Tissue-Specific Biomagnification of Polybrominated Diphenyl Ethers and Hexabromocyclododecanes in Harbor Seals from the Northwest Atlantic

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Introduction

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are widely used brominated flame retardants (BFRs) in household and commercial products and are associated with endocrine-disrupting, reproductive, and neurodevelopmental effects in animals (Alaee et al. 2003, Birnbaum & Staskal 2004). They enter marine waters from multiple sources, readily biomagnify in marine food webs, and are found at high concentrations in marine mammals (Hites 2004). Globally, the penta- and octa-BDE mixtures have been largely restricted or withdrawn, and the usage of deca-BDE is beginning to be regulated (Shaw & Kannan 2009). The global demand for HBCD, the principal BFR in polystyrene foams used in building insulation, has increased as an alternative for the banned PBDEs, and temporal studies have shown that HBCD levels are increasing in biota (Covaci et al. 2006, Stapleton et al. 2006a, Tanabe et al. 2008). Our previous work demonstrated that harbor seals *(Phoca vitulina concolor)* from the northwest Atlantic are highly contaminated by PBDEs (Shaw et al. 2008), with congener profiles exhibiting a "penta" signature dominated by BDE-47, similar to that in their prey fish (Shaw et al. 2009a). Highly brominated BDEs including BDE-209 were detected at low levels in seal blubber and whole fish tissue, indicating that these congeners are bioavailable and accumulate throughout the marine food web. Tetra- through hexa-BDEs were highly biomagnified from fish to seal blubber, but no biomagnification was observed for deca-BDE (BDE-209). Studies in rats and fish have shown that BDE-209 preferentially binds to blood proteins and migrates to perfused tissues, such as the liver (Mörck et al. 2003, Stapleton et al. 2006b, Huwe et al. 2008). Thus, blubber concentrations may represent an underestimation of the body burden and biomagnification potential of BDE-209 in seals.

The present study analyzed PBDEs (tri- to deca-BDE) and HBCD isomers in liver of 56 harbor seals and compared BFR levels and biomagnification rates with those previously detected in blubber. HBCD isomers were analyzed in blubber samples of 11 harbor seals to enable further tissue comparisons. The majority of the animals were pups, providing an opportunity to examine tissue distribution of BFRs resulting from placental and lactational transfer.

Materials and Methods

Samples. Liver samples were collected between 2001 and 2006 from 56 harbor seals (6 adult males, 22 male pups, 28 female pups) found stranded along the northwest Atlantic coast from the eastern coast of Maine to Long Island, New York (Figure 1). Blubber samples were collected from 11 harbor seals (4 adult males, 1 male pup, 6 female pups) from the same locations. Seals were weighed, standard length and axillary girth were measured. Age was estimated based on body size. Samples were stored in hexane and acetone rinsed aluminum foil at – 40°C until analysis.

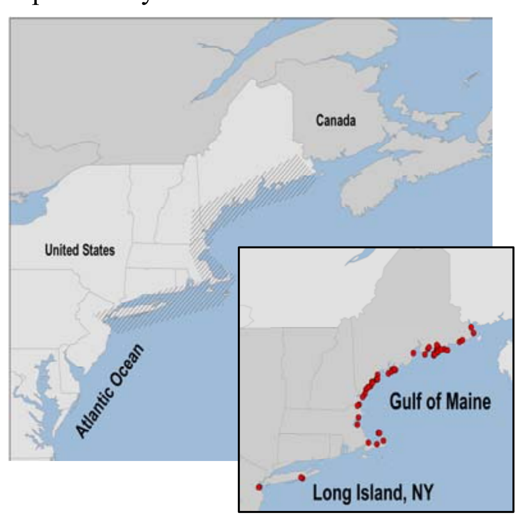

Figure 1. Map of the northwest Atlantic showing locations of harbor seals sampled in this study

Chemical Analysis. Harbor seal liver and blubber samples (2 - 2.5 g) were ground with sodium sulfate, spiked with internal standards (BDE 77, BDE 128, CB 143, ^{13}C-BDE 209 and ^{13}C-HBCDs), extracted and cleaned on acidified silica (Voorspoels et al. 2003, Covaci et al. 2008). Lipid content was determined gravimetrically. PBDEs (mono- through deca-BDEs) and HBCDs (α-, β-, and γ-isomers) were analyzed in seal tissues using the isotope dilution quantification method as described by Shaw et al. 2009b and Covaci et al. 2009. PBDEs were determined by GC-ECNI/MS or GC-EI/MS and HBCDs by LC-MS/MS.

