



Bioaccumulation of polybrominated diphenyl ethers in harbor seals from the northwest Atlantic

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ARTICLE INFO

Article history:

Received 10 June 2008

Received in revised form 14 August 2008

Accepted 2 September 2008

Available online 23 October 2008

Keywords:

Brominated flame retardants

BFRs

PBDEs

Deca-BDE (BDE-209)

Debromination

Harbor seal

ABSTRACT

Polybrominated diphenyl ethers (PBDEs) were analyzed in blubber of harbor seals (*Phoca vitulina concolor*) collected between 1991 and 2005 along the northwest Atlantic. Σ PBDE concentrations (mono- to hexa-BDEs) detected in blubber samples ($n = 42$) ranged from 80 to 25720 ng g⁻¹ lw, (overall mean 2403 ± 5406 ng g⁻¹ lw). By age, mean Σ PBDE concentrations were: 3645 ± 7388, 2945 ± 5995, 1385 ± 1265, and 326 ± 193 ng g⁻¹ lw in pups, yearlings, adult males, and adult females, respectively. Unlike the trend for PCBs, no decreasing gradient from urban to rural/remote areas was observed for PBDEs in these samples, likely reflecting inputs from local sources. No significant temporal trend was observed for PBDEs in harbor seals between 1991 and 2005, although congener profiles shifted over time. Tetra-BDE-47 was the dominant congener, followed by BDEs-99, -100, -153, -154, and -155 in varying order, suggesting exposure to the penta-BDE product. In adult males, the hexa-BDEs contributed more to the total (22%) than BDEs-99 and -100 (14%), and concentrations of BDE-155 were elevated compared with -154. Higher BDEs were detected in a subset of seals ($n = 12$) including hepta-BDE-183, the marker for the octa-BDE mixture, and octa-BDE-197, along with several unidentified hepta- and octa- congeners. BDE-209 was detected in seal blubber at concentrations ranging from 1.1 to 8 ng g⁻¹ lw, indicating that deca-BDE is bioavailable in this marine food web. This is the first study to document the accumulation of BDE-209 at measurable levels in wild harbor seals. While the PBDE patterns in blubber indicate exposure to all three BDE commercial mixtures, the data also suggest that BDE-209 debromination by seal prey fish may contribute to the loading of lower brominated congeners (hexa- to octa-BDEs) in these seals.

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1. Introduction

The brominated flame retardants polybrominated diphenyl ethers (PBDEs) are compounds that biomagnify in food webs and are associated with endocrine-disrupting and reproductive/neurodevelopmental effects in animals (Darnerud, 2003; Birnbaum and Staskal, 2004). As a result of their environmental persistence and widespread use in household and commercial products including textiles, furniture, plastics, polyurethane foams, and electronic equipment, PBDEs have become ubiquitous global contaminants, even in remote areas (Hites, 2004; de Wit et al., 2006). Marine mammals are long-lived, apex predators in marine ecosystems that accumulate relatively high PBDE levels (>1000 ng g⁻¹ lw), with congener patterns suggestive of exposure to the penta-BDE commercial mixture (Hites, 2004). Temporal studies suggest that PBDEs in marine mammals have been increasing in recent decades

(Ikonomou et al., 2002; She et al., 2002), presumably due to the increased use of these compounds in consumer products. Historically, of the three commercial PBDE mixtures, the global market for the penta-BDE formulation was dominated by North America, with usage estimated at 7100 metric tons (95%), whereas North American usage of the octa-BDE mixture was an estimated 1500 metric tons (40%) (BSEF, 2003). The penta- and octa-BDE formulations were banned from use in Europe in 2004 (European Union, 2003) and both products were subsequently withdrawn from the US market (Betts, 2008). The third commercial product, deca-BDE (consisting of >97% BDE-209), represents 83% of the global market demand (BSEF, 2003), and has only recently begun to be regulated in Europe and the US (Betts, 2008). North American usage of deca-BDE in 2001 was estimated at 24500 metric tons (44%) compared with Europe (13%) and Asia (41%). In the US, the state of Maine has enacted legislation prohibiting the use of deca-BDE in mattresses and residential upholstered furniture sold within its borders, and will extend the ban to electronics in 2010. Washington state prohibits the use of deca-BDE in mattresses and is considering a ban

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on its use in furniture and electronics. Similar legislation is pending in other US states and in Asian countries. Nevertheless, large amounts of deca-BDE have been released to the global environment and this chemical is still in high-volume use.

