result appears anomalous and could indicate laboratory contamination. Unfortunately, due to their small size and limited sample weight, the entire sample was extracted in its entirety. The nona-brominated congeners (BDE 206, 207, 208) were also detected in the millipede composite, but in ratios generally consistent with the commercial Deca-BDE product (La Guardia et al., 2006).

Firefly larvae are small and dwell on the soil surface and within the upper soil strata. Unlike the above organisms, they are predators, consuming primarily small soft-bodied invertebrates such as worms, slugs and snails. Composite samples (n=2) contained slightly higher $\sum PBDE_{3-8}$ concentrations (1890-2660 μg/kg lw) than millipedes, but less than woodlice. The calculated BDE 47/99 ratio of 2.5 was similar to the woodlice, indicating some enrichment relative to soil PBDE patterns. BDE 209 was not quantifiable in these larvae. These results are consistent with those of Xia et al. (2008) who found that median Penta-BDE burdens in aquatic carnivorous and herbivorous insects from the Hudson River (US) were comparable.

June beetle larvae were present in larger numbers at the field site where manure, rather than biosolids, was applied. This is consistent with the preference of the adult beetles to seek out organic matter enriched sites to deposit their eggs. In turn, the larvae consume this versus bulk soil. June beetle larvae collected from the sludge-applied site were scarce and appreciably smaller than those collected from the unsludged site. At the sludge-applied site, the $\sum PBDE_{3-8}$ concentration (667±279 μg/kg lw; n=3 composites) and BDE 47/99 ratio (1.5) were similar to the detritivorous millipedes. Like the millipede sample, a high concentration of BDE 209 (59,800 μg/kg lw) was detected, but in only one of the three June beetle larvae composites. This may be due in part to more heterogeneous distribution of soil burdens. No quantifiable levels of BDE 209 were detected in the June beetle larvae from the unsludged site.

Two composites of adult June beetles were collected from the sludge-applied field. The $\sum PBDE_{3-8}$ concentrations therein (1760 and 2220 μg/kg lw) were several-fold higher than those in June beetle larvae and the BDE 47/99 ratio (3.7) exceeded that of the

larvae. This may reflect some enrichment of existing body burdens during metamorphosis, as has been observed for PBDE burdens in aquatic insects (Bartrons et al., 2007). Adult beetles feed mostly on leaves of exposed plants, not detritus or soil. As noted, PBDEs were not detected in the gamagrass. No BDE 209 was quantifiable in the June beetles. As these organisms are capable fliers, our results suggest that they may be able to transport sludge-associated PBDEs offsite. Normalizing our $\sum PBDE_{3\text{-}8}$ lw burdens to the number of individuals per composite sample yielded a burden range of 585 to 740 μg $\sum PBDE_{3\text{-}8}$ per individual. By comparison, Blankenship et al. (2005) measured ∑PCBs of 340 μg/kg ww in composite samples of June and Japanese beetles from field-contaminated soil (soil ∑PCBs=6500 μg/kg ww). Reliable estimates of June beetle population densities in temperate soil ecosystems are unavailable. However, if a population of 100 larvae were assumed to inhabit this system and successfully pupate into flying adults, roughly 59 to 74 mg of $\sum PBDE_{3\text{-}8}$ could be exported. In contrast to June beetles, ground beetles are predators, consuming primarily soft-bodied invertebrates. $\sum PBDE_{3\text{-}8}$ concentrations in these (1980±562 μg/kg lw; N=3) were similar to those in the June beetles and predatory firefly larvae. However, their BDE47/99 ratio (1.2) was more comparable to the soil and earthworms. BDE 100, 153 and 154 were also detected at quantifiable levels in these beetles. BDE 209 was detected in all three replicates at substantial levels (5820±3840 μg/kg lw). This pattern of PBDE congeners is reminiscence of that observed in earthworms, which are a preferred prey item.

Crickets are omnivorous scavengers, eating plants, dead insects and other materials. They may also consume paper, textiles and polymers. The $\sum PBDE_{3\text{-}8}$ burdens in the composite crickets sampled (n=3) from the biosolids-applied field were among the

lowest of the invertebrates sampled, i.e. $\leq$ 371 µg/kg lw $\sum PBDE_{3-8}$. The BDE 47/99 ratio of < 0.3 was also the lowest of the invertebrates (Fig. 2; Table 2) and far less than that of the soil (1.0). This might indicate BDE 47 elimination. Despite the low $\sum PBDE_{3-8}$ levels, BDE 209 was quantifiable in 1 of 3 composites at 1440 µg/kg lw. As noted above, Hale et al. (2002) reported direct PBDE uptake by crickets from commercial PUF and tissue congener patterns in crickets resembling that of the treated PUF. Similar results were observed in our more controlled study of uptake by *A. domesticus* from PUF containing 9% (w/w) Penta-BDE (see Chapter 2). BDE 47/99 ratios on 28 d of that lab study were generally comparable with those calculated for field crickets in this study. PCBs have also been detected in crickets at contaminated disposal sites (Watson et al., 1985; Paine et al., 1993). Rapid uptake and elimination of benzo(a)pyrene (He et al., 1998) and PCB-47 and 77 (Li and McKee, 1992) by *A. domesticus* has also been reported. While taxonomically related and similar in physical form to crickets, grasshoppers are strictly herbivorous. Consistent with what was observed in the gamagrass, no BDE 209 or $\sum PBDE_{3-8}$ were detected at quantifiable levels in the three composite grasshopper samples from the sludge-applied field.

PBDEs were generally below quantitation limits in both the ground-feeding wolf (n=10) and web spiders (n=2), except for a single composite of the former wherein 84 µg/kg of BDE 99 and 1690 µg/kg lw of BDE 209 were detected. This outcome was unexpected, as spiders are the dominant macroinvertebrate predator in such ecosystems (Foelix, 1996). Further, Walters et al. (2008, 2009) observed appreciable PCB uptake by web-building spiders inhabiting a contaminated riparian system. On account of their trophic position, abundance and polyphagous feeding strategies, wolf spiders are believed

to be useful bioindicators of soil contamination (Wilczek et al., 2005). To cope with increased intake of dietary toxins, ground spiders are thought to have evolved more sophisticated digestive and detoxification enzyme systems (Wilczek et al., 2005). This may in part explain the relatively lower PBDE bioaccumulation in wolf spiders.

$\sum PBDE_{3-8}$ constituent BSAFs among arthropods were variable, ranging from about 0.006 for crickets to 0.3 for woodlice and ground beetles (Table 2). These are in the range of $\sum PCB$ BSAFs (e.g. 0.022) calculated for soil arthropod composites by Blankenship et al. (2005). BDE 47 BSAFs were not significantly different among woodlice and firefly larvae and the range of June beetle BSAFs (0.11-0.13) were comparable. Ground beetle BDE 47 BSAFs were significantly lower than woodlice, but about half those of June beetles and firefly larvae. BDE 47 BSAFs were lower in arthropods than in earthworms by at least a factor of two (Fig. 1; Table 2). BSAFs for BDE 47 decreased in the order worms > woodlice > June beetles ~ firefly larvae > ground beetles > June beetle larvae > millipedes > crickets. The BDE 47 BSAF in crickets was the lowest among arthropods by about a factor of 2 to 30. For BDE 100, BSAFs could only be calculated for woodlice and ground beetles. BSAFs were not significantly different between the latter, but both were significantly lower than calculated for BDE 100 in worms ($p<0.001$). For BDE 99, BSAF means and ranges among arthropods were consistently lower than those calculated for BDE 47. An exception to this was observed in crickets. For this congener, no significant differences were detected among woodlice, June beetle larvae and ground beetles (Table 2). However, in ground beetles, the BDE 99 BSAF was higher than millipedes ($n=1$) and statistically higher ($p<0.05$) than June beetle larvae. By comparison, worm BDE 99

BSAFs were about 8-fold higher than the highest calculated for arthropods (ground beetles). Woodlice and ground beetle ∑BSAFs were not significantly different, but both were higher than millipede ∑BSAFs. Worm individual congener and ∑BSAFs were significantly higher than those of all other invertebrates ($p<0.001$; ANOVA, HSD test). For Deca-BDE constituents, BSAFs were calculable only for BDE-209 in the millipede composite (10.5), June beetle larvae (5.8), crickets (0.4) and ground beetles (0.9).

Evaluating equilibrium partitioning. Equilibrium partitioning theory (EPT) predicts that relative burdens of hydrophobic organic chemicals in soil and resident organisms are controlled by fugacity, i.e. the organic carbon normalized concentrations in the biotic and abiotic phases (Di Toro et al., 1991; Achazi and Van Gestel, 2003). Under these conditions, a regression plot of lw- and TOC-normalized PBDE concentrations should be approximately isometric with zero intercept. Complicating this simplistic scenario are factors such as protracted mass transfer from slowly reversible soil organic carbon pools, short organismal life spans, xenobiotic biotransformation, equivalency of lipid/TOC partitioning and effects of food digestion on fugacity. We hypothesized that, because worms are in intimate contact with (and ingest) soil, equilibrium partitioning with soil may control $\sum PBDE_{3\text{-}8}$ uptake, whereas uptake by soil arthropods may be complicated by additional mechanism(s). To explore this, we evaluated the relationship between soil TOC and tissue lw-normalized $\sum PBDE_{3\text{-}8}$ burdens for dissimilar taxa using least squares regression (Fig. 3). For earthworms and ground beetles, organismal lw and soil TOC burdens were strongly correlated ($r^2=0.95$; $p<0.001$), whereas woodlice regressions were not ($r^2=0.37$; $p<0.06$). The slope of the fitted worm regression line approximated unity, while that of ground beetles and woodlice were much smaller.

Further insight as to uptake mechanism(s) may be obtained by examining the relationship of BSAFs to log Kow values using least squares regression (Fig. 1). Decreasing trends were apparent in all taxa. Burdens and log Kow were strongly correlated for woodlice (r^2=0.91; $p<0.05$), but less so for earthworms (r^2=0.63; $p<0.06$) and ground beetles (r^2=0.42; $p<0.24$). Interestingly, regression line slopes were more comparable between worms and woodlice, i.e. -0.47 and -0.33, respectively. The weaker worm correlation can be explained by the elevated BDE-100 burdens. The mean ground beetle BDE-209 BSAF was significantly higher than those of other congeners and was determined to be an outlier (Dixon's Q-test) and thus was omitted from the regression analysis. Increased BDE-209 bioaccumulation (and a weaker correlation between lw and TOC burdens) in ground beetles may reflect polyphagous feeding and predation on earthworms containing elevated $\sum PBDE_{3\text{-}8}$ and BDE-209 burdens. Greater relative heterogeneity in soil BDE-209 burdens may also contribute to the latter. Previous studies have shown that chemical uptake via the gut dominates over the dermal route in *E. andrei* for chemicals with log Kow > 6 (Jager et al., 2005). Far less is known about POP uptake in arthropods such as ground beetles and woodlice. Detritivores and predators will likely accumulate hydrophobic chemicals via the gut as well. Indeed, gut uptake has been shown to be an important exposure pathway in woodlice ingesting particle-associated PAHs (Achazi and Van Gestel, 2003). Obligatory transfer of POPs from the soil via air or water to dietary intermediaries, such as detritus and prey items, may further distort the original soil congener profile. Similar PCB weathering was observed in the PCB-contaminated soil system studied by Blankenship et al. (2005).

PBDE bioaccumulation and trophic relationships. Stable isotope analyses can provide insights into nutrient sources and trophic relationships. Here, soil, vegetation and grasshoppers occupied lower positions with respect to $\delta^{15}N$ at both the sludged and unsludged sites (Fig 4A & B). $\delta^{15}N$ enrichment in samples from the sludged site was appreciably higher than in those from the sludge-applied site. The former site was forested before being cleared only about two years before our study commenced. Hence, that addition of manure may not have been significant enough to inflate $\delta^{15}N$ values. Choi et al. (2005) reported $\delta^{15}N$ enrichment values of 2.2 to 2.8 for soil from a Mid-Atlantic forest, similar to that for our unsludged site soil (Fig 4A). Studies of soil isotope enrichment following land application of sewage sludges are limited. However, the mean $\delta^{15}N$ enrichment in weathered sludge samples obtained from the biosolids-applied site is comparable to that reported for land-applied biosolids (Wang et al., 2004), for bulk sewage sludge used as combustion feedstock (Arenillas et al., 2005) and for coastal zones receiving sewage outfall (Costanzo et al., 2001). $\delta^{15}N$ enrichment in some terrestrial arthropods inhabiting a sewage-influenced wetland has also been reported (Fair and Heikoop, 2006). However, there, higher %RSDs were noted for $\delta^{15}N$ mean values in arthropods compared to birds or plants. Similarly, Van Dover et al. (1993) reported significant $\delta^{15}N$ enrichment in benthic organisms at a deepwater sludge dumpsite off the coast of New Jersey (USA). In the Wang et al. (2004) study, $\delta^{15}N$ enrichment in sampled vegetation also reflected that of the applied biosolids.

