Bioavailability of Brominated Diphenyl Ethers to the Marine Polychaete Worm, *Nereis virens*

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Introduction

Brominated diphenyl ethers (BDEs) are a class of hydrophobic organic chemicals (HOCs) produced for use as flame retardants and incorporated into a wide variety of consumer products to comply with fire safety regulations. The high frequency of detection of BDEs in both biotic and abiotic matrices globally has resulted in their classification as ubiquitous environmental contaminants. In aquatic environments BDEs are associated with particles and concentrate in sediments. Due to high rates of sediment ingestion, deposit-feeders are capable of transferring other sediment-bound HOCs such as polychlorinated biphenyls (PCBs) to aquatic food webs (e.g., Pruell et al 2000) and may be an important route of transfer for BDEs to higher trophic levels. Few studies however have investigated the bioavailability of sediment-associated BDEs.

The freshwater oligochaete *Lumbriculus variegatus* accumulated congeners in the Penta-BDE mixture from both spiked sediments and composted biosolids in laboratory studies (Leppänen and Kukkonen 2004; Ciparis and Hale 2005) and some of these same congeners were also detected in earthworms living in soils receiving historical loadings of BDE-contaminated sewage sludge and river overflow (Matscheko et al. 2002). Penta-BDE congeners also accumulated in amphipods (*Leptocheirus plumulosus*) and two species of polychaete worms (*Nereis succinea* and *Marenzelleria viridis*) living in a highly contaminated tributary of the Chesapeake Bay receiving high loadings of wastewater effluent (Klosterhaus and Baker, unpublished). In contrast to the high frequency of detection of the Penta-BDE congeners in these studies and biota in general (Hites 2004), detection of the less water soluble congeners in the Deca-BDE mixture is less common. Other studies have reported detection of BDE 209 in earthworms, fish, birds, and mammals but polychaete worms and amphipods did not accumulate detectable levels of BDE 209 (MDL 0.3-35 ng/g wet) in our laboratory studies or field collections despite exposure to highly concentrated field sediments (up to 2000 ng/g dry). BDE 209 also did not accumulate or was minimally detected in the oligochaete worms, *L. variegatus* in the laboratory study by Ciparis and Hale (2005).

Mechanisms that could potentially limit BDE 209 accumulation in deposit feeders include (1) the inability of digestive fluid to mobilize BDE 209 from the sediment matrix in the gut (i.e. sediment desorption), (2) chemical interactions in the gut which limit BDE 209 solubility, and/or (3) an inability or inefficiency of BDE 209 transfer from the gut fluid across the gut wall (i.e. steric hindrance). Our intention was to design an experiment which would provide insight into the mechanism(s) limiting the bioavailability of BDE 209 to *N. virens*. Our specific objectives were to (1) test the hypothesis that BDE 209 does not accumulate because it is too large to cross the gut wall, and (2) determine if the bioavailability of BDE 209 is influenced by the presence of other BDEs. To meet these objectives, we

conducted a 28 day bioaccumulation experiment in which we attempted to 'maximize' bioavailability by introducing BDE 209 to *N. virens* in minimally aged spiked sediments (< 48 hrs) and a no sediment treatment in which worms were exposed to BDE 209 only via spiked clam meat. In addition to exposure to Deca-BDE alone in spiked sediments, worms were also exposed to spiked sediments containing both Penta- and Deca-BDE commercial mixtures to investigate whether the presence of other BDEs influenced the accumulation of BDE 209. A time series sampling regime was also conducted for worms exposed to the spiked sediment mixture to characterize the relative bioavailability of BDE congeners in the Penta-BDE and Deca-BDE commercial mixtures through comparison of congener-specific uptake rates.