In addition to direct exposure to commercial PBDE mixtures, there is concern that metabolic debromination of higher brominated congeners may be a source of exposure and/or uptake of less brominated BDEs in marine biota. Although the fully brominated BDE 209 is often a major congener in marine sediments (de Boer et al., 2003; Zegers et al., 2003), only trace levels have been detected in marine mammals (Law et al., 2006). This has been attributed to a low uptake rate due to the large molecular size of the compound or rapid excretion after biotransformation (Boon et al., 2002). Biotransformation of BDE-209 has been demonstrated in freshwater fish, resulting in the formation of hexa- through nona-BDEs, which are more persistent and bioaccumulative than the parent compound (Stapleton et al., 2004, 2006a). Moreover, a recent field study provided evidence that freshwater fish in the wild may also possess the ability to debrominate BDE-209 to form lower brominated congeners (octa- and nona-BDEs) with similar substitution patterns (La Guardia et al., 2007). In humans, BDE-209 is more readily transformed and/or eliminated than are lower brominated diphenyl ether congeners, as the apparent half-lives of deca- to hepta-BDEs in serum increase with decreasing numbers of bromine atoms (Sjödén et al., 1999; Geyer et al., 2004; Thuresson et al., 2006). A short half-life was indicated for BDE-209 ($t_{1/2} = 15$ d) in serum of workers exposed to deca-BDE, along with a relative increase in octa- and nona-BDEs that were present at trace levels in the technical mixture, suggesting that BDE-209 may be metabolized in humans (Thuresson et al., 2006). Recently, hydroxyl-BDE congeners were shown to accumulate in human serum via metabolic pathways similar to those for OH-PCBs (Athanasiadou et al., 2008). In rats and common carp (*Cyprinus carpio*), a rapid clearance ($t_{1/2} = 2.4$ d) was indicated for BDE-209 (Sandholm et al., 2003; Stapleton et al., 2004). Mörck et al. (2003) reported that BDE-209 was metabolized, excreted, and only marginally distributed to adipose tissue in the rat, but was found in plasma and highly perfused tissues along with traces of nona-BDEs. The presence of hydroxyl and methoxy-BDE congeners in tissue and plasma suggested that rats may initially debrominate BDE-209, but then other metabolic pathways become dominant, resulting in the formation of polar metabolites that are likely to be excreted. In captive gray seals (*Halichoerus grypus*), a half-life ($t_{1/2} = 8$ –13 d) similar to that in humans was estimated for BDE-209 in serum (Thomas et al., 2005). The gray seals exposed to deca-BDE spiked food retained measurable amounts of BDE-209 in blubber stores after it was no longer detectable in blood, suggesting that seals may accumulate this congener. However, it is unclear to what extent BDE-209 uptake and accumulation may occur in wild seals.

The aim of this study was to characterize exposure to PBDEs (mono- to deca-BDEs) in blubber tissue of harbor seals (*Phoca vitulina concolor*) collected between 1991 and 2005 from the northwest Atlantic. The most abundant marine mammal species in the region, harbor seals are closely associated with polluted near-shore environments and are considered to be important indicators of coastal contamination (Shaw et al., 2005; Shaw et al., 2006; Shaw et al., 2007). This is the first study to report the bioaccumulation of BFRs in pinnipeds from the northwest Atlantic marine ecosystem.

2. Materials and methods

2.1. Samples

Blubber samples were collected from freshly dead stranded harbor seals ($n = 42$; 7 adult males, 8 adult females, 14 yearlings, 13

pups) between 1991 and 2005 along the northwest Atlantic coast from eastern Maine to Long Island, New York as part of the North-east Region Marine Mammal Stranding Program (Fig. 1). Seals were weighed, and standard length and axillary girth were measured. Age was estimated based on body size. Blubber samples were stored in a freezer at -40 °C until analysis.

