BIOACCUMULATION OF PBDES AND HBCD IN THE NORTHWEST ATLANTIC MARINE FOOD WEB

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Introduction

The brominated flame retardants (BFRs) polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are synthetic organic compounds that biomagnify in food webs and are associated with endocrine-disrupting and reproductive/ neurodevelopmental effects in animals[1-3]. As a result of their environmental persistence and widespread use in household and commercial products, PBDEs and HBCD have become ubiquitous global contaminants, even in remote areas[4-6]. Temporal studies have shown that PBDE levels are increasing in wildlife and humans, particularly in North America[4], where usage of the penta-BDE formulation has dominated the global market over the past three decades[7]. Deca-BDE (>97% BDE-209) represents 83% of the global market demand[7] and has only begun to be regulated in the United States and Europe[8]. Currently, there are no restrictions on the worldwide use of HBCD[9]. Recent attention has focused on the potential of PBDEs and HBCD to bioaccumulate and biomagnify in marine ecosystems. In Europe and Asia, HBCD levels are sharply increasing in marine biota, reflecting the usage profile of HBCD over PBDEs in these countries[6,10,11]. There is concern that, in addition to direct exposure to commercial PBDE mixtures, BDE-209 debromination may also be a source of exposure and/or uptake of PBDEs in marine food webs. Although BDE 209 is often a major congener in marine sediments[12], only trace levels have been detected in marine mammals[10,13-18]. This has been attributed to a low uptake rate due to the large molecular size of the compound or rapid excretion after biotransformation[19]. Biotransformation of BDE-209 has been demonstrated in freshwater fish, resulting in the formation of hexa- through nona-BDEs that have the potential to be more persistent and bioaccumulative than the parent compound[19,20]. Previously, we detected PBDEs at high concentrations in blubber of harbor seals *(Phoca vitulina concolor)*, apex predators in the northwest Atlantic marine ecosystem[21]. BDE 209 was detected above trace levels in some of the animals, indicating that this congener is bioavailable and can be accumulated through the marine food chain. Here we report on the analysis of PBDEs (mono-deca-BDEs) in seven teleost fish species that comprise the harbor seal diet and examine transfer trends of PBDEs from prey to predator. In addition, we analyzed HBCD in three fish species to provide an initial estimation of HBCD contamination in this ecosystem. This is the first study to document the bioaccumulation and biomagnification of BFRs in the northwest Atlantic marine food web.

Materials and methods

Samples. Prey-sized individual fish (<35 cm) of seven species known to be components of the harbor seal diet were collected by trawl along the mid-Maine coast in spring 2006, including silver hake (*Merluccius bilinearis*), white hake *(Urophycis tenuis)*, Atlantic herring (*Clupea harengus*), Atlantic mackerel (*Scomber scombrus)*, American plaice *(Hippoglossides platessoides)*, alewife (*Alosa pseudoharengu)*, and winter flounder *(Pseudopleuronectes americanus).*

Chemical Analysis. The analytical method used for determination of PBDEs in fish has been previously described[22, 23], with determination of total HBCD here being integrated. In brief, fish whole body composites were homogenized and samples were analyzed using HRGC/HRMS for 35 PBDE congeners (mono- through deca-BDE) and total HBCD following the isotope dilution quantification method. Before extraction, a mixture of ^{13}C-labelled internal standards (13 PBDE congeners (mono- through deca-BDE) and HBCD) was added to the sample. For extraction a sample amount equal to approximately 1.5 g of lipids (ranging approximately between 5 g and 100 g of fresh weight material) was homogenized with dry sodium sulphate. After liquid/ solid-extraction by means of cyclohexane/ dichloromethane a column-clean up using acid treated, activated silica gel and alumina was performed. The measurements were performed by means of high-resolution gas chromatography / high-resolution mass spectrometry (HRGC / HRMS). The identification of PBDEs and total HBCD was based on retention time and isotope ratio. Concentrations were calculated on fresh weight as well as on lipid weight (lw) basis.

Results and discussion:

PBDE and HBCD concentrations in teleost fishes. ∑PBDE concentrations in the fish samples (whole body homogenates) ranged, on average, from 18.3 (in alewife) to 81.5 ng/g, lw (in Atlantic herring), with an overall mean of 62 ± 34 ng/g, lw (Table 1). ∑PBDE levels in our samples were comparable to those reported in Atlantic cod from southern and western Norway[15], various fishes from the Belgian North Sea[24] and the eastern (North Sea) coast of UK[13]. Comparable levels were also reported in muscle tissues of marine fishes from the Florida coast[17], while concentrations 3-15 times higher were recently reported in marine fishes from the coasts of California[25] and Georgia[26].