At the unsludged site, ground beetles (also generalist predators) were also unusual in that their $\delta^{15}N$ signature was significantly lower than that of woodlice and June beetle larvae and comparable to earthworms. Increased worm predation may account for

isotopic signatures in common with the latter. This may also reflect greater omnivory in general among ground beetles within this type of soil ecosystem (McNabb et al., 2001). Relatively lower $\delta^{13}C$ enrichment among the unsludged site samples may also be explained by differences in C3 (temperate forest influences) versus C4 (gamagrass) photosynthetic pathways in vegetation prevalent at the two sites.

Invertebrate trophic relationships at the unsludged site (Fig 4A) appear to be more typical of temperate agricultural soil ecosystems (McNabb et al., 2001; Wise et al., 2006) than those within the sludge-applied system (Fig 4B). For example, wolf spiders from the unsludged site were significantly $\delta^{15}N$-enriched relative to all other organisms, indicative of their high predatory status. We originally hypothesized that the wolf spiders would, in order of increasing size, feed at the top of the insect food web. In contrast to the unsludged site, some soil-, detritus- and plant-ingesting species (e.g. earthworms, crickets, woodlice and June beetles) at the sludged site exhibited greater $\delta^{15}N$-enrichment than wolf spider size classes 1, 3 and 4. However, wolf spider class 2 and 5 and the web spiders occupied the highest trophic positions at the sludged site. The wolf4 spiders were unusual in appearing to feed at least one trophic level lower than the smaller wolf2 spiders at both sites. Wise et al. (2006) reported that small wolf spiders typically feed within detrital food webs. Thus we were surprised to find the larger wolf3 and wolf4 spiders apparently feeding at this level. This may be due to increased feeding within the detrital food web by larger wolf spiders due to lack of larger prey. Alternatively, increased organic matter input may increase detritivore biomass and thus predation by larger spiders within the detrital food web (Wise et al., 2006).

Significantly smaller ($p<0.05$; fresh weight) wolf spiders were found at the long-term sludge-amended site. This may be due in part to decreased availability of high quality prey. This, in turn, may result in more intraguild predation and cannibalism (Nielsen and Toft, 1998; McNabb et al., 2001) and general ecosystem stress (Haagvar, 1994). Such stresses may provide additional insight regarding stable isotope patterns and lack of PBDE bioaccumulation in wolf spiders (see Supporting Information for discussion). The source of this stress may arise from multiple sources, including long-term agriculture, vulnerability to predation by higher organisms such as birds, distance from habitat margins (e.g. forest cover) or other factors unconnected with sludge application.

McNabb et al. (2001) measured $\delta^{15}C$ and $\delta^{15}N$ enrichment in wolf spider size classes within a non-sludge-amended temperate agroecosystem (otherwise comparable to our own) and found no changes in $\delta^{15}N$ signatures as smaller wolf spider individuals (*Pardosa spp.*) grew to adulthood. However, increased $\delta^{13}C$ enrichment with growth was observed in that study. Comparable changes in $\delta^{13}C$ enrichment with size were observed here as well. McNabb et al (2001) also found that $\delta^{13}C$ and $\delta^{15}N$ enrichment in ground beetles (*Scarites spp.*) was similar to wolf spiders, reflective of shared predaceous feeding habits. In this study, $\delta^{15}N$ enrichment in ground beetles was not significantly different from the wolf3 spiders, but $\delta^{13}C$ enrichment was higher, suggesting a shared trophic level. $\delta^{15}N$ enrichment in woodlice, millipedes and crickets was similar, but $\delta^{13}C$ enrichment among these taxa varied. Interestingly, $\delta^{15}N$ enrichment was similar in firefly larvae, ground beetles, crickets and worms. Comparable enrichment among the predaceous taxa is consistent with their ecology. However, the enrichment in crickets

and earthworms is more difficult to explain. Clustering among the sludge-amended stable isotope signatures made interpretation challenging. Indeed, this is a recognized drawback to their use in delineating trophic relationships in complex soil food webs (McNabb et al., 2001). Trophic level identification can often be confused by both preferential and obligate dietary shifts (Eggers and Jones, 2000), with corresponding isotopic shifts occurring on short (i.e. 7-28 d) time scales (Ostrom et al. 1997). This might have occurred more frequently in the sludge-amended system. The fact that $\delta^{15}N$ enrichment was comparable in earthworms and crickets relative to predaceous arthropods in the sludge-amended system may be an indication of this.

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are increasingly measured to resolve POP bioaccumulation as a function of trophic relationships. For example, Walters et al. (2008) found that total PCB burdens were significantly correlated with $\delta^{15}N$ and $\delta^{13}C$ enrichment in web spiders within a contaminated riparian ecosystem. Though regressions there were significant, it is interesting that $\delta^{15}N$ enrichment was weakly correlated (r^2=0.15) with total PCBs. In a related riparian study, $\delta^{15}N$ enrichment in upland web building spiders (*Araneidae*) and terrestrial insects were comparable to most of our reference site arthropods (enrichment in our wolf spiders notwithstanding) (Walters et al., 2009). In our study, correlations with tissue $\sum PBDE_{3\text{-}8}$ burdens were weak, with isotope signatures revealing no clear trend of accumulation with trophic levels (Figs. S1 and S2). Regression analyses of $\sum PBDE_{3\text{-}8}$ BSAFs revealed a similar lack of correlations (data not shown). Accumulated PBDE burdens in taxonomically varied soil organisms suggest minimal accumulation in predatory taxa. Instead, proximity to, and

intimate contact with, PBDE-contaminated soil appeared more important in determining PBDE burdens in biota inhabiting this sludge-applied system.

Figures

Figure 1. Least squares regression analysis of mean calculated PBDE BSAFs and published log Kow values for earthworms, ground beetles and woodlice collected from the sludge-amended site. Ground beetle BDE-209 burdens were determined to be extreme outliers (Dixon's Q-test) and were thus omitted from regression analysis. Vertical and horizontal error bars are standard deviations from the mean. Error bars not visible are small enough to be obscured by the data point. Penta-BDE constituent log Kow data from Braekevelt et al., 2003. BDE-209 log Kow data from Wania and Dugani, 2003.

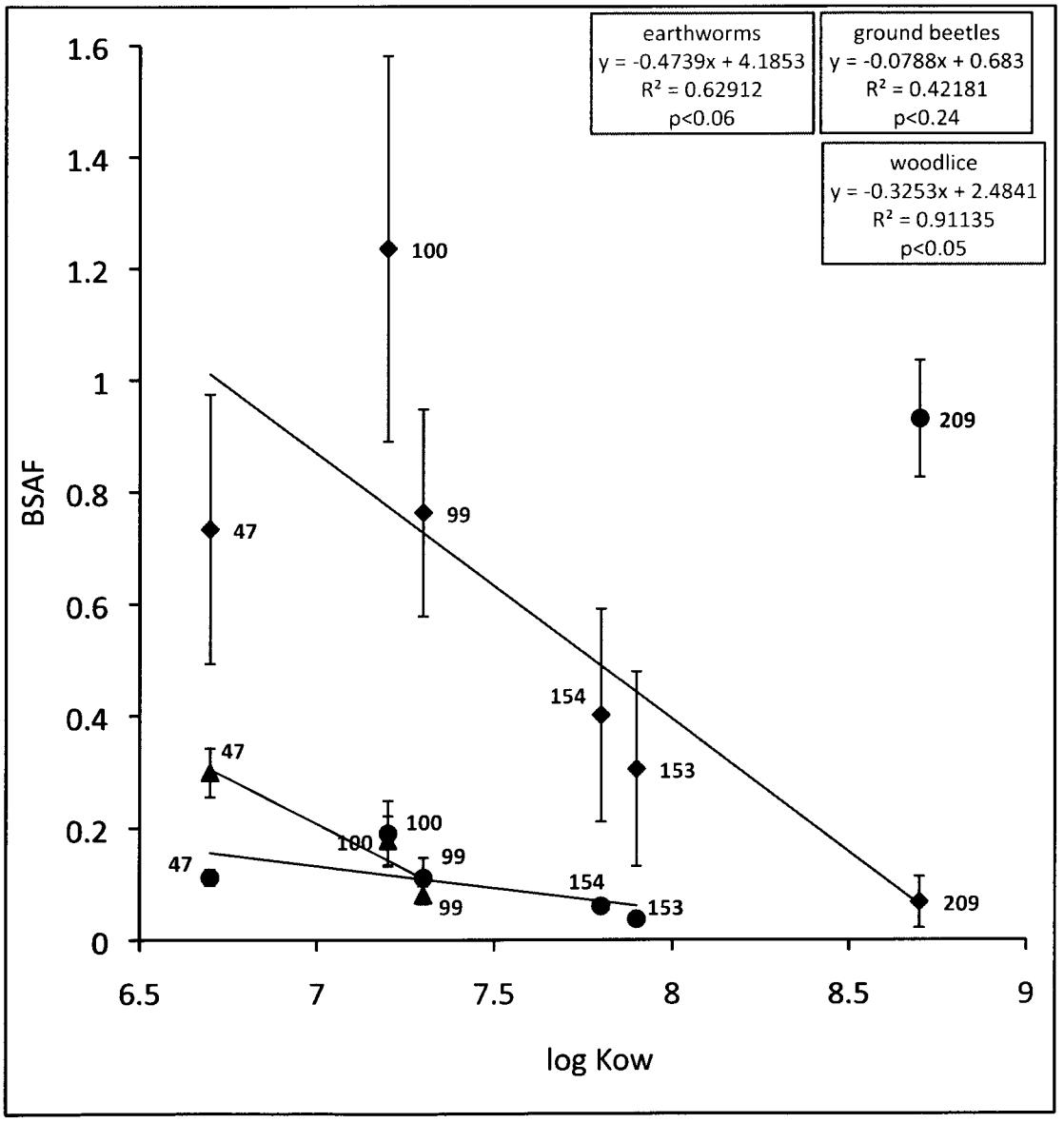

Figure 2. First two principle components of lipid- and TOC-normalized Penta-BDE constituent burdens in soil and biota from the sludge-amended site. Individual congener burdens are expressed as a percent of total PBDEs. Several worm (N=10) and soil (N=10) data points overlap. DE-71 compositioin data from La Guardia et al. 2006.