2.2. Chemical analysis

Harbor seal blubber samples were analyzed for 41 PBDE congeners (mono- through octa-BDEs and BDE 209) following the isotope dilution technique (Lee, 2007). Briefly, approximately 5 g of blubber was spiked with $^{13}\text{C}_{12}$ -labeled BDE-3, 15, 28, 47, 99, 100, 118, 153, 183, and 209 (Cambridge Isotope Laboratories, Andover, MA) and was ground with anhydrous sodium sulfate and silica gel (LSP1, Whatman Inc., Clifton, NJ) in 50 mL of 50% dichloromethane in hexane with a Polytron PT300 tissue mixer (Brinkmann Instrument Inc., Westbury, NY). The organic phase was collected and the solid residue was extracted twice more with the same solvent. The combined extract was volume-reduced and solvent-exchanged to 20 mL in hexane. An aliquot of the extract (2 mL) was used for the determination of lipid content by gravimetry. The majority of the remaining extract (16 mL) was purified by Power-Prep, a commercially available automated multi-column clean-up system (Fluid Management Systems, Waltham, MA). This system used high-capacity ABN silica, classical ABN silica (40–45% sulfuric acid by weight), basic alumina (11 g), and carbon/celite (0.34 g) columns to separate PBDEs from other classes of organic pollutants (e.g. pesticides and PCBs) and matrix interferences by fractionation. Collected PBDE fractions were volume-reduced and solvent-exchanged to 50 μL in iso-octane. The injection internal standard (1.5 μL), containing 400 ng g^{-1} 2,4,6-trichlorobiphenyl, 3,3',4,4'-tetrabromobiphenyl and 2,2',4,4',6,6'-hexabromobiphenyl, was added to the concentrated extracts prior to GC-MS analysis. Quantification of 36 mono- to hexa-BDEs was performed by HRGC-LRMS (HP 6890 GC) with a DB-XLB column, 30 m \times 0.25 mm i.d. \times 0.25 μm film thickness coupled to a mass spectrometer (HP 5972) and monitored by selected ion monitoring (SIM) at the two most intensive ions in the molecular ion or the M-2Br ion cluster. Analysis of hepta-, octa- and deca-BDE was carried out on an Rtx-5MS column, 15 m \times 0.25 mm i.d. \times 0.1 μm film thickness, coupled to a MAT-95 XP high-resolution ($R \sim 9000$) mass spectrometer and GC (Thermo Trace GC Ultra) or an Autospec Q high-resolution ($R \sim 4000$) magnetic sector mass spectrometer and HP5890GC. All PBDE concentrations were calculated using the internal standard method and were corrected by surrogate recoveries. Total (Σ) PBDEs represent the sum of all identified mono- to hexa-BDE congeners unless specified. Data are presented on a lipid weight (lw) basis.

A method blank (i.e. pharmaceutical-grade fish oil), a matrix spike (i.e. fish oil spiked with representative levels of selected PBDEs) and a duplicate seal blubber were analyzed with every batch of 10 samples. Method detection limits (MDLs) for 36 mono- to hexa-BDEs, defined as 3.14 ($p = 0.01$) times the standard deviation of seven matrix spikes (Federal Register, 1984), ranged from 0.05 to 0.86 ng g^{-1} wet wt. The limits of quantification for hepta-, octa-, and deca-BDE were estimated as 10 times the instrumental detection limits (Zhao et al., 2007), ranging from 0.006 to 0.4 ng g^{-1} wet wt. The average recovery for $^{13}\text{C}_{12}$ -BDE 3, 15, 28, 47, 99, 100, 118, 153 and 183 was 61 \pm 28%, 60 \pm 26%, 65 \pm 24%, 75 \pm 25%, 63 \pm 22%, 73 \pm 21%, 65 \pm 23%, 54 \pm 30%, and 47 \pm 21%, respectively. The laboratory-controlled spikes were used to monitor the analytical accuracy and the relative bias in measurement ranged from -8.5% to 9.9%. The method was also validated by analyzing Standard Reference Materials (a duplicate of SRM 2977) to measure the accuracy of our method (Lee, 2007). There are no certified values for PBDE congeners for SRM 2977, so we compared our measurements with results from other



Fig. 1. Map of the northwest Atlantic showing the harbor seal range and locations of stranded seals sampled in this study.

sources available (Zhu and Hites, 2003; Schantz et al., 2006). We also participated in a NIST intercomparison exercise in 2005 for PCB, pesticide, PBDE and PAH analyses. Overall, our measurements for PBDEs in SRM 2977 were comparable to the results reported by both Schantz et al. (2006) and Zhu and Hites (2003). Precision of the method was evaluated by one duplicate analysis of seal blubber per 10 samples analyzed. Across the identified congeners, relative percent difference ranged from 0.1% to 21.4%. To avoid or minimize exposure of extracts of samples to fluorescent light, amber glassware was used or transparent glassware was covered with aluminum foil during the procedures.

2.3. Statistics

Statistical analyses were performed using SPSS 14.0 Statistical Package. Concentrations below the level of detection were calculated by treating the result as half the method detection limit. Due to unequal variances and sample sizes among age classes, age effects and regional differences were tested with non-parametric Kruskal–Wallis and Mann–Whitney U tests. To exclude age-related effects, temporal trend analysis was performed using linear regression on log-transformed concentrations in yearling seals.

