Bioaccumulation and biotransformation of brominated and chlorinated contaminants and their metabolites in ringed seals (Pusa hispida) and polar bears (Ursus maritimus) from East Greenland

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Abstract

We report on the comparative bioaccumulation, biotransformation and/or biomagnification from East Greenland ringed seal (Pusa hispida) blubber to polar bear (Ursus maritimus) tissues (adipose, liver and brain) of various classes and congeners of persistent chlorinated and brominated contaminants and their metabolic by-products: polychlorinated biphenyls (PCBs), chlorodienes (CHLs), hydroxyl (OH-) and methylsulfonyl (MeSO2-) PCBs, polychlorinated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) flame retardants and OH- and methoxyl (MeO-) PBDEs, dibromodichloromethane (p,p'-DDE), 3,3',5-trichlorobenzene (MeO-PBDEs), 3,3',5,4,4'-pentamethylbenzene (PCP) and 4-OH-heptachlorostyrene (4-OH-HpCS). We detected all of the investigated contaminants in ringed seal blubber with high frequency, the main diet of East Greenland bears, with the exception of OH-PCBs and 4-OH-HpCs, which indicated that these phenolic contaminants were likely of metabolic origin and formed in the bear from accumulated PCBs and octachlorostyrene (OCS), respectively, rather than being bioaccumulated from a seal blubber diet. For all of the detectable sum of classes or individual organohalogens, in general, the ringed seal to polar bear mean BMFs for total-(α),-HBCD, ΣOH-PBDEs, ΣMeO-PBDEs and ΣOH-PBDEs indicated that these organohalogens bioaccumulate, and in some cases there was tissue-specific biomagnification, e.g., BMFs for bear adipose and liver ranged from 2 to 570. The blood-brain barrier appeared to be effective in minimizing brain accumulation as BFs were ≤ 1 in the brain, with the exception of ΣOH-PBDEs (mean BF = 93 ± 54). Unlike OH-PCB metabolites, OH-PBDEs in the bear tissues appeared to be mainly accumulated from the seal blubber rather than being metabolic formed from PBDEs in the bears. In vitro PBDE depletion assays using polar bear hepatic microsomes, wherein the rate of oxidative metabolism of PBDE congeners was very slow, supported the probability that accumulation from seals is the main source of OH-PBDEs in the bear tissues. Our findings demonstrated from ringed seal to polar bears that organohalogens biotransformation, bioaccumulation and/or biomagnification vary widely and depended on the contaminant in question. Our results show the increasing complexity of bioaccumulated and in some cases biomagnified, chlorinated and brominated contaminants and/or metabolites from the diet may be a contributing stress factor in the health of East Greenland polar bears.

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1. Introduction

Polar bears (Ursus maritimus) are among a few species in the marine food web of the circumpolar Arctic occupying the highest trophic positions along with marine mammal species such as killer whales (Orcinus Orca) (Letcher et al., 2009). Depending on the subpopulation, seal species availability and food stress situations, polar bears consume mainly the blubber of ringed seal (Pusa hispida). However, depending on the polar bear sub-population lesser amounts of harp (Phoca groenlandica), hooded (Cystophora cristata), bearded (Erginathus barbatus) and harbour (Phoca vitulina) seals may be consumed (Derocher et al., 2002; Grahl-Nielsen et al., 2003; Thiemann et al., 2008). Other marine mammals have been shown to be consumed by polar bears depending on the sub-population (Smith and Sjare, 1990; Thiemann et al., 2008). Based on stable carbon and
nitrogen isotopes as dietary tracers, Bentzen et al. (2007) inferred that for Beaufort Sea bears the dietary contribution from scavenging bowhead whale (Balaena mysticetus) carcasses was 11–26% in 2003 and 0–14% in 2004. For western Hudson Bay bears it was recently shown that over the last two decades changes in chemical tracers of diet (i.e., stable carbon isotopes and fatty acid profiles) were related to increasingly earlier ice break-up date, which suggested a dietary shift with a relative decrease in the proportion of bearded seals consumed and increases in the proportion of harbour and harp seals consumed in years with a longer period of open water. This shift in the proportions of ice-associated to open-water associated prey was largely consistent with an observed diet shift for western Hudson Bay bears using fatty acid tracers of the bears and of their prey over the 1994–2004 period (Thiemann et al., 2008). Regardless, both of these studies noted that regardless of temporal diet shifts that for polar bears from the western Hudson Bay sub-population, there was a relatively constant and high ringed seal consumption over this time period. Therefore, ringed seal likely remains as the predominant prey item for polar bears across regions, especially for the East Greenland sub-population, and thus the main source of dietary exposure to persistent and bioaccumulative organohalogen pollutants (POPs).