QA/QC was performed through the analysis of procedural blanks, a replicate sample and a standard reference material (SRM 1945, PBDEs and HBCDs in whale blubber). Method quantification limits (LOQs) for individual PBDE congeners were based on procedural blanks (3x SD) and the sample volume. LOQs for tri-hepta PBDEs range between 1 and 2 ng/g lw. LOQ for BDE 209 was variable and ranged between 5 and 35 ng/g lw. LOQs were 1.5 ng/g lw for α-HBCD and 2 ng/g lw for β- and γ-HBCD.

Statistics. Statistical analyses were performed using SPSS 14.0. Concentrations below LOQ were replaced with ½ LOQ for calculation of means and totals. Non-parametric statistical tests were used since the data were not normally distributed and sample sizes of the gender-age classes were unequal. Because of the predominance α-HBCD in seal tissues, statistics were based on the α-HBCD isomer. Concentrations are reported on a lipid weight (lw) basis.

Results and discussion

PBDE and HBCD concentrations in harbor seal tissues. PBDE congeners detected in liver of harbor seals (n=56) included: BDEs-28, -47, -49, -85, -99, -100, -153, -154, -155, -181, -183, -184, an unidentified hepta-, -191, -197, and -209. ΣPBDE (tri-octa) concentrations in seal liver ranged from 35 to 19547 ng/g lw, with an overall mean of 2671 ± 3566 ng/g lw. These levels are similar to those previously reported in blubber (range 80 to 25720; overall mean 2403 ± 5406 ng/g lw; n=42) (Shaw et al. 2008). PBDE congener profiles in liver showed a "penta-BDE" signature (BDE-47>-99>-100>153), similar to that found in blubber and in seven species of harbor seal prey fish (Shaw et al. 2009b). PBDE concentrations in these seals are an order of magnitude higher than those reported in harbor and gray seals from Europe (Kalantzi et al. 2005, Weijs et al. 2009), reflecting the heavy usage of penta-BDE in North America. Higher PBDE levels were reported in California sea lions (*Zalophus californianus)* (Stapleton et al. 2006a, Meng et al. 2009) and harbor seals from San Francisco Bay (She et al. 2002).

Of the three HBCD isomers detected in harbor seal liver, α-HBCD accounted for 95% of total HBCDs in all samples. α-HBCD concentrations in liver ranged from 2 to 279 ng/g lw, with an overall mean of 38 ± 48 ng/g lw (n=56). In blubber, only α-HBCD was detected and concentrations were three times lower than in liver (range 2 to 29; overall mean 12 ± 9.5 ng/g lw; n=11). Whereas commercial HBCD mixtures consist mainly of γ-HBCD (75–89%), α-HBCD (10–13%), and β-HBCD (1–12%) (Heeb et al. 2005), stereoisomeric profiles of HBCDs in marine biota are dominated by α-HBCD, and selective enrichment of this isomer is observed with increasing trophic level in the food web (Covaci et al. 2006). α-HBCD levels in NW Atlantic harbor seals are lower than those reported in European harbor seals (Jenssen et al. 2007, Morris et al. 2004) and northern fur seals from Japan (Tanabe et al. 2008), reflecting the low usage of this compound in North America. However, Stapleton et al. (2006a) reported that HBCD concentrations were increasing exponentially in California sea lions between 1993 and 2003, possibly indicating a shift toward greater usage of HBCD in the US.

Age and gender differences in tissue distributions. ΣPBDE concentrations in liver of the male pups were significantly higher than those in the adult male harbor seals (p=0.02) (Figure 2). Although not statistically significant, mean hepatic concentrations of α-HBCD in the male pups were more than two-fold higher in those in the adult males. This accumulation pattern is similar to that observed in blubber and shows that the highest lifetime exposure of seals to lipophilic BFRs may result from placental and lactational transfer, placing young seals at risk for adverse effects of these compounds.