3. Results and discussion

Of 41 BDEs analyzed, a total of 25 congeners were detected in harbor seal blubber (i.e., BDEs-15, -17, -25, -28, -30, -35, -37, -47, -49+71, -75, -77, -85, -99, -100, -116, -118, -119, -126, -153, -154, -155, -183, -197, and -209). In addition, several unidentified hepta- and octa-BDEs were detected in blubber (Fig. SI-1, Supplementary material). Sixteen BDEs were below the level of detection in blubber (i.e., BDE -1, -2, -3, -7, -8, -10, -11, -12, -13, -32, -33, -66, -138, -166, -181, and -190).

3.1. PBDE concentrations in harbor seals

The overall average \sum PBDE concentration (mono- to hexa-BDEs) in harbor seals was $2403 \pm 5406 \text{ ng g}^{-1} \text{ lw}$ (range 80–

25720; $n = 42$). By comparison, the mean PCB concentration previously reported in these samples was one to two orders of magnitude greater (overall mean 46540 ± 86610 , range: 636–460600 $\text{ng g}^{-1} \text{ lw}$; $n = 42$) (Shaw et al., 2007). PBDEs in seal blubber were positively correlated with PCBs ($R = 0.83$, $p < 0.001$), suggesting a parallel accumulation of these compounds through the marine food chain. Mean \sum PBDE concentrations in seals by age were (in descending order): 3645 ± 7388 , 2945 ± 5995 , 1385 ± 1265 , and $326 \pm 193 \text{ ng g}^{-1} \text{ lw}$ in pups, yearlings, adult males, and adult females, respectively (Table 1). Gender-related differences in PBDE concentrations were not observed among the younger seals but were apparent among the adults ($p = 0.01$), with adult males having four-fold higher PBDE burdens than females. This pattern reflects differences in age-dependent bioaccumulation between males and females due to placental and lactational transfer of lipophilic organic compounds from females to pups.

\sum PBDE concentrations detected in northwest Atlantic harbor seals were compared with levels reported in stranded marine mammals from various marine regions (Fig. 2). Such comparisons are necessarily limited because of the numerous sources of variability across the studies. \sum PBDE levels in these harbor seals were an order of magnitude higher than those recently reported in marine mammals collected from European waters, reflecting the greater production and use of the penta-BDE product in the US. In the younger seals, \sum PBDE concentrations (mean range 2945–3645 $\text{ng g}^{-1} \text{ lw}$) were higher than those reported in juvenile harbor seals collected from the southern North Sea during 1999–2004 (mean 490 $\text{ng g}^{-1} \text{ lw}$) (Weijs et al., 2007) and in gray seal pups (*H. grypus*) collected from eastern UK waters during 1998–2000 (290 $\text{ng g}^{-1} \text{ lw}$) (Kalantzi et al., 2005). PBDE levels in the adult males (mean 1385 $\text{ng g}^{-1} \text{ lw}$) were four- to six-fold greater than those reported in adult male harbor seals collected from the North Sea during 1999–2004 (300 $\text{ng g}^{-1} \text{ lw}$) (Weijs et al., 2007) and more recently from the Dutch Wadden Sea (232 $\text{ng g}^{-1} \text{ lw}$) (Leonards et al., 2008). However, higher PBDE levels were reported in adult male California sea lions (*Zalophus californianus*) collected during 1993–2003 (5778 $\text{ng g}^{-1} \text{ lw}$) (Stapleton et al., 2006b) and in adult male harbor seals collected during 1997–1998 from San

Table 1
PBDE concentrations (ng g⁻¹ lw) in harbor seal blubber by age class

N	% lipid	28 ^a	47	49+71	99	100	118	153	154	155	183 ^b	197 ^b	209 ^c	∑PBDE ^d
Adult M	7	63.4	1.7	904	134	49.2	0.93	210	31.3	44.9	20	21	1.1	1385
			(<0.03–6.1)	(173–2510)	(47.8–366)	(12.7–163)	(<0.04–3.0)	(35.0–626)	(2.26–125)	(0.72–144)	(12–29)	(<0.02–41)	(<0.3–2.6)	(454–3827)
Adult F	8	83.0	4.1	190	46.2	18.1	0.58	50.7	5.95	6.32	20	<0.02	0.5	326
			(<0.03–14)	(65.3–397)	(16.0–88.1)	(8.40–34.4)	(<0.04–2.0)	(3.13–146)	(1.91–17.6)	(<0.1–29.5)	20	29	(<0.2–1.1)	(131–713)
Yearling	14	61.5	8.6	1730	335	124	0.99	558	94.7	84.6	20	29	1.9	2945
			(0.7–54)	(160–12700)	(42.2–2550)	(13.1–827)	(<0.04–3.8)	(17.3–6090)	(<0.3–613)	(5.0–805)	(2.7–45)	(6.0–57)	(<0.2–7.6)	(250–23510)
Pup	13	59.1	15	2970	308	308	2.3	99.0	41.7	29.7	14	2.7	0.8	3645
			(<0.4–110)	(45.2–21800)	(19.7–1950)	(3.80–1030)	(<0.04–11)	(<0.09–715)	(<0.2–296)	(<0.1–170)	(1.7–40)	(<0.02–10)	(<0.2–2.9)	(80.2–25720)

