Bioavailability of Polybrominated Diphenyl Ethers (PBDES) in Biosolids and Spiked Sediment to the Aquatic Worm Lumbriculus variegatus

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BIOAVAILABILITY OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN BIOSOLIDS AND SPIKED SEDIMENT TO THE AQUATIC WORM

*LUMBRICULUS VARIEGATUS*

A Thesis
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Science

by
Serena Ciparis
2003
This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Science

Approved, April 2003

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ACKNOWLEDGEMENTS

I greatly appreciate the support and guidance provided by my major advisor, Dr. Robert Hale, throughout the course of my graduate studies. His insights into the design, implementation, and interpretation of this research project were invaluable. I also wish to thank my advisory committee for all of their input and advice. Dr. Herbert Austin provided continual enthusiasm for the research topic, Dr. Robert Diaz provided much needed assistance with statistics and questions about oligochaete biology, and Dr. Peter Van Veld provided expertise in the field of aquatic toxicology.

Successful completion of this research would not have been possible without generous contributions of time and knowledge by many people at VIMS. Mark LaGuardia helped me tremendously with all aspects of chemical analysis and generation of data. Ellen Harvey provided instrumentation and the willingness to instruct me on its operation. Mary Ann Vogelbein spent many hours teaching me general wet lab practice and helping me set up and break down my experiment. Twenty-four students and staff assisted with worm picking, many on more than one occasion. Without their help, I would still be in the basement of Byrd Hall!

Finally, I would like to thank my family for their love and encouragement. For the past three years my parents, grandmother, and Michael have had unwavering faith in my abilities and I will always appreciate their support.
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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are brominated aromatic compounds currently used as flame retardants in a variety of polymeric materials. PBDEs are persistent and lipophilic and appear to be ubiquitously distributed in aquatic environments. In the United States, high concentrations of PBDEs have been reported in freshwater fish and also in treated sewage sludge, referred to as biosolids, which is applied to agricultural, public, and reclaimed lands as a fertilizer and soil conditioner. Land-application of biosolids creates the potential for redistribution of PBDEs to surface waters and subsequent association with aquatic sediments. However, little is known about the bioavailability of PBDEs associated with biosolids or sediment to benthic organisms. In this study, freshwater oligochaetes, *Lumbriculus variegatus*, were exposed to composted biosolids (1600 ng/g total PBDEs) and artificial sediment spiked with commercial penta- and deca-BDE formulations (1300 ng/g total PBDEs). Uptake was studied over a 28-day exposure period and depuration was studied for 21 days after removal from the substrates. The bioaccumulation kinetics were then compared between the two exposure substrates and between eight PBDE congeners.

PBDEs were bioavailable to *L. variegatus* from both spiked artificial sediment and biosolids. However, bioaccumulation was 5-10 times greater in worms exposed to spiked artificial sediment, and patterns of uptake over time were significantly different between the exposure substrates. The differences in accumulation between the two exposure substrates were likely due to differences in substrate characteristics that affect partitioning. This highlights the importance of choosing environmentally realistic test matrices for bioaccumulation assays. Of the eight PBDE congeners studied, BDE 47 and BDE 99 were the most prevalent in oligochaetes at the end of the exposure. BDE 47 was more bioaccumulative, which coincides with patterns observed in wildlife. Depuration of BDE 99 was three times faster than that of BDE 47, which suggests that bioaccumulation is strongly correlated to elimination. Amongst the tetra- through hexa-brominated congeners, the substitution pattern appeared to have a stronger effect on overall bioaccumulation than the number of bromine substituents. The uptake of BDE 209, the dominant congener in deca-BDE, was very low. This is likely due to its large molecular size and extreme hydrophobicity. Uptake of PBDE congeners from biosolids and sediments provides a pathway for transfer to higher trophic levels, and discrimination of congeners may increase with each trophic transfer.
BIOAVAILABILITY OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN BIOSOLIDS AND SPIKED SEDIMENT TO THE AQUATIC WORM *LUMBRICULUS VARIEGATUS*
INTRODUCTION

Production and usage

Polybrominated diphenyl ethers (PBDEs) are brominated aromatic compounds used as flame retardants in polyurethane foam, plastics in electronic devices and electrical equipment, and textiles. PBDEs are classified as additive flame retardants, they are not chemically bonded with the polymer molecule. Additive flame retardants are only mixed with or dissolved into the polymer, and have the potential for limited migration out of the material throughout the lifetime of the final product (deBoer et al 2000).

Brominated flame retardants inhibit combustion through chemical decomposition. An ideal flame retardant decomposes at approximately 50% of the combustion temperature of the polymer (deBoer et al 2000). Decomposition of brominated flame retardants liberates bromine atoms. These atoms are effective reducing agents and free radical inhibitors that quench oxidation reactions and prevent the formation of flammable gases (Manchester-Neesvig 2001).

PBDEs are produced by bromination of diphenyl oxide in the presence of a catalyst. This results in products containing mixtures of PBDE congeners (deBoer et al 2000). There are 209 possible congeners, depending on the number and position of the bromine atoms on the two phenyl rings. The naming system for PBDE congeners is similar to that used for the polychlorinated biphenyls (PCBs). The general structure of a PBDE molecule is shown in Figure 1 (see page 21). Commercial formulations contain from three to ten bromine atoms and are sold as penta-, octa-, and deca-BDE. Penta-BDE is primarily utilized in polyurethane foams and contains a mixture of tri- through hexa-brominated diphenyl ethers. DE-71, the major penta-BDE product used in North
America, consists of 24-38% tetrabromodiphenyl ethers (TeBDEs) and 50-62% pentabromodiphenyl ethers (PeBDEs). Octa-BDE is used in high impact plastics and is composed of hexa- through nona-brominated diphenyl ethers. Heptabromodiphenyl ethers (HpBDEs) and octabromodiphenyl ethers (OBDEs) constitute 75-80% of octa-BDE formulations. Deca-BDE is used in textiles and high impact plastics and is 97-98% decabromodiphenyl ether, which consists of a single congener named BDE 209 (WHO 1994).

Commercial PBDE mixtures are relatively stable and PBDEs are considered to be persistent compounds. Boiling points range between 310 and 425°C and increase as bromine content of the congeners increases. The volatility of PBDE compounds is low and decreases as bromine content increases. Solubility in water is very low, less than 30 μg/L at 25°C, and decreases with increasing bromine content. The octanol-water partitioning coefficients (log K_{ow}) for PBDEs range between 4.3 and 9.9 and increase as the bromine content of the congeners increases. PBDEs are lipophilic compounds and have the potential to accumulate in organisms (WHO 1994, deBoer 2000).

PBDEs are structurally similar to both PCBs and polybrominated biphenyls (PBBs). PCBs were used as coolant-insulation fluids in transformers and capacitors, as plasticizers, and as additives to some epoxy paints. The manufacture of PCBs was banned in the United States in 1976 (Manahan 1999). PBBs were used as flame retardants, but after accidental contamination of cattle feed in Michigan in 1973, the use of PBBs in the United States was voluntarily phased out. Since 1976, all PBBs manufactured in the United States have been exported. A production ban on hexabromobiphenyl currently exists in Europe (deBoer et al 2000). In contrast to PCBs
and PBBs, PBDEs are widely used today. The annual worldwide consumption of PBDEs was approximately 40,000 metric tons in 1992 and increased to approximately 67,000 metric tons in 1999 (WHO 1994, Renner 2000a). Deca-BDE accounts for approximately 81%, penta-BDE approximately 13%, and octa-BDE less than 6% of this consumption. North America uses 98% of the 8500 metric tons of penta-BDE consumed worldwide (Renner 2000a). Recently, the apparent ubiquitous distribution of certain PBDE congeners in the environment has led the European Commission to propose a ban on the production and use of penta-BDE by 2003. North America has not proposed any restrictions on PBDE usage.

Environmental occurrence

PBDEs have been detected in marine organisms from many areas of the world. Early studies of PBDEs detected the compounds in seals, guillemots, and white-tailed sea eagles from the Baltic Sea, North Sea, and Arctic Ocean at concentrations ranging from 10-370 ng/g lipid (Jansson and Asplund 1987). Later studies quantified the congeners 2,2',4,4'-TeBDE (BDE 47), 2,2',4,4',5-PeBDE (BDE 99) and 2,2',4,4',6-PeBDE (BDE 100) in the previously mentioned samples, and also in herring, seals, and guillemots from the Arctic Ocean, North Sea, and the Baltic Sea (Sellstrom et al 1993 and 1996). A long-term study of the blubber of ringed seals from the Canadian Arctic indicated that concentrations of tetra- through hexa-BDEs have increased exponentially between 1981 and 2000 to levels of 5 ng/g lipid. BDE 47, 99, and 100 were the most prevalent congeners in these samples (Ikonomou et al 2002). Nineteen tetra- through hexa-brominated diphenyl ethers were identified in the blubber of long-finned pilot whales.
caught off of the Faroe Islands in the Atlantic. Concentrations ranged from 843-3160 ng/g lipid total PBDEs. BDE 47 and BDE 99 were predominant in all samples (Lindstrom et al 1999). PBDEs have been found in sperm whale blubber at concentrations of approximately 100 ng/g lipid. This indicates that PBDEs have reached deep ocean waters, since sperm whales are not usually found in shelf seas and typically feed at depths of 400-1200 m or more (deBoer et al 1998). PBDEs have also been detected in marine organisms from coastal environments. Total PBDE concentrations ranged from 200-2269 ng/g lipid in hepatopancreas of dungeness crabs and blubber of harbor porpoises from estuarine environments in British Columbia. BDE 47 was the dominant congener in all samples (Ikonomou et al 2000). BDE 47 was also detected in blubber samples from harbor seals in San Francisco Bay at concentrations ranging from 46-6682 ng/g lipid (She et al 2000). Thus, it appears that PBDEs are present in marine organisms from shallow- and deep-water marine environments and that BDE 47 is the dominant congener in tissues of these organisms.

PBDEs have also been detected in freshwater environments, often at high concentrations. In 1981, tri- through hexa-brominated diphenyl ethers were found in fish and eel samples from the Swedish River Viskan in the vicinity of plastic and textile industries. Since then, the congeners BDE 47, 99, and 100 have been quantified in freshwater fish from both industrialized and remote areas in Sweden, with samples from heavily populated areas containing higher levels of PBDEs than pristine areas (Sellstrom et al 1993 and 1996). Despite the heavy usage of PBDEs in North America, little research had been conducted on these compounds in aquatic environments until relatively recently. Loganathan et al (1995) detected PBDEs in muscle tissue of carp from the
Buffalo River, New York. TeBDEs accounted for 94-96% of the total PBDE concentrations and older fish contained higher concentrations of PBDEs than younger fish. PBDEs have been detected in fish and birds from the Great Lakes region. Lake trout from Lake Ontario, Lake Huron, and Lake Superior had total PBDE concentrations ranging from 135-545 ng/g lipid (Alaee et al 1999). Coho and Chinook salmon from Lake Michigan tributaries had a mean PBDE concentration of 2440 ng/g lipid (Manchester-Neesvig 2001). Concentrations of BDE 47, 99, and 100 in herring gull eggs collected from colonies throughout the Great Lakes region increased exponentially from 1983 to 2000 to maximum concentrations of 400-1100 ng/g wet weight (Norstrom et al 2002). In the three Great Lakes studies, BDE 47 was the dominant congener in all analyzed samples. In Virginia, BDE 47 was detected in 89% of fish samples from rivers and reservoirs in the Roanoke and Dan River watersheds, and over half of the 334 fish samples analyzed contained >100 ng/g lipid of BDE 47 (Hale et al 2001a). BDE 47 was the most abundant congener out of the tetra- through hexa-BDEs that contributed to total PBDE concentrations. The highest total PBDE concentration was 47,900 ng/g lipid in a carp from the Hyco River, located in a region right in textile and furniture manufacturing activities (Hale et al 2001a). Thus, PBDEs appear to be present in relatively high concentrations in some freshwater fish in both Europe and North America and congener patterns are similar to those found in marine organisms.

The presence of these compounds in freshwater and marine fish creates potential exposure routes for organisms occupying higher trophic levels. Detection of PBDEs in marine mammals and fish-eating birds supports this idea. PBDEs have also been detected in human samples. A study of Latvian and Swedish men found a significant
correlation between the level of consumption of fatty fish and plasma levels of BDE 47 (Sjodin et al 2000). Levels of BDE 47 increased by 13% with each additional fish meal per month, which was the largest relative effect of fatty fish consumption out of approximately 25 halogenated organic contaminants studied. PBDEs have also been detected in human adipose tissue and breast milk, and BDE 47 was the dominant congener in these matrices (She et al 2000, Noren and Meironyte 2000). Because of its high fat content, breast milk is an excellent medium for monitoring levels of organohalogen compounds in humans over time. Noren and Meironyte (2000) identified an exponential increase in PBDEs in breast milk of Swedish women over the period of 1972-1997, with concentrations doubling approximately every 5 years. This was opposite of the trends of both PCBs and DDT, which are decreasing in human breast milk over time. By the end of the study, PBDE levels in breast milk had reached 4 ng/g lipid, which is still relatively low compared to concentrations of PCBs and DDT. Preliminary studies of breast milk of North American women also showed an exponential increase in PBDEs, with concentrations doubling every 2-5 years. However, the body burden of American and Canadian women appears to be 40 times higher than that of women in Sweden (Betts 2002). Studies of marine mammals have found decreased levels of contaminants in females relative to males, suggesting lactational losses. This trend has been observed for PBDEs in both long-finned pilot whales from the Atlantic and ringed seals from the Canadian Arctic (Lindstrom et al 1999, Ikonomou et al 2002). Thus, PBDEs in the environment appear to be bioavailable to organisms occupying higher trophic levels and increasing concentrations have the potential to threaten offspring by exposure to PBDEs through breast milk.
Toxicity

Toxicological studies of PBDEs are limited. The acute toxicity of PBDEs is very low. For rats fed commercial formulations of penta-, octa-, and deca-BDE, LD$_{50}$s were >1g/kg body weight, which are similar to that of PCBs (WHO 1994, deBoer 2000). However, PBDEs appear to have the potential to negatively affect hepatic and endocrine system function. Increased liver weights and histological alterations, including enlargement and granular appearance of hepatic parenchymal cells, were observed in rats fed octa- and penta-BDE. Both octa- and penta-BDE induced hepatic microsomal enzymes and increased cytochrome P450 at doses as low as 0.78 umol/kg of body weight (WHO 1994). Penta-BDEs have been shown to increase cytochrome P450 to a higher extent than Octa-BDEs (deBoer et al 2000). When BDE 47 alone was fed to rats at doses of 6 and 18 mg/kg body weight, induction of the CYP2B class of P450s was as strong as that of PCBs, but induction of CYP1A1 and CYP1A2 was less than that of the PCBs (deBoer et al 2000). The induction of cytochrome P450 and microsomal enzymes can lead to the production of hydroxylated metabolites.

