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In Situ Accumulation of HBCD, PBDEs, and Several Alternative Flame-Retardants in the Bivalve (*Corbicula fluminea*) and Gastropod (*Elimia proxima*)

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Ⓢ *Supporting Information*

ABSTRACT: Alternative brominated flame-retardants (BFRs), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4,5-tetrabromophthalate (TBPH), 1,2-*bis*(2,4,6-tribromophenoxy) ethane (BTBPE) and decabromo-diphenyl ethane (DBDPE), are now being detected in the environment. However, contaminant bioavailability is influenced by the organisms' ecology (i.e., route of uptake) and in situ environmental factors. We observed that the filter-feeding bivalve (*Corbicula fluminea*) and grazing gastropod (*Elimia proxima*), collected downstream from a textile manufacturing outfall, exhibited TBB, TBPH, and BTBPE concentrations from 152 to 2230 ng g^{-1} lipid weight (lw). These species also contained additional BFRs. Maximum levels of total hexabromocyclododecane diastereomers ($\sum$HBCDs) in these species were 363 000 and 151 000 ng g^{-1} lw, and those of polybrominated diphenyl ethers ($\sum$PBDEs) were 64 900 and 47 200 ng g^{-1} lw, respectively. These concentrations are among the highest reported to date worldwide. While BDE-209 was once thought to be nonbioavailable and resistant to degradation, it was the dominant BFR present and likely debromination products were detected. Contributions of α- and β-HBCD were higher in tissues than sediments, consistent with γ-HBCD bioisomerization. Mollusk bioaccumulation factors were similar between HBCD and PBDEs with 4 to 6 bromines, but factors for TBB, TBPH, and BTBPE were lower. Despite different feeding strategies, the bivalves and gastropods exhibited similar BFR water and sediment accumulation factors.

INTRODUCTION

Brominated flame retardants (BFRs) are commonly added to constituent polymers in electronics, furniture, and textiles. With regard to the environmental properties and distribution, polybrominated diphenyl ethers (PBDEs) have been the most studied. Due to their bioaccumulation potential, persistence, and associated detrimental health effects following exposure, manufacturing of two of three PBDE formulations (i.e., Penta- and Octa-BDE) were discontinued in the U.S. in December 2004 [1] and added to the Persistent Organic Pollutants list of the Stockholm Convention in May 2009.[2] In July 2008, the third and most widely used PBDE formulation, Deca-BDE, was banned in electrical equipment in the EU [3] and will be phased out of U.S. production by the end of 2013.[4] A fourth BFR, hexabromocyclododecane (HBCD), primarily used in polystyrene insulation boards and to a lesser extent in textiles, is also considered persistent and bioaccumulative and may cause reproductive, developmental, and neurological disorders.[5,6] Under the EU's REACH program, HBCD will be phased-out of European commerce by mid-2015. In the U.S., HBCD is now under evaluation by the U.S. EPA.[7] In order to meet fire safety standards, new consumer products will need to contain alternative flame retardants. However, the environmental fate of many replacement products remains inadequately investigated.[8] Recently, several Penta- and Octa-BDE replacement products (2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and 2-ethyl-hexyl 2,3,4,5-tetrabromophthalate (TBPH), components of Firemaster-550 (Chemtura Corp., USA) and 1,2-*bis* (2,4,6-tribromophenoxy) ethane (BTBPE), a component of Fire-master-680 (Chemtura Corp., USA)) were observed in household dust, sewage sludge, aquatic biota, and sedi-ments.[8−10] Decabromodiphenyl ethane (DBDPE), offered as an alternative to Deca-BDE, has similar chemical structure and properties. Like Deca-BDE, DBDPE has also been detected in sewage sludge, air, sediments, fish and birds.[11,12] This compound, along with TBB, TBPH, and BTBPE, has also been reported in house and office dust.[13]

Table 1. PBDEs, Alternative BFRs and HBCD Concentrations in Sediments and Mollusks (*Corbicula fluminea* and *Elimia proxima*)