^a Mean (range). Values below detection limit were replaced with 1/2 DL for calculation of means.

^b Total n = 12 (2 adult M, 1 adult F, 4 yearlings, 5 pups).

^c Total n = 20 (3 adult M, 3 adult F, 7 yearlings, 7 pups).

^d Sum of 36 mono- to hexa-BDEs.

Francisco Bay (5135 ng g⁻¹ lw) (She et al., 2002). PBDE concentrations in the younger harbor seals were higher than those recently reported in various cetacean species, with the exception of the very high levels reported in dolphins collected during 1995–2001 in UK waters (7657 and 10478 ng g⁻¹ lw, respectively) (Law et al., 2003, 2005). Collectively, these data indicate that northwest Atlantic harbor seals are at the high end of the concentration range reported for PBDEs in marine mammals around the world. Although many PBDEs can cause deleterious effects in animals (Birnbaum and Stasakal, 2004), the threshold level for toxic effects in marine mammals is unknown (see discussion of toxicological implications in Supplementary material).

3.2. BDE congener profiles

Ten BDE congeners accounted for the majority (98%) of the total PBDE content in harbor seal blubber samples (Fig. 3). BDE-47 was the dominant congener in all samples, contributing 62–73% of the PBDE content, followed by the penta-BDEs-99 (11–15%) and -100 (3–6%), and the hexa-BDEs -153, -154, and -155 (5–22%), in varying order. These congeners are generally dominant in marine mammals worldwide (Watanabe and Sakai, 2003) and suggest exposure to the technical penta-BDE mixture consisting of approximately 25% and 50% BDE-47 and BDE-99, respectively (La Guardia et al., 2006). Age-related differences in congener profiles were apparent, reflecting differences in exposure/uptake and metabolic capacity for individual BDEs between adults and pups. The pups had the highest concentrations of BDE-47 (mean 2974 ng g⁻¹ lw), accounting for 73% of the total PBDE content, followed by the penta-BDEs-99 and -100 (18%), while the hexa-BDEs-153, -154, and -155 accounted for only 5% of the total (Table 1). In contrast, BDE-47 concentrations in the adult females (mean 190 ng g⁻¹ lw) accounted for 58% of the total PBDE content, whereas the penta- and hexa-BDEs contributed 21% and 16% to the total, respectively. Although these were not mother–pup pairs, the accumulation pattern in the pups suggests efficient placental and lactational transfer of BDE-47 and to a lesser degree, BDEs-99, and -100, but very limited transfer of the hexa-BDEs. In grey seals, maternal transfer efficiency was shown to decline with increasing degree of bromination of the molecule, as a function of increasing *K*_{ow} values (Ikonomou and Addison, 2008). This may be a consequence of molecular size of higher BDEs, which may limit diffusion and lipid/water partitioning in females during lactation.

Concentrations of the tri-BDE-28 and tetra-BDE-49 were very low in seal blubber (range 1.1–14.5 ng g⁻¹ lw) (Table 1). Based on their structural similarity to PCBs, it follows that these BDEs would be easily metabolized in adult seals, as they have vicinal H atoms at the *ortho*–*meta* and *meta*–*para* positions (Boon et al., 1997), whereas hexa-BDEs accumulate with age, reflecting their recalcitrance to P450 metabolism. In the adult males, the hexa-BDEs-153, -154, and -155 contributed proportionally more (22%) to the total than BDEs-99 and -100 (14%), and concentrations of BDE-155 were elevated compared with BDE-154. BDE-155 has rarely been reported in marine mammals (Stapleton et al., 2006b). This congener is present at only 0.2–0.7% in technical mixtures (La Guardia et al., 2006) and was identified, along with BDE-154, as a specific debromination product of BDE-209 in fish (Stapleton et al., 2006a).