Mean HBCD concentrations detected in alewife, mackerel, and herring (7.6, 14, and 23 ng/g, lw, respectively) were comparable to those reported in Atlantic cod from the Norwegian coast[15] and skipjack tuna from Asian waters[27], but were generally lower than levels reported in marine fishes from industrialized areas of Europe[6]. These differences likely reflect the greater market demand for HBCD in Europe (9,500 metric tons in 2001) relative to America (2,800 metric tons) and Asia (3,900 metric tons)[7]. However, a recent report of increasing HBCD levels in California sea lions between 1993 and 2003 may be indicative of a changing usage profile for HBCD over PBDEs in the US[9]. In view of the toxicity and increasing global use of HBCD, there is a need for more information on the loading and biomagnification of this flame retardant compound in marine ecosystems.

PBDE concentrations in harbor seals. ∑PBDE concentrations (mono-hexa-BDEs) detected in harbor seal blubber samples (n=42) ranged from 80 to 25720 ng/g, lw (overall mean 2403 ± 5406 ng/g. lw). By age, mean ∑PBDE levels were (in descending order): 3645, 2945, 1385, and 326 ng/g, lw in pups, yearlings, adult males, and adult females, respectively[21]. ∑PBDE concentrations in the younger seals were an order of magnitude higher than those reported in juvenile harbor seals collected from the southern North Sea during 1999-2004[28] and gray seal pups collected from eastern UK waters during 1998-2000[29]. ∑PBDE concentrations in the adult males were four-fold greater than those reported in adult male harbor seals collected from the North Sea during 1999-2004[28], but lower than those reported in adult male California sea lions collected during 1993-2003[9]. We observed no significant temporal trend in ∑PBDE concentrations in harbor seals between 1991 and 2005. Similarly, Stapleton et al. (2006)[9] reported the lack of a temporal trend in PBDE concentrations in California sea lions between 1993 and 2003. Kajiwara et al. (2004)[30] reported a 150-fold increase in ∑PBDEs in northern fur seals from the Japanese coast between 1972 and 1994, but levels had decreased by 50% between 1994 and 1998. Collectively, the data suggest that PBDE levels were increasing in pinnipeds between the 1970s and the mid-1990s, but may have stabilized over the past decade.

PBDE congener profiles in seals and fish. BDE 47 was the dominant congener in all samples, accounting for 62-73% and 45-68% of the total PBDE content in seals and fish, respectively, in most cases, followed by BDEs-99 and 100 in varying order (Figure 1). In the seal pups, BDE-47 contributed 73% of the total PBDE content, while the hexa-BDEs accounted for only 5% of the total. In the adult females, BDE-47 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 maternal transfer of BDE-47 and very limited transfer of the hexa-BDEs. Maternal transfer efficiency in seals declines with increasing degree of bromination of the molecule, as a function of increasing K_{ow} values[31]. This may be a consequence of molecular size of higher BDEs, which may limit diffusion and lipid/water partitioning in females during lactation. In adult males, hexa-BDEs accounted for more of the total PBDE (22%) than BDEs-99 and -100 (14%), and levels of BDE-155 were elevated compared with -154. Overall, the fish contained a greater proportion of tri- and tetra BDEs and the penta BDE-100 in their tissues than are present in technical penta-BDE mixtures or in seals. BDE-49 accounts for only 0.4 – 0.7% of the technical mixture, but contributed 3-16% of the PBDE mass in fish tissues. In the trophic transfer from fish to harbor seals, the tetra BDEs -49, -66, and -75 were detected in every fish sample and accounted for 3-19% of the PBDE content, while these congeners never exceeded 2% of the total in the seals. These different distributions reflect differences in dietary exposure and species-specific metabolism for individual congeners between fish and seals. Higher brominated congeners were detected in two fish species: hepta-BDE-183, the marker for the octa-BDE mixture, was detected in plaice, and in mackerel, hepta-BDEs 181 and -183, octa-BDEs-197 and -203, and nona-BDE-207 were detected. In harbor seals, hepta-BDE-183 and octa-BDE-197 were detected along with other unidentified hepta- and octa- congeners. BDE-209 was detected in both fish and seal tissue at measurable concentrations, indicating that BDE-209 is bioavailable and is transferred from fish to seals. Whereas the presence

of BDE-47 and -99 in these samples is suggestive of exposure to the penta-BDE mixture, the occurrence of higher BDEs indicates exposure to the octa-BDE and deca-BDE technical mixtures.

