POLYBROMINATED DIPHENYL ETHERS (PBDES) IN HARBOR SEALS (*PHOCA VITULINA CONCOLOR*) FROM THE NORTHWESTERN ATLANTIC

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Introduction

Brominated flame retardants, especially the polybrominated diphenyl ethers (PBDEs), are persistent organic pollutants (POPs) that biomagnify and are associated with endocrine-disrupting and neurodevelopmental effects in animals.[1] As a result of their lipophilicity and widespread use in household products including textiles, furniture, and electronics, PBDEs are now ubiquitous and have been found in tissues of humans and wildlife, even in remote areas. Temporal trends for PBDEs suggest that concentrations were increasing in biota and human milk between the 1970s and late 1990s.[2,3] These levels appear to be leveling off in some industrialized areas of Europe, but they are increasing exponentially in North America, particularly in the United States, where PBDEs are still in extensive production and use.[4,5] Recent studies show that concentrations in marine mammals from the Canadian Arctic are very low at ~5 ng/g lipid weight (lw), but they have increased since the early 1980s with a doubling time of ~7 years.[6] Marine mammals from other regions have current PBDE levels of ~1000 ng/g, lw, with higher concentrations occurring in animals living close to industrialized areas.[7-10] These concentrations are also increasing with a doubling time of ~5 years, similar to the doubling time observed in people.[2]

Studies on the occurrence of PBDEs in marine mammals have focused on Europe, the Canadian Arctic, the St. Lawrence estuary, and the US Pacific coast.[3-10] The occurrence of these compounds in marine mammals from the US northwestern Atlantic coast has been scarcely reported.[11] This is one of the most industrialized regions in the world, and environmental contamination has been a concern since at least the 1950s. At the top of the food chain, harbor seals (*Phoca vitulina concolor*) inhabit near-shore waters and are an important sentinel species for coastal contamination. Central to their migratory range, the Gulf of Maine is a shallow, semi-enclosed sea receiving significant riverine, urban, agricultural, and industrial pollutant inputs from large urban centers in the Northeast as well as via long-range atmospheric transport. PCB burdens in these seals were previously reported to be relatively high on a global scale, similar to levels reported in seals from polluted regions of Europe and Asia.[12] Here we report, for the first time, the presence of PBDE congeners in harbor seals from the northwestern Atlantic.

Methods and Materials

Samples. Blubber samples were collected between 1991 and 2005 from 26 stranded harbor seals (6 adult males, 12 yearlings, 8 pups) along the northwestern Atlantic coast at locations ranging from Maine to Long Island, New York (Figure 1). Seals were weighed, and standard length and axillary girth were measured. Age was estimated based on body size. Condition indices were calculated by dividing axillary girth/standard length and body weight/standard length. Blubber samples were stored in a freezer at -40°C until analysis.

Chemical Analysis. Harbor seal blubber samples were analyzed for 36 PBDE congeners (IUPAC numbers 1, 2, 3, 7, 8, 10, 11, 12, 13, 15, 17, 25, 28, 30, 32, 33, 35, 37, 47, 49+71, 66, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, and 166) following the isotope dilution quantification method. Seal samples

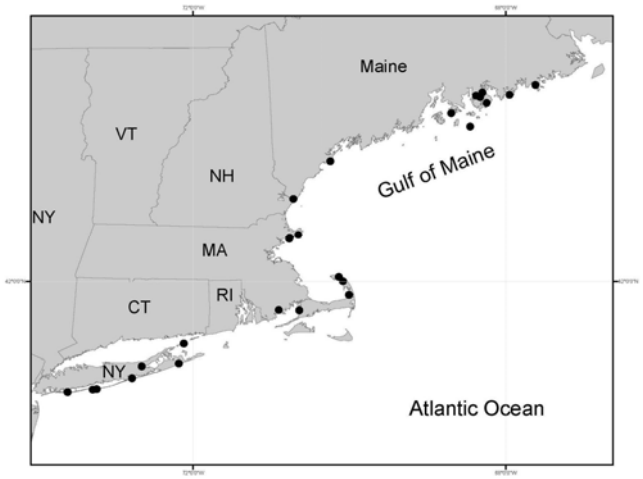

Figure 1. Map of the northwestern Atlantic showing sampling locations of harbor seals