3.3. Higher brominated congeners

Hepta- and octa-BDEs were analyzed in a subset (*n* = 12) of our harbor seal samples. BDE-183, considered a marker compound for the octa-mix PBDE formulation, was detected in all samples at average concentrations of 17.6 ng g⁻¹ lw (range 1.7–45). This indicates that components of the octa-BDE mixture, which was princi-

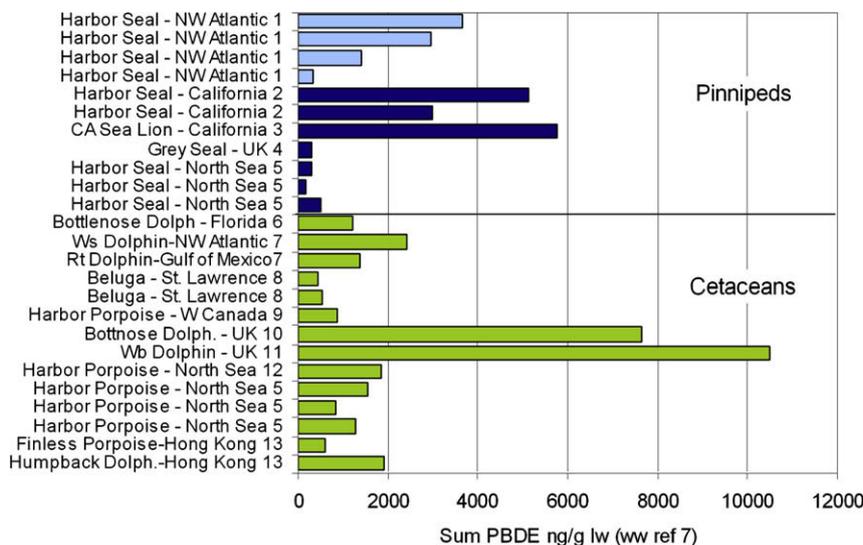


Fig. 2. Global comparison of total PBDE levels in marine mammals from around the world. References: (1) This study; (2) She et al., 2002; (3) Stapleton et al., 2006b; (4) Kalantzi et al., 2005; (5) Weijs et al., 2007; (6) Johnson-Restrepo et al., 2005; (7) Tuerk et al., 2005; (8) Lebeuf et al., 2004; (9) Ikonomou et al., 2002; (10) Law et al., 2005; (11) Law et al., 2003; (12) Beineke et al., 2005; (13) Ramu et al., 2005. Data are in ng g^{-1} lw except for Ref. (7) (ng g^{-1} wet wt).

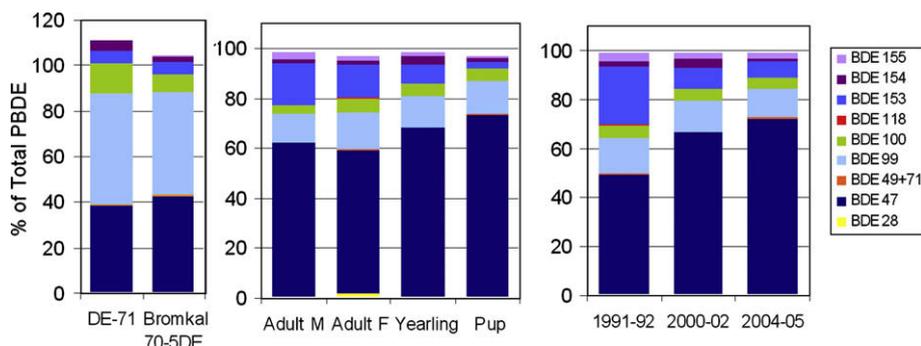


Fig. 3. BDE congener profiles in penta-BDE mixtures (La Guardia et al., 2006) (a), in harbor seal blubber by age class (b), and in yearling blubber samples from 1991 to 2005 (c).

pally used in molded parts of computers, televisions, car parts and other products, have contaminated this marine food web. In addition, octa-BDE-197 was detected in 58% of the samples (mean 24 ng g^{-1} lw, range 0.02–57), along with several unidentified hepta- and octa-congeners (Table 1; Fig. SI-1, Supplementary material). There is evidence that in fish, these congeners along with other higher BDEs are also metabolic debromination products of BDE-209 (Stapleton et al., 2006a). In a BDE-209 dietary exposure experiment using freshwater fish, Stapleton et al., 2006a reported that common carp (*C. carpio*) debrominated BDE-209 to form one octa- (BDE-202), two heptas-(BDEs-179 and -188), two hexas-(BDEs-155, -154), and an unidentified hexa-BDE. Rainbow trout (*Oncorhynchus mykiss*) debrominated BDE-209 to form several hepta- (BDEs-199, -184, -179, and -183), octa- (BDEs-202, -201, -204/197, -203, and -196), and nona- (BDEs-208, -207, and -206). Similar patterns of octa- and nona-BDEs were identified in freshwater fish and crustaceans exposed to BDE-209 in wastewater effluent, suggesting that BDE-209 debromination may also occur in wild fish in the aquatic environment (La Guardia et al., 2007). Thus, while the presence of tetra- to hexa-BDEs in seal blubber probably represents direct exposure to components of the penta-BDE mixture, the occurrence of hepta- and octa-BDEs suggests the additional contribution of metabolic processes in the fish prey of harbor seals and/or recent exposure to the octa- and deca-BDE mixtures, since a