Polar bears from East Greenland have been documented to accumulate some of the highest levels of brominated and chlorinated persistent organic pollutants (POPs) in their adipose tissue and/or blood relative to bears from other circumpolar populations. Elevated levels of polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), as well as metabolites and by-products including methylsulfonyl-PCB (MeSO2-PCB) metabolites of PCBs, and DDT and chlordane metabolites have been reported (Gebbink et al., 2008a, b; Letcher et al., 2006; Muir et al., 2006; Norstrom et al., 1998; Verreault et al., 2005a).

The bioaccumulation of an organohalogen POPs in wildlife is determined by the uptake and retention from all applicable exposure routes (primarily through ingestion) (MacDonald and Bewers, 1996). Biomagnification refers to the tendency of a contaminant to become increasingly concentrated at successively higher trophic levels of a food web, and is indicated by a biomagnification factor (BMF; ratio of concentration in predator to prey; Muir et al., 1988) greater than unity. The biomagnification of organohalogenated chemicals as legacy POPs (e.g., PCBs and several classes of OC pesticides) has been studied in ringed seal (blubber) relative to polar bear (fat) from Canadian and Alaskan populations (Bentzen et al., 2008; Letcher et al., 1998; Muir et al., 1988; Kucklick et al., 2002). MeSO2-PCBs and 3-MeSO2-p,p′-DDE, which are known metabolites of PCBs and p,p′-DDE, respectively, have been found to be formed in both ringed seals and polar bears, but have also been shown to accumulate from ringed seals to polar bears from the Resolute Bay area (Letcher et al., 1998). Muir et al. (2006) recently reported that for female bears from circumpolar populations, the BMFs (from seal blubber) for individual PBDE congeners ranged from 0.2 (BDE154) to 130 (BDE153). For East Greenland females, using ringed seal blubber data from Vorkamp et al. (2004), the BMFs reported for individual PBDE congeners ranged from 0.2 (BDE154) to 130 (BDE153). For East Greenland females, using ringed seal blubber data from Vorkamp et al. (2004), the BMFs reported for individual PBDE congeners ranged from 0.2 (BDE154) to 130 (BDE153). For East Greenland females, using ringed seal (blubber) relative to polar bear (fat) from Canadian and Alaskan populations, Vorkamp et al., 2002). MeSO2-PCBs and 3-MeSO2-p,p′-DDE, which are known metabolites of PCBs and p,p′-DDE, respectively, have been found to be formed in both ringed seals and polar bears, but have also been shown to accumulate from ringed seals to polar bears from the Resolute Bay area (Letcher et al., 1998). Muir et al. (2006) recently reported that for female bears from circumpolar populations, the BMFs (from seal blubber) for individual PBDE congeners ranged from 0.2 (BDE154) to 130 (BDE153). For East Greenland females, using ringed seal blubber data from Vorkamp et al. (2004), the BMFs reported for individual PBDE congeners ranged from 0.2 (BDE154) to 8.8 (BDE153). A recent study on PBDEs in male Svalbard polar bears and ringed seals showed a contrast in BMFs for individual PBDE congeners, which ranged from 0.16 (BDE28) to 5.2 (BDE153) (Sermon et al., 2006). These results illustrate the challenges in accurately assessing the biomagnification of contaminants in studies on polar bears and other wildlife from different regions, considering the variation in factors such as sex, age class, diet, collection season and year and the number of congeners comprising the sum concentration of a given organohalogen compound.