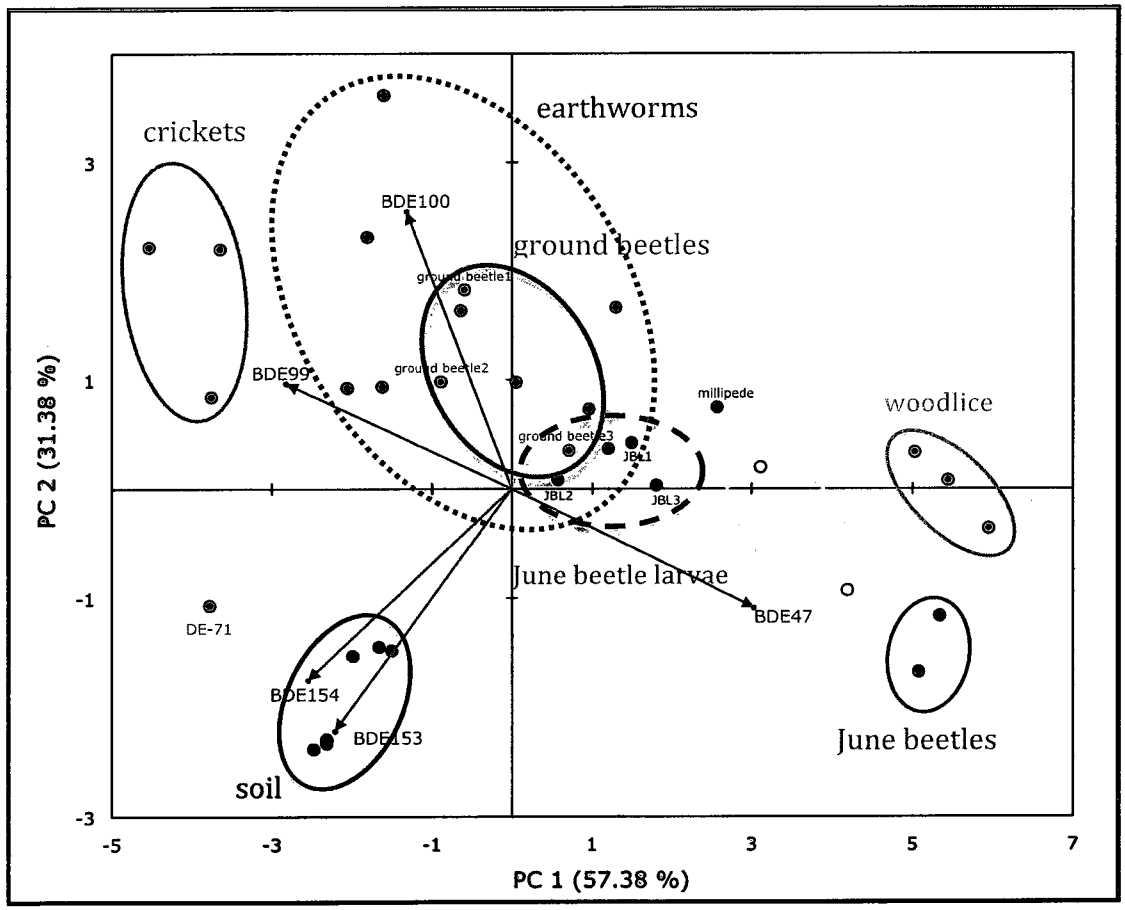

Figure 3. Least squares regression analysis of mean tissue (lipid basis) and soil (TOC basis) PBDE burdens for earthworms (N=10), ground beetles (N=3) and woodlice (N=3) from the sludge-amended site. BDE-209 data points were omitted from the worm (N=5) and ground beetle (N=3) regressions. Vertical and horizontal error bars are standard deviations from the means.

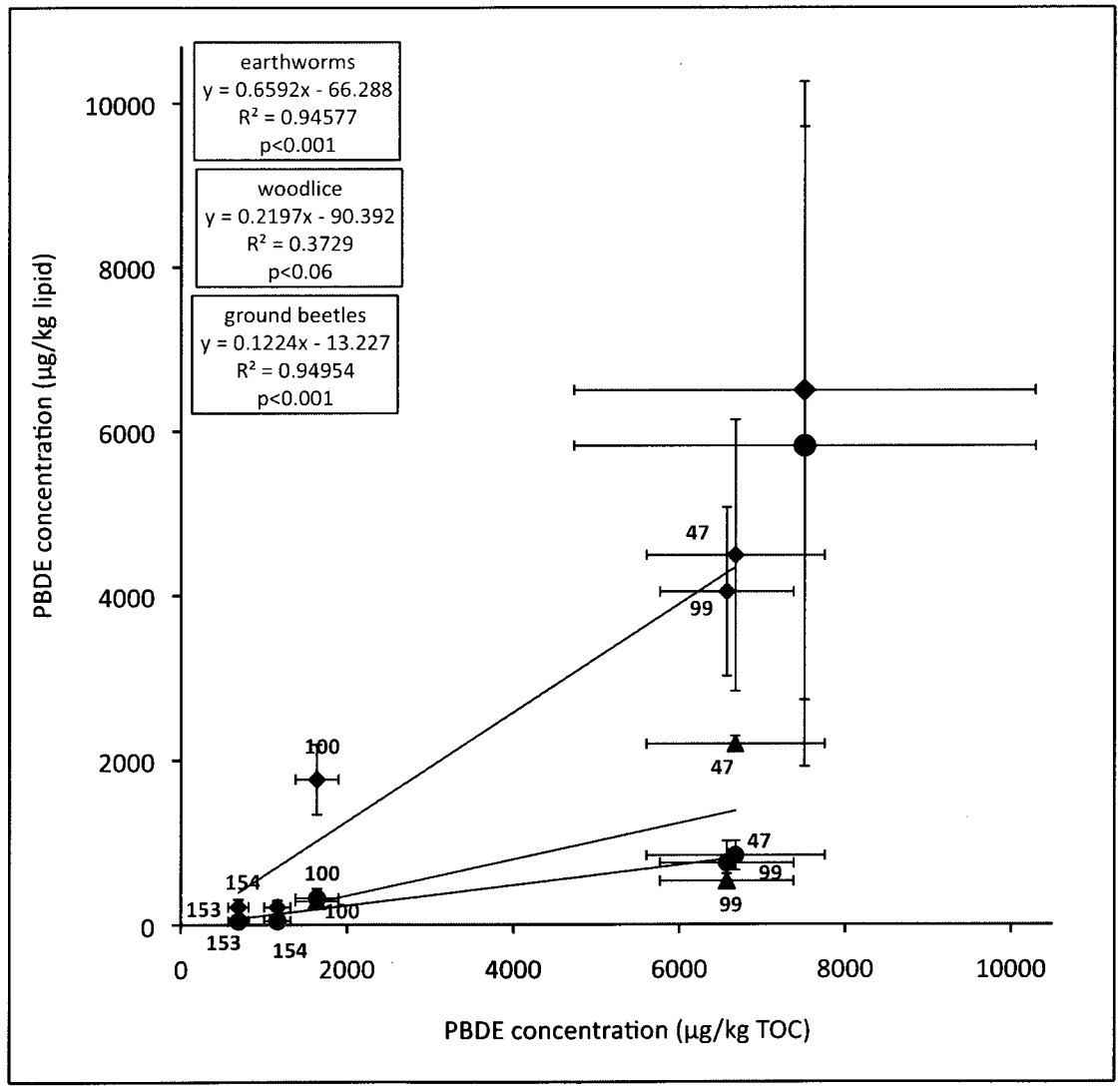

Figure 4. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures for samples collected from the reference **(plot A)** and sludge-amended **(plot B)** field sites. Vegetation samples are composites of Orchard Grass (*Dactylis glomerata L.*; reference site) and Eastern Gamagrass (*Tripsacum dactyloides*; sludge-applied site) shoots collected just above the soil surface. Soil samples are composites of soil parcels from which collocated earthworms were extracted. For wolf spiders: wolf1<0.1 g; wolf2=0.1-0.2 g; wolf3=0.2-0.3 g; wolf4=0.3-0.4 g; wolf5>0.4 g. Error bars are standard devations from the mean values. PUF data point is the mean of sub-samples of PUF taken from furniture cushioning and carpet underlayment samples from multiple locations within the US (N=10).

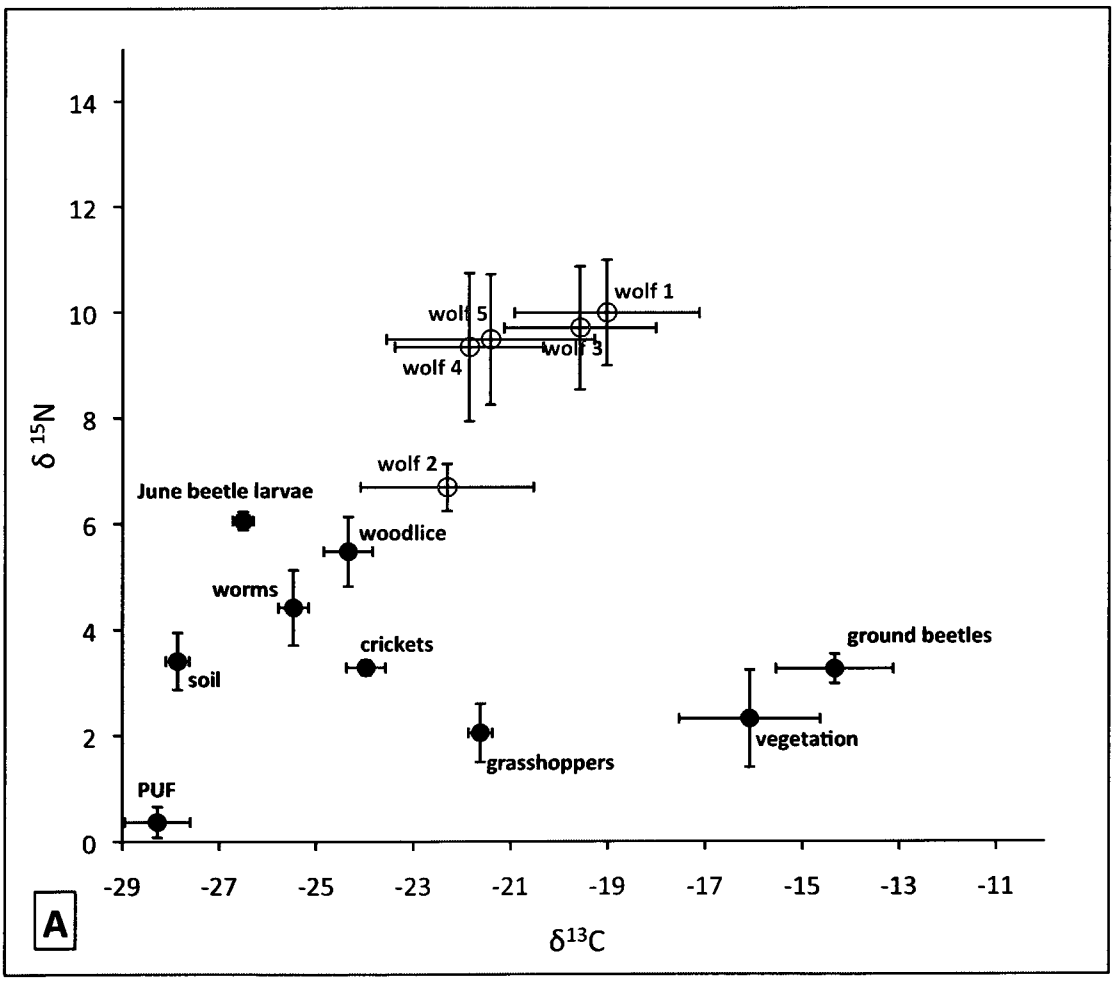

Figure 4 (Plot B).

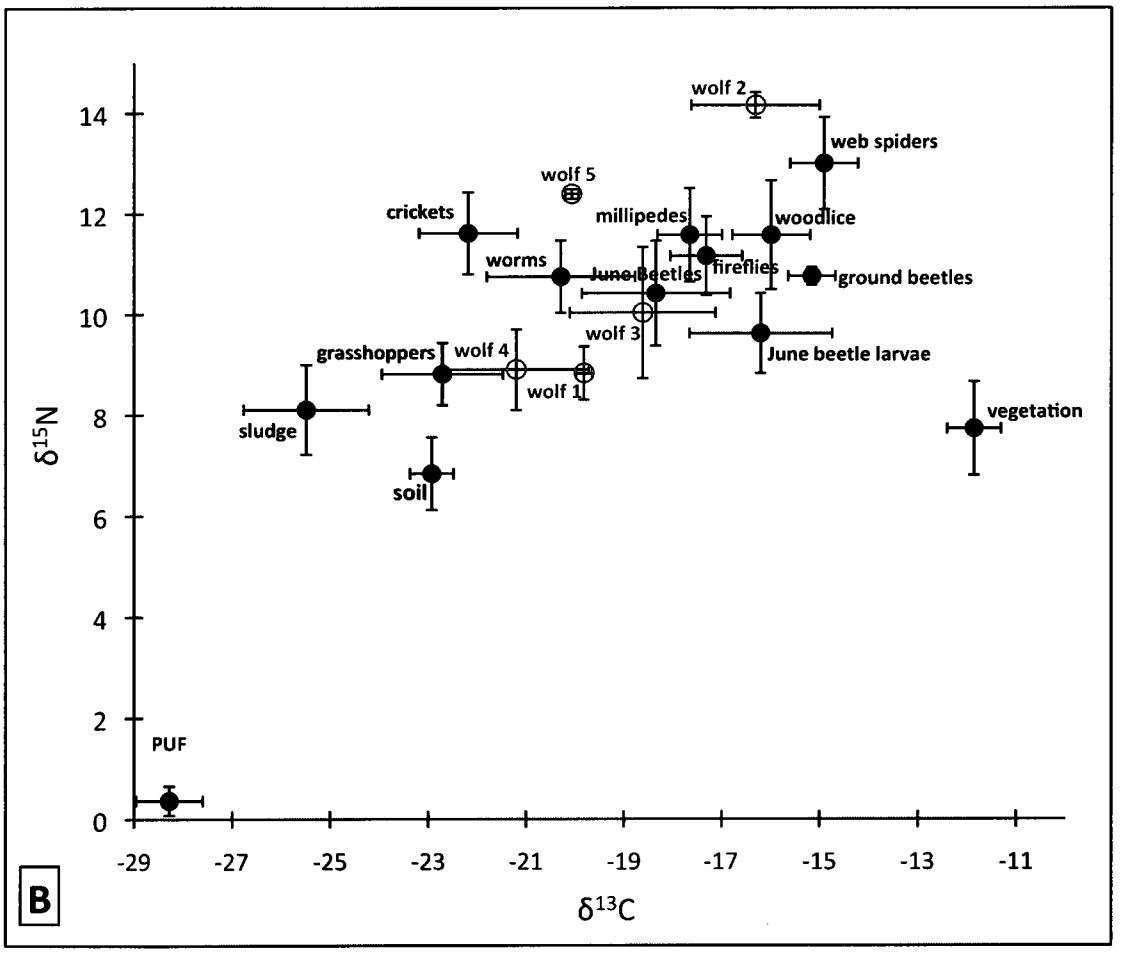

Table 1. Mean (±S.D.) PBDE burdens (μg/kg) for soil (TOC basis) and biota (lipid basis) from the sludge-amended site.