The most serious effects of PBDEs appear to be on the endocrine system, and these compounds are characterized as endocrine disruptors. PBDEs have the potential to affect the thyroid hormonal system. Such effects are important because these hormones play a crucial role in the development of many organs, such as the brain. One of the ways in which organohalogen contaminants are known to interact with the thyroid hormone system is through interference with thyroid hormone transport (deBoer et al 2000). A recent study determined that lower brominated PBDE congeners were able to compete with the thyroid hormone thyroxine (T4) binding to transthyretin (TTR) in vitro after
metabolic conversion by induced rat liver microsomes. Synthetic hydroxylated PBDEs demonstrated similar effects (Meerts et al 2000). TTR is one of the thyroid hormone-binding transport proteins in the plasma of vertebrate species. Meerts et al (2000) suggested that the binding of hydroxylated PBDEs to TTR may also be involved in facilitated transfer of these compounds across the placenta and blood-brain barrier, leading to accumulation in the fetus, especially the brain. Another recent study by Meerts et al (2001) demonstrated that both lower brominated PBDE congeners and hydroxylated PBDEs induce the estrogen receptor signal transduction pathway \textit{in vitro}. The estrogenic potencies are in the same range as bisphenol A. Xenoestrogens may disrupt normal endocrine function, which can lead to reproductive failure and cancer of estrogen-sensitive tissues in humans and wildlife (Meerts et al 2001). Similarly to other polyhalogenated aromatic hydrocarbons, PBDEs appear to have the potential to disrupt endocrine systems, especially after hydroxylation (deBoer et al 2000). However, data on the effects of PBDEs on organisms in the environment are virtually non-existent.

Potential sources and mechanisms of transport

The direct source of PBDEs in environmental samples is unclear and requires further investigation. There are no known natural sources of PBDEs. However, certain species of marine sponges produce methoxylated brominated diphenyl ethers and polybrominated phenoxy phenols (Renner 2000b). Both the production of PBDEs and the process of incorporating the flame retardants into final products create the potential for emission of PBDEs into air and wastewater. Leaching from recycled, landfilled, or incinerated materials is another possible source of PBDEs in the environment (deBoer et
al 2000). Weathering and subsequent disintegration of products containing PBDEs, such as polyurethane foam seat cushions, is beginning to receive more attention. Flame-retarded polyurethane foams are up to 30% penta-BDE by weight. Foams tend to crumble with age and may be a source of lower-brominated congeners to the environment (Hale et al 2002). The potential for deca-BDE (BDE 209) to decompose into lower-brominated congeners in the environment has also been suggested (Watanabe et al 1987a, WHO 1994). However, environmental degradation pathways require further investigation.

Long-range transport by air is one distribution mechanism for PBDEs. TeBDEs and PeBDEs were detected in air samples from rural locations without any industrial activities in Sweden (Sellstrom et al 1996). The authors concluded that atmospheric transport was one of the reasons for widespread distribution of PBDEs in Sweden and the Arctic, and was the only explanation for the detection of PBDEs in whitefish from a pristine mountain lake in the same study. Atmospheric transport was also cited as the explanation for rising PBDE concentrations in ringed seals from the Canadian Arctic (Ikonomou et al 2002). In a recent study by Strandberg et al (2001), PBDEs were detected in urban, rural, and remote sites near the Great Lakes in the United States, providing further support for the idea that PBDEs can be transported through the atmosphere to remote areas. BDE 47 and BDE 99 dominated the congener distribution of PBDEs in all samples. BDE 209 was only detected in one urban air sample. Thus, the importance of atmospheric transport may vary as a function of the bromination and relative volatility of the different congeners.
Sewage sludge

Another potential mechanism for distribution of PBDEs in the environment is the disposal of sewage sludge. Sewage sludge is a semi-solid, organic-rich material produced during wastewater treatment. Organic chemicals can enter wastewater from human wastes, consumer products, street runoff, and industrial processes. During the sewage treatment process, organic compounds such as PBDEs may partition onto solids due to their hydrophobic/lipophilic properties. This can result in enrichment of PBDEs in sewage sludge solids (Rogers 1996). PBDEs have been detected in sewage sludge from both Europe and the United States. In Sweden, sewage sludge samples were collected from the same sewage treatment plant during a dry period and a rainy period. The PBDE levels in both sludge samples were similar, suggesting that the primary PBDE sources to this matrix are household and industrial effluents, not washout from the atmosphere (Nylund et al 1992). In the United States, PBDEs were detected in 11 sewage sludge samples destined for land application from four regions of the country. Total tetra- through hexa-BDE concentrations were 1100-2290 µg/kg dry weight and were consistently high regardless of source location or method of sludge processing. The PBDE congener profile was similar to that of the penta-BDE commercial product DE-71. BDE 209 was also detected in the sludges but concentrations varied greatly, between 85 and 4,890 µg/kg dry weight, from site to site (Hale et al 2001b). The source(s) of PBDEs to sewage sludge has not yet been determined.

Land application of sewage sludge is an increasingly popular method of disposal that reintroduces the sludge into the environment as fertilizer (USEPA 1999). Sewage sludge is rich in nutrients and organic carbon and has the potential to increase soil quality
and productivity. The sewage sludge is spread on the soil surface or incorporated or injected into the soil. Over half of the sewage sludge generated in the United States is applied to land, which was equal to approximately 4 million dry tons in 1998 (USEPA 1999). Prior to application, sewage sludge is subjected to one or more stabilization processes in order to reduce odor, pathogens, and volatile solids content. The most common methods of sludge treatment include anaerobic digestion, aerobic digestion, composting, alkali (lime) stabilization, and heat drying. Treated sewage sludges destined for land application are commonly referred to as “biosolids”. Biosolids are applied to various types of land including agricultural fields, forests, parks, golf courses, and mine reclamation sites. Certain types of biosolids are also used as fertilizers and/or soil conditioners by landscapers, nurseries, and homeowners. Under the USEPA’s Part 503 Biosolids Rule, biosolids can only be land applied if metals and pathogens are below specified levels (USEPA 1999). Biosolids are classified in terms of pathogen levels. “Class A” biosolids undergo treatment that reduces pathogens below detectable levels and can be applied without pathogen-related restrictions. “Class B” biosolids are subject to specific use conditions that minimize the potential for animal or human contact until environmental factors have reduced pathogen levels (USEPA 1999). Persistent organic chemicals in biosolids have generally received less attention due to improvements in industrial pre-treatment of wastewater and discontinued production and usage of notorious toxic organic chemicals such as PCBs and DDT (Chaney et al 1996).

The application of biosolids to the soil creates the potential for PBDEs to be transported to surface waters by runoff, aeolian transport, or misapplication (McLeod and Hegg 1984). PBDEs may remain associated with biosolids organic matter or leach out of
the biosolids matrix. As PBDEs are hydrophobic, most will be sorbed to non-polar surfaces of particles. Therefore, the concentration of PBDEs in suspended solids and sediments is expected to be much greater than the concentration in the water column of surface waters that receive input from biosolids treated sites.

Bioavailability

PBDEs that become associated with aquatic sediments are potentially bioavailable to aquatic organisms. Bioavailability of sediment-associated contaminants is defined as the fraction of the contaminant that is available for uptake by aquatic organisms. Bioconcentration is uptake from an aqueous solution and bioaccumulation is uptake from all environmental sources, including overlying water, pore water, food, and sediment particles. Sellstrom et al (1998) reported fish to sediment ratios ranging between 6.6 and 30 for BDE 47, 99, and 100 in fish from Swedish rivers containing sediment contaminated with PBDEs. Similar fish to sediment ratios, ranging between 16 and 38, were documented in Hadley Lake, Indiana, USA (Dodder et al 2002). These data suggest that PBDEs may be bioavailable from sediments. However, benthic organisms would provide a better indication of the bioavailability of sediment-associated PBDEs and would likely accumulate higher burdens than water column organisms. Deposit feeding benthos are maximally exposed to sediment-associated contaminants through overlying water, pore-water, and ingested sediment particles. Few studies have investigated PBDE concentrations in benthic organisms. Field surveys by Watanabe et al (1987b) and Allchin et al (1999) involved the analysis of both sediments and mollusks. The PBDE concentrations in the mollusks appeared to be higher than in the sediments, but no direct
correlations were established. Thus, it appears as if further study of the bioavailability of PBDEs to benthic organisms is needed.

Benthic organisms can be important links in aquatic food chains and may be a source of PBDEs to higher trophic levels. Biomagnification refers to the process by which tissue concentrations of an accumulated chemical increase as the chemical passes through several trophic levels. The potential for PBDEs to biomagnify up the food chain has been documented in samples from the Baltic Sea. Concentrations of BDE 47, 99, and 100 in guillemot eggs and grey seal blubber were between 4.3 and 19 times greater than in herring, their major food item (Sellstrom et al 1996). Discrimination of different PBDE congeners may also occur at various trophic levels. Bioaccumulation models have predicted that substances with log $K_{ow}$ values between 5 and 7 bioaccumulate in organisms and biomagnify through the food chain to the highest degree (Thomann 1989). TeBDEs and PeBDEs have log $K_{ow}$ values ranging between 5.8 and 7, and these compounds appear to dominate in environmental samples (deBoer et al 2000). In a laboratory feeding study in which zebrafish were fed chironomids contaminated with a commercial formulation of penta-BDE, the magnification potential of PBDE congeners appeared to be determined by both the number and relative positions of bromine substituents (Andersson 1999). Thus, further study of PBDE accumulation in benthic organisms is important due to the potential for selective accumulation of PBDE congeners from contaminated sediment and subsequent transfer of these congeners to higher trophic levels.
Bioaccumulation assays

To obtain a direct measure of bioaccumulation of contaminants from sediment, laboratory tests have been developed in which organisms are exposed to sediments under controlled conditions. While not entirely representative of environmental conditions, these tests have the advantage of providing direct, quantifiable evidence of the biological consequences of sediment contamination (USEPA 2000). There are two common approaches to assessing bioaccumulation of sediment-associated organic contaminants. The first approach compares the concentration of the contaminant in the organism to the concentration in the sediment. This approach works under the assumption that steady-state conditions have been achieved (Landrum and Robbins 1990). The second approach relates the contaminant flux into the organism to the contaminant concentration in the sediment and involves measuring the kinetics of accumulation, uptake and elimination over time. Examination of accumulation over time allows insight into the time required to achieve steady-state. If apparent steady-state is not achieved during the course of the exposure, kinetic relationships can then be used to calculate steady-state concentrations (Landrum and Robbins 1990). In addition, effects of changes in exposure conditions on bioaccumulation can be evaluated using kinetic studies (Landrum et al 1996).

The selection of an ideal benthic organism for a bioaccumulation test depends on many factors including ecological relevance, incorporation of all pertinent routes of exposure, tolerance of a wide range of physical sediment characteristics, and sufficient biomass for chemical analyses (Phipps et al 1993). Oligochaete worms (Annelida) fulfill all of these requirements. In freshwater environments, oligochaetes occupy sandy to muddy substrates where they usually burrow in the substrate or reside in bottom debris.
Many aquatic oligochaetes continuously ingest large volumes of sediment to obtain adequate nutrition (Brinkhurst and Cook 1974). Freshwater oligochaetes have a high caloric value and are important energy sources for higher trophic levels (Diaz 1980, Phipps et al 1993). Oligochaetes are relatively tolerant of certain classes of contaminants, which is a positive attribute for bioaccumulation testing. Oligochaetes also have sufficient biomass to support subsequent analytical determinations with a logistically realistic number of organisms. Finally, many species of oligochaetes can be easily cultured in the laboratory (Phipps et al 1993).

The standard test organism used by the USEPA for assessing the bioaccumulation of sediment-associated contaminants in freshwater systems is the aquatic oligochaete _Lumbriculus variegatus_ (Annelida: Oligochaeta: Lumbriculida: Lumbriculidae). This oligochaete is globally distributed and can be found in streams, rivers, ponds, lakes, and marshes (Spencer 1980, Phipps et al 1993, Lesiuk and Drewes 1999, Verdonschot 1999). _L. variegatus_ typically dwells in silty to sandy sediments at water depths ranging from less than 1 m to 60 m. The anterior portion of the body is usually buried in the sediment and the posterior portion extends out of the sediment for respiratory exchange. The worms feed on sediment-associated organic material and egested material is deposited on the sediment surface (Phipps et al 1993, Leppanen and Kukkonen 1998a). _L. variegatus_ can reproduce sexually, but fragmentation (architomy) is the most common mode of reproduction (Cook 1969, Phipps et al 1993, Leppanen and Kukkonen 1998a). Worms typically divide into two parts after reaching a certain size, approximately 9 mg wet weight, and then either the anterior or posterior portion of the worm is regenerated over a period of 2-7 days (Leppanen and Kukkonen 1998a). _L. variegatus_ is also capable of
autotomy, in which body segments can be discarded in response to injury or mechanical
stimulation. This results in small body fragments that can regenerate missing head and/or
tail segments to form new individuals (Lesiuk and Drewes 1999). *L. variegatus* is easily
cultured in the laboratory. Newly hatched worms have not been observed in cultures,
which consist of adults of various sizes (Phipps et al 1993). Adult worms range from 30-
90 mm in length and from 4-12 mg in weight (Leppanen and Kukkonen 1998a, Lesiuk
and Drewes 1999, USEPA 2000). Laboratory tests have indicated that *L. variegatus* is
relatively insensitive to sediment physiochemical properties such as grain size
distribution, organic carbon content, and mineralogical composition. Without feeding, *L.
variegatus* demonstrated 100% survival and some reproduction in clean sediments with a
range of physiochemical characteristics (Ankley et al 1994).