Biomagnification of PBDEs. This study clearly demonstrates that many PBDEs are able to bioaccumulate and biomagnify in this marine ecosystem, as concentrations in harbor seal blubber were two orders of magnitude higher than those in their fish prey. Biomagnification factors (BMFs) for ∑PBDEs from fish to seals ranged, on average, from 17 to 76, indicating a high potential for biomagnification of PBDEs throughout the food web (Table 2). Comparable BMFs for ∑PBDEs were reported between teleost fishes and bottlenose dolphins along the Florida coast[17], between fish and harbor seals in the North Sea[13], and between polar cod and Arctic ringed seals[16]. BDE-47 was biomagnified throughout this food web, while BDE-99 was biomagnified to a lesser degree from three fish species (winter flounder, plaice, and white hake). From fish to seals, there was a lack of biomagnification of the tetra BDEs-49, -66, and -75, and very little biomagnification of the tri-BDE-28, indicating an efficient metabolism for these congeners in the seals. The hexa-BDEs-153 and -155 were highly biomagnified in harbor seal blubber, with BMFs ranging from 148 to 677 and 12 to 236, respectively. BDE-155 is present at only 0.2 - 0.7% in technical penta-BDE mixtures[32] and was identified, along with other hexa-BDEs, as a specific debromination product of BDE-209 in fish[20]. There are also indications that in fish, several octa- and nona-BDEs are metabolic debromination products of BDE-209[19, 20]. In this study, these octa- and nona-BDEs were detected in 26-39% of the prey fish of harbor seals. Thus, it is plausible that BDE-209 debromination processes in these fish may be contributing to the accumulated load of persistent, lower-substituted BDE congeners in seals. BDE-209 was detected in three fish species (plaice, mackerel and white hake) at levels ranging from 0.2 to 4 ng/g, lw. Similar BDE-209 levels were detected in harbor seal blubber (1.1 to 8 ng/g, lw)[21], indicating that BDE 209 is bioavailable in this marine food web and can be accumulated at measurable levels in seals in the wild. This is consistent with the recent observation that gray seals fed deca-BDE spiked food accumulated BDE-209 in blubber[32]. The levels in the gray seals (2 to 8 ng/g, 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 marine food web.

Whereas the results of this study indicate that BDE-209 may be accumulated from fish to seals, the biomagnification potential was low (BMF≤1). A short half-life (8 to 13 days) was indicated for BDE-209 in serum of gray seals[33], suggesting rapid clearance of this compound in seal blood; however, low levels of BDE-209 remained in blubber stores of the animals long after cessation of exposure. 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 the fish is unclear. It is also possible that BDE-209 may be partitioning to tissues other than blubber in the harbor seal. Studies in rats and fish have shown that the highest concentrations of BDE-209 are found in plasma and other highly perfused tissues, such as the liver[20, 34]. In view of the widespread use of deca-BDE, there is a clear need for more information on the kinetics and accumulation of BDE-209 and its derivatives in marine ecosystems.

Acknowledgments
The authors gratefully acknowledge the Maine Department of Marine Resources Trawl Survey for assistance in the collection of fish samples from the Gulf of Maine. This work was funded by the National Oceanic and Atmospheric Administration (NOAA).

References:
1. Darnerud P.O. *Environ Inter* 2003*;* 29: 841-853.
2. Birnbaum L. S. and Staskal D. F. *Environ Health Perspect* 2004; 112: 9-17.
3. Mariussen E., Fonnum F. *Neurochem Inter* 2003 ; 43: 533–542.
4. Hites R. A. *Environ Sci Technol* 2004; 38: 945-956.
5. de Wit C. A., Alaee M. and Muir D. C. G. *Chemosphere* 2006; 64: 209-233.
6. Covaci A., Gerecke A. C., Law R. J., Voorspoels S., Kohler M., Heeb N. V., Leslie H., Allchin C. R. and De Boer J. *Environ Sci Technol* 2006; 40: 3679-3688.
7. Bromine Science and Environmental Forum (BSEF). 2003. http://www.bsef-site.com/doc/BFR_vol2001.doc.
8. Betts K. *Environ Health Perspect* 2008; 116: A 210.
9. Stapleton H. M., Dodder N. G., Kucklick J. R., Reddy C. M., Schantz M. M., Becker P. R., Gulland F., Porter B. J. and Wise S. A. *Mar Pollut Bull* 2006; 52: 522-531.
10. Law R. J., Allchin C. R., de Boer J., Covaci A., Herzke D., Lepom P., Morris S., Tronczynski J. and de Wit C. A. *Chemosphere* 2006; 64: 187-208.
11. Tanabe S., Ramu K., Isobe T. and Takahashi S. *J Environ Monit* 2008; 10: 188-197.