were thawed, and cut to remove hair and skin. Blubber (5 g approx.) was spiked with ^{13}C-labeled PBDE congeners (PBDE 3, 15, 28, 47, 99, 100, 118 and 153) and extracted by homogenization with solvent followed by centrifugation as described previously.[13] An aliquot of the extract (10%) was used for the determination of lipid content by gravimetry. The remaining extract 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 analytes of interest from matrix interferences. The configuration of the system allowed us to collect different fractions at different steps of the purification process. Collected PBDE fractions were volume-reduced and solvent-exchanged to isooctane to 50 µL. The injection internal standard (3.5 µL), containing 400 ng/g PCB 30 and 400 ng/g 3,3',4,4'-tetrabromobiphenyl was added to the concentrated extracts prior to injection onto the GC system.

Analyses were performed by GC-MS, using an HP 6890 gas chromatograph with a 30-m length × 0.25-mm i.d. × 0.25-µm film thickness DB-XLB fused-silica column (J & W Scientific Inc., Folsom, CA) connected to an HP 5972 mass spectrometer. PBDE congeners were monitored by selected ion monitoring (SIM) at the two most intensive ions in the molecular ion cluster or M-2Br ion cluster. All PBDE concentrations were calculated using the internal standard method and were corrected by surrogate recoveries. MDLs for individual PBDE congeners ranged from 0.05 to 0.86 ng/g wet wt, depending on the congener. All analyses were conducted in accordance with Wadsworth Center's Quality Assurance and Quality Control protocols. Concentrations are reported on a lipid weight (lw) basis. Sixteen congeners (BDEs 1, 2, 3, 7, 10, 11, 12, 13, 32, 33, 35, 37, 66, 126, 138, and 166) were below the limit of detection in these samples. In cases where congeners were reported as non-detects, concentrations were calculated by treating the result as if half the detection limit. The variables were log normalized prior to statistical analysis.

Results and Discussion

Of 20 PBDE congeners detected in harbor seal blubber, 10 congeners (IUPAC numbers 28, 47, 49+71, 99, 100, 118, 119, 153, 154, and 155) accounted for 99.1% of the ∑PBDE, which ranged from 96 to 25739 ng/g, lw (mean: 3512 ng/g, lw). The highest concentration (25739 ng/g, lw) was found in a female pup from mid-coast Maine sampled in 2005. BDE 47 dominated the congener profiles, contributing 67%, 58%, and 81% of the total PBDE concentration in adults, yearlings, and pups, respectively. Profiles of the six major PBDEs in these seals (Figure 2) differ from those previously reported for most marine mammals, which are in the descending order: BDE 47>99>100>154>153.[2] In our adult and yearling samples, BDE 153 was the second most prevalent congener and the profile followed the order: BDE 47>153>99>100>154>155. This pattern is similar to that observed in non-occupationally exposed people.[2] The harbor seal pups in this study exhibited a profile distinct from that of the adult males and yearlings: BDE 47>99>100>153>154>155. The differences in profiles could reflect differences in feeding behavior between pups and older seals; adults and yearlings are exposed to PBDEs in their fish prey, while the pups obtain their contaminant burdens almost entirely from their mothers through placental and lactational transfer. The predominance of lower brominated BDE congeners in the pups may be similar to the pattern observed for other lipophilic POPs (PCBs) in which an apparent lipid barrier between mothers and their pups protects nursing pups from accumulating higher chlorinated congeners.[14]