*Lumbriculus variegatus* has been successfully used in bioaccumulation assays for
a number of different compounds. For example, kinetic studies involving the exposure of
*L. variegatus* to sediments spiked with the polycyclic aromatic hydrocarbons (PAHs)
pyrene and benzo[a]pyrene, indicated that apparent steady state was achieved within
seven days (Kukkonen and Landrum 1994 and 1995a). Results of a study of the
bioaccumulation of chlorinated paraffins from spiked sediments by *L. variegatus*
suggested that accumulation was strongly influenced by compound structure, chlorine
content and log $K_{ow}$. Bioaccumulation decreased with increasing chlorine content and
log $K_{ow}$ (Fisk et al 1998). This pattern was also observed in a study of the accumulation
of polychlorinated dibenzo-*$p$-dioxins from spiked sediment by *L. variegatus*.
Tetrachlorodibenzo-*$p$-dioxin (log $K_{ow}$ 6.8) reached apparent steady state within 28-days,
but octachlorodibenzo-*$p$-dioxin (log $K_{ow}$ 8.2) did not (Loonen et al 1997).
When the accumulation of PCBs from river sediments was compared between laboratory-exposed *L. variegatus* (28-day exposure) and field-collected oligochaetes, the best correspondence was for PCB homologues with six chlorines or less. Field-collected oligochaetes tended to have higher concentrations of the heptachlororinated through decachlorinated congeners (log $K_{ow}$ 7-8). It was hypothesized that this was due to longer equilibration times for field-collected organisms (Ankley et al 1992).

Like many of the compounds used in previous studies with *L. variegatus*, PBDEs are halogenated organic compounds. *Lumbriculus variegatus* appears to be a viable organism for use in a bioaccumulation assay with PBDEs, and bioaccumulation of the different PBDE congeners by *L. variegatus* will likely follow the pattern of the compounds described previously.

**Objectives**

The main objective of this study was to assess the extent to which PBDE congeners associated with land-applied biosolids are bioavailable to *Lumbriculus variegatus*. Bioaccumulation kinetics of PBDEs were compared between worms exposed to biosolids and worms exposed to sediments spiked with a mixture of PBDE congeners. This allowed assessment of differences in uptake between a simple and a complex matrix and provided a relative measure of bioavailability of PBDEs from biosolids. Uptake and elimination were also compared between different PBDE congeners in order to assess the potential for preferential uptake of specific congeners at lower trophic levels.
METHODS AND MATERIALS

Organisms

Adult *Lumbriculus variegatus* were obtained from Aquatic Research Organisms Co., Hampton, NH. Worms were cultured under static conditions in either 20 or 40 L aquaria under artificial light at a 15 h light: 9 h dark photoperiod. Aquaria contained approximately 1-2 cm of commercially available “play” sand, sieved to a particle size between 63 um and 1 mm. Aquaria were filled to half volume with Gloucester Point well water (pH 8, alkalinity 100 mg/L, hardness 115 mg/L) that was aerated and allowed to come to room temperature (20-22°C) prior to use. Worms in 20 L aquaria were fed 1 g of trout chow twice per week and worms in 40 L aquaria were fed 1.5 g trout chow twice per week. The water in the aquaria was changed on the day after feeding. Dissolved oxygen concentrations were measured once per week using a YSI Dissolved Oxygen Meter (Yellow Springs, OH). With aeration, concentrations were maintained above 8 mg/L at the surface of the sand. Under these culture conditions, the biomass of *L. variegatus* doubled approximately every 30 days.

Substrates

The choice of experimental substrates was determined on the basis of small-scale preliminary experiments designed to determine the tolerance limits of *Lumbriculus variegatus*. Tests were conducted for 10 and 28 days with survival and behavior as endpoints. Exposures were conducted in 350 ml glass jars containing 100 ml of substrate and 200 ml of well water. Overlying water was aerated and changed twice daily using a
siphon. Ten *L. variegatus* were added to each jar on Day 0. Behavior of the worms was observed daily. At the end of the test, worms were removed from the substrates using a No. 50 US standard brass sieve (300 μm), counted, and the number of survivors was recorded. Worms were cleaned, placed in clean well water, and observed at 12, 16, 20, and 24 h to assess the time required for gut content clearance.

Composted biosolids, a “Class A” material, was chosen as the sewage sludge matrix. Preliminary exposures indicated that *L. variegatus* remained buried and had 100% survival for up to 28 days in composted biosolids. Two 40 lb bags of composted biosolids were purchased from a local nursery. Prior to purchase, the biosolids had been anaerobically digested, mixed with wood chips and recycled paper, and windrow composted. The composted biosolids were sieved through a 2 mm mesh to remove large debris and thoroughly homogenized by hand mixing. Sieved biosolids were stored in plastic lidded buckets at 4°C. Prior to conducting experiments, the biosolids were analyzed for percent water content, percent total organic carbon (TOC), and concentrations of eight PBDE congeners (Tables 1 and 2).

Artificial sediment was used as reference sediment. Preliminary exposures indicated that *L. variegatus* remained buried and had 100% survival for up to 28 days in artificial sediment composed of 69% sand, 15% peat moss, 15% kaolin clay, and 1% dolomite. Artificial sediment was also spiked with known concentrations of selected PBDE congeners and used as an exposure medium. Although not entirely representative of environmental conditions, the use of formulated sediments reduces substrate physiochemical variability and eliminates interferences caused by indigenous organisms and contaminants (Egeler et al 1997, USEPA 2000).
Figure 1. General structure of a PBDE molecule. Each phenyl ring can have a maximum of five bromine substituents. The numbers represent the locations of the bromine substituents and are used in naming PBDE congeners. On each phenyl ring, positions 2- and 6- are ortho-, positions 3- and 5- are meta-, and position 4- is para- to the oxygen linkage.

Table 1. Names and structures of the eight PBDE congeners included in chemical analyses. Numbering of bromine substituents begins at the oxygen linkage.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE 49</td>
<td>2,2',4,5'-TeBDE</td>
</tr>
<tr>
<td>BDE 47</td>
<td>2,2',4,4'-TeBDE</td>
</tr>
<tr>
<td>BDE 100</td>
<td>2,2',4,4',6-PeBDE</td>
</tr>
<tr>
<td>BDE 99</td>
<td>2,2',4,4',5-PeBDE</td>
</tr>
<tr>
<td>BDE 85</td>
<td>2,2',3,4,4'-PeBDE</td>
</tr>
<tr>
<td>BDE 154</td>
<td>2,2',4,4',5,6'-HxBDE</td>
</tr>
<tr>
<td>BDE 153</td>
<td>2,2',4,4',5,5'-HxBDE</td>
</tr>
<tr>
<td>BDE 209</td>
<td>DeBDE</td>
</tr>
</tbody>
</table>
Table 2. Mean concentrations (+ one standard deviation) of PBDE congeners in the composted biosolids and spiked artificial sediment (AS). The concentrations of tetra- through hexa-BDEs and BDE 209 in the composted biosolids (n=4) were used to establish target concentrations for spiking the artificial sediment. The expected concentrations for the tetra- through hexa-BDEs were determined based on the composition of DE-71 and the amount spiked into the artificial sediment matrix. BDE 49 was not detected in DE-71. Pre-aging concentrations were determined in subsamples (n=8) taken immediately after the spiked sediment components were mixed together. BDE 209 was not quantified in the pre-aging subsamples due to problems with the GC/ELCD. Post-aging concentrations were determined in subsamples (n=8) taken after the spiked artificial sediment had been aged for six weeks.

<table>
<thead>
<tr>
<th></th>
<th>Composted Biosolids (ng/g)</th>
<th>Spiked AS Expected (ng/g)</th>
<th>Spiked AS Pre-aging (ng/g)</th>
<th>Spiked AS Post-aging (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE 49</td>
<td>59.2 (5.4)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>BDE 47</td>
<td>411 (33)</td>
<td>452</td>
<td>360 (24)</td>
<td>251 (23)</td>
</tr>
<tr>
<td>BDE 100</td>
<td>81.0 (5.6)</td>
<td>127</td>
<td>136 (9.1)</td>
<td>94.0 (8.1)</td>
</tr>
<tr>
<td>BDE 99</td>
<td>473 (28)</td>
<td>887</td>
<td>667 (26)</td>
<td>507 (52)</td>
</tr>
<tr>
<td>BDE 85</td>
<td>30.8 (3.7)</td>
<td>30</td>
<td>44.1 (3.8)</td>
<td>33.9 (2.5)</td>
</tr>
<tr>
<td>BDE 154</td>
<td>32.7 (3.1)</td>
<td>52</td>
<td>50.0 (2.3)</td>
<td>34.5 (2.4)</td>
</tr>
<tr>
<td>BDE 153</td>
<td>38.8 (4.1)</td>
<td>68</td>
<td>62.9 (2.1)</td>
<td>39.8 (3.4)</td>
</tr>
<tr>
<td>Total</td>
<td>1127</td>
<td>1616</td>
<td>1320</td>
<td>960</td>
</tr>
<tr>
<td>BDE 209</td>
<td>335 (36)</td>
<td>538</td>
<td>---</td>
<td>261 (32)</td>
</tr>
</tbody>
</table>
The artificial sediment was prepared using a variation of the methods published by Egeler et al (1997). The peat moss was ground into a fine powder using a small meat grinder. Deionized water was added in excess to the powder and the solution was stirred in a 30 L glass container for approximately 48 h with a motor and propeller unit. This step was performed in order to hydrate the peat moss so that it would not separate out of the sediment when submerged in water. The hydrated peat moss was weighed after excess water was removed and subsamples were taken to determine percent water content. The sand, peat moss, clay, and dolomite were then mixed on a dry weight basis using a hand-held electric drill equipped with a stainless steel shaft and propeller. Artificial sediment for each set of replicates was mixed separately for manageability. Artificial sediments were stored in 4 L glass jars at 4°C prior to use. Subsamples were collected and analyzed for percent water content, percent TOC, and background PBDE concentrations, which were below the detection limit in reference artificial sediment.

**Sediment spiking**

Artificial sediment was spiked with PBDE congeners at target concentrations similar to the concentrations determined in the composted biosolids. The target concentrations were 1500 ng/g tetra- through hexa-BDEs and 500 ng/g BDE 209 on a dry weight basis. The commercial penta-BDE product DE-71 (Great Lakes Chemical Corporation, IN), was used for the tetra-through hexa-BDE congeners spike, and is primarily composed of the congeners BDE 47, 100, 99, 85, 154, and 153 (Table 1). BDE 209, 97% purity, was obtained from Fluka Chemika, Switzerland. Stock solutions of the two PBDE formulations were prepared and had concentrations of 630 µg/ml DE-71 in
hexane and 294 μg/ml BDE 209 in diethyl ether. The concentrations of both solutions were verified before they were used for spiking.

The artificial sediment was spiked with the PBDE congeners by coating the sand fraction. The sand, sieved to a particle size between 63 μm and 1 mm, was weighed into eight 4 L glass jars. Each jar contained 1400 g of sand and was spiked with 3000 μg of DE-71 and 1000 μg of BDE 209 using 250 ml of hexane as a carrier solvent. The 250 ml of hexane was poured over the sand and the jars were vigorously shaken to distribute the solvent. The jars were then placed in a fume hood and shaken every 2 h for a 12 h period. The sand was dry and the odor of hexane could not be detected after 24 h of evaporation. A subsample of sand was collected from each jar and analyzed to verify PBDE congener concentrations before it was mixed with other sediment constituents. After verification, the artificial sediment was prepared as described previously. Prior to use, the spiked artificial sediment was stored for 6 weeks at 4°C in order to allow the spiked PBDEs to partition within the sediment matrix. Aging has been recommended due to the potential effects of contaminant partitioning into organic matter on bioavailability (Northcott et al 2000, USEPA 2000). A 6-week storage period also allowed the spiked artificial sediment to more closely mimic the composted biosolids, which had been both windrow composted and stored in a bag, increasing the equilibration time of the PBDEs within the compost matrix. Subsamples of the spiked artificial sediment were analyzed before and after equilibration for determination of PBDE congener concentrations (Table 2). Subsamples were also analyzed for percent water content and TOC.
Bioaccumulation assay

Exposures of *Lumbriculus variegatus* were conducted following the USEPA guidelines for a 28-day sediment bioaccumulation test (USEPA 2000). The kinetic experiment was designed to include four sampling times for uptake during the 28-day exposure period and three sampling times for depuration after the 28-day exposure period. Exposure chambers were 4 L glass jars containing 1 L of substrate and 3 L of overlying water. Three replicate chambers were tested for each of the three substrates for each of the four uptake sampling times. For each of the three depuration sampling times, three replicate chambers of biosolids and spiked artificial sediment and one chamber of reference artificial sediment were tested. Thus, a total of 57 test chambers were prepared at the beginning of the exposure.

Substrates were placed in the jars two days before adding organisms (Day –2). Substrates were first homogenized using a hand-held electric drill equipped with a stainless steel shaft and propeller. Approximately 1 L of substrate was distributed into each jar using a stainless steel spatula. The weight of substrate in each jar was recorded and a subsample from each treatment was collected for determination of PBDE congener concentrations. A total of 21 “Day 0” subsamples were collected, including seven samples of biosolids, seven samples of spiked artificial sediment, and five samples of reference artificial sediment.