short half-life (months) is indicated for higher BDE congeners (Geyer et al., 2004).

Although BDE-209 is generally the dominant BDE congener detected in marine sediments (de Boer et al., 2003), it is detected only at trace levels in marine mammals, and contributes very little to the PBDE content in marine organisms (Law et al., 2006). BDE-209 was detected in 25% of the 20 harbor seal blubber samples analyzed at concentrations ranging from 1 to 8 ng g^{-1} lw. To our knowledge, this is the first report of measurable BDE 209 concentrations (above trace levels) in a wild marine mammal species, and indicates that BDE 209 is bioavailable in the marine food web. This finding is consistent with results of a laboratory exposure study (Thomas et al., 2005) in which gray seals (*H. grypus*) were fed deca-BDE spiked oil capsules ($12 \mu\text{g d}^{-1}$) for 1 month. At the end of the study (after 29 d on a deca-free diet), 11–15% of the ingested BDE-209 was stored in the blubber. The blubber concentrations in the gray seals ($2\text{--}8 \text{ ng g}^{-1}$ lw) continuously exposed to deca-BDE were similar to those in our harbor seals, suggesting that more or less continuous exposure may be occurring through the food web.

The primary exposure route for harbor seals is via ingestion of teleost fishes, which in turn are likely exposed to BDE-209 via ingestion of sediment-associated prey organisms (zooplankton, benthic invertebrates, echinoderms, marine worms, and flatfishes).

However, BDE-209 does not appear to biomagnify from teleost fish to seals ($BMF \leq 1$) (Jenssen et al., 2007). A short half-life for BDE-209 was indicated in serum of gray seals, suggesting rapid clearance in seal blood; however, low levels of BDE-209 remained in blubber stores long after cessation of exposure (Thomas et al., 2005). Whether the lack of biomagnification of BDE-209 is a result of a low uptake rate for this large molecule into blubber or efficient debromination processes in fish is unclear. It is also possible that BDE-209 may be partitioning to tissues other than blubber in the harbor seal. In the rat, BDE-209 is only marginally distributed to adipose tissue but may be associated with blood proteins, and thus migrates to plasma-rich tissues such as the liver (Mörck et al., 2003).

3.4. Spatial trends

Considered relatively non-migratory, northwest Atlantic harbor seals nevertheless make seasonal movements along the coast from Maine southward to the coast of New Jersey (NMFS stock Assessment, 2007). As high trophic-level feeders in coastal and estuarine environments they are exposed to a variety of contaminated habitats and prey across their range. To examine spatial trends, we compared PBDE concentrations in blubber of adult males from the industrialized southern area (Massachusetts to New York) with those from the lightly populated, rural northern area (central and eastern Maine). Mean concentrations of Σ PBDEs in harbor seal blubber from the southern and northern areas were 1600 and 1000 ng g⁻¹ lw, respectively. A Mann–Whitney *U* test suggested no difference in PBDE levels between the two subregions ($p = 0.72$), nor was there a significant difference in BDE congener profiles. Unlike the trend for PCBs in harbor seals, which decreased with increasing latitude as a function of distance from sources (Shaw et al., 2007), a south to north (urban–rural–remote) decreasing gradient was not observed for PBDEs in this study. This is consistent with the different continental distribution patterns reported for atmospheric PCBs and PBDEs in North America (Shen et al., 2006). Whereas atmospheric PCB distribution is strongly related with population density and follows an urban–rural–remote gradient, the spatial distribution observed for PBDEs is clearly not related to proximity to urban and industrial centers. It is believed that factories manufacturing products containing brominated flame retardants, the use and recycling of PBDE-treated polymers and products, and waste incineration constitute major point sources of PBDEs (Watanabe and Sakai, 2003). Recent studies have highlighted the importance of uncontrolled incineration as a local source of environmental PBDEs in remote sites (Shen et al., 2006; Athanasiadou et al., 2008) as well as to atmospheric emissions and thus long-range transport of PBDEs to non-urban areas (Law et al., 2006). In addition, waste water treatment plants (WWTPs) and the application of PBDE-contaminated sewage sludge to agricultural lands also increases the possibility of subsequent remobilization of these compounds in rural environments. Recent data indicate that the major source of PBDEs in sewage sludge from both industrial and background locations is from diffuse leaching from products into wastewater streams from users, households, and industries generally (de Wit, 2002; Law et al., 2006). A recent study reported high PBDE levels (5750–29000 ng g⁻¹ lw) in fish collected from the Penobscot River downstream from a WWTP in a rural area of central Maine (Anderson and MacRae, 2006) along the northern part of the harbor seal range. High PBDE levels were also found in biosolids applied to agricultural land directly and in composted materials in the area. This WWTP is one of four contributing to land spreading and composting of biosolids, providing a significant route to move PBDEs into the aquatic environment through runoff and leaching. Thus, it is likely that a south to north spatial trend in PBDE concentra-