PCB, OC and/or PBDE concentrations have shown significant correlations with changes in immune, endocrine, reproductive and organ histopathological biomarkers, suggesting possible exposure–effect linkages in polar bears from the Svalbard and East Greenland sub-populations (Letcher et al., 2009; Sonne et al., 2006a, b). However, there is presently a dearth of information on the bioaccumulation and/or biomagnification from the diet to polar bears from any subpopulation, for several emerging chlorinated and brominated POPs that have been recently identified in polar bears, specifically those of the phenolic variety (Braune et al., 2005; Letcher et al., 2009; Sandala et al., 2004; Sandau et al., 2000). In the case of hydroxylated (OH) PCBs, they have been shown to be metabolites of accumulated PCBs in comparative studies on captive sled dogs (Canis familiaris) and the diet they were fed (Verreault et al., 2009a, b). However, persistent OH-PCBs have been reported to have log Kow values from 5.9 to 7.2 and thus are sufficiently lipophilic and have the potential to accumulate from the diet and persist in (selected) tissues of exposed biota (Malmberg, 2004). We recently identified and characterized in tissues of East Greenland polar bears OH-PCB, OH-PBDE and OH-PBB congeners, and 4-OH-heptachlorostyrene (4-OH-HpCS); however, it was not clear as to the relative importance of dietary accumulation versus metabolic formation in bears from bioaccumulated PCB, PBDE, PBB and octachlorostyrene (OCS) precursors, respectively (Gebbink et al., 2008a, b). In the present study, we examined the comparative bioaccumulation (including biotransformation) and/or biomagnification among a diverse suite of chlorinated and brominated contaminants in ringed seals to polar bears from East Greenland.

2. Materials and methods

2.1. Sample collection

Adipose tissue, brain and liver samples were collected from adult male (n = 10; ages 6–16 years) and female (n = 10; ages 6–23 years) polar bears in the Ittoqqortoormiit/Scoresby Sound area in central East Greenland between 69°00′ N and 74°00′ N and within the area of research of Bentzen et al. (2004). All samples were collected in November 2003 and March 2004, which are described in detail elsewhere (Gebbink et al., 2008a, b; Dietz et al., 2006; Verreault et al., 2005a). The age of all the individuals was determined by counting annual growth layers in the cementum of an I3 tooth after decalcification, thin sectioning (14 μm) and staining with toluidine blue (Gebbink et al., 2008a, b and references therein). Blubber tissues from n = 6 female and n = 9 male ringed seals that were collected between August 2001 and January 2002 in the same area as for the polar bear tissues. The ringed seals had a mean age of 5.4 years, which ranged from 2 to 11 years old. All tissues were taken <12 h post mortem and stored in polyethylene bags. All samples were frozen and kept at temperatures of −5 to −20 °C at the time of sampling, and stored at −20 °C until chemical analysis. Polar bear sampling was conducted under Greenland research licenses/permits. Both ringed seal and polar bear samples were transported to Canada under a valid Danish CITES export permit (# RE 0813–457/04) and a veterinary import permit (# A-2005-03018-3) issued by the Canadian Food Inspection Agency.