Surprisingly large differences in tissue distribution of BFRs were observed between the male and female pups. Hepatic ΣPBDE concentrations in the male pups (mean 4400 ng/g lw) were two-fold higher than those in the female pups (1680 ng/g lw) ($p=0.02$) (Figure 2). In addition, levels of the BDE congeners -28, -47, -49, -99, -153, and -155 were significantly higher in liver of the male pups. In blubber, although not statistically significant, mean levels of ΣPBDEs and the major BDE congeners were ten times higher in female pups than in the male pups. Hepatic concentrations of α-HBCD were also higher in male pups ($p=0.03$), whereas no significant gender differences were observed for α-HBCD in blubber (Figure 2). The first weeks of life represent a period of rapid growth, during which harbor seal pups almost triple their birth weight and lay down layers of blubber prior to weaning. The exact ages of the pups were unknown, thus we used biometric data to examine the possible influence of growth on hepatic BFR concentrations. No correlations between PBDEs or α-HBCD and body weight, body length, or lipid content of the samples were found for male or female pups. Nevertheless, this finding was interesting, and is suggestive of possible gender differences in metabolism and elimination/sequestration of PBDEs and α-HBCD among young seals.

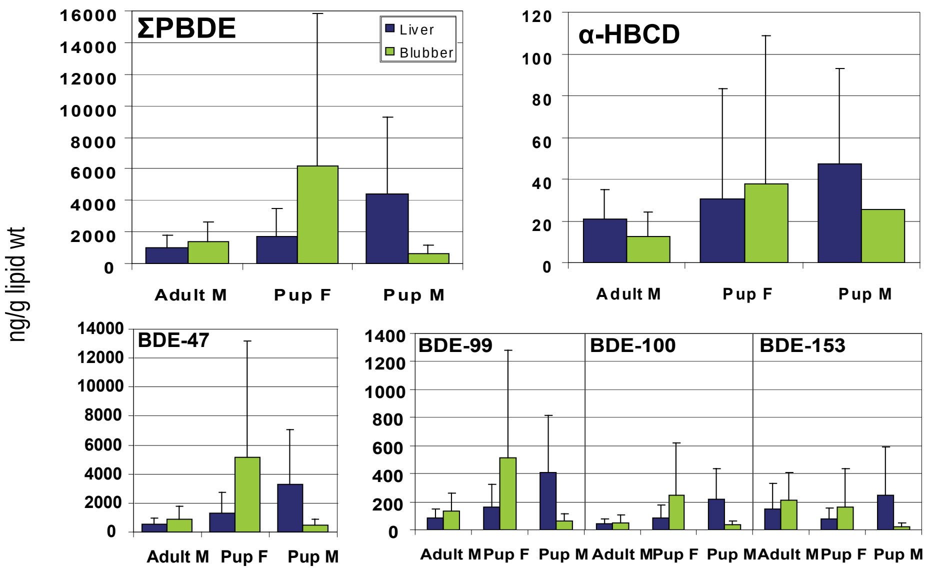

Figure 2. ΣPBDE, α-HBCD, and dominant BDE congeners in harbor seal liver and blubber

Spatial and temporal trends. ΣPBDE and α-HBCD concentrations in male/female pups from the more industrialized southern area (New York, Massachusetts) did not differ from those in seals from the less populated northern areas (Maine). This lack of a spatial trend probably reflects the presence of diffuse local sources (e.g., waste water treatment plants, sewage sludge applications, landfill leachate) across the harbor seal range. To assess temporal trends, concentrations in pups were plotted against the year of sampling (Figure 3). Results show a lack of a temporal trend for ΣPBDEs in male or female pups from 2001 to 2006, which is consistent with our findings in blubber (Shaw et al. 2008) and with the lack of a trend observed in other studies over the same time period (Stapleton et al. 2006a, Tuerk et al. 2005, Tanabe et al. 2008). 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. In contrast with the increasing trend for HBCD reported in California sea lions (Stapleton et al 2006a), we found no temporal trend for α-HBCD concentrations in harbor seal pups from the northwest Atlantic.