Arrangement of the exposure jars was determined using a random number generator. Overlying water was added to each jar on the day before adding organisms (Day –1) using a siphon. Overlying water was the same on-site well water used for culturing the worms. During the exposure, water was delivered to the jars using a flow-
through system. Well water was run through a series of 10 μm and 1 μm filter cartridges to remove particulates and was heated to 21°C with an in-line heater. The water then entered a 40 L head tank in which the volume was controlled by a float and ball-valve mechanism. Water was then delivered to two plexi-glass flow splitters via Tygon® tubing. The water level in each flow splitter remained constant, and was calibrated using a glass standpipe held in place by a silicone stopper. Water was delivered to each jar approximately 5 cm above the substrate surface via Tygon® microbore tubing. Excess water flowed out of the top of the jar. Flow rate through the tubing was 5-7 ml/min. At this flow rate, water was renewed approximately two times per day. Water was aerated in the flow splitters by airstones and in each jar via airlines equipped with glass Pasteur pipets.

*Lumbriculus variegatus* were removed from the culture substrate and placed in 10L aquaria containing aerated well water on the day prior to stocking the test chambers (Day -1). In the absence of substrate, *L. variegatus* clump together. This behavior was helpful when transferring organisms to the test chambers. Worms were transferred to test chambers on Day 0. Clumps of worms were gently removed from the water with a stainless steel spatula, lightly touched against the sides of the aquarium to remove excess water, and placed in a tared aluminum weighing pan. Approximately 2.8 g of wet worms (2.6-3.0 g) were transferred into each test jar. This wet weight represents approximately 200-300 individuals and approximately 0.3 g dry weight. Ten subsamples of worms (2.6-3.0 g each) were taken on Day 0 for determination of background levels of PBDEs. Samples of the culture substrate and the trout chow used to feed the cultures were also collected for residue analysis, and did not have detectable concentrations of PBDEs.
Behavior of the oligochaetes in the substrate and the general condition of the test system were evaluated daily. The water temperature in each flow splitter was monitored daily. The dissolved oxygen concentration and temperature of the overlying water near the surface of the substrate were measured in each jar every 3 days. Conductivity and pH of the overlying water were monitored every 7 days. Alkalinity, hardness, and total ammonia were measured every 14 days. Subsamples of the biosolids and spiked artificial sediment were collected on Days 21 and 28 for evaluation of potential changes in PBDE concentrations over time.

*Lumbriculus variegatus* were removed from the exposure substrates for analysis of PBDE uptake on Days 7, 14, 21, and 28 of the bioaccumulation assay. Worms were collected from each jar by washing the substrate through a No. 50 US standard brass sieve (300 μm). Sieved substrates were transferred to clear glass pans and worms were removed using disposable plastic bulb pipets. Once isolated, *L. variegatus* were cleaned of any remaining debris and placed in 2 L beakers containing aerated well water only. The beakers were placed back in the flow-through system for approximately 21 h to allow the worms to clear their gut contents. Although shorter purge times have been suggested, observations during preliminary experiments indicated that 20+ h were required for the majority of worms to egest all of the biosolids contained in their guts. After the purge period, *L. variegatus* were cleaned of debris, transferred to a tared weigh pan using a stainless steel spatula, and excess water was removed using a kimwipe. Samples were weighed, placed in solvent-rinsed glass jars, and immediately frozen.

For the depuration portion of the study, *L. variegatus* were removed from all remaining jars after approximately 28 days of exposure. Worms were removed from
spiked artificial sediment, biosolids, and clean artificial sediment on Day 30. Ideally, the worms would have all been removed on Day 28, but this was not possible due to the laborious nature of sample processing. Methods for isolating the worms from the substrates were the same as the methods used for the uptake portion of the experiment. Once isolated, *L. variegatus* were cleaned of any remaining debris and transferred directly into 4 L jars containing approximately 100 ml of clean sand (particle size between 63 um and 1 mm). The jars were placed into the flow-through system under the same conditions as described previously. The worms were fed a supplementary diet of approximately 0.2 g trout chow per jar every 5 days. The three depuration sampling points occurred 5, 12, and 21 days after the worms were placed in clean sand. *L. variegatus* were removed from the sand by washing with well water. Once isolated, the worms were placed in 2 L beakers containing only aerated well water for approximately 21 h to allow for clearance of gut contents. After the purge period, *L. variegatus* were cleaned of debris, transferred to a tared weigh pan using a stainless steel spatula, and excess water was removed using a kimwipe. Samples were weighed, placed in solvent-rinsed glass jars, and immediately frozen.

**Chemical analysis**

Worm tissue and substrates were freeze-dried and homogenized. Prior to extraction, dry worm tissue (0.25-0.45 g) and substrates (5 g) were mixed with 30-35 g Na$_2$SO$_4$, and spiked with 500 ng of PCB 204 (Ultra Scientific, North Kingstown, RI) as a surrogate standard to account for losses of PBDEs during sample preparation. Analytical blanks (50 g Na$_2$SO$_4$) were processed concurrently with worm tissue and substrates.
Samples were subjected to enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA) involving two 5 min. cycles of 60% vessel flush with dichloromethane at 100°C and 1000 psi. Substrate extracts were then purified by size exclusion chromatography to remove high molecular weight compounds, such as humics and lipids. An Envirosep® size exclusion column (Phenomenex, Torrence, CA) was eluted with dichloromethane at 5 ml/min., and the fraction eluting between 15 and 24 min. was collected. Worm tissue extracts were not subjected to this purification step because of very small sample size. The extracts of both worm tissue and substrates were solvent exchanged to hexane and subjected to silica gel chromatography to separate the PBDEs from other endogenous organic compounds that could interfere with detection and quantification. Solid-phase extraction columns containing 2 g silica gel (International Sorbent Technology, Mid Glamorgan, UK) were eluted with 3.5 ml of hexane, which was discarded. The columns were then eluted with 6.5 ml of 60:40 hexane:dichloromethane, which was collected. The collected fraction was solvent exchanged to hexane and spiked with the internal standards p-terphenyl (5 ug) and decachlorodiphenyl ether (348 ng) (Ultra Scientific, North Kingstown, RI).

The congeners BDE 49, 47, 100, 99, 85, 154 and 153 (Table 1) in the purified extracts were separated, identified, and quantified on a gas chromatograph (GC) equipped with a mass spectrometric (MS) detector (Varian Saturn 2000 ion trap, Walnut Creek, CA). The injector temperature was held at 330°C and injections were 1.5 ul in splitless mode with activation of the splitless injector purge valve occurring after 0.45 min. Helium was used as the carrier gas. The GC was equipped with a 15 m DB-5 column with a film thickness of 0.25 um and 0.33 mm i.d. (J&W Scientific, Folsom, CA).
column temperature was held at 75°C for 1 min., programmed to 350°C at 10°C/min., and then held for 4.5 min. The MS detector was operated in full-scan electron ionization (EI) mode. Calibration with perfluorotributylamine (FC43) was performed at the beginning of each sample queue. The GC-MS interface and the ion trap were maintained at 330 and 250°C respectively. The emission current of the filament was 15μA. The scan range was 50-650 m/z and the multiplier set voltage was 1950 volts. The instrument was operated at a resolution of 1. Area counts of tetra- through hexa-BDEs were determined by summing the areas of the two major ions (m/z) for each congener. The m/z were: 326 (M⁺-Br₂+2) and 486 (M⁺+4) for tetra-BDEs, 404 (M⁺-Br₂+2) and 406 (M⁺-Br₂+4) for penta-BDEs, and 484 (M⁺-Br₂+4) and 643 (M⁺-H⁺) for hexa-BDEs. Area counts of the surrogate and internal standards were also determined using the areas of major ions. The m/z were 430 (M⁺+4) for PCB 204 (surrogate) and 230 (M⁺) for p-terphenyl (internal).

Standards containing known amounts of BDE 49, 47, 100, 99, 85, 154, and 153 (Cambridge Isotope Laboratories, Andover, MA), PCB 204, and p-terphenyl were used to positively identify and quantify individual PBDE congeners. The standards were used to construct five-point calibration curves for each PBDE congener. For each standard, the area count of each PBDE congener was divided by the area count of p-terphenyl and plotted against the concentration of the congener in the standard. Equations were generated using linear regression and were used to calculate concentrations of individual PBDE congeners in the extracts from the area count ratios of the congener and p-terphenyl. The lowest quantification standard for all analyzed PBDE congeners was 40 ng/ml. The detector response was not linear at lower concentrations even though standards as low as 10 ng/ml could be detected. Resulting quantitation limits were
approximately 85 ng/g for worm tissue and 8 ng/g for substrates. PCB 204 concentrations were determined in the same manner as the PBDE congeners. PBDE concentrations in each sample were corrected for percent recovery of PCB 204. Mean percent recoveries ± one standard deviation were 92 ± 9% for worm tissue and 92 ± 16% for substrates.

BDE 209 in the purified extracts was separated and quantified on a GC equipped with a halogen-selective electrolytic conductivity (ELCD) detector (Varian 3400, Sugar Land, TX). The carrier gas was helium and injections were 1.5 μl on-column. The injector temperature was held at 95°C for 0.1 min. then ramped at 180°C/min. to 350°C and held for 10 min. The GC column was 15 m DB-5HT with a 0.1 μm film thickness and 0.25 mm i.d. (J&W Scientific, Folsom, CA). The initial column temperature was held at 120°C for 3 min. then ramped at 10°C/min. to 350°C and held for 10 min. The ELCD detector temperature was 350°C and the detector vent was open for 2.5 min. after each injection. External standards containing known amounts of BDE 209 (Fluka Chemika, Switzerland) and decachlorodiphenyl ether were used for quantification. For each standard, the area count of BDE 209 was divided by the area count of decachlorodiphenyl ether and plotted against the concentration of BDE 209 in the standard. Equations were generated using linear regression and were used to calculate concentrations of BDE 209 the extracts from the area count ratios of BDE 209 and decachlorodiphenyl ether. The lowest standard used for quantification was 95 ng/ml. Standards as low as 48 ng/ml could be detected at three times the system noise, but the response of the detector was not linear. Quantitation limits were approximately 190 ng/g.
for worm tissue and 20 ng/g for substrates. Concentrations of BDE 209 were corrected for percent recovery of PCB 204 as described previously.

Data analysis

For the substrate data, concentrations of each congener were compared between spiked artificial sediment and composted biosolids using Student’s t-test. A one-factor analysis of variance (ANOVA) was used to compare percent TOC between exposure substrates and to compare the concentration of each PBDE congener in the substrates between Days 0, 21, and 28 of the exposure. For the oligochaete data, a one-factor ANOVA was used to compare dry weights of worm tissue between sampling days for each substrate treatment. For the uptake phase of the exposure, treatments were considered stable when mean dry weights were not significantly different between sampling days, and dry weights were then pooled and compared between substrate treatments using a one-factor ANOVA. All concentrations of PBDE congeners determined in worm tissue are reported on a dry weight basis. Lipid content of worm tissue was not determined due to the small available sample size and related concerns about analyte quantitation limits. The concentrations of individual PBDE congeners in worm tissue were plotted against the sampling day. A one-factor ANOVA was used to compare the concentrations of each PBDE congener between sampling days during the 28-day exposure. Slopes of uptake curves for each PBDE congener were determined by linear regression of concentration versus time and were compared between substrates using Student’s t-test. Tissue concentrations of each PBDE congener were pooled over all sampling days during the 28-day exposure and were compared between substrates.
using Student’s t-test. All statistical tests were conducted at the $\alpha=0.05$ level of significance. When significant differences were indicated by an ANOVA, pairwise comparisons were made using Tukey’s studentized range (HSD) test.

Depuration rate constants were determined for depuration phase data by linear regression of natural log of the worm tissue concentration ($\ln C_{\text{worm}}$) versus time. Regression results were fitted to the natural log-transformed first-order depuration model:

$$\ln C_{\text{worm}} = -k_2 \times \text{time} + b,$$

where $k_2$ is the depuration rate constant (days$^{-1}$) and $b$ is the y-intercept. The $k_2$ values for individual PBDE congeners were compared between the exposure substrates using Student’s t-test ($\alpha=0.05$). Depuration half-lives ($t_{1/2}$) were calculated using the equation: $t_{1/2} = -\ln(0.5)/k_2$, and theoretical times to reach 90% of steady-state tissue concentrations ($t_{90}$) based on the depuration rate constants were calculated using the equation: $t_{90} = -\ln(0.1)/k_2$.

Bioaccumulation factors (BAFs) were calculated by dividing the mean PBDE congener concentration in the worm tissue over the 28-day exposure period (ng/g dry weight) by the concentration in the exposure substrate on Day 0 (ng/g dry weight). BAFs were also calculated using TOC-normalized Day 0 substrate concentrations (ng/g dry weight$_{oc}$). If possible, uptake rate coefficients ($k_1$) were estimated by fitting the results of nonlinear regression of the uptake data to a first-order bioaccumulation model:

$$C_t = (k_1/k_2)*C_s*(1-e^{-k_2*t}),$$

where $t =$ time (days), $C_t =$ concentration in worm tissue at time $t$ (ng/g), $C_s =$ concentration in substrate (ng/g), $k_2 =$ elimination rate constant (days$^{-1}$), and $k_1 =$ uptake rate constant (ng/g worm/(ng/g sediment * day)). Kinetic bioaccumulation factors (BAF$_{\text{kin}}$) were calculated using the kinetic rate constants: $\text{BAF}_{\text{kin}} = k_1/k_2$. The BAF$_{\text{kin}}$ and the BAF were then compared.
RESULTS

Substrates

Percent TOC was statistically similar between the three exposure substrates. Mean percent TOC ± standard error for each substrate was: 7.4 ± 0.4 % for reference artificial sediment, 7.3 ± 0.3 % for spiked artificial sediment, and 7.9 ± 0.5 % for composted biosolids.

Reference artificial sediment collected on Day 0 did not have detectable concentrations of any of the measured PBDE congeners. The detection limit for all PBDE congeners in substrates was approximately 1 ng/g. BDE 49 was not detected in the spiked artificial sediment on Days 0, 21, or 28. However, BDE 49 was detected and quantified in the composted biosolids on all three days. Mean Day 0 concentrations of all measured PBDE congeners except BDE 85 and 154 were significantly higher (p<0.05, t-test) in the composted biosolids than in the spiked artificial sediment (Figure 2).