		PBDE burdens (μg/kg)												
sample matrix	*N*	47	100	99	154	153	$\Sigma_{3\text{-}8}$PBDEs	209	$\Sigma_{9\text{-}10}$PBDEs	lipid(%)[e]	204(%)	$\Sigma_{3\text{-}8}$PBDE QL[f]	$\Sigma_{9\text{-}10}$PBDE QL[f]	ecology
[b]soil	10	6680±1074	1637±259	6577±805	692±122	146±23	17635±2332	7512±2773	7512±2773	1.5-1.9[a]	82-103	53-76[a]	1112-1669	--
vegetation	3	<QL	<QL	<QL	<QL	<QL	--	<QL	--	40.1-42.7[a]	78-86	16-31[a]	346-717	--
[b]earthworms	10	4483±1651	1761±767	4039±1026	213±90	210±81	10284±2665	6494±4119	6494±4119	7.8-11.1	42-92	132-221	3149-8570	detritovore
millipedes	1	391	<QL	198	<QL	<QL	589	64701	86471	8.7-9.6	79-96	166-184	2511-2819	detritovore
woodlice	3	2190±96	286±50	525±87	<QL	<QL	3001±227	<QL	--	7.5-10.6	81-90	151-214	3206-4286	detritovore
June beetle larvae	3	403±188	<QL	264±102	<QL	<QL	667±279	59844	59844	2.2-22.1	68-72	184-212	2224-13395	herbivore
June beetles	2	1370-1773	<QL	387-442	<QL	<QL	1757-2215	<QL	--	9.5-10.1	89-93	357-381	8541-8886	herbivore
grasshoppers	3	<QL	<QL	<QL	<QL	<QL	--	<QL	--	9.1-12	57-71	51-64	1138-2203	herbivore
[c]crickets	3	53-55	<QL	146-316	<QL	<QL	199-371	1440	1440	13.3-18.6	70-84	50-83	771-1255	omnivore
firefly larvae	2	1352-1895	<QL	538-768	<QL	<QL	1890-2662	<QL	--	5.3-5.9	90-93	344-431	5875-6408	predator
ground beetles	3	837±179	318±118	744±266	41±6	43±7	1984±562	5815±3843	5815±3844	13.8-16.7	52-62	38-40	536-595	predator
[d]web spiders	2	<QL	<QL	<QL	<QL	<QL	--	<QL	--	12.3-14.7	90-93	125-153	2123-2382	predator
[e]wolf spiders		--	--	--	--	--	--	--	--	--	--	--	--	--
wolf1	2	<QL	<QL	<QL	<QL	<QL	--	<QL	--	7.7-9	67-87	152-220	2644-3688	--
[e]wolf2	2	<QL	<QL	82	<QL	<QL	82	1693	1693	7.3-11.5	65-82	72-85	1296-1464	--
wolf3	2	<QL	<QL	<QL	<QL	<QL	--	<QL	--	8.1-10.6	69-84	139-149	2444-2879	--
wolf4	2	<QL	<QL	<QL	<QL	<QL	--	<QL	--	9.2-13.8	82-83	195-301	3554-5351	--
wolf5	2	<QL	<QL	<QL	<QL	<QL	--	<QL	--	11.2-15.5	65-79	69-103	1288-4647	--

QL=quantitation limit.

[a]TOC reported for soil and vegetation; QLs reported on TOC basis.

[b]Only n=8 soil samples analyzed for BDE-209, as two soil samples were lost during processing subsequent to analysis of Penta-BDE constituents. BDE-209 was quantifiable in n=5 worm samples. BDE-206, 207 and 208 were detectable in several soil and worm replicates but below QL in all.

[c]BDE-47 was quantifiable in only n=2 samples. BDE-99 was quantifiable in all samples.

[d]Web spider samples are composites of black widows, garden spiders, spiny and long-jawed orb weavers and tangle web spiders (see Experimental Section for taxonomy).

[e]Wolf spider samples are each composites of approximately 12-70 individuals; BDE-99 and 209 were quantifiable in only a single sample replicate; wolf1<0.1g; wolf2=0.1-0.2g; wolf3=0.2-0.3g; wolf4=0.3-0.4; wolf5>0.4g.

[f]Lipid values are calculated on a dry weight basis and are reported as the range among samples.

Additional Notes: BDE-209 was quantifiable in only in n=1 June beetle larvae replicate; BDE-206, 207 and 208 were detectable in n=1 June beetle larvae but below QL. BDE-209 was quantifiable only in n=1 cricket replicate. BDE-209 was below QL but detectable in firefly larvae replicates. BDE-206, 207 and 208 were detectable in all ground beetle replicates but below QL.

Table 2. Mean (±S.D.) PBDE biota-sediment bioaccumulation factors (BSAFs) and congener ratios of major Penta-BDE constituents calculated for biota from the sludge-amended site. BSAFs were computed as the ratio of lipid- to TOC-normalized PBDE burdens. (--) BSAFs and PBDE ratios not computed for PBDE tissue burdens below quantitation limit (QL). Ranges are reported where sample n < 3, or PBDEs below QL in at least one sample replicate. June beetle composite sample replicates each contained three individuals.

		PBDE BSAFs							PBDE ratio	
sample	N	47	100	99	154	153	$\Sigma_{3\text{-}8}$PBDE	209	47/99	100/99
soil	10	--	--	--	--	--	--	--	1.1±0.03	0.3±0.01
earthworms	10	0.7±0.2	1.2±0.3	0.8±0.2	0.4±0.2	0.3±0.2	0.7±0.2	0.07±0.01	1.1±0.3	0.4±0.1
millipedes	1	0.05	--	0.03	--	--	0.03	10.5	2.01	--
woodlice	3	0.3±0.04	0.2±0.04	0.08±0.02	--	--	0.2±0.03	--	4.3±0.7	0.5±0.01
June beetle larvae	3	0.07±0.04	--	0.05±0.03	--	--	0.06±0.04	5.8	1.5±0.1	--
June beetles	2	0.21-0.24	--	0.067-0.069	--	--	0.11-0.13	--	3.5-4.1	--
grasshoppers	3	--	--	--	--	--	--	--	--	--
crickets	3	0.006-0.009	--	0.03-0.05	--	--	0.01-0.02	0.4	0.19-0.37	--
firefly larvae	2	0.21-0.26		0.09-0.12	--	--	0.12-0.15	--	2.47-2.52	--
ground beetles	3	0.1±0.01	0.2±0.06	0.1±0.04	0.06±0.01	0.04±0.01	0.1±0.02	0.9±0.1	1.2±0.2	0.4±0.05
web spiders	2	--	--	--	--	--	--	--	--	--
wolf spiders	--	--	--	--	--	--	--	--	--	--
wolf1	2	--	--	--	--	--	--	--	--	--
wolf2	2	--	--	0.01	--	--	0.01	0.2	--	--
wolf3	2	--	--	--	--	--	--	--	--	--
wolf4	2	--	--	--	--	--			--	--
wolf5	2	--	--	--	--	--			--	--

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APPENDIX I (Chapter 1)

Polybrominated Diphenyl Ether (PBDE) Accumulation in Earthworms After Exposure to Sewage Sludge, Polyurethane Foam Microparticles and Neat Chemical-Amended Soils

Chapter 1 Supporting Information

Experimental

PBDE analysis. Freeze-dried biosolids, soil and worm samples were spiked with a surrogate standard (PCB 204) to monitor analyte recoveries and then subjected to enhanced solvent extraction (Dionex ASE 200; Sunnyvale, CA) using methylene chloride. Total extractable lipids in earthworms were estimated by evaporating 10% of the total extract to constant weight. Solvent extracts were purified by elution from an EnviroSep® size-exclusion column (Phenomenex; Torrance, CA) with methylene chloride, followed by polarity-based purification on 2 g silica gel solid-phase extraction (SPE) columns (EnviroPrep®; Honeywell Burdick & Jackson; Muskegon, MI).

Following extract solvent exchange to hexane and addition of an internal quantitation standard, decachlorodiphenyl ether (DCDE; Ultra Scientific; Kingston, RI), PBDEs were separated on a gas chromatograph (GC; Varian 3400; Sugar Land, TX) equipped with a 15 m DB-5 column (J&W Scientific; Folsom, CA; 0.25 μm film, 0.32 mm ID) and Varian 8200 CX autosampler. The GC carrier gas was helium. Injections were made in the splitless mode. Initial GC column temperature was held at 90°C for 2 min and then programmed to 320°C at 4°C/min and held for 10 min. Analyte detection was achieved using electron impact MS with selected ion monitoring (MS; Varian 4D ion-trap; Sugar Land, TX). MS-based quantitation was accomplished from five point calibration curves by summing the areas of the three major ions of each PBDE congener versus that of the DCDE internal standard. PBDE congeners assessed were BDE 28, 47, 85, 99, 100, 153, 154 and 183 (Cambridge Isotope Laboratories; Andover, MA).

Biosoilds bioassay QA/QC. PBDEs were not detected in quantifiable levels in the

components used to produce the artificial soil (AS) and in earthworms prior to the experiments. Subsamples of these materials were initially spiked with the targeted PBDEs, as well a recovery surrogate (PCB 204) and analyzed as described above. PBDE recovery from all materials was quantitative (∑PBDEs = 55-93%; PCB 204 = 82-91%). BDE 28 recovery was the lowest (55-64%). A procedural blank (ignited sodium sulfate) was analyzed with each batch of 10 samples. The method quantitation limit (QL) was established using the lowest concentration PBDE quantitation standard (16 ng/ml) yielding a mean S/N ratio of ≥ 3 in substrates, biota and procedural blanks. QLs were normalized to individual sample dry and lipid weights and corrected for recovery of the surrogate standard, PCB-204. QL ranges of 23-32 μg/kg dw (211-262 μg/kg lipid), 20-32 μg/kg dw (186-262 μg/kg lipid) and 15-20 μg/kg dw (182-248 μg/kg lipid) were established for earthworms exposed to (spiked artificial soil) SAS-, (composted biosolids) CB- and (anaerobically digested biosolids) ADB-amended soil, respectively. In the same order, soil substrate QLs were 3.7-4.6 μg/kg dw (32-80 μg/kg TOC), 3.7-4.2 μg/kg dw (33-73 μg/kg OC) and 4-39 μg/kg dw (30-46 μg/kg TOC), respectively. The passive sampling device (PSD) QL was 5 μg/kg dw. All PBDE data were corrected for PCB 204 recoveries. These ranged from 89-103%, 86-101% and 49-98% for earthworms exposed to ADB, CB and SAS soil, respectively (N=72); 73-105%, 69-88% and 45-74% for ADB, CB and AS, respectively (N=72); 69-92% for artificial soil controls (N=9); 76-100% for sludge-exposed PSDs (N=24) and 43-89% for procedural blanks (N=16) (ignited sodium sulfate and DCM-extracted PSDs).

