CHLORINATED AND BROMINATED CONTAMINANTS AND THEIR METABOLIC/DEGRADATION PRODUCTS: BIOACCUMULATION AND DISTRIBUTION IN EAST GREENLAND POLAR BEARS (*URSUS MARITIMUS*) AND RINGED SEALS (*PHOCA HISPIDA*)

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Introduction

Congeners of the additive flame retardants, polybrominated diphenyl ethers (PBDEs) and hexbromocyclododecane (HBCD) have recently been reported in the fat of the apical Arctic predator, the polar bear (*Ursus maritimus*), as well as in the blubber of their ringed seal (*Phoca hispida*) prey, from Scoresby Sounds area of East Greenland, and in animals from Svalbard and areas of the Canadian high Arctic.[1-5] Similar to polychlorinated biphenyl (PCB) concentrations, East Greenland and Svalbard bears and seals were shown to contain higher concentrations relative to other populations in the circumpolar western hemisphere.[6]

Polar bears have demonstrated a high metabolic capacity, via cytochrome P450 (CYP) enzyme-mediated processes, towards chlorinated contaminants such as PCBs[7]. In both polar bears and ringed seal from the Canadian Arctic and East Greenland, OH-PCB metabolites and other chlorinated phenolic compounds have been found in the blood.[8,9] In the case of bears, OH-PCBs often exceed PCB concentrations in blood, which is likely due to effective, competitive binding with thyroid hormones and thyroid hormone transport protein (e.g., transthyretin).[9] In the case of East Greenland bears, levels of OH-PCBs have been reported to be ten times or higher than PCB levels.[8] A few OH-PBDE and/or methoxy- (MeO-) PBDE congeners have been reported in the blood of Arctic mammals and birds.[10,11] It has been hypothesized that like OH-PCBs, OH-PBDEs would be present in bears as oxidative metabolic products, via comparable CYP enzyme-mediated formation processes from PBDEs and favourable, e.g. TTR binding affinity and retention. However, we recently showed that OH-PBDEs and MeO-PBDEs are of very low importance as contaminants in the blood of Svalbard bears.[10]

The bioaccumulation of PCBs, PBDEs and HBCD has recently been reported in polar bear fat relative to ringed seal blubber for several circumpolar populations from the western hemisphere, including East Greenland.[1] It has also been reported that MeO-PBDE and some OH-PBDE congeners present in the tissue of higher tropic level species in marine environments may be the result of the bioaccumulation of natural products produced by marine organisms such as sponges and algae.[12,13] However, there are no known reports on the bioaccumulation or tissue distribution of OH-PCBs, OH-PBDEs or other potential halogenated phenolic contaminants (HPCs) in polar bears. This is in addition to the general dearth of information on the metabolic fate (e.g., oxidative and via dehalogenation) of PCBs and brominated flame retardants like PBDEs[14].

We presently investigated the relative bioaccumulation, metabolism and tissue distribution of PBDEs, HBCD and PCBs, and degradation products or analogues, OH-PCBs and OH-PBDEs, as well as other unexpected halogenated phenolic contaminants, in the liver, blood, brain and fat of polar bears and the blubber of ringed seal prey from an East Greenland population.