2.2. Contaminant analyses

The extraction and clean-up of polar bear adipose tissue, brain and liver for PCBs, OC pesticides, BFRs and MeSO2- and OH-metabolites is described in complete detail elsewhere (Gebbink et al., 2008a, b). The extraction and clean-up of the ringed seal blubber was identical to the extraction and clean-up of the polar bear adipose tissue. Briefly, around 0.5 g of ringed seal blubber sample was spiked with internal standards, which included a 13C12-labeled PCB congener mixture (6 congeners for PCBs and OCs), 3-MeSO2-2-Ch2-3′,4′,5′,5′-pentancB (for MeSO2-PCBs/ p,p′-DDE), BDE30 and 71 (for PBDEs, MeO-PBDEs, total-(α)-HBCD and PBBS), a 13C12-labeled OH-PCB congener mixture (4 congeners) for OH-PCBs, PCP and 4-OH-HpCS) and 2-OH-BDE28 (for OH-PBDEs and OH-PBBS) and extracted with organic solvents. Lipid content was determined gravimetrically, and lipids were removed from the extract by liquid–liquid partitioning with H2SO4. Neutral and phenolic POPs were extracted from the acid with hexanes.

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After dilution with H2O, the MeSO2-metabolites were extracted from the acid with DCM and cleaned up on a KOH/silica column. Neutrals were separated from phenolics by KOH partitioning. The MeSO2-PCBs/DDE and neutrals were recombined and cleaned on a Florisil column and the MeSO2-PCBs/DDE were subsequently cleaned up on a basic alumina column. The phenolic fraction was methylated using diazomethane and cleaned on an acid/silica column. Details about the gas chromatography-mass spectrometry (either in the electron impact or electron capture negative ionization modes) parameters including the specific congeners monitored (see footnotes in Table 1 as well), as well as the congeners and class-specific contaminant concentrations, have been thoroughly described and reported in Gebbink et al. (2008a,b). PCBs and OC pesticides were quantified using an external standard approach; all the other contaminants were quantified using an internal standard approach.

2.3. Microsomal in vitro PBDE metabolism

Using available hepatic microsomes from polar bear, oxidative metabolism was assessed for several PBDE congeners and α-HBCD found in polar bears. Obtaining enzymatically viable liver tissue from field collections of the present East Greenland polar bears was not possible. As an alternative, hepatic microsomes were used from the liver of Canadian polar bears that had been collected between 1992 and 1994 near Resolute Bay, Northwest Territories and had been stored continuously at NWRC between 5 and 8°C. These microsomes were prepared ca. 1995 for optimal preservation of oxidative cytochrome P450 monooxygenase (CYP450) catalytic activity (Letcher et al., 1996), and the EROD activity was determined according to Letcher et al. (1996). A re-analysis of the EROD activity (representative of general CYP isoenzyme catalytic activity) just prior to the present study showed that it was at 60% (626 pmol min⁻¹ mg⁻¹) of the former rate (1056 pmol min⁻¹ mg⁻¹). Thus, the microsomes were still possessed high catalytic activity.

Procedures for oxidative metabolism in vitro PBDE metabolism (individual congeners) assay have been described elsewhere (McKinnney et al., 2006b). Briefly, the hepatic polar bear microsomes (1 mg total protein) were incubated in triplicate with individual PBDE congeners (BDE-47, -99, -100, -118, -138, -153, -154, -183, -209, and α-HBCD) at 10 μg/ml. All incubations contained CB-153 as the internal/ recovery standard and negative control and BDE-15 as the positive control. CB-153 is a highly recalcitrant PCB congener and has been shown previously not to be significantly depleted in the time frame of the assay when using seal or beluga whale hepatic microsomal assays (Li et al., 2003; McKinnney et al., 2006b).