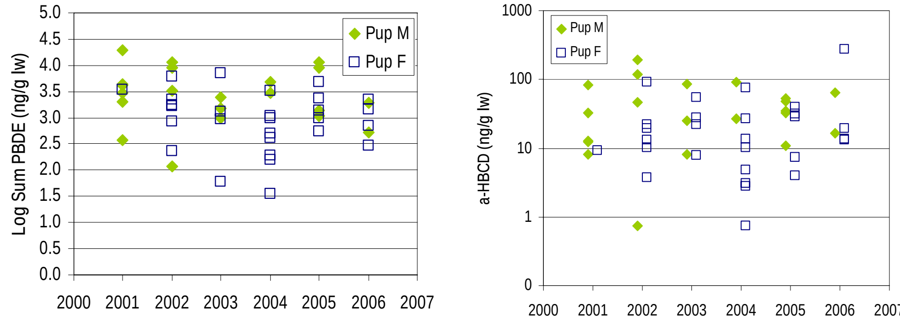

Figure 3. Lack of a temporal trend in hepatic concentrations of ΣPBDEs and α-HBCD from 2001 to 2006 for male (◊) and female pups (□)

Biomagnification of BFRs in harbor seal tissues. PBDEs (tri- to hexa-BDEs) were highly biomagnified through the food web, with average biomagnification factors (BMFs) from fish to adult seal livers ranging from 14 to 54. A similar, high biomagnification potential was previously reported for tri- through hexa-BDEs from fish to seal blubber (BMFs 17 to 76), whereas no biomagnification was found for BDE-209 (BMF≤1) (Shaw et al. 2008). Although detection frequency was low (<20%), the maximum BDE-209 concentrations in seal livers (range 14-40 ng/g lw) were five times higher than those in blubber (1-8 ng/g lw) (Figure 4), which is consistent with observations that deca-BDE preferentially accumulates in perfused tissues (Mörck et al. 2003, Stapleton et al. 2006b, Voorspoels et al. 2006, Huwe et al. 2008, Kunisue et al. 2008). Moreover, BDE-209 levels in adult seal liver samples were up to ten times higher than in fish tissue, suggesting possible biomagnification of deca-BDE (Figure 5). Similarly, α-HBCD was slightly biomagnified from fish to seal liver (average BMFs 1 to 3), but not from fish to seal blubber. These findings suggest that the biomagnification of PBDEs and HBCDs in marine food webs should be evaluated on a tissue-specific basis.

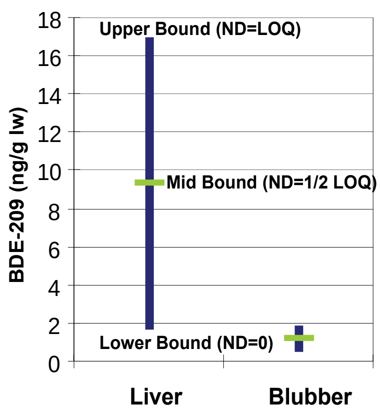

Figure 4. BDE-209 levels in harbor seal tissues

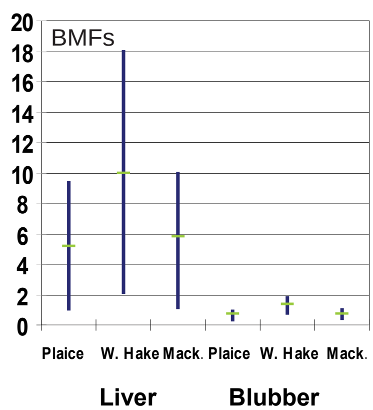

Figure 5. BMFs of BDE-209 from fish to adult seal tissues

Most of the seals in this study were pups, implying that BDE-209 and HBCD are subject to placental and lactational transfer, possibly placing pups at risk for endocrine disruption, developmental neurotoxicity and other adverse effects. Given the large reservoirs of BDE-209 in marine sediments and ongoing inputs of deca-BDE and HBCD, the biomagnification of these compounds in marine mammals is of concern.

Acknowledgements

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