However, with the exception of BDE 47, differences in concentrations of PBDE congeners between the two exposure substrates were less than 30%. In the spiked artificial sediment, mean concentrations of BDE 99 and 85 were significantly higher on Day 0 than on Days 21 and 28 (p<0.01, ANOVA, Tukey's HSD). Mean concentrations of all other measured PBDE congeners were statistically similar between the three days (Figure 3). In the composted biosolids, the mean concentration of BDE 85 was significantly higher on Day 0 than on Days 21 and 28 (p<0.01, ANOVA, Tukey's HSD). Mean concentrations of all other measured PBDE congeners were similar between the three sampling days (Figure 3). However, statistical comparisons of concentrations
Figure 2. Mean concentrations of PBDE congeners in Day 0 subsamples of spiked artificial sediment (AS, n=7) and composted biosolids (n=7). Error bars represent standard error of the mean. An * indicates a significant difference (p<0.05) in concentrations between the substrates. BDE 49 was not detected in the spiked artificial sediment.
Figure 3. Mean concentrations of PBDE congeners in spiked artificial sediment (top) and biosolids (bottom) on Days 0, 21, and 28 of the exposure. Error bars represent standard error of the mean. An * indicates a significant difference (p<0.05) in concentrations between Day 0 (n=7) and Days 21 (n=2) and 28 (n=2). BDE 49 was not detected (ND) in spiked artificial sediment.
between Days 0, 21, and 28 for both substrates were weakened by differing numbers of replicates. Sample sizes were n=7 for Day 0 substrates and n=2 for substrates collected on Days 21 and 28.

**Oligochaetes**

Throughout the 28-day exposure, *Lumbriculus variegatus* remained buried in all substrate treatments and active feeding on substrates was observed. Dry weights of worms recovered from each treatment were used to assess the condition of *L. variegatus* during the exposure (Figure 4). Mean dry weights of worms exposed to the reference artificial sediment decreased significantly between Days 0 and 7 (p<0.003, ANOVA, Tukey’s HSD), but mean dry weights were not significantly different between Days 7 and 28. Mean dry weights of worms exposed to spiked artificial sediment decreased significantly between Days 0, 7, and 14 (p<0.0001, ANOVA, Tukey’s HSD), but mean dry weights were not significantly different between Days 14 and 28. Mean dry weights of worms exposed to composted biosolids were not significantly different throughout the 28-day exposure period. Therefore, between Days 14 and 28 all substrate treatments were stable. Dry weights of worms on Days 14, 21, and 28 were pooled and compared between exposure substrates. Mean dry weights of worms were significantly different between the three exposure substrates (p<0.0001, ANOVA, Tukey’s HSD), and followed the pattern: composted biosolids > reference artificial sediment > spiked artificial sediment. During the depuration phase of the experiment, worms were fed a supplementary diet of trout chow, and there was a significant increase in mean dry weights between subsequent sampling days for worms removed from spiked artificial
Figure 4. Mean dry weights of worms exposed to reference artificial sediment, spiked artificial sediment, and composted biosolids on each sampling day. Dry weights represent approximately 200-300 organisms. Error bars represent standard error of the mean. The dotted line represents the beginning of the depuration phase of the study.
sediment (p<0.0001, ANOVA, Tukey’s HSD) and worms removed from composted biosolids (p<0.0002, ANOVA, Tukey’s HSD).

Uptake of PBDEs

*Lumbriculus variegatus* collected on Day 0 of the experiment did not have detectable concentrations of any of the measured PBDE congeners. The detection limit for all PBDE congeners for worm tissue was approximately 20 ng/g. Worms exposed to reference artificial sediment did not have detectable concentrations of any of the measured PBDE congeners on any of the sampling days. Therefore, worms collected on Day 0 and worms exposed to reference sediments were not included in statistical analyses.

BDE 47, 100, and 99 were rapidly accumulated from both spiked artificial sediment and composted biosolids by *L. variegatus*, essentially reaching maximum concentrations by the first day of sampling (Day 7) following initiation of exposure (Figure 5). Mean concentrations of BDE 47, 100, and 99 in worms exposed to biosolids were not significantly different between sampling days, indicating that apparent steady state between uptake and elimination was achieved by Day 7 of the 28-day exposure. However, in worms exposed to spiked artificial sediment there was a significant decrease in the concentrations of BDE 47, 100, and 99 between subsequent uptake phase sampling days (p<0.05, ANOVA, Tukey’s HSD). Patterns of uptake over time were compared between substrates by linear regression of concentration versus time. Regression coefficients (slopes) were significantly different between exposure substrates for BDE 47, 100, and 99 (p<0.01, t-test). When the effect of time was removed by pooling tissue
Figure 5. Mean concentrations of BDE 47, 100, and 99 in worms exposed to either spiked artificial sediment (AS) or composted biosolids on each sampling day. Error bars represent standard error of the mean. The dotted line represents the beginning of the depuration phase of the study.
concentrations of each congener over all uptake phase sampling times, mean concentrations of BDE 47, 100, and 99 were significantly greater (p<0.001, t-test) in worms exposed to spiked artificial sediment than in worms exposed to biosolids (Figure 6).

BDE 85, 154, and 153 were also rapidly accumulated from spiked artificial sediment by *L. variegatus*, although concentrations were much lower than BDE 47, 100, and 99 (Figure 7). Mean concentrations were not statistically different between sampling days during the uptake phase of the exposure. BDE 85, 154, and 153 were detected in worms exposed to biosolids, but concentrations were not above the quantitation limit (85 ng/g), and uptake could not be statistically compared between sampling days or between substrates. BDE 49 was detected in worms exposed to both spiked artificial sediment and composted biosolids, but concentrations were not above the quantitation limit (85 ng/g) and were not included in data analyses.

BDE 209 was not detected in worms exposed to biosolids but was detected in worms exposed to spiked artificial sediment. Concentrations were below the quantitation limit (190 ng/g), but estimates of concentrations were used to assess relative bioavailability. The concentrations were estimated by comparing the area count of BDE 209 in the lowest standard that could be detected at three times the system noise (48 ng/ml) to area counts of BDE 209 in extracts of worm tissue. Area counts were similar between all four sampling days of the 28-day exposure. The estimated mean concentration of BDE 209 in worm tissue on each sampling day during the uptake phase of the experiment was 80 ng/g dry weight, which is between three and thirty times lower
Figure 6. Concentrations of BDE 47, 100, and 99, pooled over all sampling times during the 28-day exposure, in worms exposed to either spiked artificial sediment (AS) or composted biosolids. Within each box, the dotted line represents the mean and the solid line represents the median. The top and bottom perimeters of the box are the 75th and 25th percentiles, respectively. Error bars represent the 90th and 10th percentiles. Dots represent the 95th and 5th percentiles.
BDE 47

BDE 100

BDE 99

Spiked AS  Biosolids

Exposure substrate

Conc. (ng/g)

Spiked AS  Biosolids
Figure 7. Concentrations of BDE 85, 154, and 153 in worms exposed to spiked artificial sediment on each sampling day. Error bars represent standard error of the mean. The dotted line represents the beginning of the depuration phase. Concentrations of BDE 85 and 153 were below the quantitation limit during the depuration phase of the study (***) and accurate concentrations could not be determined.
than the concentrations of tetra- through hexa-BDE congeners in worms exposed to spiked artificial sediment.

**Depuration of PBDEs**

Elimination of PBDE congeners was apparent in worms collected during the depuration phase of the study. Elimination rates appeared to be similar between substrate treatments, but different between PBDE congeners (Figures 5 and 7). PBDE concentrations on Days 28, 35, 42, and 51 were used in calculation of depuration rate constants ($k_2$). Linear regressions of natural log concentration versus time were performed when concentrations remained above the quantitation limit for at least three sampling days. Data were a good fit to the natural log transformed first-order depuration model ($0.80 < R^2 < 0.96$). Regression coefficients ($k_2$) were significantly different from zero ($p < 0.0001$) for BDE 47 and 99 in worms exposed to biosolids and for BDE 47, 99, 100 and 154 in worms exposed to spiked artificial sediment (Table 3). Depuration rate constants determined for BDE 47 and 99 were not statistically different when compared between worms exposed to the two different substrates. The depuration rate constants determined for BDE 99 were nearly three times greater than the rate constants determined for BDE 47 for worms from both exposure substrates, and the calculated 99% confidence intervals did not overlap. Depuration rate constants were similar between BDE 47, 100, and 154 for worms exposed to spiked artificial sediment and there was overlap of the calculated 99% confidence intervals for the three rate constants. Estimated $t_{1/2}$ values for PBDE congeners were: 7.5 days for BDE 99 and 23 days for BDE 47, 100, and 154.
Table 3. Depuration rate constants (days$^{-1}$) and 99% confidence intervals for PBDE congeners in worms exposed to either spiked artificial sediment or composted biosolids. Depuration rate constants were determined by linear regression of natural-log concentration versus time for depuration phase data. Reported rate constants were significantly different from zero (p<0.0001).

<table>
<thead>
<tr>
<th></th>
<th>Spiked Artificial Sediment</th>
<th>Composted Biosolids</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE 47</td>
<td>0.031 (0.015, 0.047)</td>
<td>0.033 (0.019, 0.047)</td>
</tr>
<tr>
<td>BDE 100</td>
<td>0.028 (0.014, 0.042)</td>
<td></td>
</tr>
<tr>
<td>BDE 99</td>
<td>0.092 (0.073, 0.11)</td>
<td>0.093 (0.062, 0.12)</td>
</tr>
<tr>
<td>BDE 154</td>
<td>0.032 (0.016, 0.048)</td>
<td></td>
</tr>
</tbody>
</table>
Based only on depuration rate constants, the estimated times to reach 90% of steady-state tissue concentrations were: 25 days for BDE 99 and 75 days for BDE 47, 100, and 154.

Bioaccumulation factors

Due to the decrease in PBDE body-burdens over time in worms exposed to spiked artificial sediment, worm tissue concentrations were averaged over the 28-day exposure in order to provide a best estimate of bioaccumulation factors (BAFs). Concentrations were also averaged over the 28-day exposure for worms exposed to composted biosolids, but the effects of averaging were minimal because concentrations were not significantly different between sampling days.

BAFs were calculated for BDE 47, 100 and 99 in worms exposed to biosolids and for BDE 47, 100, 99, 85, 154, and 153 in worms exposed to spiked artificial sediment (Figure 8). BAFs for BDE 47, 100, and 99 were five to ten times higher for worms exposed to spiked artificial sediment than for worms exposed to biosolids. For both exposure substrates, BAFs were two times higher for BDE 47 than for BDE 99. The absolute uncertainty was calculated for each BAF value using the error associated with the mean PBDE congener concentrations in both worm tissue and substrates (Christian 1994). This value was then used to determine a range for each BAF value. For worms exposed to biosolids, the ranges of calculated BAF values were very small, and did not overlap between BDE 47, 100, and 99. For worms exposed to spiked artificial sediment, the ranges in BAF values for BDE 47, 100, and 154 were between 6 and 12 and the ranges in BAF values for BDE 99, 85, and 153 were between 3 and 6. Thus, there was
Figure 8. Bioaccumulation factors (BAFs) for PBDE congeners in worms exposed to either spiked artificial sediment or composted biosolids. BAFs were calculated by dividing the mean concentration in worm tissue over the 28-day exposure (ng/g dry weight) by the Day 0 substrate concentration (ng/g dry weight). The error bars represent absolute uncertainties, the range in BAF, calculated using propagation of error.
apparent similarity of BAFs within and a two-fold difference in BAFs between these two arbitrary groups of PBDE congeners. Normalization of substrate concentrations to TOC reduced the calculated BAF values by a factor of 12-13, but did not change the magnitude of the difference in BAFs between exposure substrates (Figure 9). As noted previously, worm data could not be expressed on a lipid basis due to the small amount of available tissue and the need to retain the entire extract for PBDE analysis.

In general, uptake data were a poor fit to the first-order bioaccumulation model. Only data for BDE 99 in worms exposed to biosolids could be fitted to the model (Figure 10). The regression was significant (p<0.0001), and the regression coefficient was 522.2, resulting in a $k_1$ value of 0.08 ng/g worm/(ng/g sediment*day). However, because of the fixed $k_2$ value, initial uptake was not well described by the nonlinear regression. The calculated BAF$_{kin}$ was 0.90, which is equal to the calculated BAF, indicating that apparent steady-state was achieved during the 28-day exposure.
Figure 9. Bioaccumulation factors (BAFs) for PBDE congeners in worms exposed to either spiked artificial sediment or composted biosolids. BAFs were calculated by dividing the mean concentration in worm tissue over the 28-day exposure (ng/g dry weight) by the TOC-normalized Day 0 substrate concentrations (ng/g dry weight). The error bars represent absolute uncertainties, the range in BAF, calculated using propagation of error. Normalization of substrate concentrations to TOC did not change the effect of exposure substrates on calculated BAFs.
Figure 10. Uptake of BDE 99 in worms exposed to composted biosolids over time. Data points are mean concentrations of BDE 99 on each sampling day. Error bars represent standard error of the mean. The solid line represents nonlinear regression of the data using the equation: \( \text{Conc. (ng/g)} = a \cdot (1 - e^{-k_2 \cdot \text{time}}) \), where \( k_2 \) is the depuration rate constant. The regression was significant (\( p<0.0001 \)) with an \( R^2 \) of 0.95 and a regression coefficient of \( a=522.2 \). However, because of the fixed \( k_2 \) value, initial uptake was not well described by the nonlinear regression.
DISCUSSION

Comparisons between exposure substrates

Tetra- through hexa-BDEs were bioavailable to *Lumbriculus variegatus* from both spiked artificial sediment and composted biosolids. However, the degree of bioavailability differed between the two substrates. The concentrations of BDE 47, 100, and 99 were significantly higher in worms exposed to spiked artificial sediment than in worms exposed to composted biosolids. Concentrations of BDE 85, 154, and 153 exceeded the quantitation limit in worms exposed to spiked artificial sediment, but concentrations could not be quantified in worms exposed to composted biosolids. The calculated BAFs for BDE 47, 100, and 99 were five to ten times higher in worms exposed to spiked artificial sediment than in worms exposed to composted biosolids. Thus, despite the higher PBDE concentrations in the composted biosolids, the accumulation of PBDEs was greater from the spiked artificial sediment. This supports the idea that chemical measurements of contaminants in sediment do not always reflect the fraction that is bioavailable to benthic organisms (Landrum and Robbins 1990).