Materials and Methods

Uncontaminated *Nereis virens* were exposed to five sediment treatments and three clamfood treatments for 28 days. Worms in the sediment treatments were not fed and worms in the clam feeding treatments were kept in water only with no sediment present. The sediment treatments were: Deca-BDE spiked sediment, a mixed Deca-BDE/ Penta-BDE/ PCB 209 spiked sediment treatment, PCB 209 spiked sediment, naturally contaminated field sediment, and a solvent control sediment. The clam feeding treatments were Deca-BDE spiked clams, PCB 209 spiked clams, and control clams with no chemicals added. The target concentration for Deca-BDE and PCB 209 in the individual and mixed spiked sediment treatments and the clam treatments was 2500 ng/g dry sediment or wet weight clam and was designed to approximate the BDE 209 concentration in the field sediment treatment. PCB 209 was used as a positive control for sediment and clam meat ingestion since it accumulated in *N. virens* in a previous study. An abiotic control treatment containing Deca-BDE and PCB 209 at the same target concentrations was also set up to track any changes in sediment chemical concentrations over 28 days. Sediment and worms were sampled on days 0 and 28. Worms in the mixed spiked sediment treatment were also sampled on days 2, 4, 8, and 16. Worms were allowed to purge their gut contents for 5 hrs in clean sediment followed by 19 hrs in seawater. Worms and sediment were extracted via accelerated solvent extraction and interfering compounds were removed via alumina and Florisil. A total of 34 BDEs were quantified using programmed temperature vaporization injection in a GC-MS operated in negative chemical ionization mode. PCBs were quantified using GC-µECD.

Results and Discussion

Despite exposure to similar sediment concentrations, BDE 209 was not available to worms exposed to field sediments (< 0.3 ng/g wet) whereas worms exposed to the Deca-BDE spiked sediments consistently accumulated BDE 209 well above detection limits. Other components of the Deca-BDE mixture were either detectable at very low levels (BDEs 197, 207, and 208 0.02-0.08 ng/g wet) or not detected (BDEs 196 and 206 < 0.06 ng/g wet) in worms exposed to concentrations of 0.5-40 ng/g dry in the field sediment. Worms exposed to Deca-BDE spiked sediments containing 1-190 ng/g dry of these same congeners consistently accumulated levels well above detection (0.1-3 ng/g wet weight). Bioaccumulation factors (BAFs) for BDE 209 from both spiked sediment and the spiked clams were < 0.05, indicating very low availability of this congener, even under high exposure conditions. The availabilities of BDE 209 and PCB 209 were similar, with BAFs for both congeners 3-4 times higher in the clam exposure compared to the spiked sediment exposure. These results demonstrate that BDE 209 and other congeners in the Deca-BDE mixture are capable of crossing the gut wall and accumulating in *Nereis virens* when presented to the organism in an artificially bioavailable form, but that accumulation from naturally-

contaminated field sediment is in some way constrained. Together with observations that BDE 209 detection in aquatic species is generally sporadic (usually near point sources) and concentrations are usually low when detected, these results indicate that characteristics of the sediment matrix in aquatic environments render BDE 209 unavailable for uptake to any great extent by aquatic organisms.