		sediments (ng g^{-1} TOC)				*Corbicula fluminea* (ng g^{-1} lipid)				*Elimia proxima* (ng g^{-1} lipid)			
sample matrix	location site [distance downstream of outfall (km)]	outfall	16.8 km	25.2 km	44.6 km	outfall	16.8 km	25.2 km	44.6 km	outfall	16.8 km	25.2 km	44.6 km
polybrominated diphenyl ethers (PBDEs)													
BDE-17	2,2',4-tri BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-28	2,4,4'-tri BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-47	2,2',4,4'-tetra BDE	4470	699	309	123	5230	2980	2650	1592	8660	795	958	601
BDE-49	2,2',4,5'-tetra BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-66	2,3',4,4'-tetra BDE	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-100	2,2',4,4',6-penta BDE	602	159	174	68	2030	1730	1060	804	2040	277	291	106
BDE-99	2,2',4,4',5-penta BDE	6870	1290	901	153	4080	2600	2500	1448	10 600	821	1420	518
BDE-85	2,2',3,4,4'-penta BDE	120	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-154	2,2',4,4',5,6'-hexa BDE	70	7	17	nd	113	56	nd	40	432	24	53	nd
BDE-153	2,2',4,4',5,5'-hexa BDE	301	112	121	nd	403	128	140	70	682	nd	nd	nd
BDE-183	2,2',3,4,4',5,6-hepta BDE	146	20	17	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDE-202	2,2',3,3',5,5',6,6'-octa BDE	nd	nd	nd	nd	56	32	nd	nd	nd	nd	7	nd
BDE-201	2,2',3,3',4,5,5',6-octa BDE	19	nd	14	nd	47	31	nd	10	nd	nd	6	nd
BDE-197	2,2',3,3',4,4',6,6'-octa BDE	100	37	38	nd	51	26	nd	nd	52	nd	8	nd
BDE-203	2,2',3,4,4',5,5',6-octa BDE	380	120	146	nd	111	47	nd	nd	79	nd	10	nd
BDE-196	2,2',3,3',4,4',5,6-octa BDE	554	136	153	283	99	35	nd	nd	70	nd	11	nd
BDE-208	2,2',3,3',4,5,5',6,6'-nona BDE	1300	559	1030	363	1220	111	78	20	341	45	73	19
BDE-207	2,2',3,3',4,4',5,6,6'-nona BDE	3860	1360	2140	731	2740	325	270	54	752	103	167	59
BDE-206	2,2',3,3',4,4',5,5',6-nona BDE	12 500	3160	7690	1070	4990	411	347	75	751	114	211	76
BDE-209	decabromodiphenyl ether	298 000	169 000	215 000	61 500	43 700	11 400	12 000	2388	22 700	2700	2500	2200
NBFRs: alternatives to Penta- and Octa-BDEs													
TBB	2-ethylhexyl 2,3,4,5-tetrabromobenzoate	3850	598	326	80	2220	nd	nd	nd	1740	nd	nd	nd
TBPH	di (2-ethylhexyl)-2,3,4,5-tetrabromophthalate	19 200	3120	3570	2000	1370	816	nd	nd	380	nd	99	36
BTBPE	1,2-bis (2,4,6-tribromophenoxy) ethane	2000	223	229	77	153	112	nd	nd	303	nd	nd	nd
% rec. BDE-166	2,3,4,4',5,6-hexa BDE	95%	129%	108%	87%	121%	120%	85%	93%	88%	71%	85%	88%
hexabromocyclododecane (HBCD)[a]													
α-HBCD	α-1,2,5,6,9,10-HBCD	16 400	2250	2410	351	43 900	16 900	9400	***	15 200	8160	5390	4020
β-HBCD	β-1,2,5,6,9,10-HBCD	35 300	4590	3840	641	109 000	35 400	32 100	***	28 600	11 900	6500	4530
γ-HBCD	γ-1,2,5,6,9,10-HBCD	338 000	76 500	46 000	11 200	210 000	26 800	77 900	***	107 000	34 800	23 900	12 800
% rec C13-α-HBCD	α-1,2,5,6,9,10-HB[^{13}C$_{12}$]CD	75%	61%	87%	93%	87%	100%	108%	***	74%	70%	67%	77%
%TOC or % lipid (dry weight based)		1.10%	1.60%	0.61%	0.41%	10.90%	10.45%	14.67%	11.89%	7.83%	6.00%	10.13%	9.29%

[a] *** Sample compromised and not analyzed. nd = not detected.