Materials and Methods

All the female polar bear and male and female ringed seal tissues were collected in the Ittoqqortoormiit/Scoresby Sound area in central East Greenland and were stored at -20ºC or below until analysis. Procedures for the determination of PCBs, PBDEs, total-(α) HBCD, MeO-PBDEs, OH-PBDEs, OH-PCBs, pentachlorophenol (PCP) and 4-OH-heptachlorostyrene (4-OH-HpCS) have been described elsewhere for all tissues, for the brain modification had been made[1,10,11] Soxhlet extraction was used as well as liquid-liquid partitioning with H_2SO_4 was used to remove lipids from the brain. Phenolics were separated from neutrals by KOH partitioning. The neutral fraction was cleaned up by 1.2% deactivated Florisil column chromatography, one fraction contained PCBs, PBDEs, total-(α) HBCD and MeO-PBDEs. In the phenolic fraction, contaminants were methylated to MeO-derivatives, and were further cleaned up using a 22% H_2SO_4/silica column. All brominated compounds were analyzed by GC-MS(ECNI) in SIM mode for the bromine ion and it's isotope (*m/z* 79 and 81). All other compounds were quantified using an internal standard approach. All tissues were analyzed for 13 PBDE congener (BDE-17, -28, -47, -66, -85, -99, -100, -138, -153, -154, -183, -190 and -209), 15 MeO-PBDE congeners (4'-MeO-BDE17, 6'-MeO-BDE17, 2'-MeO-BDE28, 4-MeO-BDE42, 3-MeO-BDE47, 5-MeO-BDE47, 6-MeO-BDE47, 4'-MeO-BDE49, 6'-MeO-BDE49, 2'-MeO-BDE68, 6-MeO-BDE85, 6-MeO-BDE90, 6-MeO-BDE99, 2-MeO-BDE123, 6-MeO-BDE137) and 14 OH-PBDE congeners (see Figure 1). Recoveries were 90%, 84% and 53% for PBDEs (and PCBs and MeO-PBDEs), OH-PCBs and OH-PBDEs, respectively.

Viable liver tissue from East Greenland polar bears was not collected. Microsomes were prepared for optimal oxidative metabolism from Canadian polar bear liver collected between 1992 and 1994 near Resolute Bay, Northwest Territories and stored at -80°C.5 Procedures for oxidative metabolism *in vitro* assay have been described elsewhere.[15] The microsomes (1mg protein) were exposed in triplicate to individual PBDE congeners (BDE-15, -47, -99, -100, -138, -153, -154, -183, -209, and α-HBCD) at 10 μg/ml, all containing CB-153 as internal standard. The 2'-OH-BDE28 was added as IS for the phenolic fraction before extraction with MTBE/*n*-hexane. Phenolics were separated from neutrals by KOH partitioning. Each assay included BDE-15 as a positive control. The assays included n=3 controls for each BDE congener, instead of NADPH regenerating system solutions a buffer was added. The results are reported as the fraction of PBDE congener depleted (metabolized).

Results and Discussion

The highest Σ-PBDE levels in male and female polar bears were found in liver (1434±1301 ng/g lipid weight) followed by fat (112±118 ng/g lw), blood (93±24 ng/g lw) and brain (15±11 ng/g lw). BDE-47 was the most abundant congener in the polar bear tissues (32-85% of ΣPBDE) as well is ringed seal blubber (48% of ΣPBDE). Σ-MeO-PBDE and Σ-OH-PBDE were found at lower concentrations compared to the parent PBDEs. As indicated by the bioaccumulation factors (BAFs), several PBDEs bioaccumulate from ringed seal to polar bear, although the accumulation favours the liver of polar bear (Table 1).

Table 1. Bioaccumulation factors (BAFs) of PBDE congeners and total (α)-HBCD in female polar bear (n=10) tissues relative to ringed seal blubber (n=15, 9 male, 6 female).*

Tissue	BDE28	BDE47	BDE99	BDE100	BDE138	BDE153	BDE183	Total-HBCD
Fat	0.04	0.41	0.05	0.11	---	1.60	0.44	1.20
Blood	---	0.31	0.06	0.05	---	0.48	---	---
Brain	---	0.07	0.01	---	---	0.14	---	---
liver	0.47	3.90	3.40	2.80	14	7.10	---	---

***** If no BAF value is listed, than the congener could not be quantified in either the polar bear or ringed seal tissues.

In contrast to PCBs and PBDEs, ΣOH-PCB concentrations found in bears were at highest levels in blood (827±353 ng/g wet weight) >> liver (322±176 ng/g ww) > fat (59±46 ng/g ww) > brain (11±7 ng/g ww), which indicates the importance of protein association as the primary mechanism by which these HPCs persistent in the polar bear (Table 2). Furthermore, the blood-brain barrier appears to effectively at hindering deposition of PBDEs in the brain. The trend was similar for 4-OH-HpCS concentrations. ΣMeO-PBDEs accumulated mainly in fat, while the majority of ΣOH-PBDEs retain in the blood in polar bears.