12. de Boer J., Wester P.G., van der Horst A., Leonards PEG. *Environ Pollut* 2003; 122: 63-74.
13. Boon J. P., Lewis W. E., Tjoen-A-Choy M. R., Allchin C. R., Law R. J., de Boer J., Hallers-Tjabbes C. C. T. and Zegers B. N. *Environ Sci Technol* 2002; 36: 4025-4032.
14. Verreault J., Gabrielsen G. W., Chu S., Muir D. C. G., Andersen M., Hamaed A. and Letcher R. J. *Environ Sci Technol* 2005; 39: 6021-6028.
15. Jenssen B. M., Sørmo E. G., Baek K., Bytingsvik J., Gaustad H., Ruus A. and Skaare J. U. *Environ Health Perspect* 2007; 115 (suppl 1): 35-41.
16. Sørmo E. G., Salmer M. P., Jenssen B. M., Hop H., Bæk K., Kovacs K. M., Lydersen C., Falk-Petersen S., Gabrielsen G. W., Lie E. and Skaare J. U. *Environ Tox Chem* 2006; 25: 2502-2511.
17. Johnson-Restrepo B., Kannan K., Addink R. and Adams D. H. *Environ Sci Technol* 2005; 39: 8243-8250.
18. Ikonomou M. G., Rayne S. and Addison R. F. *Environ Sci Technol* 2002; 36: 1886-1892.
19. Stapleton H. M., Alaee M., Letcher R. J. and Baker J. E. *Environ Sci Technol* 2004; 38: 112-119.
20. Stapleton H. M., Brazil B., Holbrook R. D., Mitchelmore C. L., Benedict R., Konstantinov A. and Potter D. *Environ Sci Technol* 2006; 40: 4653-4658.
21. Shaw S. D., Brenner D., Berger M. L., Fang F., Hong C.-S., Storm R., Hilker D. and O'Keefe P. *Organohalogen Comp* 2007; 69: 829-832.
22. Päpke O. and Herrmann T. *Organohalogen Comp* 2004; 66: 3971-3976.
23. Päpke O, Fürst P, Herrmann T. *Talanta* 2004; 63: 1203-1211.
24. Voorspoels S., Covaci A. and Schepens P. *Organohalogen Comp* 2003; 61: 279-282.
25. Brown F. R., Winkler J., Visita P., Dhaliwal J. and Petreas M. *Chemosphere* 2006; 64: 276-286.
26. Sajwan K. S., Kumar K. S., Nune S., Fowler A., Richardson J. P. and Loganathan B. G. *Toxicol Environ Chem* 2008; 90: 81-96.
27. Ueno D., Alaee. M., Marvin C., Muir D.C.G. , Macinnis G., Reiner E., Crozier P., Furdui V.I., Subramanian A., Fillmann G., Lam P.K.S., Zheng G.J., Muchtar M., Razak H., Prudente M. Chung K.-H, Tanabe S. *Environ Pollut* 2006; 144 :238-247.
28. Weijs L., Gheorghe A., Dirtu A. C., Das K., Reijnders P. J. H., Neels H., Blust R. and Covaci A. *Organohalogen Comp* 2007; 69: 825-828.
29. Kalantzi O. I., Hall A. J., Thomas G. O. and Jones K. C. *Chemosphere* 2005; 58: 345-354.
30. Kajiwara N., Ueno D., Takahashi A., Baba N. and Tanabe S. *Environ Sci Technol* 2004; 38: 3804-3809.
31. Ikonomou, M.L. and Addison, R.F. *Mar Env Research* 2008; In Press doi:10.1016/j.marenvres.2008.02.004.
32. La Guardia M. J., Hale R. C. and Harvey E. *Environ Sci Technol* 2006; 40: 6247-6254.
33. Thomas G. O., Moss S. E. W., Asplund L. and Hall A. J. *Environ Pollut* 2005; 133: 581-586.
34. Mörck A., Hakk H., Örn U. and Wehler E. K. *Drug Metab Disp* 2003; 31: 900-907.