Toxic effects are mediated by contaminant bioavailability, which is controlled by the properties of the chemical and in situ environmental factors. Bioaccumulation in the real world is influenced by many factors. Their interplay may be too complex for simple laboratory studies to encompass. For example, lipophilic compounds (e.g., PBDEs) partition into organic-rich sediments. Benthic organisms that ingest or are in close contact with such sediments may be at greater risk of exposure. However, contaminants accumulated by such biota will eventually be redistributed to others via predation, scavenging, and other mechanisms. In freshwater environments mollusks constitute one of the largest groups, in terms of biomass, and serve as an important food source for aquatic and terrestrial predators. Over 650 species of gastropods and 300 species of bivalves inhabit freshwater systems in North America. Major molluscan feeding strategies include periphytan/detritus grazing/shredding (e.g., many gastropods) and filter-feeding (e.g., many bivalves). Bivalves have been long utilized for in situ monitoring of chemical body burdens (e.g., NOAA's Mussel Watch Program). However, 71% of native U.S. freshwater mussels are currently at risk of extinction.[14] Gastropods have also been used with increasing frequency for contaminants and effects monitoring and some are particularly sensitive to impacts such as imposex.[15] As an alternative to sacrificing native species, sampling of non-native invasive species, e.g., asian clams (*Corbicula sp.*), has been suggested for chemical exposures studies. Past studies have examined acute and chronic exposures to single and multichemical stressors including metals, surfactants, pesticides, and industrial or municipal effluents in the laboratory and the field.[14] While *Corbicula* spp. is native to Africa, Australia, and Asia, they have become established in parts of Europe and 41 U.S. states, including Hawaii.[16] This wide distribution offers the opportunity for in situ regional, continental, and intercontinental biomonitoring research using a single test organism.

On the basis of data from the U.S. EPA's Toxics Release Inventory (TRI), we previously identified a North Carolina textile-manufacturer as a likely substantial contributor of Deca-BDE to a municipal wastewater treatment plant (WWTP) and local surface waters.[17] We hypothesized that replacement BFRs may also be used at this facility and accumulate in local sediments and mollusks (e.g., *Corbicula fluminea* and *Elimia proxima*). The BFRs examined here included: HBCD, constituent congeners of the Penta-, Octa-, and Deca-BDE formulations and several PBDE alternatives (i.e., TBB, TBPH, BTBPE, and DBDPE). While laboratory-derived bioaccumulation factors are valuable for evaluating uptake potential, use of in situ exposed organisms allows consideration of bioaccumulation potential under environmentally realistic conditions. Hence, our goal was to evaluate in situ bioaccumulation of PBDE, HBCD, and alternative BFRs in two benthic invertebrates (*Corbicula fluminea* and native *Elimia proxima*) with differing feeding strategies (i.e., grazing vs filter feeding).

EXPERIMENTAL SECTION

The bivalve *Corbicula fluminea*, gastropod *Elimia proxima* and surficial sediments were collected in July 2009, from the Yadkin River, downstream of a North Carolina WWTP outfall. This treatment facility was constructed in 1968 to service a local textile mill. This WWTP was upgraded in 2003 to allow treatment of up to 16 million liters per day. According to their National Pollutant Discharge Elimination System (NPDES) permit this facility is owned and operated by a local textile

manufacturer and treats ∼92% industrial process wastewater and ∼8% domestic sewage. Treatment includes bar and fine screening, aeration, dual clarifiers, aerobic digesters, and sludge drying beds. Mollusks and sediments were obtained at the outfall, 16.8, 25.2, and 44.6 km downstream and 0.2 km upstream (sediment only) of the outfall. Samples were collected in precleaned 1 L glass jars with Teflon lids and stored at <4 °C. At the laboratory, mollusks were rinsed with reagent grade water, shells were removed, and tissue was placed into stainless steel pans. Sediment and tissue were freeze-dried and stored at <0 °C until analyzed. Two sediment aliquots from each collection site were analyzed for total organic carbon (TOC). TOC means reported in Table 1, %RSD range 5−37%. TOC was determined by combustion and infrared detection (CE-440, Exeter Analytical, North Chelmsford, MA).

For BFR analysis dried samples (20 g sediment and ∼2 g tissue) were subjected to accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA) with methylene chloride. Surrogate standards (200 ng of 2,3,4,4′, 5,6-hexabromodiphenyl ether (BDE-166); Cambridge Isotope Laboratories, Inc., Andover, MA and 200 ng of ^{13}C-labeled α-HBCD; Wellington Laboratories, Ontario, Canada) were added to each sample prior to extraction. Extracts were purified by size exclusion chromatography (SEC, Envirosep-ABC, 350 × 21.1 mm. column; Phenomenex, Torrance, CA). Each post-SEC extract was reduced in volume, added to the top of a solid phase 2-g silica glass extraction column (Isolute, International Sorbent Tech.; Hengoed Mid Glamorgan, U.K.) and eluted with 3.5-mL hexane (fraction one), followed by 6.5 mL of 60:40 hexane/DCM and then 8 mL DCM (fraction two). The second fraction, containing the compounds of interest, was reduced in volume and solvent exchanged to hexane. Decachlorodiphenyl ether (DCDE; 100 ng; Ultra Scientific, North Kingstown, RI) was then added as the internal standard and the extracts were analyzed for PBDEs, TBB, TBPH, BTBPE, and DBDPE. Identification and quantitation were done by gas chromatography-selective ion monitoring mass spectrometry (GC/SIM−MS). For HBCD analysis, extracts were solvent exchanged to methanol and spiked with 100 ng of d18-labeled α-HBCD (Wellington Laboratories, Ontario, Canada), as an internal standard. These were then analyzed for α-, β-, and γ- isomers by ultraperformance liquid chromatography tandem mass spectrometry (UPLC−MS/MS). Surrogate recoveries and method blanks were evaluated along with sample analyses. Results were surrogate recovery corrected. Recoveries ranged from 71 to 129% for BDE-166 and 61 to 108% for ^{13}C-α-HBCD (Table 1). Blanks did not exhibit any targeted compounds above the quantitation limit (QL) of 1 ng g^{-1}, dw. (Details of instrumentation and operating conditions for GC/SIM-MS and UPLC−MS/MS, along with quality assurance procedures and BFR matrix-spiking experiments and results can be found in the Supporting Information, SI, section. Spiking results are listed in Table S1.) Statistical analyses were performed using SigmaStat for Windows, version 2.0. Analysis of variance (ANOVA) was used to examine differences ($P < 0.05$) between bivalve and gastropod BFR bioaccumulation factors (BAFs).