It is likely that different substrate characteristics contributed to the difference in bioavailability of tetra- through hexa-BDEs between spiked artificial sediment and composted biosolids. For burrowing deposit feeders such as *L. variegatus*, the accumulation of sediment-associated contaminants is primarily from exposure to interstitial water and ingestion of sediment. Uptake from pore water and ingested sediment were not distinguished in this study, but regardless of the route of uptake, accumulation of contaminants by benthic organisms is regulated in part by contaminant-
sediment interactions, such as adsorption and desorption (Leppanen and Kukkonen 2000a). Significant correlations have been established between amounts of sediment-associated contaminants solubilized by clean seawater and amounts solubilized by digestive fluids of marine invertebrates, although solubilization was much greater during digestive attack (Mayer et al 1996). Therefore, sediment characteristics that increase the sorptive strength of the contaminant to sediment particles will decrease overall bioavailability through a decreased tendency for desorption into pore water and/or digestive fluids.

One sediment characteristic that can affect bioavailability is organic matter composition. The organic matter component of sediment can sorb hydrophobic contaminants and reduce bioavailability (Landrum and Robbins 1990). Organic matter content is typically measured as total organic carbon (TOC). Percent TOC was not significantly different between spiked artificial sediment and composted biosolids. In addition, when BAF values were normalized to percent TOC, the magnitude of the difference in BAFs between the exposure substrates did not change. However, TOC content is not a measure of organic matter composition. Standley (1997) exposed L. variegatus to four sediments spiked with dieldrin and found that normalization to TOC and lipid content reduced differences in bioaccumulation factors from 38- to 8-fold between the sediments. The remaining difference in bioaccumulation was attributed to variations in sediment composition. DeWitt et al (1992) exposed burrowing amphipods to fluoranthene-spiked sediments, in which the amount of particulate organic carbon was the same but the source of organic matter differed, and found significantly different toxicity among the five organic-source treatments. Both the molecular structure and the
surface area comprising the various forms of organic carbon can affect the strength of sorption of contaminants (Landrum et al 1996). The only source of organic matter in the spiked artificial sediment was peat moss, which consists of slightly decomposed, dehydrated remains of acid bog plants. The composted biosolids was composed of sewage sludge mixed with wood chips and recycled paper. The primary source of organic matter in sewage sludge is human excreta, which is a complex mixture of carbohydrates, lipids, proteins, breakdown products, and humic material. Live and dead microorganisms are a significant contribution to the organic matter content of sewage sludge. In addition, organic material can enter the sewage treatment process from consumer products, street runoff, and industrial effluents (Rogers 1996). The complexity of the organic component of the composted biosolids probably increased the sorption capacity for organic contaminants relative to the spiked artificial sediment, which may have contributed to the difference in bioavailability of PBDEs from the two substrates.

Another sediment characteristic that can influence bioavailability is the duration of contact between sediment particles and the contaminant. Contaminants can become more tightly bound to sediment particles with increased contact time. In partitioning experiments, the increased binding has been described as movement from a readily reversible to a slowly exchangeable particle pool (Landrum et al 1992). This results in a decreased potential for the contaminant to desorb from sediment particles and become available for uptake by an organism. Loonen et al (1997) exposed L. variegatus to sediments spiked with tetrachlorodibenzo-p-dioxin (TCDD) and octachlorodibenzo-p-dioxin (OCDD) and found a 1.5 to 2-fold decrease in BAFs when the exposure was repeated after aging the sediment for 21 months. Increasing the sediment-chemical
contact time from 3 to 60 days reduced the uptake rate coefficients 2 to 6-fold for amphipods exposed to sediments spiked with polycyclic aromatic hydrocarbons (PAHs) (Landrum et al 1992). In this study, the PBDE-spiked artificial sediment was aged for six weeks prior to use in the bioaccumulation assay in order to allow the PBDEs to partition and equilibrate within the sediment matrix. The chemically extractable concentrations of all of the measured PBDE congeners decreased between the analysis immediately after spiking and the analysis after the six-week aging period, indicating increased binding to sediment particles. However, when compared to composted biosolids, the aging time for the spiked artificial sediment was relatively short. Prior to composting, the sewage sludge was subjected to anaerobic digestion. In order to reduce pathogens and volatile solids, sewage sludge must remain in the digester for a mean cell residence time of 15 to 60 days depending on the temperature. After digestion, the biosolids were subjected to windrow composting for approximately 60 days. Finally, the composted biosolids were stored in a bag for an unknown amount of time prior to purchase. Therefore, the duration of contact between PBDEs and the substrate was at least twice as long for the composted biosolids than for the spiked artificial sediment. The greater aging of the composted biosolids may have resulted in tighter binding of the PBDEs to substrate particles and subsequently lower bioavailability relative to the spiked artificial sediment.

In addition to the difference in overall bioavailability between the spiked artificial sediment and the composted biosolids, the kinetics of PBDE uptake were significantly different between the two substrates. In the spiked artificial sediment, the significant decreases in the concentrations of BDE 47, 100, and 99 in \textit{L. variegatus} over the course of the 28-day exposure may have resulted from changes in the spiked artificial
sediment matrix. One potential change is depletion of the readily bioavailable fraction of PBDEs. Ingested material was found to be the major route of accumulation of pyrene in *L. variegatus*, but pyrene sorbed to particles was found to be much less bioavailable than pyrene in pore water (Leppanen and Kukkonen 1998b). Uptake from pore water can occur either through the integument or through ingestion during non-selective feeding. However, the concentrations in the pore water can become depleted over time if the accumulation rate of the contaminant from the water is greater than the desorption rate, and ingestion becomes more competitive (Kukkonen and Landrum 1994). A decline in the accumulation of PBDEs from spiked sediment over the course of the exposure may have resulted from depletion of the readily available PBDEs in pore water and an increasing dependence on ingestion, which is a less efficient route of uptake. The concentrations of BDE 47, 100, and 99 in worms exposed to composted biosolids did not decrease during the exposure. Ingestion is thought to be of increased importance in accumulation of compounds that are more strongly sorbed to substrate particles. Once ingested, the sediment is subjected to physical mixing and attack by digestive fluids, which act to solubilize contaminants (Leppanen and Kukkonen 2000a). Possibly, increased sorption to particles reduced the importance of uptake from pore water in accumulation of PBDEs from the composted biosolids, and ingestion was the dominant route of accumulation throughout the exposure. Thus, no decreases in tissue concentrations due to depletion of PBDEs in pore water were observed during the uptake phase of the experiment.

Changes in partitioning of PBDEs within the sediment matrix is another possible cause of the decrease in accumulation of BDE 47, 100, and 99 over time in worms.
exposed to spiked artificial sediment. Over the course of the exposure, there may have been increased sorption of the PBDEs to the slowly reversible particle pool, resulting in a decreased potential for desorption into pore water and/or digestive fluids and subsequent flux into *L. variegatus*. Landrum and Robbins (1990) have suggested that it may take months to years for the fraction of contaminant residing in the readily reversible and resistant particle pools to achieve steady state. The six-week aging period in this study may not have been long enough for equilibration to occur. A decrease in bioavailability of pyrene to *L. variegatus* from spiked sediment aged for 1.5 months was partially attributed to a change in pyrene partitioning during the 28-day exposure (Kukkonen and Landrum 1994). The time for partitioning of PBDEs between different particle pools was much longer in the composted biosolids matrix than in the spiked artificial sediment. This may help to explain why the concentrations of BDE 47, 100, and 99 in *L. variegatus* exposed to composted biosolids did not decrease over the course of the exposure.

Changes in the composition of the organic fraction of the sediment can also alter the bioavailability of organic contaminants over time (Landrum et al 1992). For the spiked artificial sediment, the aging process was initiated immediately after the sediment components were mixed together and involved storage of the spiked artificial sediment at 4°C in the dark. Storage of sediments at 4°C is recommended in order to reduce biological activity (Northcott and Jones 2000). Therefore, the peat moss component of the sediment probably exited the aging process relatively unchanged. However, the bioaccumulation assay was conducted at 21°C. An increase in microbial activity throughout the 28-day exposure may have caused a significant change in the organic matter composition of the spiked artificial sediment over time, through breakdown of the
peat moss, and resulted in a decrease in PBDE bioavailability. Sediment diagenesis results in the formation of high-molecular weight compounds such as humic substances, and organic matter that has been subjected to microbial activity may have a larger sorptive capacity for organic contaminants than fresh plant matter (DeWitt et al. 1992). The biosolids had been subjected to both anaerobic digestion and aerobic digestion (composting) prior to entry into the exposure system and the organic component of the substrate was probably very stable. Therefore, changes in organic matter composition were not likely to occur and no effect on the bioavailability of PBDEs was observed in the composted biosolids over the course of the exposure.

The apparent decreases in the bioavailability of BDE 47, 100, and 99 to *L. variegatus* from the spiked artificial sediment over the course of the 28-day exposure, as indicated by the decline in body burdens, were not directly reflected in the chemical analysis of the sediment. The measured concentrations of BDE 47 and BDE 100 in the spiked artificial sediment were not significantly different between Days 0, 21, and 28. Previous research has indicated that bioavailability may decline up to an order of magnitude faster than chemical extractability (Landrum 1989). This is most likely due to the fact that uptake by organisms is regulated by solubilization of the contaminant by water and digestive fluids, compared to chemical extraction which utilizes non-polar solvents to solubilize hydrophobic organic compounds. Thus, subtle changes in the strength of association of contaminants with sediment particles will affect bioavailability first.

The measured concentration of BDE 99 in the spiked artificial sediment was significantly lower on Days 21 and 28 than on Day 0. The decrease in the BDE 99
concentrations over the course of the experiment may have been due to either degradation or increased binding to the substrate over time. The chemical extractability of BDE 99 decreased over the six-week aging period prior to the bioaccumulation assay, indicating that the binding strength was changing over time. It is possible that the binding was still changing between Days 0 and 21 of the exposure and that the change was strong enough to affect the chemical extractability. However, the reason for differential partitioning of BDE 99 relative to the other congeners is unclear. The concentration of BDE 99 in the spiked artificial sediment was higher than that of any other congener. The concentration of contaminants can affect their partitioning among the sediment particles (Landrum et al 2002). In addition, the structure of BDE 99 may have affected its partitioning behavior within the spiked artificial sediment matrix (see following discussion). It is also possible that degradation of BDE 99 occurred between Days 0 and 21 of the exposure.

Debromination of BDE 99 was not indicated by apparent increases in the concentrations of the measured tetra-BDEs, BDE 47 and BDE 49, in the spiked artificial sediment over the course of the exposure. Analyses for other potential metabolites were not conducted, therefore microbial degradation of BDE 99 in the spiked artificial sediment cannot be ruled out. However, the concentration of BDE 99 in the composted biosolids did not decrease over the course of the exposure, and susceptibility of this congener to degradation would have likely been reflected in both substrates.

The apparent decrease in BDE 99 in the spiked artificial sediment did not affect the overall BAF. Calculation of BAFs for Days 21 and 28 using their respective worm and sediment concentrations resulted in values that were within the range associated with the reported BAF for BDE 99. The concentration of BDE 85 was significantly lower on
Days 21 and 28 in both substrates. However, bioavailability of BDE 85 could only be assessed for worms exposed to spiked artificial sediment and there was no significant decrease in worm tissue concentrations over time. As for BDE 99, the process that caused the decrease in chemical extractability of BDE 85 requires further investigation.

The original source of PBDEs to the substrates may have affected the partitioning of PBDEs between substrate particles, pore water and digestive fluids, and may have contributed to differences in accumulation between worms exposed to spiked artificial sediment and worms exposed to composted biosolids. In the spiked artificial sediment, the tetra- through hexa-BDEs originated from a commercial penta-BDE formulation (DE-71) that was dissolved in hexane and introduced to the sand fraction. In the composted biosolids, the PBDEs were inherent in the matrix, the source of PBDEs occurred prior to the sewage treatment process. While determining the exact source of PBDEs to the composted biosolids was beyond the scope of this study, it is important to note that a substantial portion of the measured tetra- through hexa-BDEs may have been associated with a polymer matrix, such as polyurethane foam (Hale et al 2002). Association with a polymer matrix may not affect the chemical extractability of PBDEs from biosolids, but could potentially decrease the availability for uptake by L. variegatus through a decreased potential for desorption into pore water and/or digestive fluids. Strong association with a polymer matrix would also decrease the potential for changes in partitioning to occur over the course of the 28-day exposure. Thus, the source of PBDEs in the exposure substrates may have also contributed to the difference in the uptake kinetics.
In addition to sediment-chemical interactions, organism behavior can strongly affect bioavailability and bioaccumulation kinetics. Worms remained buried throughout the 28-day exposure in all substrates, and reduced exposure due to sediment avoidance did not contribute to differences in overall accumulation or uptake over time.

Bioturbation by *L. variegatus* can result from burrowing and feeding activity, which involves ingestion of particles at depth and egestion at the sediment surface (Landrum and Robbins 1990). Loss of PBDEs to the overlying water due to bioturbation did not appear to occur, since the substrate concentrations of all but two congeners remained constant throughout the exposure in both spiked artificial sediment and composted biosolids. Loss to overlying water would have been reflected in all congeners, or if selective loss were to occur, it would have been most severe for BDE 47, the most water-soluble congener studied.