Polyurethane foam (PUF) microparticle-amended soil bioassay QA/QC. The lowest concentration PBDE standard (16 ng/ml) yielding a S/N ratio of ≥ 3 was used to establish

quantitation limit (QL) ranges of 19.7-25.6 μg/kg dw (347.8-470.6 μg/kg lipid) for earthworm tissues, 22.9-32 μg/kg dw (210.5-262.3 μg/kg C) for Penta-spiked artificial soil, 25.6 μg/kg dw (40.6-45.7 μg/kg C) for the PUF microparticle-amended soil and 80 μg/kg dw (133.3 μg/kg C) for PUF replicates. All PBDE data were corrected for PCB-204 recoveries (tissue 71±16% (N=9); PUF 102±10% (N=3); PUF-amended soil 88±11% (N=9); Penta-spiked soil 76±9% (N=9); control soils/tissue 79±14% (N=12)).

Tables and Figures

Table S1. Mean (±S.D.) PBDE burdens (µg/kg dw) determined for biosolid-amended soil treatments and their parent bulk biosolids. Amended soil concentrations reported for 28 d treatments only, as 14 d levels were not statistically different ($p<0.05$; ANOVA). (--)=not included in spiking mixture. < QL=below quantitation limit. [a]anaerobically digested sludge as received from POTW (N=3); [b]composted biosolid (N=3).

	substrate PBDE concentrations (µg/kg dw)										
	ADB-amended soil			CB-amended soil			Penta-BDE-spiked soil			ADB[a]	CB[b]
PBDE	low	medium	high	low	medium	high	low	medium	high	bulk	bulk
47	37±3	57±10	86±7	25±1	50±4	94±1	248±15	1130±55	1909±203	1808±107	411±33
85	<QL	<QL	<QL	<QL	<QL	<QL	20±2	94±9	208±13	403±32	31±7
99	29±2	40±10	57±9	18±1	35±2	66±3	277±11	1059±86	2251±42	2119±223	473±28
100	6±2	8±2	16±2	5±1	9±1	17±1	65±3	312±24	581±46	521±46	81±4
153	<QL	<QL	<QL	4±1	5±1	8±1	34±3	150±14	343±23	407±35	39±4
154	<QL	<QL	<QL	<QL	<QL	6±1	32±1	153±13	341±6	300±31	33±3
183	<QL	<QL	<QL	<QL	<QL	5±1	--	--	--	216±27	31±4
ΣPBDE	72±7	105±22	169±18	48±4	94±9	196±8	676±36	2898±204	5633±334	5558±440	1126±79

Table S2. Mean (±S.D.) whole body PBDE burdens (μg/kg lipid) in earthworms exposed to ADB- and CB-amended soils and Penta-BDE-spiked AS (N=3 each treatment). (--)=congener not added to the spiking mixture. <QL = below quantitation limit.

	ADB-exposed earthworms					
	14d			28d		
PBDE	low	medium	high	low	medium	high
47	6500±620	6300±500	6700±670	5700±630	6900±400	6400±900
85	<QL	<QL	<QL	<QL	<QL	<QL
99	3200±290	3200±290	3200±370	2600±240	3800±500	3500±500
100	790±70	890±270	750±70	690±130	900±50	900±160
153	<QL	<QL	<QL	<QL	<QL	<QL
154	<QL	<QL	<QL	<QL	<QL	<QL
183	220±50	200±60	220±10	320±50	490±50	450±80
ΣPBDE	10700±500	10600±1200	10900±1000	9000±990	11800±900	11000±1500
	CB-exposed earthworms					
PBDE	low	medium	high	low	medium	high
47	3400±470	4800±250	4000±400	3900±230	5700±220	7500±770
85	<QL	<QL	<QL	<QL	<QL	<QL
99	1800±400	2800±630	2800±300	2600±280	3300±450	4300±420
100	550±110	810±132	750±80	770±70	970±100	1200±110
153	230±40	200±50	220±20	201±29	200±20	240±40
154	<QL	<QL	<QL	<QL	<QL	<QL
183	260±70	250±20	170±10	<QL	170±40	260±40
ΣPBDE	6300±1100	8800±920	7900±600	6400±560	10300±770	13500±1300
	Penta-BDE-spiked AS-exposed earthworms					
PBDE	low	medium	high	low	medium	high
47	14200±970	10500±20400	197000±8020	37000±4700	105000±16700	285000±14600
85	620±110	3300±510	7500±600	2400±370	11800±2600	23000±5300
99	14800±2230	122000±31000	245000±16000	14500±2020	168000±51000	420000±14000
100	3400±370	18000±3000	52000±4700	8100±1200	46000±4100	81000±3300
153	540±70	2800±530	5600±270	960±150	6500±1900	13600±1870
154	600±200	3200±800	6700±130	1200±220	7500±1600	15000±2200
183	--	--	--	--	--	--
ΣPBDE	34070±3673	254000±56000	514000±29000	64300±8200	345000±42000	837000±18000

Table S3. Mean (±S.D.) PBDE congener ratios calculated for earthworms (lipid-normalized; N=3) and corresponding biosolid-amended soils and Penta-BDE-spiked AS treatments (TOC-normalized; N=3 each).

	ADB-amended soil					
	earthworms					
	14d			28d		
BDE ratio	low	medium	high	low	medium	high
47/99	2.1±0.4	2.0±0.1	2.1±0.2	2.2±0.04	1.8±0.2	1.8±0.03
100/99	0.3±0.01	0.3±0.1	0.2±0.01	0.3±0.02	0.2±0.02	0.3±0.02
	substrate					
47/99	1.4±0.05	1.7±0.1	1.8±0.04	1.3±0.1	1.5±0.1	1.5±0.2
100/99	0.2±0.02	0.3±0.1	0.2±0.05	0.2±0.09	0.2±0.01	0.3±0.02

	CB-amended soil					
	earthworms					
BDE ratio	low	medium	high	low	medium	high
47/99	1.9±0.2	1.8±0.4	1.5±0.2	1.5±0.1	1.8±0.2	1.8±0.1
100/99	0.3±0.01	0.3±0.02	0.3±0.1	0.3±0.02	0.3±0.01	0.3±0.01
	substrate					
47/99	1.3±0.1	1.4±0.1	1.4±0.1	1.4±0.1	1.4±0.2	1.4±0.1
100/99	0.3±0.02	0.3±0.01	0.3±0.01	0.3±0.01	0.3±0.01	0.3±0.02

	Penta-BDE-spiked AS					
	earthworms					
	14d			28d		
BDE ratio	low	medium	high	low	medium	high
47/99	1.0±0.1	0.9±0.1	0.8±0.02	2.6±0.2	0.7±0.2	0.7±0.1
100/99	0.2±0.01	0.2±0.02	0.2±0.01	0.6±0.04	0.3±0.1	0.2±0.001
	substrate					
47/99	0.9±0.01	1.1±0.04	0.9±0.1	0.9±0.09	1.1±0.1	0.9±0.1
100/99	0.2±0.002	0.3±0.02	0.3±0.01	0.2±0.01	0.3±0.1	0.3±0.02

Table S4. Mean (±S.D.) PBDE concentrations in PUF- and SAS-exposed earthworms and the corresponding PUF-amended soil and SAS exposure substrates (N=3).

	mean whole body (mg/kg lipid) and soil substrate (mg/kg dw) PBDE burdens						
	PUF-exposed worms			SAS-exposed worms		exposure soil substrate	
PBDE	7d	14d	28d	14d	28d	PUF-amended soil	SAS
47	790±99	1100±23	1500±120	200±8	290±15	27.4±2.8	1.9±0.2
85	68±13	80±24	140±9	8±1	23±5	2.8±0.6	0.2±0.01
99	820±110	1170±94	1620±160	250±20	420±14	38.5±2.9	2.3±0.04
100	140±24	198±35	310±60	52±5	81±3	6.1±0.7	0.6±0.05
153	53±15	52±8	92±7	6.0±0.3	14±2	4.8±0.6	0.3±0.02
154	24±6	32±11	70±8	7.0±0.1	15±2	3.6±0.4	0.3±0.006
ΣPBDE	1890±240	2630±100	3740±330	510±30	840±20	83.3±8	5.6±0.3

Table S5. Mean (±S.D.) PBDE biota-soil accumulation factors (BSAFs; lipid:TOC normalized) for worms exposed to three doses of ADB- and CB-amended soil and Penta-BDE-spiked artificial soil. <QL used to indicate that BSAFs were not computed due to PBDE congener below QL in substrate and/or tissue. (--)=not included in the spiking mixture.

	ADB-exposed earthworms					
	14d			28d		
PBDE	low	medium	high	low	medium	high
47	16.9±3.2	11.8±2.8	9.8±2.4	20.4±2.1	11.8±3.9	6.4±1.9
85	<QL	<QL	<QL	<QL	<QL	<QL
99	11.1±0.7	9.8±3.4	8.3±2.5	12.1±2.3	9.7±4.7	5.4±1.6
100	13.0±1.0	10.2±4.1	9.2±1.3	17.7±4.7	10.9±3.4	4.8±1.3
153	<QL	<QL	<QL	<QL	<QL	<QL
154	<QL	<QL	<QL	<QL	<QL	<QL
183	<QL	<QL	<QL	<QL	<QL	<QL

	CB-exposed earthworms					
	14d			28d		
PBDE	low	medium	high	low	medium	high
47	12.4±2.1	7.8±0.9	4.3±0.3	13.4±1.1	12.6±0.8	5.2±1.0
85	<QL	<QL	<QL	<QL	<QL	<QL
99	8.7±1.3	6.7±2.4	4.2±0.7	12.3±1.5	10.2±0.9	4.2±0.6
100	10.1±1.7	7.4±2.3	4.3±0.9	13.5±2.0	11.5±0.7	4.5±0.9
153	9.4±2.6	4.3±1.5	2.6±0.6	5.7±1.2	6.1±0.8	2.1±0.2
154	<QL	<QL	2.2±0.4	<QL	<QL	1.6±0.4
183	<QL	<QL	7.6±1.2	<QL	<QL	3.5±1.6

	Penta-BDE-spiked AS-exposed earthworms					
	14d			28d		
PBDE	low	medium	high	low	medium	high
47	4.4±1	5.1±0.6	11.6±1.7	13.0±0.3	6.8±0.3	16.7±2.8
85	2.7±0.9	1.9±0.2	4.2±0.6	10.6±1.5	8.9±2.4	12.2±2.6
99	3.9±1.1	6.3±1.1	13.0±1.4	4.5±0.4	10.6±1.8	20.7±2.0
100	4.1±1.1	3.3±0.5	10.7±1.1	10.7±0.3	11.0±1.3	15.5±2.9
153	1.4±0.4	0.9±0.1	1.9±0.2	2.4±0.1	3.4±1.4	4.4±0.6
154	1.5±0.7	1.0±0.3	2.3±0.3	3.3±0.6	3.8±1.3	4.8±0.6
183	--	--	--	--	--	--

Table S6. Mean (±S.D.) PBDE BSAFs for earthworms exposed to the PUF-amended (83.3 mg/kg dw) and Penta-BDE-spiked (5.6 mg/kg dw) artificial soil substrates.

earthworm BSAFs					
	PUF-amended soil			Penta-BDE-spiked soil	
PBDE	7d	14d	28d	14d	28d
47	17.8±3.5	24.5±2.4	33.4±3.3	11.7±1.7	16.8±2.8
85	14.6±3.9	16.6±3.8	30.7±2.9	4.2±0.6	12.2±2.6
99	13±2.4	18.5±2.6	25.6±2.1	13±1.4	20.7±2
100	13.7±1.8	19.6±1.5	30.2±3.1	10.8±1.1	15.6±2.9
153	6.9±2.7	6.6±0.4	11.8±2.4	2±0.2	4.4±0.6
154	3.9±0.7	5.3±1.3	11.9±2.4	2.3±0.3	4.9±0.6