RESULTS AND DISCUSSION

Polybrominated Diphenyl Ethers. PBDEs were not detected above the 1 ng g^{-1} (dw) QL in sediments from the site 0.2 km upstream from the WWTP outfall. However, sediments at the outfall exhibited 16 of 20-targeted PBDE congeners, totaling 329 000 ng g^{-1} (normalized to TOC)

(Table 1). This concentration rivals those in sediments from the highly industrialized and populated Pearl River Delta (PRD), P.R. China, previously reported as the highest concentration worldwide.[18,19] Concentrations of $\sum$PBDEs detected in *Corbicula fluminea* and *Elimia proxima* collected at the outfall were also considerable, totaling 64 900 and 47 200 ng g^{-1} lipid weight (lw), respectively (Table 1). BDE-17, -28, -49, and -66 were not detected. PBDE sediment and mollusk profiles were dominated by congeners of the Deca-formulation and their $\sum$PBDEs decreased with distance downstream from the outfall. However, there was a notable spike in $\sum$PBDEs sediment concentration at the 23.2 km site, i.e., 228 000 ng g^{-1} TOC. This pattern was also observed in the gastropods and bivalves and may have resulted from historical changes in local industrial BFR release rates, or past river flow events (e.g., flooding or WWTP pass through). Chen et al.[20] recently documented a temporal decrease in PBDE concentrations in finfish in another North Carolina riverine system, the Hyco that exhibited extensive PBDE contamination. There, the PBDEs were believed to have entered via a municipal WWTP, following release from textiles and plastics manufacturing. PBDEs were also detected in sediments and mollusks at our most downstream station, 44.6 km from the outfall (Table 1). Concentrations at this site also exceeded those of sediment and marine mussels (*Perna viridis*) of the PRD.[19]

Relative abundance of PBDE congeners in sediments and mollusks in our study were BDE-209 $\gg$ −206 > −99, −207, −47 > −208 and −100. The major constituent congener of Deca-BDE,[21] BDE-209, contributed 90 to 96% of the sediment total PBDE concentration. According to the U.S. EPA's TRI the on-site wastewater treatment facility released 6000 kg of BDE-209 to surface waters between 2000 and 2004.[17] However, TRI estimates are often not based on actual chemical measurements. Hence, their accuracy is uncertain. The log K_{ow} of BDE-209 exceeds 12 (Table S1 of the SI) indicating extreme hydrophobicity. In surface waters and wastewater streams it is largely particulate bound. In WWTPs, BDE-209 will primarily reside in sewage sludge.[22] However, treated effluents also contain entrained solids. These may be released to receiving streams and any PBDE-laden particles may eventually become incorporated in sediments. Sediments in proximity to wastewater outfalls have previously been observed to be dominated by BDE-209.[23,24] In the present study, BDE-209 was the major PBDE congener detected in both bivalves and gastropods collected at the outfall. However, the percent contribution of BDE-209 to total PBDEs was lower (37 to 67%) than in sediments. This likely relates to its strong association with particulates and attendant lower bioavailability. Moon et al.[25] reported a similar percentage contribution of BDE-209 in marine bivalves from Korean waters. In contrast, BDE-47, -99, and -100 contributed 18 to 59% of total PBDEs in these mollusks, while their contributions in sediments ranged from only 0.5 to 3.6%. This greater bioaccumulation of the lower brominated diphenyl ethers in mollusks is likely due to their intermediate log K_{ow} (<8). Chen et al.[20] reported that BDE-209 contributed less than 1% to the $\sum$PBDE in several finfish species from the Hyco River. The lower BDE-209 in finfish may be due in part to its metabolic debromination to lower brominated PBDEs (e.g., BDE-154 and -202) or decoupling of direct exposure from the sediments. This phenomenon has also been previously reported in finfish by others.[26,27]