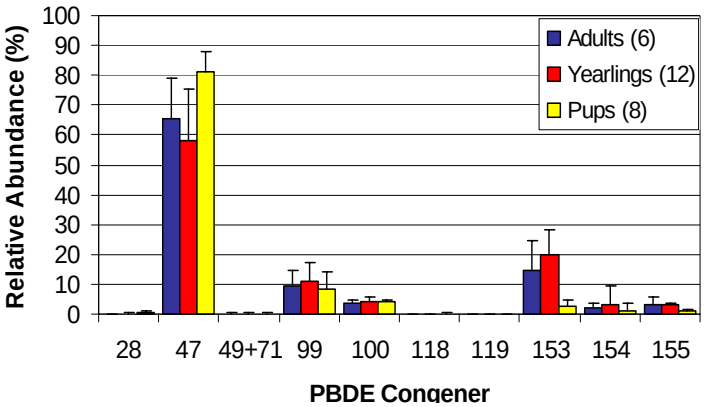

Figure 2. Profile of predominant PBDEs in harbor seals

Influence of Age, Gender, and Condition. No significant differences were found in ∑PBDE concentrations between males and females or between seals of different age classes. The highest concentrations were found in the pups, (5378 ± 9152 ng/g, lw), followed by the yearlings (3254 ± 6473 ng/g, lw) and adult males (1540 ± 1315 ng/g, lw). For the younger seals, the accumulation pattern is similar to that observed for other lipophilic POPs (*e.g.*, PCBs, DDTs). Newborn pups accumulate high levels during gestation and lactation, whereas yearling seals exhibit a

growth dilution of their original body burden. However, the lower PBDE concentrations found in our adult males compared with yearlings is in contrast with the age-dependent increase of lipophilic POPs generally observed in male seals. In our previous study, PCB burdens were nearly identical in the adults males and pups from this region.[12]

Condition indices calculated for harbor seals in this study (weight/length) increased significantly with age ($F_{2,19}$=27.2, p<0.001), but were not significantly correlated with PBDE concentrations. Using ANCOVA to examine the effect of age on PBDE levels while controlling for condition, we found only a marginally significant effect of condition on PBDE burdens among pups and yearlings (p=0.07) and no significant effect of age. Lipid content (%) of the harbor seal blubber samples was positively correlated with condition (R2=0.19, p=0.05) and negatively correlated with wet weight blubber concentrations of ∑PBDE (R2=0.16, p<0.05) and BDE 99 (R2=0.32, p<0.01). Lipid content was highly variable, indicating that some of the animals were in poor nutritive condition. In samples from two pups and one yearling, the lipids were very low (<20%), and PBDE concentrations in these seals were elevated (range of three: 12228-25739 ng/g, lw) compared with levels in the remaining samples. However, when these animals were removed from the analysis, PBDE concentrations in some pups and yearlings still remained relatively high (range: 96-3310 ng/g, lw).