Table S7. Mean (±S.D.) solvent extractable lipid content of ADB-, CB- and SAS- and artificial soil (AS) control-exposed earthworms (N=3 all treatments). Differences in extractable lipids and earthworm wet weights were not significantly different with dose or time within a given substrate treatment ($p>0.05$; ANOVA). However, 28 d ADB- and CB-exposed extractable lipids were both significantly higher than those of the 28 d Penta-BDE-spiked AS- and AS control-exposed treatments ($p<0.05$). ADB- and CB-exposed extractable lipids were not significantly different from each other, regardless of exposure time ($p<0.05$). Penta-BDE-spiked AS- and AS control-exposed extractable lipids were significantly lower in 28 d compared to 14 d treatments. For wet weights, ADB-exposed worms were significantly higher than CB-, Penta-BDE-spiked AS- and AS control-exposed worms (N=3 all treatments).

earthworm extractable lipids (%)

	ADB-amended soil			CB-amended soil			Penta-BDE-spiked AS			AS
time	low	medium	high	low	medium	high	low	medium	high	controls
14d	9.0±0.9	8.7±0.9	8.2±0.6	10.3±1.0	10.4±1.2	13.4±1.4	10.1±0.5	11.2±1.2	10.7±0.2	11.9±1.6
28d	10.4±0.5	9.3±0.7	11.2±1.0	9.8±0.2	11.8±0.7	11.6±0.6	8.6±0.7	8.2±1.4	8.4±1.2	7.8±0.4

earthworm wet weights (g)

	ADB-amended soil			CB-amended soil			Penta-BDE-spiked SAS			AS
time	low	medium	high	low	medium	high	low	medium	high	controls
14d	4.5±0.6	4.7±0.7	4.2±0.3	2.8±0.4	3.4±0.6	2.5±0.1	3.3±0.3	2.8±0.3	3.3±0.4	3.1±0.5
28d	4.3±0.4	4.4±0.3	4.1±0.5	2.7±0.3	2.6±0.4	2.4±0.5	3.2±0.5	3.5±0.3	3.3±0.5	2.8±0.4

Table S8. Mean (±S.D.) percent total extractable lipid content of earthworms exposed to the PUF-amended soil mixture and Penta-BDE-spiked and control artificial soil. (--) = lost sample replicates.

	earthworm total extractable lipid (%)		
time	PUF-amended soil	Penta-BDE-spiked soil	unspiked control soil
7d	5.8±0.8	--	9.8±1.3
14d	5.8±0.6	10.7±0.1	7.6±0.8
28d	5.4±0.2	8.4±1.2	8.4±1.1

Table S9. Mean (±S.D.) PSD PBDE burdens (μg/kg PSD) versus those of the corresponding ADB-amended soil treatments (μg/kg dw). [a]Only 28 d ADB-amended soil treatment values shown as PBDE burdens were not significantly different from 14 d treatment burdens. BDE-85, 154, 153 and 183 were below QL in all treatments. PBDEs were not detectable (S/N<3) in PSDs immersed in non-amended control AS treatments. All N=3.

ADB-amended soil PSD and corresponding soil substrate PBDE burdens

	14d PSDs			28 d PSDs			ADB-amended soil[a]		
PBDEs	low	medium	high	low	medium	high	low	medium	high
47	35±10	32±5	52±5	54±7	66±7	104±5	37±3	57±10	86±7
100	4±1	3±1	6±1	3±1	5±1	10±1	6±2	8±2	16±2
99	11±2	10±2	17±2	15±2	13±1	27±2	29±2	40±10	57±9
ΣPBDEs	50±14	45±6	74±5	71±8	84±7	142±2	72±7	105±22	169±18

Table S10. Mean (±S.D.) PSD accumulation factors (PAFs) for PBDEs in the ADB-amended soil treatments (N=3). PAFs are the ratio of PSD concentrations (µg/kg PSD dw) to substrate concentrations (µg/kg TOC) that were above quantitation limits.

	PSD accumulation factors (PAFs)					
	14d			28d		
PBDEs	low	medium	high	low	medium	high
47	0.09±0.02	0.07±0.02	0.08±0.01	0.2±0.06	0.1±0.03	0.1±0.02
100	0.07±0.02	0.03±0.01	0.07±0.01	0.07±0.02	0.06±0.02	0.05±0.01
99	0.04±0.01	0.03±0.01	0.04±0.01	0.07±0.01	0.03±0.01	0.04±0.01
ΣPBDEs	0.07±0.02	0.05±0.02	0.06±0.01	0.1±0.03	0.08±0.02	0.08±0.01

Figure S1. Relative 28 d ∑Penta-BDE uptake (dry weight basis) by *E. fetida* from the PUF-amended and solvent-spiked soil (this study; red bars) and solvent-spiked natural soil derived from 28d data reported by Liang et al. (2010) (green bars) and ShuZhen et al. (2010) (blue bars). Error bars represent standard deviations from the means. Replicate statistics were not reported in the ShuZhen et al. (2010) study.

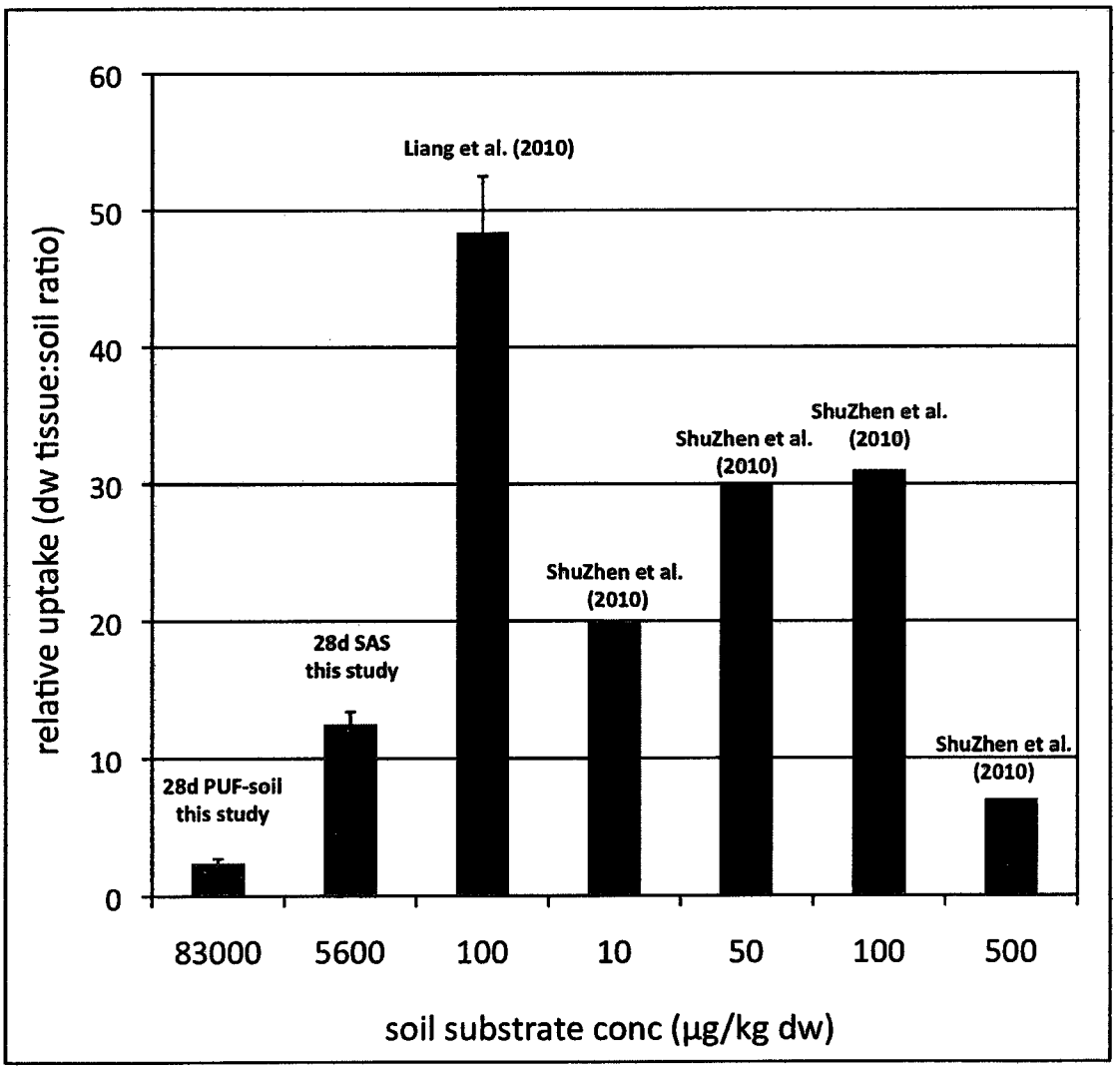


Figure S2. Regression analysis of mean 28 d worm (µg/kg lipid) congener burdens versus literature log Kow values (Braekevelt et al., 2003). Low- and high-dose biosolids-exposed worms and low-dose SAS-exposed worms are depicted. SAS-exposed (676 µg/kg dw) worm burdens are plotted on secondary y-axis (green). Congener concentrations were not plotted if <QL. Error bars represent standard deviations from the means (N=3).

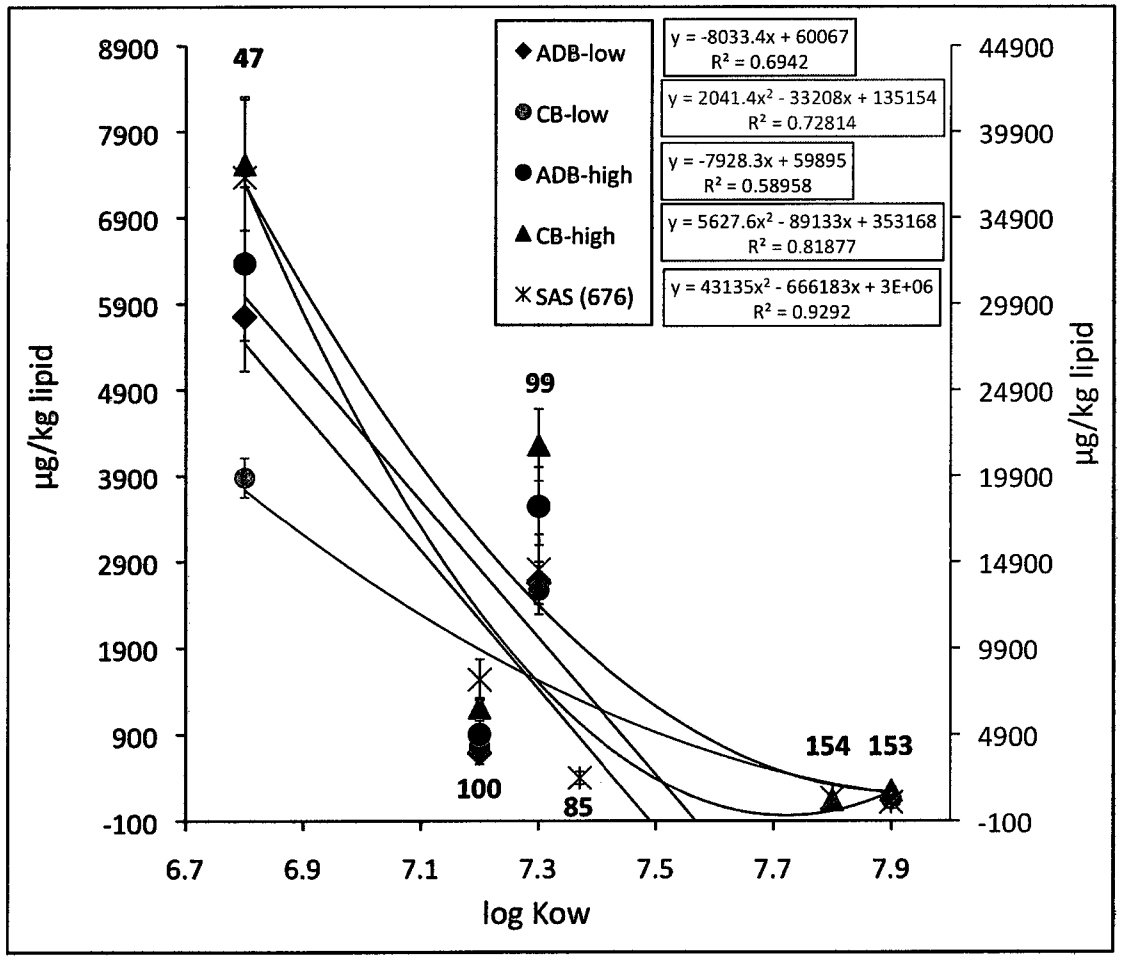
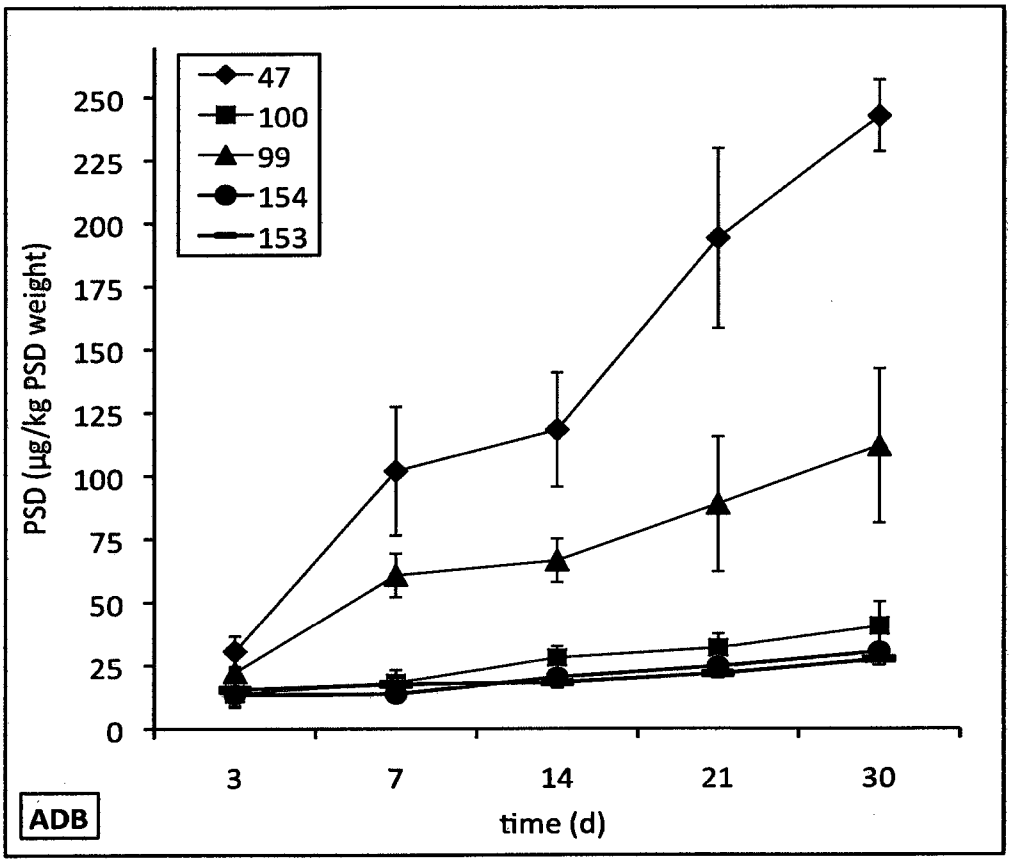


