

# PBDES AND HBCD IN HARBOR SEALS AND THEIR PREY IN THE NORTHWEST ATLANTIC 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<sup>1-3</sup>. As a result of their environmental persistence and widespread use in household and commercial products, PBDEs and HBCD have become ubiquitous global contaminants found in air, water, fish, birds, marine mammals, and human tissues, even in remote areas<sup>4-6</sup>. PBDE levels have been increasing in biota and humans over the last 30 years, particularly in the United States, where the penta- and octa-BDE formulations were withdrawn from the market following the banning of these compounds by the European Union in 2004. Deca-BDE (>97% BDE-209), represents 83% of the 2001 global market demand<sup>7</sup>, and has only recently begun to be regulated<sup>8</sup>. Currently, there are no restrictions on the use of HBCD<sup>9</sup>. Recent attention has focused on the potential of PBDEs, including BDE-209, 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<sup>6,10,11</sup>. Recent data indicate a similar increasing trend for HBCD in California sea lions<sup>9</sup>, possibly reflecting a shift toward greater usage of HBCD in the US. Although BDE 209 is often a major congener in marine sediments<sup>12</sup>, only trace levels have been detected in marine mammals<sup>10,13-18</sup>. This has been attributed to a low uptake rate due to the large molecular size of the compound<sup>19</sup>. Uptake and biotransformation of BDE-209 has been demonstrated in fish, resulting in the formation of lower brominated PBDEs (hexa- through nona-BDEs) that have the potential to be more persistent, and bioaccumulative than the parent compound<sup>19,20</sup>. In our previous work, we detected PBDEs at high concentrations in blubber of harbor seals (*Phoca vitulina concolor*), apex predators in the northwestern Atlantic ecosystem<sup>21</sup>. 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 web. Here we report on the analysis of PBDEs (mono-deca-BDEs) in seven teleost fish species 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. To our knowledge, this is the first report of the occurrence of BFRs in the northwestern 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 pseudoharengus*), and winter flounder (*Pseudopleuronectes americanus*).

**Chemical Analysis.** The analytical method used for determination of PBDEs in fish has been previously described<sup>22,23</sup>, 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 <sup>13</sup>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 basis.

## Results and discussion:

**PBDE and HBCD concentrations in teleost fishes.**  $\Sigma$ 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  $62 \pm 34$  ng/g, lw (Table 1).  $\Sigma$ PBDE levels in our samples were comparable to those reported in Atlantic cod from southern and western Norway<sup>15</sup>, various fishes from the Belgian North Sea<sup>24</sup> and the eastern (North Sea) coast of UK<sup>13</sup>. Comparable levels were also reported in muscle tissues of marine fishes from the Florida coast<sup>17</sup>, while concentrations 3-15 times higher were recently reported in marine fishes from the coasts of California<sup>25</sup> and Georgia<sup>26</sup>.

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<sup>15</sup> and skipjack tuna from Asian waters<sup>27</sup>, but were generally lower than levels reported in marine fishes from industrialized areas of Europe<sup>6</sup>. 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)<sup>7</sup>. 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<sup>9</sup>. 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.** Mean  $\Sigma$ PBDE concentrations (mono- through hepta-BDEs) in blubber of northwestern Atlantic harbor seals collected between 1991 and 2005 were 3659, 2958, 1393, and 332 ng/g, lw, in pups, yearlings, adult males and females, respectively<sup>21</sup>.  $\Sigma$ PBDE concentrations in the younger seals (~3000-4000 ng/g, lw) were an order of magnitude higher than those reported in juvenile harbor seals collected from the southern North Sea during 1999-2004<sup>28</sup> and gray seal pups collected from eastern UK waters during 1998-2000<sup>29</sup>. 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<sup>28</sup>, but lower than those reported in adult male California sea lions collected during 1993-2003<sup>9</sup>. We observed no significant temporal trend in  $\Sigma$ PBDE concentrations in harbor seals between 1991 and 2005. Similarly, Stapleton et al. (2006a)<sup>9</sup> reported the lack of a temporal trend in PBDE concentrations in California sea lions between 1993 and 2003. Kajiwara et al. (2004)<sup>30</sup> reported a 150-fold increase in  $\Sigma$ 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 in industrialized areas 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 60-75% 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). Congener profiles in the seals varied by age. In pups BDE-47 contributed 75%, while the hexa-BDEs accounted for only 5% of the total, indicating that the pups may initially retain lower brominated BDEs following lactational exposure. In adult males, hexa-BDEs contributed relatively more to the total than the penta-BDEs (22 versus 14%), and levels of BDE-155 were elevated compared with -154. Overall, the fish contained a greater proportion of tri- and tetra BDEs and less of 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 plaice (hepta-BDE-183) and mackerel (hepta-BDEs 181 and -183, octa-BDEs-197 and -203, and nona-BDE-207). In harbor seals, hepta-BDEs were below the limits of detection in blubber, (octa- and nona-BDEs were not analyzed). BDE-209 was detected in fish and seal blubber at similar concentrations, indicating that BDE 209 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 food web, as concentrations in harbor seal blubber were two orders of magnitude higher than those in their fish prey. Biomagnification factors (BMFs) for  $\Sigma$ PBDEs from fish to seals ranged, on average, from 17 to 76, indicating a high potential for biomagnification of PBDEs throughout this food web (Table 2).