Alternative BFRs: TBB, TBPH, BTBPE, and DBDPE. In light of market pressures and regulatory actions to reduce PBDE usage, BFR manufacturers have promoted alternatives to meet mandated fire safety standards. Of the four replacements sought, TBB, TBPH, and BTBPE were detected in sediments at the textile WWTP outfall, at 3850, 19 200, and 2000 ng g^{-1} TOC, respectively (Table 1). None of these substitutes were detected (>1 ng g^{-1}, dw) in sediments collected at our upstream site. DBDPE was not detected in any of our mollusk or sediment samples. Its absence may be due to the continued usage of Deca-BDE or the use of other alternatives, e.g., HBCD. Chen et al.[20] reported an increase in the abundance of HBCDs in finfish at another North Carolina riverine site impacted by textile-related discharges. We detected TBB, TBPH, and BTBPE in all of the sediments collected downstream from the WWTP outfall. At the 44.6 km sampling site sediment concentrations were 80, 2000, and 77 ng g^{-1} TOC, respectively. Four formulations of TBB, TBPH, and BTBPE are manufactured by Chemtura Corp. USA. The product FF-680 consists of 100% BTBPE by weight according to the manufacturer's Material Safety Data Sheet (MSDS). Firemaster-550 (FM-550), Firemaster BZ-54 (BZ-54), and DP-45 are mixtures of TBB and TBPH. Of these three formulations, only DP-45 lists compositional details on its MSDS sheet, i.e., >91% TBPH and 2−6% TBB, by weight. Components of the other two are designated as trade secrets. However, FM-550 and BZ-54 have been reported to contain TBB and TBPH.[9,28] According to Stapleton et al.[9] FM-550s TBB to TBPH ratio is 4:1. FM-550 also is reported to contain isopropylated triaryl phosphate and triphenylphosphate at approximately 50% by weight.[9] In the sediments collected at the outfall and downstream, TBPH was the primary alternative BFR we detected, comprising 77 to 93% of the total. On the basis of the available product information, this suggests that manufacturers in this area may have used primarily DP-45. However, TBPH has been reported to be less vulnerable to photodegradation and to possess a half-life twice that of TBB.[28] The change in the TBPH/TBB ratio from 5:1 at the outfall to 25:1 at the 44.6 km downstream site may result from degradative processes. However, local BFR product usage and composition may also be factors.

TBB, TBPH, and BTBPE were also detected in the bivalve and gastropod samples collected at the outfall (Table 1). In these biota, TBB was the most abundant alternative BFR detected (2220 and 1740 ng g^{-1} lw in bivalves and gastropods, respectively), followed by TBPH (1370 and 380 ng g^{-1} lw) and BTBPE (153 and 303 ng g^{-1} lw). TBB has a less extreme log K_{ow} (8.75) than BTBPE (9.15) or TBPH (11.95) (Table S2 of the SI) which suggests a greater bioaccumulation potential. Interestingly, TBB was only detected in the mollusk samples collected at the outfall, but BTBPE and TBPH were also observed in the bivalves collected at the 16.8 km downstream site (112 and 816 ng g^{-1} lw, respectively). TBPH was detected in both bivalve and gastropod samples from the 44.6 km site, 37 and 36 ng g^{-1} lw, respectively. The sediment concentration of TBPH was 25-fold higher at this site than TBB or BTBPE.

Hexabromocyclododecane. Data on HBCD levels in the North American environment, and the U.S. in particular, are limited. While its use here is generally assumed to be lower than in the EU, this presumption is based on outdated 2001 market demand statistics. These indicated that in North America HBCD consumption is less than 10% of PBDEs and constitute less than 17% of the global HBCD market demand.[29] However, 2006 U.S. production/import volumes were between 4535 and 22 679 tonnes.[30] This represents a 2- to 10-fold