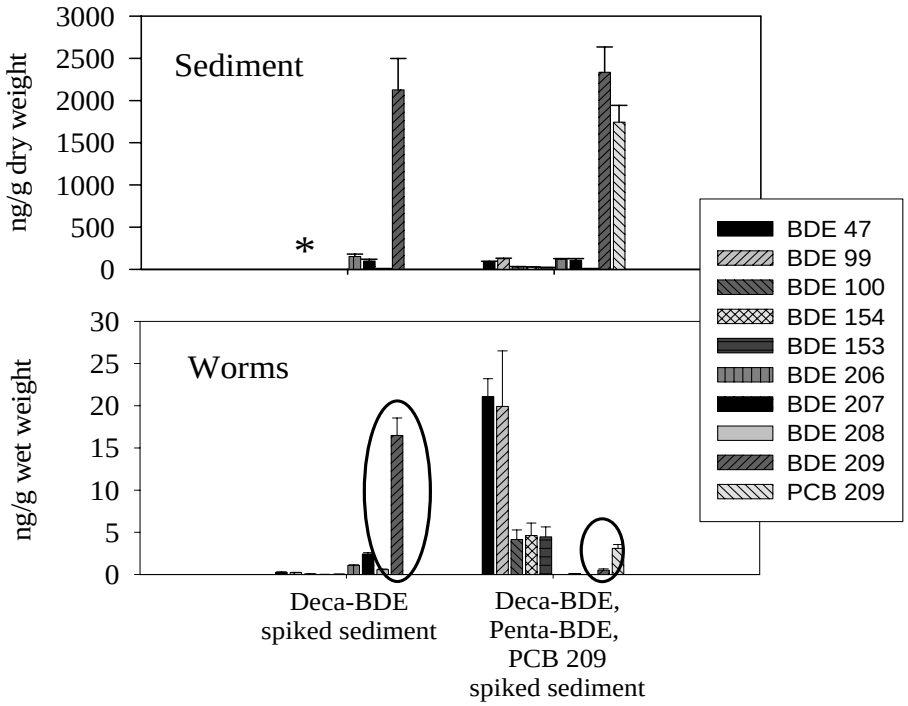

Figure 1. Mean concentrations of Penta- and Deca-BDE congeners and PCB 209 in spiked sediments and worms after 28 days of exposure. Error bars represent one standard deviation of the mean. * Penta-BDEs below detection in Deca-BDE spiked sediments (MDLs 0.02-0.2 ng/g dry weight).

Compared to spiked sediments containing only Deca-BDE, BDE 209 was much less bioavailable to *N. virens* when sediments also contained Penta-BDE and PCB 209 (Figure 1). Selective uptake was evident, with concentrations of BDE 209 30 times lower in worms exposed to the contaminant mixture compared to worms exposed to Deca-BDE alone, even though concentrations of BDE 209 were similar in both spiked sediment treatments. Penta-BDE congeners were selectively accumulated over the Deca-BDE congeners and PCB 209 despite Penta-BDE concentrations in the sediment that were approximately 20 times lower. Much lower accumulation of BDE 209 from the spiked sediment mixture may have been the result of competition with the lower brominated Penta-BDE congeners and PCB 209 for space in digestive fluid micelles. Although BDE solubilization in gut fluid has not yet been investigated, solubilization in micelles is likely required before accumulation can occur, similar to other sediment-bound HOCs (e.g. Weston and Mayer 1998). The Penta-BDE congeners are smaller and less hydrophobic

than BDE 209 which may have enabled them to outcompete the larger and heavier BDE 209 for micellar space. Weaker association of the Penta-BDE congeners to sediment particles may enable these congeners to be more easily desorbed in the gut, thus allowing them to saturate digestive micelles and limit the solubility of BDE 209 in the gut. Competition for space and saturation of digestive micelles was previously suggested to have occurred in the gut fluid of the polychaete *Arenicola marina* (Voparil and Mayer 2000) when less solubilization of PAH from highly contaminated field sediment in the gut fluid was observed compared to when pure PAH solid was added directly to the fluid. In vitro gut fluid incubations of BDE contaminated sediments would assist in clarifying the mechanism(s) responsible for our results.

BDE concentrations in worms exposed to the spiked sediment mixture increased over time with BDEs 47, 99> 100, 153, 154>28/33, 66, 85/155, 209. Uptake rates generally decreased with increasing K_{ow}. For the congeners consistently detected by day 28, biota-sediment accumulation factors (BSAFs) ranged from 0.0003-0.6 and 0.3-1.5 for worms exposed to the spiked sediment mixture and field sediments, respectively. BSAFs generally decreased as log K_{ow} increased from 6-10, though BSAFs for the predominant Penta-BDE congeners were similar.

Acknowledgements

We thank Rob Hale for contributing the DE-71 mixture and Greg Ziegler and Eileen Beard for help with sediment collection. Funding was provided by the Maryland Department of the Environment.

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