The CYP450 catalytic assay was initiated by the addition of NADPH regenerating system solutions [50 μl of Solution A and 10 μl of Solution B, (Gentest San Jose, CA, USA)] and the catalytic activity was terminated after 90 min by the addition of 0.5 M NaOH. The 2'-OH-BDE28 congener was added as the IS for the brominated phenolic fraction prior to the extraction of the incubation medium with MTBE/n-hexanes. Phenolics were separated from neutrals by aqueous KOH partitioning. Each set of assays included negative controls (n = 3) wherein buffer was added instead of the NADPH regenerating system. The results are reported as the fraction of PBDE congener depleted (metabolized). For each incubation, the results for the parent PBDE congeners were first internal standard corrected (Eq. (1)), the RatiocB153 to the samples (to which NADPH was added) was then compared to the RatioCB153 of the controls (to which NADPH was not added) (Eq. (2)).

\[
\text{Ratio}_{\text{B153}} = \frac{\text{PeakArea}(\text{BDE} - X)}{\text{PeakArea}(\text{CB153})}
\]

\[
\text{Fraction Remaining} = \frac{\text{Ratio}_{\text{B153}}(\text{Sample})}{\text{Ratio}_{\text{B153}}(\text{Control})}
\]

2.4. Quality assurance and control

Quality assurance and quality control for contaminant and metabolite determinations included laboratory method blanks, matrix (IS) spikes, and calibration standard injections. Traces of BDE-47, -99 and -100 found systematically in the method blanks during the PBDE analysis were low compared to levels in the samples, and the samples were thus background subtracted. The method limits of quantification (MLOQs) for PCBs, OC pesticides and PBDEs were around 0.1 ng/g wet wt for all the tissues, for MeSO2-PCBs, OH-PCB and OH-PBDE the MLOQs were calculated based on the laboratory method blanks, matrix (IS) or the catalytic activity was terminated after 90 min by the addition of 0.5 M NaOH. The 2'-OH-BDE28 congener was added as the IS for the brominated phenolic fraction prior to the extraction of the incubation medium with MTBE/n-hexanes. Phenolics were separated from neutrals by aqueous KOH partitioning. Each set of assays included negative controls (n = 3) wherein buffer was added instead of the NADPH regenerating system. The results are reported as the fraction of PBDE congener depleted (metabolized). For each incubation, the results for the parent PBDE congeners were first internal standard corrected (Eq. (1)), the RatiocB153 to the samples (to which NADPH was added) was then compared to the RatioCB153 of the controls (to which NADPH was not added) (Eq. (2)).

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\text{Fraction Remaining} = \frac{\text{Ratio}_{\text{B153}}(\text{Sample})}{\text{Ratio}_{\text{B153}}(\text{Control})}
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2.5. Data analysis

Concentrations of all of the organohalogen compounds are reported on a lipid weight (lw) basis and used to determine the BMFs of polar bear liver.
3. Results and discussion

3.1. Organochlorines and organobromines in ringed seal blubber

In general, there was no significant difference (p=0.09) in the levels among the sums of the individual POP classes (Table 1) in the blubber of male versus female ringed seals. Therefore, male and female ringed seals were viewed as a single sample set representing the diet of East Greenland polar bears. Among fourteen individual organochlorines or sum of classes of organochlorines, the 2,3PCB concentration was highest, followed by p,p′-DDE and 2,3CHL in the ringed seal blubber (Table 1). Mean levels of POPs were higher in females than in males reported in East Greenland ringed seals sampled in 2001 (Vorkamp et al., 2004).