Figure S3. Mean (±S.D.) uptake of Penta-BDE congeners with time by passive sampling devices (μg/kg PSD) buried in 100% ADB. Error bars represent standard deviations from the means (N=3). Uptake of BDE-47 and 99 are significantly different for all but t=3 d time point ($p<0.05$; ANOVA with Tukey's HSD test). Concentrations of BDE-100, 154 and 153 are not significantly different at any time point. PBDEs not detectable (S/N<3) in PSDs immersed in 100% artificial soil (AS) controls.



APPENDIX II (Chapter 2)

Accumulation of Brominated Flame Retardants by Insects From Polyurethane Foam Derived from Household Furniture

Chapter 2 Supporting Information

Figure S1. Means of BDE 47/99 and BDE 100/99 congener ratios (red and green bars) in depurated crickets allowed access to PBDE-treated PUF. These are compared with ratios calculated for sub-samples of PUF (n=4) used here and the US Penta-BDE formulation, DE-71 (La Guardia et al., 2006). Error bars are standard deviations from treatment means. Bars with different lower case letters are statistically different ($p<0.05$; paired two sample t-test assuming unequal variance).

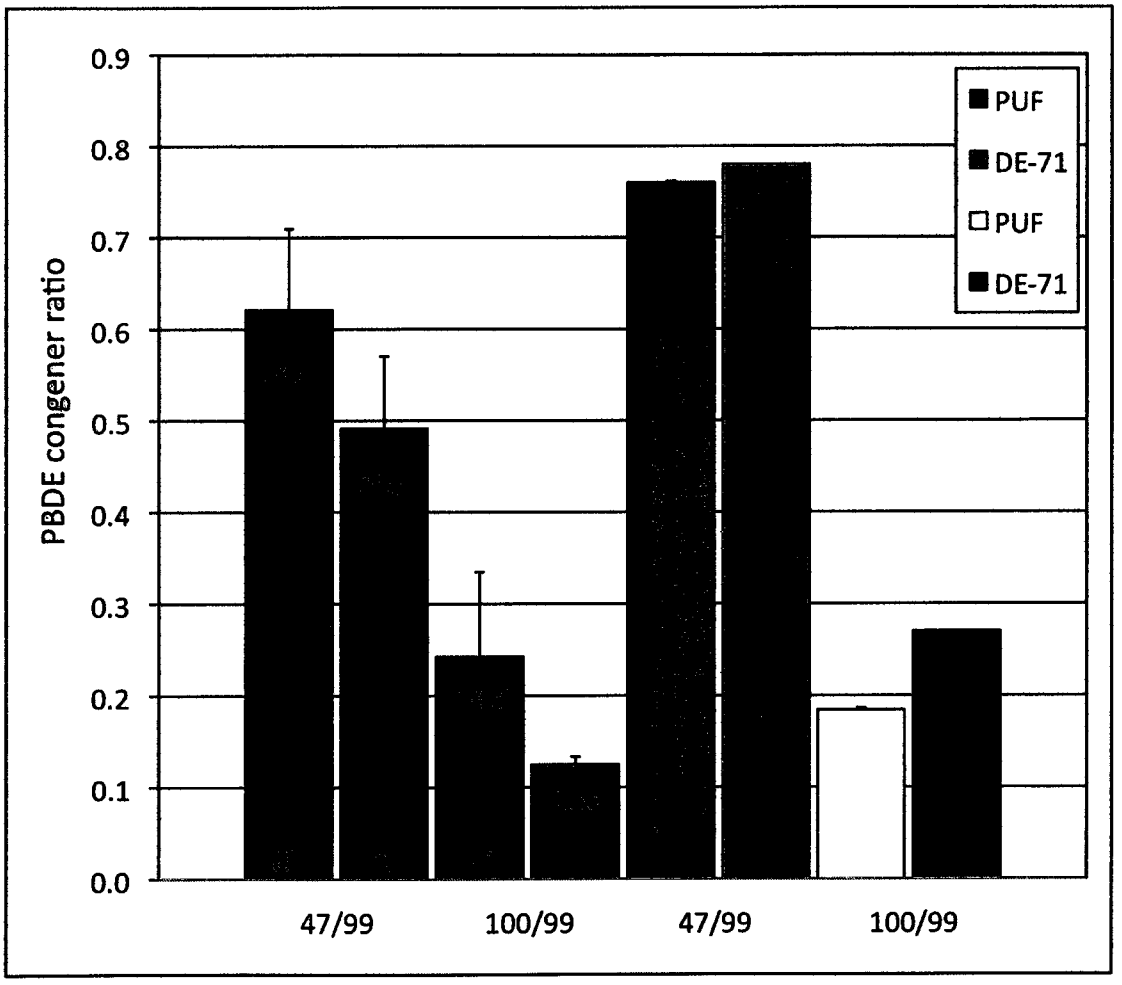


Figure S2. Mean growth (cm) for crickets exposed to Penta-treated PUF for 14- and 28-d. For all treatments, cricket growth was not statistically different ($p>0.05$) from non-exposed controls.

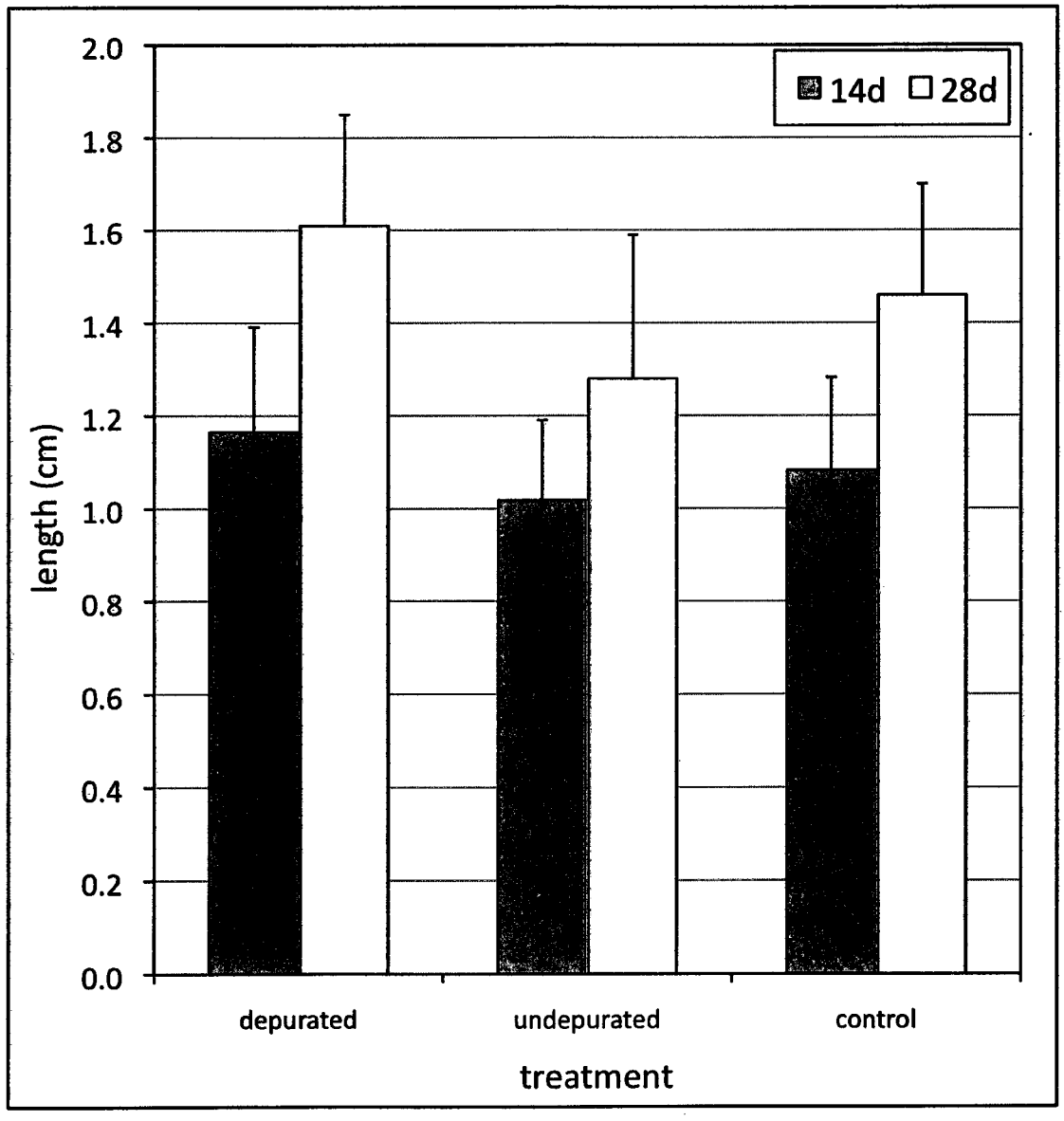


Table S1. Mean (±S.D.) whole body tissue PBDE burdens in depurated and undepurated crickets exposed to PBDE-treated PUF.

whole body tissue PBDE burdens (mg/kg lipid)				
	depurated		undepurated	
PBDE	14d	28d	14d	28d
47	3.4±0.8	3.4±0.7	2.2±0.9	28.8±2.3
100	1.3±0.1	0.7±0.2	0.5±0.2	5.5±0.9
99	5.7±1.7	6.9±1.7	3.3±0.7	39.9±10.1
154	1.6±0.5	0.5±0.1	0.7±0.1	4.1±0.3
153	2.9±0.8	0.7±0.1	0.5±0.1	2.3±0.2
ΣPBDE	14.9±2.5	13.4±3.8	7.3±1.9	80.6±11.1

APPENDIX III (Chapter 3)

Accumulation of Polybrominated Diphenyl Ether (PBDE) Flame Retardants in an Agricultural Soil Ecosystem Receiving Long-Term Sewage Sludge Amendments

Chapter 3 Supporting Information

Experimental

Paired soil-earthworm and vegetation sampling. Random linear transects within the fields were selected for sampling. Approximately 1 m^2 quadrats were established along transects at random intervals. The distance along a selected transect to a sampling quadrat was also determined randomly. If ~10 g earthworm mass was not immediately available, transects were expanded in 1 m^2 increments until such a mass was obtained.