increase in HBCD demand from 2001 to 2006. It is possible that current North American HBCD consumption may now rival that of the EU (9840 tonnes for 2010).[31] In our outfall sediment ΣHBCD (α-, β-, and γ-HBCD) was the most abundant BFR at 390 000 ng g^{-1} TOC, total (Table 1). ΣHBCD was also detected at every collection site downstream from the outfall, ranging from 88 300 to 12 200 ng g^{-1} TOC. However, HBCD was not detected (>1 ng g^{-1}, dry weight) at the upstream site. The technical HBCD formulation consists of three diastereomers: γ-HBCD isomer (75−89%), followed by β-HBCD (10−13%) and α-HBCD (1−12%).[5] However, HBCD-treated finished products (e.g., insulation boards and textiles) may contain higher proportion of the α-diastereomers than that of commercial HBCD mixtures.[32,33] It has been reported that γ-HBCD can isomerize at temperatures above 160 °C, forming α-HBCD, e.g. during polystyrene production; and the drying and curing of treated textiles.[33] Isomerization of β- and γ- to α-HBCD has also been observed in aquatic organisms.[11] HBCD in most marine mammals has been reported to be >80% α-HBCD.[32,34] The isomer composition reported in house dust [presumably released from finished products, e.g., building materials (EPS and XPS) and textiles] is 33% α-, 11% β- and 56% γ-HBCD.[35] Similar HBCD isomer profiles were observed in sewage sludge (42% α-, 6% β-, and 52% γ-HBCD) from a municipal WWTP that received wastewater from an automotive interiors manufacturer.[10] Differing degradation rates between isomers have also been reported for sewage sludge and sediments.[36,37] For example, Davis et al.[37] observed β- and γ-HBCD degradation rates in sludge 28% to 47% lower than those in freshwater sediments. However, the γ-isomer is typically the most abundant HBCD stereoisomer reported in sediments.[32,34] In our sediment sample, γ-HBCD represented >87% of ΣHBCD set, (Table 1). The release of the technical HBCD product (75−89% γ-HBCD) to waste streams by industries that utilize HBCD in their products and from HBCD production and processing facilities may explain this pattern.[20] However, γ-HBCD's 2-fold greater degradation rate contrasts with the γ-HBCD-dominated sediment isomer profiles observed tens of km downstream from the presumed sources.

ΣHBCD was also the most abundant BFR detected in the biota samples. At the outfall ΣHBCD concentrations in the gastropods and bivalves were 151 000 and 363 000 ng g^{-1} lw, respectively. At the 44.6 km site, the ΣHBCD concentration in gastropods was 21 400 ng g^{-1} lw. (Unfortunately, the bivalve sample from the 44.6 km site was compromised during HBCD sample preparation and was not analyzed.) The dominant diastereomer, γ-HBCD, constituted 34 to 71% of ΣHBCD in every biota sample (except for the bivalve sample collected at the 16.8 km site). Although their log K_{ow}s [range 5.07−5.47 (Table S2 of the SI)] would indicate similar bioavailability, the percent contribution of α- and β-HBCD compared to γ-HBCD was higher in the mollusks (29−66%) than in the sediments (8.1−13%) at these collection sites. This may reflect biologically mediated isomerization of γ-HBCD in aquatic invertebrates.[32] Alternatively, it may relate to lower bioavailability of γ-HBCD, which exhibits the lowest water solubility of the three diastereomers (i.e., 48, 15, and 2 μg L^{-1} for α-, β- and γ-HBCD, respectively[38]).

Biota-Sediment Accumulation Factors (BSAFs). The BSAF is a useful tool for assessing the bioaccumulation of lipophilic compounds in aquatic environments. It is defined as follows: BSAF = C_t /C_s; where C_t = a substance's lipid-

normalized steady-state concentration in tissue of an aquatic organism and C_s = its sediment organic carbon-normalized concentration. A BSAF exceeding 1.0 implies that bioaccumulation has occurred.[39] Laboratory-derived BSAF measurements are often used to predict tissue concentrations in wildlife. However, since ecosystems are complex and rarely at steady state, BSAFs in the environment often vary more than laboratory-determined estimates.[40,41] Feeding strategies and biotransformation capabilities may further impact BSAFs. However, strong correlations from site- and species-specific determinations have been observed between log transformed BSAF and log n-octanol/water partition coefficient (log K_{ow}) values for nonionic halogenated organic compounds (e.g., PBDEs). These often exhibit a parabolic behavior, with maximum BSAF values for compounds with log K_{ow} values between 6 and 8 (e.g., BDE-47, -99, -100).[42] (See SI Table S2 for log K_{ow} values.)