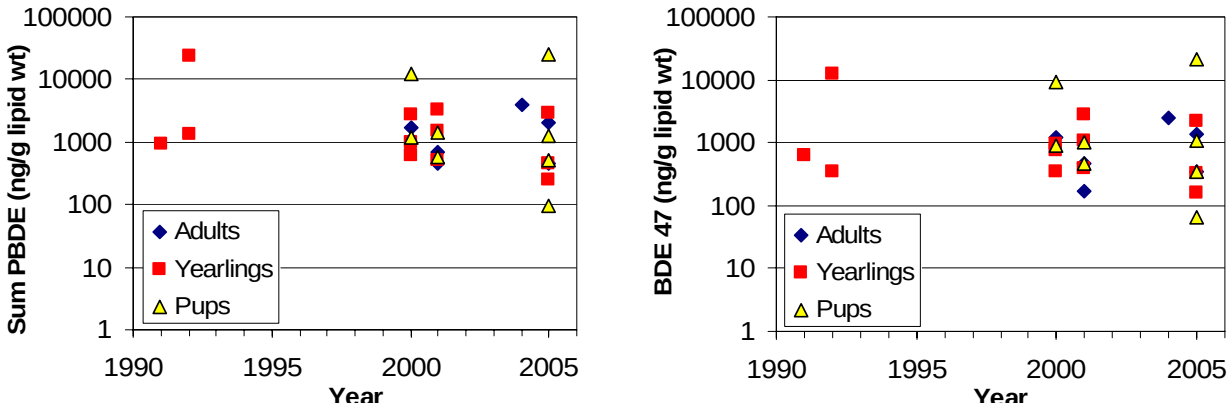

Figure 3. Total PBDE and BDE 47 concentrations (ng/g, lipid wt) in harbor seals from 1991-2005.

Temporal and Spatial Trends. In contrast with most studies examining temporal trends of PBDEs in marine mammals,[2] we found no significant time trend in ∑PBDE concentrations in harbor seals between 1991 and 2005 (Figure 3). Median ∑PBDE concentrations in our samples were 1346, 1153, and 892 ng/g, lw in 1991-92, 2000-01, and 2004-05, respectively. A similar lack of a time trend was reported for organochlorine concentrations (PCBs and DDTs) in harbor seals from this region between 1991 and 2001.[12] Interestingly, Tuerk et al.[11] also found no significant temporal trend in PBDE concentrations in stranded white-sided dolphins from the northwestern Atlantic between 1993 and 2000.

PBDE congener profiles in our samples tended to shift over time. BDE 47 concentrations were increasing and BDE 153 concentrations were decreasing between 1991 and 2000; these trends leveled off between 2000 and 2005. BDE 99 concentrations increased only slightly from 2000 to 2005. Similarly, Ikonomou et al.[6] 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.[2] The meaning of these changes is not clear, but may reflect changes in the use or the composition of the various commercial PBDE products over the years.

There was no significant spatial trend in PBDE concentrations in our samples. This result is interesting because harbor seals from the northwestern Atlantic are believed to be from one population and undergo seasonal migrations throughout their range. Because of their coastal distribution and trophic position along with the influence of the semi-enclosed character of the waters, these seals integrate their exposure to pollution sources from large urban and industrial centers in the northeastern US as well as from long-range atmospheric transport. A similar lack of a spatial

trend in organochlorine concentrations (PCBs, DDTs, chlordanes) was recently reported in harbor seals from this region.[12]

Global Comparisons. Compared with other studies of marine mammals, PBDE concentrations found in northwestern Atlantic harbor seals are approximately at the middle of the contamination spectrum on a global scale. PBDE concentrations detected in the pups and yearlings are higher than those reported in stranded harbor seals from the North Sea and the St. Lawrence Estuary,[8] and similar to those reported in harbor seals from San Francisco Bay in the mid- to late 1990s.[10] Levels in our younger seals are lower than the very high levels found in dolphins from UK waters[7-9] but are an order of magnitude higher than levels reported in St. Lawrence beluga whales.[15] PBDE concentrations detected in the adult male harbor seals are similar to those reported in stranded dolphins along the Gulf of Mexico, Florida.[11] While possible differences in species-specific metabolic capacity, nutritive state, and other factors necessarily limit the utility of comparisons between studies of stranded and free-ranging seals, the PBDE concentrations detected in the harbor seal pups and yearlings in this study exceed the concentration range of 61 – 1500 ng/g, lw, associated with elevated thyroid hormone levels in live-captured gray seal pups and juveniles from UK waters.[16]

To our knowledge, this is the first report of PBDE concentrations in pinnipeds from the US northwestern Atlantic. The long-term effects of PBDEs in marine mammals are presently unknown. Elevated PBDE concentrations in the younger seals in this study are of concern, as they may be more vulnerable to the toxic effects of lipophilic POPs than adults.[17] In view of the magnitude of current PBDE production and ongoing use in the US, further research is needed on a larger sample size to elucidate temporal trends and potential health effects of PBDE exposure in these seals.

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