Although these HPCs possess lipophilic properties (e.g., log K_{OW} values of 4 to 5) that would suggest they can potentially bioaccumulate, and in contrast to PCBs and PBDEs, they are of much lesser importance as contaminants in fat in both bear and seal. Furthermore, given the lack of detection in ringed seal blubber, and in contrast to the bioaccumulative PCP, OH-PCBs and 4-OH-HpCS are clearly metabolites of PCBs and octachlorostyrene (OCS), respectively, that are formed in the bear.

Table 2. Bioaccumulation factors (BAFs) of halogenated phenolic compounds (HPCs) and MeO-PBDEs in female polar bear (n=10) tissues relative to ringed seal blubber (n=15, 9 male, 6 female).*

Tissue	PCP	4-OH-HpCS	ΣOH-PCB	6-OH-BDE47	6-MeO-BDE47	6-MeO-BDE85
Fat	1.70	---	---	1.90	0.24	---
Blood	1.00	---	---	---	0.32	12
Brain	0.10	---	---	---	---	---
liver	0.20	---	---	---	---	---

*The 4-OH-HpCS and ΣOH-PCBs were not quantifiable in ringed seal blubber. All other compounds were detected in ringed seal blubber and at least one of the polar bear tissues. With the exception of 4-OH-HpCS and ΣOH-PCBs, if there is no BAF value, than the compound was not detected in polar bear tissue.

In contrast to OH-PCBs, OH-PBDEs and MeO-PBDEs are of minimal importance in bears or seals as PBDE metabolites or bioaccumulated organohalogens from seal to bear (Table 2). Thus, PBDEs do not appear to be favourable substrates for CYP enzymes that would otherwise mediate the formation of OH-PBDE metabolites in polar bears. The BAFs of the four OH- and MeO-PBDE that could be identified and quantified (anthropogenic, natural products and/or metabolites) demonstrated that these *ortho*-OH-substituted congeners are likely accumulated natural products in both seals and bears (Table 2).

The lack of apparent PBDE oxidative metabolism in the bears was further corroborated by the results from the preliminary, hepatic oxidative microsomal *in vitro* assay results that showed no *in situ* OH-PBDE formation from example BDE-47 (Figure 1).

In contrast to the BDE congeners, the low BAF for α–HBCD suggests that the flame retardant is susceptible to CYP-mediated oxidative metabolism (Table 1). This appeared to be corroborated by the preliminary results from the 90 min *in vitro* hepatic microsomal assay, where the positive control, BDE-15, and α-HBCD were depleted 42% and 25%, respectively. However, no oxidative metabolites of BDE-15 and α-HBCD have as yet been determined.

Finally, we unexpectedly detected in the GC-MS(ECNI) mass spectra (not shown) three other major, brominated phenolic compounds that are may be OH-PBB metabolites of accumulated PBBs or accumulated OH-PBBs, which we detected in both polar bear.

In summary, there are large contrasts in the source, fate and accumulation of difference classes of chlorinated and brominated phenolics, several of which were previously unknown in the tissues of polar bears and ringed seals.

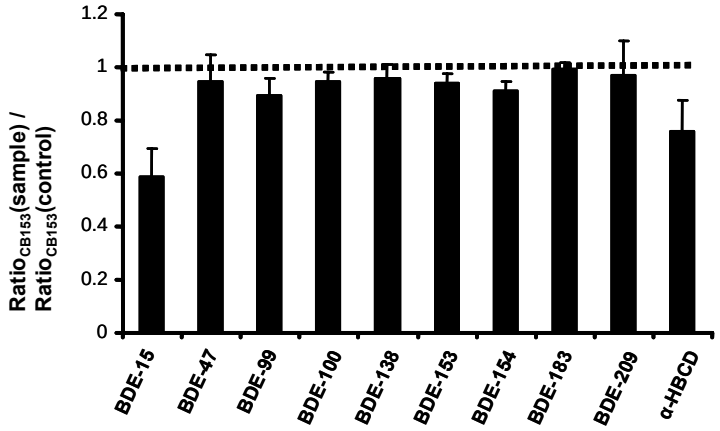

Figure 1. Fraction of the BDE congener concentration remaining after a 90 min *in vitro* assay using hepatic microsomes from polar bear (n=3 intra-day replicates plus n=2 inter-day sets of replicates). The dotted lines signify no depletion.

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