Bioturbation can also alter the partitioning of contaminants among particles through disturbance of sediment aggregates (Landrum et al 1996). For compounds that are less strongly sorbed to particles, mixing of sediments can reduce bioavailability by increasing the potential for sequestering of the contaminant into more resistant particle pools (Van Hoof et al 2001). Landrum (1989) saw an accelerated reduction in bioavailability when organisms were present compared to sediment aging alone and partly attributed the difference to bioturbation. Thus, bioturbation may have contributed to the reduction in bioavailability of PBDEs from the spiked artificial sediment over the course of the 28-day exposure, but cannot account for the difference in overall accumulation of PBDEs between the spiked artificial sediment and the composted biosolids.
The physiological condition of the organisms during an exposure can affect bioaccumulation kinetics. The dry weights of worms collected from each treatment were used as a measure of the condition of *L. variegatus* throughout the 28-day exposure. There was a significant decrease in the dry weights of worms exposed to spiked artificial sediment until Day 14 of the exposure. The stabilization of weights by Day 14 suggests that the initial decrease was not caused by acute toxicity. The weights of worms exposed to reference artificial sediment decreased significantly between Days 0 and 7, and then stabilized. The decrease in worm dry weights in both artificial sediment treatments reflects poorer nutritional conditions of the sediment relative to the culture conditions (Kukkonen and Landrum 1994). The lipid content of organisms is another measure of physiological conditions during an exposure. Lipids act as storage sites for hydrophobic contaminants and changes in lipid content can significantly affect apparent bioaccumulation (Kukkonen and Landrum 1994). The lipid content of *L. variegatus* was not measured in this study, but lipid content is expected to correlate with dry weight. The lipid content of worms exposed to the spiked artificial sediment may have decreased during the uptake portion of the exposure and contributed to the apparent decrease in accumulation of PBDEs over time. Kukkonen and Landrum (1994) found that normalization to lipid content eliminated an apparent decrease in pyrene concentration over time that was observed for wet weight-normalized data. The dry weights of *L. variegatus* exposed to composted biosolids were not significantly different between any of the sampling days during the uptake phase of the exposure. Thus, changes in lipid content and resulting impacts on uptake kinetics were probably minimal.
The nutritional value of sediment can affect the feeding rate of deposit feeders, and feeding rate can affect the bioavailability of contaminants and bioaccumulation kinetics. The dry weights of worms exposed to composted biosolids were significantly higher than the dry weights of worms exposed to the artificial sediment treatments, which suggests that the composted biosolids substrate was nutritionally superior to the artificial sediment. The difference in nutritional value was likely due to the difference in the composition of organic matter between the substrates. In sediments with lower nutritional quality, organisms must ingest more material in order to fulfill energy needs (Leppanen and Kukkonen 1998c). Therefore, it is probable that the feeding rates of worms exposed to spiked artificial sediment were higher than those of worms exposed to composted biosolids. One suggested effect of a higher feeding rate is higher body burdens of contaminants, resulting from increased exposure through ingested sediment (Kukkonen and Landrum 1994). A difference in feeding rates between worms exposed to spiked artificial sediment and worms exposed to composted biosolids may have contributed to the difference in PBDE accumulation between the substrate treatments. In contrast, it has also been suggested that increased feeding rates decrease the time available for desorption of contaminants from sediment particles and can result in decreased accumulation (Leppanen and Kukkonen 2000a). If the feeding rate of worms exposed to spiked artificial sediment increased during the 28-day exposure due to poor nutritional quality of the sediment, this increase could have contributed to the decrease in the PBDE concentration in the worm tissue over time. Unfortunately, the actual effects of feeding behavior on the bioaccumulation of PBDEs from the spiked artificial sediment
and composted biosolids cannot be determined since quantitative data on feeding activity, such as egestion rates, were not collected.

In contrast to the uptake kinetics, potential differences in substrate characteristics and worm physiology between the spiked artificial sediment and the composted biosolids did not affect the depuration kinetics. Depuration rate constants were not significantly different between worms exposed to spiked artificial sediment and worms exposed to composted biosolids, although only depuration rate constants for BDE 47 and BDE 99 could be compared between the two substrates. In both the uptake and depuration phase of the exposure, worms purged their gut contents in clean well water prior to analysis. Depuration rate constants were based only on elimination of PBDEs from worm tissue, there was no initial pulse in elimination due to gut contents. Thus, effects of the exposure substrates were removed.

Biotransformation may have contributed to the elimination of PBDEs from worm tissue but without conducting the analysis for potential PBDE metabolites, it is difficult to assess if biotransformation occurred. The fact that depuration rate constants were not statistically different when compared between worms exposed to spiked artificial sediment and worms exposed to composted biosolids suggests that any contribution of metabolism to elimination of PBDEs did not differ between the substrate treatments. Thus, the difference in accumulation kinetics between the two substrates was probably not due to greater metabolism of PBDEs by worms exposed to the spiked artificial sediment.

For both exposure substrates, the applicability of the first-order bioaccumulation model to the data was limited. The first-order bioaccumulation model is only valid if the
bioavailability of a compound remains constant over the course of the exposure. The
decrease in accumulation over time in worms exposed to spiked artificial sediment
suggests a change in the bioavailable fraction of PBDEs in the substrate. In addition, the
significant weight loss of worms exposed to spiked artificial sediment indicates a
physiological change in the organisms over time, which may have affected PBDE
accumulation. Thus, the first-order bioaccumulation model could not be used to estimate
uptake rate coefficients and kinetic bioaccumulation factors for worms exposed to spiked
artificial sediment.

For worms exposed to composted biosolids, there was no apparent change in
physiology or decrease in the bioavailable fraction of PBDEs, and steady-state
concentrations appeared to be achieved by Day 7 of the exposure. The linear portion of
the uptake curve was not captured, and without sampling points between Days 0 and 7,
the shape of the uptake curve leading up to steady-state concentrations is uncertain. Data
for BDE 47 were a poor fit to the first-order bioaccumulation model because of rapid
uptake and very slow elimination. Data for BDE 99 were a relatively good fit to the
model overall, but the initial uptake was not well described, which appears to be related
to the calculated depuration rate constant. Estimated times to achieve 90% of steady-
state concentrations were approximately 75 days for BDE 47 and approximately 25 days
for BDE 99. These estimates were calculated using the depuration rate constants, under
the assumption that as the depuration rate increases, the time required to achieve steady-
state between uptake and elimination decreases. There is an apparent disconnect between
the predicted and measured times to achieve steady state, the depuration rate constants
are too low to have allowed steady-state to have been achieved within the first seven days of exposure.

During the depuration phase of the study, the dry weights of worms from both substrate treatments increased significantly on subsequent sampling days. This increase was most likely due to the supplemental diet of trout chow. A corresponding increase in the lipid content of the worms may have also occurred, and an increase in lipid content can decrease the measured depuration rates of hydrophobic compounds in invertebrates (Landrum et al 1988, Fisk et al 1998). If an increase in lipid content occurred between each sampling day, then normalization to lipid content would have enhanced the decrease in PBDE concentration over time, and the depuration rate constants would have been higher. Thus, the calculated depuration rate constants may be underestimates, which would affect their applicability in the first-order bioaccumulation model. An increase in worm weights may have also caused an apparent decrease in tissue concentrations over time due to dilution. Reproduction may have occurred during the depuration phase of the study, since oligochaetes reproduce by fragmentation after reaching a certain size (Leppanen and Kukkonen 1998a). However, both growth dilution and reproduction would have caused an apparent increase in the depuration rate constants (Landrum et al 2002).

If nutritional conditions did affect the calculated depuration rate constants, the effects were similar between the substrate treatments, as indicated by the statistically similar depuration rate constants for BDE 47 and BDE 99 when compared between worms exposed to spiked artificial sediment and worms exposed to composted biosolids.
In addition, the comparison of depuration rate constants for individual PBDE congeners within a substrate treatment would not be affected by nutritional conditions.

Comparisons between individual PBDE congeners

The BAF is typically calculated using steady-state tissue concentrations and is used as a quantitative measure of bioavailability. For worms exposed to spiked artificial sediment, apparent steady-state concentrations were not achieved during the 28-day exposure and could not be estimated using the first-order bioaccumulation model. The BAFs for each PBDE congener were instead calculated using the mean of the tissue concentrations measured during the 28-day exposure, and a range for each value was calculated using the error associated with the mean. Thus, the calculated BAFs are not necessarily representative of the true accumulation potential for sediment-associated PBDEs, but provide a mechanism for comparison of the relative accumulation of the individual PBDE congeners. For worms exposed to composted biosolids, BAFs for each PBDE congener were also calculated using the mean of the tissue concentrations measured during the 28-day exposure. However, since apparent steady-state concentrations were achieved by Day 7 of the exposure, the range of each BAF was very small and the values are more representative of the true accumulation potential of biosolids-associated PBDE congeners. As previously discussed, the exposure substrate strongly affected the magnitude of the calculated BAFs for each PBDE congener. However, the relative accumulation of the different PBDE congeners appears to have been similar between the two exposure substrates.
Out of the eight congeners studied, BDE 47 and BDE 99 accumulated to the highest concentrations in worms exposed to spiked artificial sediment and worms exposed to composted biosolids. For worms from both substrate treatments, the calculated BAF for BDE 47 was two times the BAF for BDE 99. In reports of tetra- through hexa-BDEs in wildlife, BDE 47 is typically the dominant congener (Lindstrom et al 1999, Manchester-Neesvig et al 2001, Hale et al 2001a, Ikonomou et al 2002). However, in commercial penta-BDE formulations such as DE-71 and Bromkal 70-DE, the contribution of BDE 99 is typically greater than or similar to that of BDE 47 (Hale et al 2001a, Sjodin et al 1998). The differential accumulation of BDE 47 and BDE 99 by *L. variegatus* supports the disparity in congener patterns between commercial penta-BDE formulations and biota.

The differential accumulation of the two congeners resulted in a change in the relative abundance of BDE 47 and BDE 99 in the worms compared to the exposure substrates. In both substrates the concentration of BDE 99 was greater than the concentration of BDE 47, but throughout the 28-day exposure, worm tissue concentrations of BDE 47 were equal to or greater than concentrations of BDE 99. Differences in the relative abundance of individual components of chemical mixtures in benthic invertebrates relative to sediment have been observed for PCB congeners in both field-collected and laboratory-exposed oligochaetes and for chlorinated paraffins in laboratory-exposed *L. variegatus* (Oliver 1984, Bremle and Ewald 1995, Fisk et al 1998). This may alter the availability of different congeners for uptake by the next trophic level. In a laboratory study in which zebrafish were fed chironomids spiked with PBDEs, the biomagnification factor (BMF) for BDE 47 was over ten times higher than the BMF for
BDE 99 (Andersson et al 1999). This supports the accumulation pattern observed for *L. variegatus*, and suggests that differences in the PBDE congener pattern between sediments and biota may increase with each trophic transfer.

The greater accumulation of BDE 47 compared to BDE 99 may be related to either differential uptake or elimination of the congeners by *L. variegatus*. As previously discussed, routes of uptake for sediment-associated contaminants include both pore water and ingested sediment. Because it has one less bromine substituent, BDE 47 is more water-soluble than BDE 99. Greater water solubility increases the potential for desorption from sediment particles into pore water. Thus, the amount of BDE 47 available for uptake via pore water is expected to be greater than the amount of BDE 99. The difference in water solubility between the two congeners is reflected in the difference in their octanol-water partition coefficients (log \( K_{ow} \)). The range in log \( K_{ow} \) for tetra-BDEs is 5.87 to 6.16 and the range in log \( K_{ow} \) for penta-BDEs is 6.64 to 6.97 (WHO 1994). The log \( K_{ow} \) value of a compound is a measure of its hydrophobicity and hence, the tendency for association with sediment particles. As the log \( K_{ow} \) value increases, sorption to sediment particles increases. This results in a decrease in the rate of desorption into interstitial water and lower assimilation efficiency from ingested particles (Landrum and Robbins 1990).

Uptake via particle ingestion is dependent on assimilation efficiency, which is the portion of the contaminant in the food that is retained by the organism (Kukkonen and Landrum 1995b). In a laboratory study in which pike were fed rainbow trout spiked with PBDE congeners, the uptake efficiencies of BDE 47 and BDE 99 were approximately 90% and 60%, respectively (Burreau et al 1997). The nutritional quality of the food can
affect the assimilation efficiency, and the exact values would probably differ between a pike feeding on a trout and a worm feeding on the sediment (Landrum et al 1996). However, the difference in uptake efficiencies suggests that upon ingestion, BDE 47 is more available for uptake than BDE 99.

The greater accumulation of BDE 47 compared to BDE 99 may also be related to differential elimination of the congeners. When compounds are eliminated more quickly, the overall accumulation will be lower. For worms exposed to spiked artificial sediment and worms exposed to composted biosolids, the depuration rate constant for BDE 99 was three times the depuration rate constant for BDE 47, indicating that the elimination was three times faster for BDE 99. Based on the idea that the affinity of a compound for lipids increases with increasing hydrophobicity, elimination rates are expected to decrease with increasing log $K_{ow}$ (Landrum and Robbins 1990). However, in this study, depuration was faster for the compound with the higher log $K_{ow}$. Elimination can be either passive or active, and processes can include diffusion and biotransformation (Spacie et al 1995). Lipophilicity would likely have the greatest effect on depuration rates if passive diffusion was the dominant mechanism of elimination. In a study in which mussels were exposed to PCBs and PBDEs, the bioaccumulation factors and depuration rate constants were similar for BDE 47 and BDE 99 (Gustafsson et al 1999). The depuration rate constants decreased with increasing hydrophobicity for the PCBs and elimination of BDE 47 was comparable to the PCB congeners of similar hydrophobicity. However, the elimination of BDE 99 was faster than the elimination of PCBs with similar hydrophobicity. This suggests that active transport of BDE 99 out of mussel tissues was
occurring, although the activities of biotransformation enzymes in mollusks are low relative to other invertebrates and fish (Di Giulio et al 1995).

Field surveys of freshwater fish have indicated that the contribution of BDE 99 to the total PBDE body burden appears to be reduced in common carp, suggesting the potential for metabolism of BDE 99 by this species (Hale et al 2001a, Dodder et al 2002). The biotransformation capabilities of *L. variegatus* are poorly understood, but previous research has suggested that this species is capable of metabolizing benzo[a]pyrene and chlorinated paraffins (Leppanen and Kukkonen 2000b, Fisk et al 1998). *L. variegatus* may also be able to metabolize PBDEs, and the greater elimination of BDE 99 may have been due to biotransformation of this congener. However, further study of biotransformation mechanisms for PBDEs and analyses of potential metabolites are needed in order to support this suggestion.