Table 1: Summary of mean PBDE and HBCD concentrations (ng/g, lipid wt) in teleost fishes (whole body homogenates) and in blubber of harbor seals from the NW Atlantic coast

Species	N	% lipid	28	47	49	66	75	99	100	153	154	155	∑BDE	HBCD
WF	1	0.6	0.6	34.8	1.4	0.7	0.1	2.5	6.4	0.8	2.0	1.3	52.3	n.a.
AH	6	1.1	0.5	40.1	12.6	0.9	0.9	6.9	6.7	0.4	1.4	0.6	81.5	22.9
AMP	1	0.9	0.6	42.3	3.1	0.4	0.1	3.9	7.0	1.2	2.8	3.6	68.6	n.a.
WH	2	1.0	0.4	24.9	4.7	0.06	0.08	0.6	7.1	0.3	1.9	0.3	42.2	n.a.
ALE	2	10.2	0.4	8.3	1.6	0.3	0.2	3.6	1.6	0.3	0.7	0.2	18.3	7.6
MAC	4	10.3	1.3	19.8	3.8	1.1	0.4	7.5	4.05	1.4	1.4	0.5	69.3	14.0
SH	1	2.2	0.8	17.7	4.5	0.3	0.09	6.3	4.0	0.3	2.2	0.9	38.3	n.a.
HS	7	63.4	1.7	904	1.50	0.05	0.05	134	49.2	210	31.3	44.9	1385	n.a.

n.a.=not analyzed. Species: WF = winter flounder, AH = Atlantic herring, AMP = American plaice, WH = white hake, ALE = alewife, MAC = Atlantic mackerel, SH = silver hake (whiting), HS = harbor seal (adult males)

Table 2: Biomagnification factors (BMFs) for PBDEs from teleost fishes to adult male harbor seals

Species	28	47	49	66	75	99	100	153	154	155	∑PBDE
WF	3.1	26.0	1.03	0.07	0.3	53.2	7.7	269	15.9	34.3	26.6
AH	3.2	22.5	0.1	0.06	0.05	19.5	7.3	467	22.5	73.6	17.1
AMP	3.0	21.4	0.5	0.1	0.4	33.9	7.0	174	11.3	12.4	20.3
WH	4.5	36.3	0.3	0.8	0.6	213	6.9	593	16.2	145	33.0
ALE	4.0	109	0.9	0.1	0.3	37.2	29.8	700	447	236	76.5
MAC	1.4	45.7	0.4	0.04	0.1	17.9	12.1	148	21.9	86.3	20.1
SH	2.1	51.1	0.3	0.1	0.6	21.3	12.4	677	14.1	47.8	36.4

Species: WF = winter flounder, AH = Atlantic herring, AMP = American plaice, WH = white hake, ALE = alewife, MAC = Atlantic mackerel, SH = silver hake (whiting)

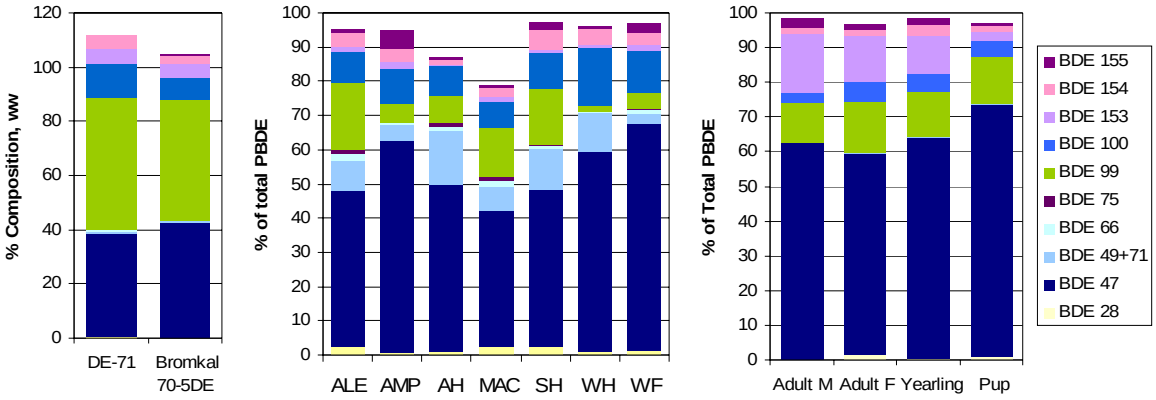

Figure 1: PBDE congener profiles in penta-BDE technical mixtures (a), whole fish by species (b), and harbor seal blubber by age class (c). ALE = alewife, AMP = American plaice, AH = Atlantic herring, MAC = mackerel, SH = silver hake, WH = white hake, WF = winter flounder