QA/QC. Prior to analyzing field-collected biota samples, earthworms (n=3) and crickets (n=3) were collected from uncontaminated sites and spiked with a PBDE mixture containing the PBDEs of interest, as well as a surrogate standard (PCB-204). Samples were extracted and analyzed according to previously reported protocols (LaGuardia et al. 2006, 2007; Chen et al., 2008). Samples were analyzed as described above to ensure quantitative recovery of all PBDEs and PCB-204. PBDE congeners were recovered in quantitative yields ($\sum$PBDEs = 49-108%; PCB-204 = 71-93%), with the exception of the most volatile congener, BDE-28 (49-58%).

Blanks (solvent-extracted PSDs and ignited sodium sulfate; N=1, respectively) were transported to the field during each sampling trip (total N=8 PSDs and sodium sulfate, respectively, for both sites), placed on the ground and exposed to the air during sampling to track any PBDE contamination arising from field sampling activities. Field blanks were analyzed as described above. PBDEs were not detected in any of the field blanks and recovery of PCB-204 from field blanks was excellent, i.e. 85-103% (N=8). A procedural blank (ignited sodium sulfate) was analyzed with each batch of 10 samples and was manipulated in the same way as all other samples. For the stable isotope

analyses, replicate analytical blanks (ignited sand and empty tin capsules) were included with each set of 15 samples submitted for analysis.

Multi-component PBDE and PCB quantitation standards at eight and six serial dilutions, respectively, were run with each batch of 25 samples analyzed. QA/QC protocols for analysis of octa-, nona- and deca-BDEs on the ECNI GC-MS system have been previously reported (LaGuardia, 2006). All PBDE concentrations were normalized to total extractable lipid for biota and total organic carbon (TOC) for soils and vegetation. All PBDE data were corrected for recovery of the surrogate compound, PCB-204.

The analyte detection limits for this study were defined as the quantitation limits (QLs). These were established using the lowest concentration PBDE quantitation standard (16 ng/ml for Penta- and Octa-BDE constituents and 156 ng/ml for Deca-BDE constituents) yielding a mean S/N ratio of ≥ 3 in spiked procedural blanks (n=9) and biota matrices (n=6). QLs were also established for each sample using the lowest PBDE quantitation standard normalized to individual sample weights (dw and lipid basis) and corrected for recovery of the surrogate standard, PCB-204.

Carbon and nitrogen stable isotope analysis. To identify trophic positions, all samples were sub-sampled for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope determinations. Sub-samples were homogenized further by grinding them to a fine powder-like consistency using a solvent-rinsed coffee grinder. Soil sub-samples were fumigated with concentrated hydrochloric acid in a desiccator overnight to neutralize carbonates. Sub-samples were submitted to the University of California Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu) for analysis.

Discussion

Evaluating ecological stress within the sludge-amended site. To explore further the concept of systemic stress within the wolf spider population at the sludge-amended site, we examined underlying statistical distributions of whole body fresh weight data for all individuals collected at both sites within several days of sampling during late September 2009 (n=347 sludge-amended site; n=245 reference site). Abundance distributions of healthy species are log-normally distributed (Gray and Mirza, 1979). This has been observed in a variety of ecosystem types and deviations from this distribution have been used to quantify systemic stress in soil arthropod communities (Haagvar, 1994). We applied this statistical approach to wolf spider fresh weights to determine whether the sludge-amended population may be stressed relative to the less disturbed reference population.

Fresh weight data were first log-transformed to determine whether the underlying statistical distribution was skewed from the lognormal distribution. Statistical tests were then conducted on both raw and log-transformed data to test for statistical differences. Log-transformed fresh weights from the reference population were log-normally distributed ($p>0.05$; Shapiro Wilks test), while log-transformed fresh weights from the sludge-amended population were significantly skewed from the lognormal distribution ($p<0.004$; Shapiro Wilks test). Fresh weights from the sludge-amended site were significantly lower ($p<0.0001$, Shapiro Wilks test) than reference site fresh weights as well.

These results suggest that the sludge-amended wolf spider population may be stressed relative to the reference site population. This may impact how resident biota

copes with contaminant exposure. Multiple factors may contribute to this, including long-term sludge applications, farming activities, abundance of low quality prey etc. The sludge-amended site, though historically a non-tilled site, is subjected to physical disturbance via harvesting and periodic disking. In contrast, the reference site receives frequent applications of animal manure and is only mowed periodically to control vegetative succession. Owing to their distance apart (~ 160 km), distinct microclimates may also contribute. More studies are needed to better understand these results within the context of PBDE bioaccumulation within the sludge-amended site.

Figure S1. Regression analysis of mean log-transformed ∑PBDEs (lipid basis) and mean $\delta^{13}C$ enrichment for arthropods with incurred quantifiable PBDE burdens.

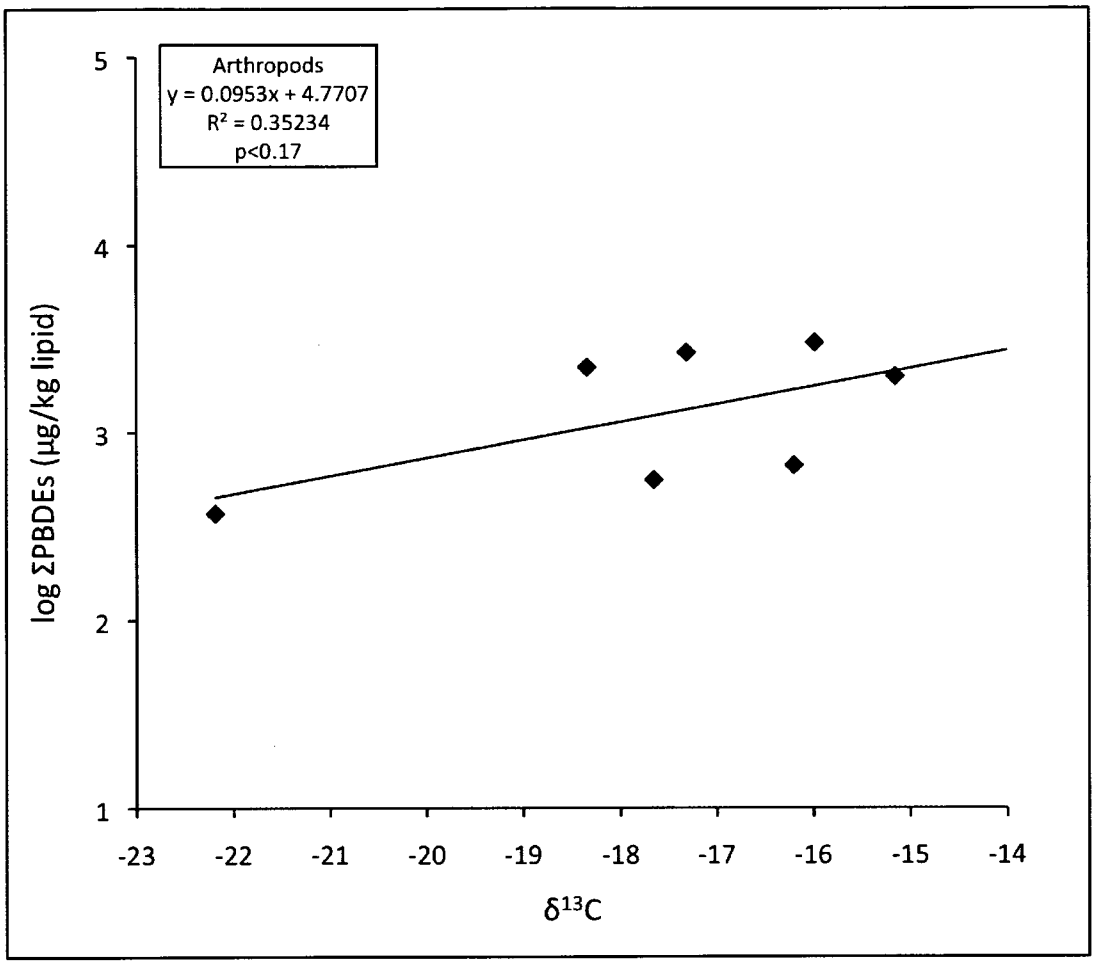
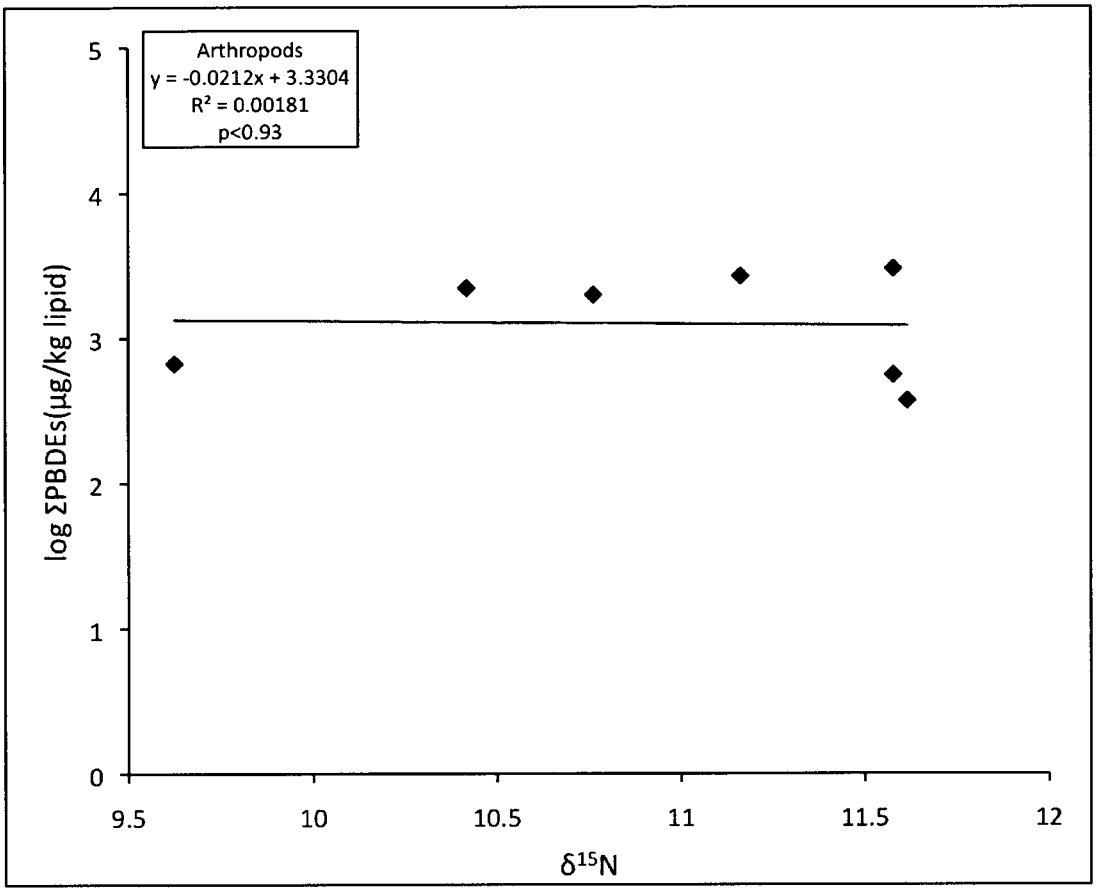


Figure S2. Regression analysis of mean log-transformed ∑PBDEs (lipid basis) and $\delta^{15}N$ enrichment for arthropods with incurred quantifiable PBDE burdens.



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VITA

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