The difference in the BAFs for BDE 47 and BDE 99 suggests that the number of bromine substituents affects the relative bioaccumulation of PBDE congeners. However, a difference in the relative accumulation of BDE 100 and BDE 99 was also observed. In worms from both the spiked artificial sediment and the composted biosolids, the BAF for BDE 100 was greater than the BAF for BDE 99. The magnitude of the difference was greater for worms exposed to spiked artificial sediment, but for both exposure substrates, the BAF ranges for the two congeners did not overlap. Similar results were found when zebrafish were exposed to PBDE-spiked food. The food contained high concentrations of both BDE 100 and BDE 99, but the BMF for BDE 100 was approximately five times greater than the BMF for BDE 99 (Andersson et al 1999). In the environment, BDE 99 and BDE 100 are found at similar levels in biota, even though the concentration of BDE
100 is much lower than BDE 99 in commercial penta-formulations (Hale et al 2001a, Manchester-Neesvig 2001, Ikonomou et al 2002). This may be related to greater accumulation of BDE 100, as seen in *L. variegatus*. BDE 100 and BDE 99 are both penta-BDEs, and the only structural difference between these two congeners is the position of one bromine atom. Thus, it appears as if the position of the bromine substituents affects the relative accumulation of PBDE congeners.

The idea that the position of the bromine substituents affects the relative bioaccumulation of PBDE congeners is further supported by comparisons of all six BAFs calculated for tetra- through hexa-BDEs in worms exposed to the spiked artificial sediment. The BAFs for BDE 47, 100, and 154 were higher than the BAFs for BDE 99, 85, and 153, and the range in BAFs did not overlap between the two groups. Stapleton et al (2002) observed a similar pattern of accumulation in carp exposed to a mixture of PBDE congeners via spiked food. BDE 47, 100, and 154 were accumulated rapidly, but there was little to no accumulation of BDE 99 and 153. BDE 85 was not accumulated by zebrafish exposed to PBDE-spiked food (Andersson et al 1999) and this congener is not typically reported in biota (Hale et al 2001a, Manchester-Neesvig et al 2001, Dodder et al 2002, Ikonomou et al 2002).

Both groups contain penta- and hexa-BDEs, and the differential accumulation between the two groups appears to be related to the positioning of the bromines on the phenyl rings. The penta- and hexa-BDEs for which BAFs were calculated have four bromines in the same position as BDE 47. Relative to BDE 47, BDE 85 has an additional bromine in the 3- position, BDE 99 has an additional bromine in the 5- position, and BDE 153 has additional bromines in the 5- and 5'- positions. Thus, the similarity between
BDE 99, 85, and 153 appears to be meta-substitution. Relative to BDE 47, BDE 100 has an additional bromine in the 6-position, and BDE 154 has additional bromines in the 5- and 6'-positions. Ortho-substitution appears to be the similarity between BDE 100 and BDE 154. Thus, the substitution pattern of penta- and hexa-BDE congeners appears to have a greater affect on relative bioaccumulation than the number of bromine substituents.

The effect of substitution pattern on bioaccumulation may be related to differential elimination. The depuration rate constants for BDE 100 and BDE 154 were similar to BDE 47, as observed for the BAFs. Thus, the elimination rate of BDE 99 was approximately three times as fast as the elimination rates of both BDE 100 and BDE 154. As previously stated, the only difference between BDE 100 and BDE 99 is the position of one bromine atom. The ortho-substituent appears to decrease the potential for elimination relative to the meta-substituent. The similarity in the depuration constants for BDE 100 and BDE 154 may be related to their ortho-substituents. A depuration rate constant could not be calculated for BDE 153, but elimination of this congener was likely similar to BDE 99. As observed for BDE 99, the elimination of BDE 153 by blue mussels was more rapid than expected based on its log $K_{ow}$, suggesting that active elimination was occurring (Gustafsson et al 1999). In addition to lower body burdens of BDE 99, field-collected carp also tend to have low body burdens of BDE 153 relative to other fish (Hale et al 2001a, Dodder et al 2002). This suggests parallel processes between BDE 99 and BDE 153. If the greater elimination of BDE 99 by $L. variegatus$ was due to biotransformation, as previously suggested, it appears as if penta- and hexa-BDE congeners with meta-substitution are more susceptible than those with ortho-
substitution. Biotransformation increased elimination rates of certain PCB congeners by the polychaete *Nerites diversicolor*, and susceptibility of the congeners to biotransformation appeared to be dependent on the position of the chlorine atoms (Goerke and Weber 1990). Therefore, it is possible that the susceptibility of different PBDE congeners to biotransformation may be affected by the position of the bromine substituents.

Biotransformation of PBDEs has not been thoroughly examined, but the potential for enzymatic debromination of certain congeners has been suggested. Stapleton et al (2002) exposed common carp to BDE 183 (2,2',3,4,4',5',6-HpBDE) via spiked food and observed no accumulation over a 25-day period. However, BDE 154 increased in carp tissue throughout the duration of the exposure, suggesting that debromination of BDE 183 to BDE 154 occurred. This conversion would result from loss of the bromine from position 3-, a meta- position. The results of another feeding study with carp suggested that debromination of BDE 99 and BDE 153 may have contributed to an unusually high assimilation efficiency (>95%) of BDE 47 (Stapleton et al 2002). The debromination pathway: BDE 153 to BDE 99 to BDE 47 would involve loss of bromines from meta-positions, 5'- and 5- respectively. Reductive dechlorination of PCB congeners has been observed in environmental samples and the susceptibility of congeners to dechlorination depends on the position of the chlorine atoms. Meta- substituted PCB congeners are most susceptible and ortho- substituted PCB congeners are least susceptible to dechlorination (Rhee et al 1993). BDE 154 can be converted to BDE 100 through loss of a bromine from a meta- position (5-), but conversion of BDE 100 to BDE 47 would require loss of a bromine from an ortho- position (6-). Debromination of BDE 47 would
require loss of a bromine from either an ortho- or a para- position. Thus, debromination appears to be more likely for BDE 99 and BDE 153 than for BDE 47 and BDE 100. However, the previously mentioned feeding studies do not indicate whether debromination of the congeners occurred in the tissue of the fish or in the digestive tract, prior to uptake.

If debromination was taking place in *L. variegatus*, body burdens of BDE 47, the most probable debromination product, would be expected to increase over time due to the introduction of a source additional to the substrates. This was not observed in worms exposed to either spiked artificial sediment or composted biosolids. The pattern of uptake over time for BDE 47 was similar to BDE 99 in worms from both exposure substrates, suggesting that the loss of one congener did not result in a significant gain of another congener. Thus, apparent differences in elimination of meta- and ortho-substituted congeners cannot simply be attributed to enzymatic debromination and other pathways need to be considered. Om and Klasson-Wehler (1998) found that rats and mice possess the limited ability to biotransform BDE 47 to hydroxylated metabolites. Hydroxylated metabolites are of particular interest due to their increased endocrine disrupting potential *in vitro* relative to parent PBDEs (Meerts et al 2000 and 2001). However, the metabolic pathway, susceptibility of other congeners, and capabilities of other species are unknown at this time.

Although there appears to be a strong effect of elimination on the relative bioaccumulation of the penta- and hexa-BDEs, the substitution pattern may have also caused differential uptake of the congeners. The physical properties of PBDEs are strongly affected by the number of bromine substituents, and the differential
accumulation of congeners with the same number of bromines was probably not related to differences in water solubility and/or the tendency for adsorption to sediment particles. However, the substitution pattern may have affected the association with active sites of organic matter and caused differences in binding strength and desorption potential among the congeners. The binding strength of the meta-substituted penta-BDEs, BDE 99 and BDE 85, may have been stronger than that of the ortho-substituted penta-BDE, BDE 100. This would help to explain the differential accumulation of the penta-BDEs by *L. variegatus* and may also account for the decrease in chemical extractability of BDE 99 and BDE 85 in the spiked artificial sediment over the course of the exposure.

The substitution pattern may have also affected the potential for absorption by intestinal epithelial cells upon desorption into digestive fluids. During digestion, hydrophobic contaminants remain closely associated with lipids. Burreau et al (1997) observed relatively efficient dietary assimilation of BDE 99 and BDE 153 by pike. Both congeners have an effective molecular cross-section (ECS) greater than 9.5 angstroms, the postulated size limit for diffusive uptake. These authors suggested that mediated uptake of lipids by proteins in intestinal epithelial cell membranes may facilitate the uptake of contaminants with an ECS greater than 9.5 angstroms. A mechanism for the mediated uptake was not proposed, but it is possible that the substitution pattern of the penta- and hexa-BDE congeners can affect the efficiency of transport across the cell membrane. However, further study of the assimilation efficiencies of other congeners, such as BDE 100 and BDE 154, is needed in order to support this idea.

Although molecular size and lipophilicity do not appear to inhibit the uptake of penta- and hexa-BDEs, the very low uptake of deca-BDE (BDE 209) was probably
related to its physical properties. BDE 209 was only detected in worms exposed to spiked artificial sediment, and the estimated BAF was 0.3, which is approximately 15 times less than the BAFs for BDE 99, 85, and 153. The log $K_{ow}$ of BDE 209 is 9.97, and based on lipophilicity, the potential for desorption of BDE 209 from sediment particles is much lower than that of the other congeners. In addition, the very large molecular size of BDE 209 may impede its transport across cell membranes (Sellstrom et al 1998). In a study by Kierkegaard et al (1999) rainbow trout were exposed to a commercial deca-BDE formulation via spiked food and accumulation of BDE 209 in muscle tissue was very low, less than 0.15%. BDE 209 is typically not found in biota samples, but trace concentrations have been observed in both fish and shellfish from areas with highly contaminated sediment (Sellstrom et al 1998, Watanabe et al 1987b). Thus, the very low uptake of BDE 209 relative to other congeners in worms exposed to spiked artificial sediment is consistent with previous studies. However, because the low concentrations could not be accurately quantified, the exact degree of BDE 209 bioaccumulation is unknown.

BDE 49 was detected in worms exposed to spiked artificial sediment and worms exposed to composted biosolids, but concentrations were not above the quantitation limit. The concentration of BDE 49 in the composted biosolids was similar to concentrations of BDE 85, 154, and 153, which were also detected but not quantified in worms from this exposure substrate. BDE 49 was not detected in the spiked artificial sediment over the course of the exposure. The presence of BDE 49 in composted biosolids most likely results from either accumulation of a minor component of commercial penta-BDE formulations over time or debromination of another congener. Either explanation can
also account for the detection of BDE 49 in worms exposed to spiked artificial sediment even though this congener was not detected in the substrate. Formation of BDE 49 from debromination of BDE 99 is possible, but requires the loss of a bromine from an ortho-position (4-). Thus, it is more likely that BDE 49 is a minor component of DE-71. BDE 49 has been reported in biota and appears to be bioavailable (Hale et al 2001a, Ikonomou et al 2002). However, the degree of bioavailability of BDE 49 to *L. variegatus* from spiked artificial sediment and composted biosolids could not be determined in this study.
CONCLUSIONS

PBDEs were bioavailable to *Lumbriculus variegatus* from composted biosolids, which suggests that if land-applied biosolids enter aquatic environments through either runoff or misapplication, they can be a potential source of PBDEs to benthic organisms. These results also imply that terrestrial oligochaetes inhabiting soils receiving biosolids applications can potentially accumulate PBDEs, due to burrowing and feeding habits similar to *L. variegatus*. PBDEs were also bioavailable to *L. variegatus* from spiked artificial sediment, which suggests that benthic organisms inhabiting PBDE-contaminated sediments can accumulate these compounds. The effects of substrate characteristics on bioaccumulation highlight the importance of choosing an environmentally realistic test matrix when conducting bioaccumulation tests.

The differential accumulation of PBDE congeners by *L. variegatus* reflects patterns observed in wildlife and other laboratory studies. Accumulation of PBDE congeners appears to be related to both uptake and elimination. The substitution pattern of penta- and hexa-BDE congeners appears to have a greater effect on accumulation than the number of bromine substituents. Thus, discrimination of congeners can begin with benthic organisms and differences in congener patterns between biota and sediments may increase with each trophic transfer.
FUTURE RESEARCH

This study is the first to investigate the bioavailability of biosolids-associated PBDEs to aquatic oligochaetes. Field validation through investigations of body burdens in benthic organisms inhabiting streams that receive runoff from biosolids-treated sites would support the suggestion that land-applied biosolids can be a source of PBDEs to benthic organisms. Identification of biochemical markers in biosolids would facilitate determination of sites impacted by biosolids inputs.

The behavior of PBDEs in sediments and uptake by benthic organisms are not well understood. Bioavailability of PBDEs to benthic organisms from contaminated sediments in the environment may be significantly reduced compared to a spiked matrix and additional field and/or laboratory investigations should be conducted. Experiments involving partitioning of PBDEs between sediment particles and water or simulated digestive fluids would provide insight into desorption kinetics and potential differential availability of individual congeners. The potential for degradation of individual PBDE congeners in both aerobic and anaerobic sediments should also be examined.

Uptake and elimination of individual PBDE congeners by organisms also requires further study. Elimination appeared to have a strong effect on overall accumulation, and the potential for biotransformation of individual congeners should be investigated. Biotransformation by organisms occupying lower trophic levels is particularly interesting due to the potential for trophic transfer of metabolites. Measurement of assimilation efficiencies of different PBDE congeners could provide information on the processes governing accumulation by separating the effects of uptake and elimination.
Accumulation of PBDEs from sediments by benthic organisms implies that these compounds will be available for uptake by the next trophic level. Biphasic studies in which benthic organisms are exposed to a PBDE contaminated substrate and then fed to fish would support this idea and allow comparisons of congener patterns with each increase in trophic level.
LITERATURE CITED


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