Log BSAFs determined for the BFRs we detected in *Corbicula fluminea* and *Elimia proxima* ranged from −1.9 to 1.1. A parabolic relationship was observed between log BSAF values and corresponding log K_{ow} values for both mollusks (Figure 1a,b), consistent with previously published observations. However, BASFs for α- and β-HBCD isomers exceeded expectations, suggesting preferential accumulation of α- and β-HBCD in bivalves and gastropods. In contrast, the BASF values for γ- HBCD were <0. Our log BSAF values for the PBDEs
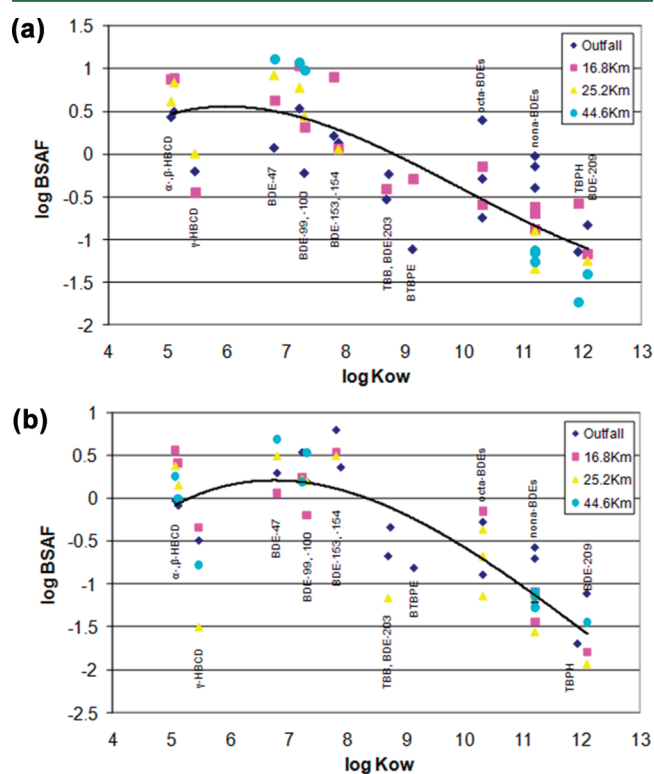
Figure 1. (a) Relationship between log biota-sediment accumulation factors (log BSAF) in *Corbicula fluminea* and log K_{ow} for several flame-retardants. A log BSAF > 0 indicates greater accumulation in tissues than sediments. Solid line indicates best fit polynomial third order, r^2 = 0.677. (b) Relationship between log biota-sediment accumulation factors (log BSAF) in *Elimia proxima* and log K_{ow} for several flame-retardants. A log BSAF > 0 indicates greater accumulation in tissues than sediments. Solid line indicates best fit polynomial third order, r^2 = 0.677.

were approximately half those previously reported [42] for the field-exposed marine mussel (*Modiolus modiolus*); but were closer to reported log BSAF values of PCBs. This may relate to discrepancies in the species assessed or ecological or environmental differences in the two studies. In both studies, however, a parabolic pattern was apparent for the PBDEs, with maximum BSAF values for congeners with log K_{ow} between 6 and 8 (i.e., BDE-47, -99, -100, -153, and -154). At the collection site adjacent to the Yadkin River textile outfall, BSAFs for BFRs with log K_{ow} < 10 were lower than those at the other stations; potentially indicating greater bioavailability with distance downstream (Figure 1a). This was also observed by Debruyn et al. [42] for PCBs in mussels collected downstream from a municipal wastewater outfall. In our study, BFRs with log K_{ow} values >8 (PBDEs with greater than seven bromines, TBB, TBPH, and BTBPE) exhibited negative log BSAFs, suggesting low bioavailability for both bivalves and gastropods. Despite differences in feeding strategies, a strong correlation was observed for log BSAF values between bivalves and gastropods (r^2 of 0.772, slope 0.875) (Figure S1 of the SI), indicating similar BFR accumulation in these mollusks (ANOVA, P > 0.05).