tions in these harbor seals may be obscured by local sources in the northern portion of the range.

3.5. Temporal trends

In this study, the harbor seals had been sampled from 1991 to 2005, providing an opportunity to assess possible changes in Σ PBDE levels over the past 14 years which may reflect the increased production and use of these compounds. To exclude the influence of age and gender, we compared Σ PBDE concentrations only in the yearlings ($n = 14$). A significant temporal trend was not observed in Σ PBDE concentrations in these harbor seals between 1991 and 2005. Similarly, Stapleton et al. (2006b) reported the lack of any temporal trend in Σ PBDE concentrations in California sea lions between 1993 and 2003, although levels of hexabromocyclododecane (HBCD) were increasing. Another recent study (Tuerk et al., 2005) also observed no temporal trend for PBDEs in Atlantic white-sided dolphins (*Lagenorhynchus acutus*) between 1993 and 2000. Previous studies reported that Σ PBDEs were increasing in harbor seals from San Francisco Bay between 1989 and 1998 (She et al., 2002), and in Arctic ringed seals between 1981 and 2000 (Ikonomou et al., 2002). Kajiwarra et al. (2004) reported a 150-fold increase in Σ PBDEs in adult female northern fur seals (*Callorhinus ursinus*) collected from the Japanese coast between 1972 and 1994, but a 50% decrease in levels between 1994 and 1998. Collectively, the data suggest that PBDE levels were increasing in marine mammals between the 1970s and the mid-1990s, but may have stabilized or reached equilibrium over the past decade.

Whereas Σ PBDE concentrations remained stable in blubber, congener compositions shifted from 1991 to 2005 (Fig. 3). Percent contribution of BDE-47 increased while BDE-153 concentrations decreased between 1991 and 2000; these trends leveled off between 2000 and 2005. BDE-99 concentrations only decreased slightly from 1991 to 2000. Similarly, Ikonomou et al. (2002) reported that concentrations of BDEs-47, -99, and -100 were increasing in male ringed seals from the Canadian Arctic between 1981 and 1996, but increases in the levels of BDE-99 were slowing considerably between 1996 and 2000. Similar changes were reported in gull egg samples from the Great Lakes between 1981 and 2000 (Norstrom et al., 2002). These changes probably reflect differences in the use or the composition of the various commercial PBDE formulations over the years. However, these harbor seals feed opportunistically on diverse fish species along an extended coastline. Since different fish species possess different metabolic potential for BDE congeners and exhibit different BDE profiles (Hale et al., 2001; Anderson and MacRae, 2006), the congener profile shifts in the seals may also reflect changes in the harbor seal diet over time.

4. Conclusions

The results of this study indicate that exposure to components of the penta-, octa-, and deca-BDE mixtures is significant for this population of harbor seals. Whereas the presence of tetra- to hexa-BDEs in blubber likely represents direct exposure to the penta-BDE product, the occurrence of higher BDEs suggests recent exposure to acta- and deca-BDE mixtures and/or the contribution of metabolic processes in fish to the loading of persistent BDEs in the seals. The uptake and accumulation of BDE-209 in marine food webs is of concern, since large amounts of deca-BDE are still in use.

Acknowledgments

The authors are grateful to Kirk Trabant, Marine Environmental Research Institute, and members of the NOAA/NMFS Northeast

Region Stranding Network for providing harbor seal tissues for this study. We also thank Patrick O'Keefe and Robin Storm for assistance with sample preparation for chemical analysis. This work was supported by the National Oceanographic and Atmospheric Administration (NOAA).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2008.09.016.

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