Comparable BMFs for  $\Sigma$ PBDEs were reported between teleost fishes and bottlenose dolphins along the Florida coast<sup>17</sup>, between fish and harbor seals in the North Sea<sup>13</sup>, and, between polar cod and Arctic ringed seals<sup>16</sup>.

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). BDE-47 and BDE-99 constitute approximately 25% and 50% of the technical penta-BDE mixture; respectively, and large volumes have been released to the environment<sup>4</sup>. 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, likely indicating an efficient metabolism for these congeners in the seals. The hexa-BDEs 153 and 155 were highly biomagnified in harbor seal blubber. BDE-155 is present at only 0.2 - 0.7% in technical penta-BDE mixtures<sup>31</sup> and was identified, along with other hexa-BDEs, as a specific debromination product of BDE-209 in fish<sup>20</sup>. Although not analyzed in the seals, octa-BDEs -197 and -203 and nona-BDE-207 were detected in 26-39% of the fish samples. There are indications that in fish, octa- and nona-BDEs, along with the hexa-BDEs, are metabolic debromination products of BDE-209<sup>19, 20</sup>. Thus, it is plausible that BDE-209 debromination processes in the fish may be contributing to the accumulated load of these congeners in seals. BDE 209 was detected in three fish species (plaice, mackerel and white hake) at levels ranging from 0.2 to 8.2 ng/g, lw. Similar BDE-209 levels were detected in harbor seal blubber (1 to 8 ng/g, lw)<sup>21</sup>, indicating that BDE 209 is bioavailable in the marine food web and can be accumulated at measurable levels in seals in the wild. A recent study demonstrated that gray seals fed deca-BDE spiked food accumulated BDE-209 in blubber<sup>32</sup>. The levels in the gray seals (2 -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 food web.

Whereas the results of this study indicate that BDE-209 may be accumulated from fish to seals, the biomagnification potential was low. A short half-life (8 to 13 days) was indicated for BDE-209 in serum of gray seals<sup>32</sup>, 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<sup>20, 33</sup>. In view of the potential toxicity and widespread global use of deca-BDE, there is a clear need for more information on the bioaccumulation and biomagnification potential of BDE-209 and its derivatives in marine ecosystems.

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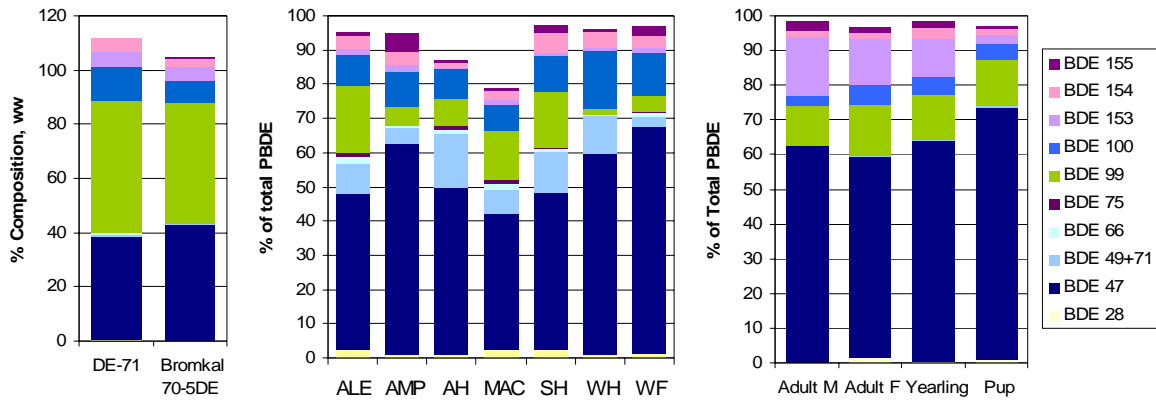
**Table 1:** Summary of mean PBDE and HBCD concentrations (ng/g, lipid wt) in teleost fishes (whole body homogenates) and in blubber of harbor seals from the NW Atlantic coast

Species	N	% lipid	28	47	49	66	75	99	100	153	154	155	ΣBDE	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	1393	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, HS = harbor seal (adult males).

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

Species	28	47	49	66	75	99	100	153	154	155	$\Sigma$ 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



**Figure 1:** PBDE congener profiles in penta-BDE technical mixtures (La Guardia 2006) (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