Bioaccumulation Factors (BAFs). The BAF_w is defined as the ratio of a substance's lipid-normalized concentration in an aquatic organism (C_t) to its organic carbon-normalized concentration in sediment pore water (C_w), i.e., $BAF_w = C_t/C_w$.[39] Sediment pore water concentrations (C_w) may be estimated from $C_w = C_s/f_{oc}K_{oc}$, where f_{oc} is the organic carbon fraction of the sediment. (Organic carbon−water partition coefficients (K_{oc}) are listed in Table S2 of the SI.) Hydrophobic compounds (compounds with log K_{oc} > 4) in the aquatic environment partition preferentially into sediment organic matter. If the log BAF_w of a chemical is equal to its log K_{oc}, then partitioning is considered to be controlled by passive diffusion. This is indicated by the dotted-line in Figure 2. However, the
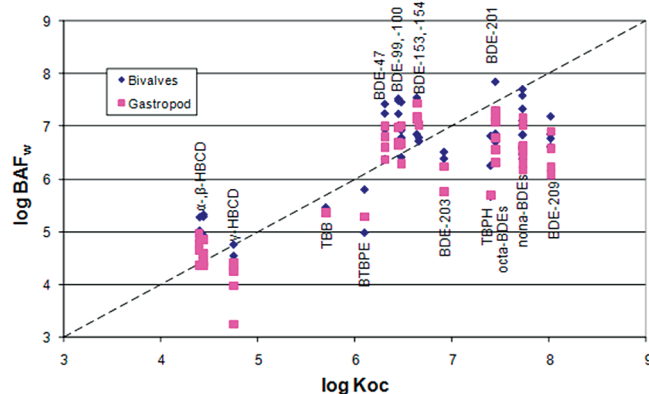
Figure 2. Relationship between log bioaccumulation factors (log BAF_w) and log Koc for various BFRs in *Corbicula fluminea* (bivalve) and *Elimia proxima* (gastropod). Dotted line indicates accumulation according to the equilibrium partitioning theory.

composition of the organic matter (e.g., black carbon), as well as environmental conditions (e.g., salinity), can also influence the bioavailability of chemicals.[43,44] Lower than predicted accumulation was observed in both bivalves and gastropods in our study, i.e., compounds with a log K_{oc} > 6.9 (PBDEs with eight or more bromines and TBPH) fell below the indicated dotted line (Figure 2). An exception was BDE-201 in bivalves collected at the outfall. A possible explanation may be in vivo

formation of BDE-201 via debromination of higher brominated diphenyl ethers such as BDE-209. Roberts et al. [45] exposed BDE-209 to carp hepatic microsomes which formed BDE-201 and other PBDEs and Ikonomou et al. [46] suggested that mechanisms other than biomagnification may be the cause for the unexpected PBDE congeners they observed in aquatic biota. We also observed a greater than predicted accumulation potential for tetra-, penta-, and hexa-BDEs indicated by their log BAF_w exceeding their log K_{oc} (Figure 2.). Different patterns were also observed for the HBCD isomers (Figure 2). The log BAF_w of γ-HBCD was less than predicted. In contrast, the log BAF_w for α- and β-HBCD suggested higher bioavailability. However, the log K_{oc} of the latter two isomers are slightly lower (<0.4) than that of γ-HBCD. As previously mentioned, α-HBCD can be formed by the bioisomerization of γ-HBCD and this may be a factor in the latter's depletion. The log BAF_w for TBB and BTBPE were also below the predicted accumulation (dotted-line). TBB and BTBPE have log K_{oc} values less than those of tetra-, penta-, and hexa-PBDEs; indicating lower sediment organic carbon associations. While this may also indicate metabolic alteration of these compounds, brominated metabolites of TBB, TBPH, and BTBPE were not observed. However, hydroxylated and debrominated degradates have been observed in BTBPE-dosed laboratory rats.[47] TBB and TBPH have also been reported to undergo photodegradation [28] and possible TBB debromination products have been detected in sewage sludge.[10] As was the case for the BSAFs, the slope of the line comparing BAF_ws between the two mollusk species approximated 1.0 (slope 0.980, r^2 of 0.858) (Figure S2 of the SI), indicating similar BFR accumulations (ANOVA, P > 0.05), despite different feeding strategies.

While PBDE congeners with four to six bromines exhibited the highest BSAFs and BAF_ws, PBDEs with 8−10 bromines (e.g., BDE-209) still appeared bioavailable. Due to the substantial local discharge of the highly brominated congeners, they contributed 40 to 82%, by weight, of the $\sum$PBDE body burden of the mollusks. The log BSAFs of α- and β-HBCD were comparable to PBDEs with 4 to 6 bromines (e.g., BDE-47, -99, and -154), although their accumulation may have been augmented by isomerization of γ-HBCD. BSAFs and BAFs for BTBPE, TBB, and TBPH were generally an order of magnitude lower than those of BDE-47, -99, -100, -153, and -154 (constituents of the commercial Penta-formulation) indicating lower potential for accumulation. However, several octabrominated BDE congeners appeared to bioaccumulate. Several of the alternative BFRs appeared to be environmentally persistent, as indicated by their detection (e.g., TBPH 2000 ng g^{-1} TOC) in sediments 44.6 km downstream from their probable point of release. In conclusion, as the usage of HBCD, TBB, TBPH, and BTBPE may be increasing due to recent and pending restrictions on PBDEs, further research is needed on their sources, release mechanisms, environmental fate and distribution, as well as potential biological effects.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Additional details on method development, quality assurance, matrix-spiking experiments, and results along with Tables S1 and S2 and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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