Of the OH-PCBs or 4,4′-OH-PCAs analyzed there were no detectable compounds in the blubber of the present East Greenland ringed seals (Table 1). This is consistent with the findings of Sandu et al. (2000) who reported a mean 2,3CHL concentration (n=5) of 0.081 ng/g ww and non-detectable 4,4′-OH-PCAs (0.05 ng/g lw) in the plasma of ringed seals from Kuumajua, Quebec, Canada (1999). Non-detectable OH-PCB and 4,4′-OH-PCAs residues in the blubber of the present East Greenland ringed seals indicated that bioaccumulation of OH-PCB and 4,4′-OHHcs from consumption of ringed seal blubber is most probably not a source of the levels in bears. The lack of OH-PCB and 4,4′-OHHcs in the present study may be accounted for by low concentration (0.05 ng/g lw), and thus, too low concentrations to be detectable. OHHcs and OCS, respectively. Although this is likely related to limited deposition and storage of these phenolics in blubber, it is possible that some contribution of the OH-PCB and 4,4′-OHHcs levels in bears was the result of accumulation and biomagnification of non-detectable levels found in the seal blubber (Table 1). Furthermore, perhaps a proportion of these phenolics came from consumption of seal blood, which is a body compartment where a large portion of the body burden resides (Geckinli et al., 2008b). Routti et al. (2008) recently showed that OH-PCBs (mainly 4-OH-CB107 and 4-OH-CB108) are present in the plasma of ringed seal from Svalbard and the Baltic Sea, and likely metabolites formed from precursor PCBs in the seal. In the present study PCP was low but quantifiable in ringed seal blubber (0.01 ± 0.01 ng/g lw), and thus, two sources of PCP in the bear tissue are possible, 1) accumulation from seal blubber and 2) hexachlorobenzene (HCB) metabolism to PCP in bears. HCB has been shown to be metabolized to PCP in dosed laboratory rats (Remer, 1988).

In East Greenland ringed seal blubber, the mean 3MeSO2-p,p′-DDE was similar in concentration (mean 36.2 ± 5 ng/g lw) (Table 1) and congener pattern to previously reported values in Canadian ringed seals sampled at Resolute Bay in 1993 (Letcher et al., 1998). A metabolite of p,p′-DDE, 3-MeSO2-p,p′-DDE, was detected in ringed seal blubber and found at levels that were comparable to 3-MeSO2-p,p′-DDE concentrations in Canadian ringed seals (Table 1) (Letcher et al., 1998). The finding of 3MeSO2-PCBs and 3-MeSO2-p,p′-DDE in the ringed seal blubber indicates that biomagnification and biomethylation are both possible sources of these MeSO2-metabolites in polar bears.

The mean 2PBD concentration (149 ± 67 ng/lw; Table 1) in ringed seal blubber was close to the means for 3,4-MeO-PCBs in the adipose tissue of adult seals on East Greenland ringed seals collected in 2001 (Vorkamp et al., 2004). Also, the PBDE congeners pattern in the present seal blubber showed that 55–85% of the 2PBD concentration was comprised of BDE-47 with much lesser proportions of BDE-99 (1–15%), 100 (<10%) and -133 (<4%). This was comparable to the pattern found in East Greenland and Canadian ringed seal blubber (Letcher et al., 1998; Gebbink et al., 2008b). Relative to the present ringed seal, similar total (α)-OHBCD levels were 2.5 times ringed seal blubber captured in 2003 (Sarrmo et al., 2006).

Of the congeners monitored (Table 1), only very low levels of one OH-PCB congener (6-OH-BDE47: 0.7 ± 0.5 ng/lw) and three MeO-PCB congeners (6-MeO-BDE47, 2-MeO-BDE99 and 6-MeO-BDE105) were detected in the ringed seal blubber. The 6-OH-BDE47, 6-MeO-BDE47 and 2-MeO-BDE68 congeners have been identified as natural products produced by marine sponges (Carter and Faulkner, 1981; Fu and Schmitz, 1996) and have been detected in red algae and blue mussels from the Baltic Sea (Malmvärn et al., 2005). The 6-MeO-BDE68 congener is also ortho-MeO-substituted and is likely also of natural origin, while all natural OH- and MeO-PCB congeners identified to date have been ortho-Me-O substituted. The 6-OH-BDE47 congener is most likely of a natural source as the capacity of ringed seals towards oxidative metabolism of PBDEs is likely to be low as exemplified by the lack of detectable OH-PCBs and 4,4′-OH-HPCs in the present ringed seal blubber (this study) or low levels of mainly ortho-substituted OHBCDs in ringed seal plasma from Svalbard, Canadian high Arctic or the Baltic Sea (<2.0 ng/g ww) (Routti et al., 2008; Sandau et al., 2009).

Three identified OH-PBB congeners, one tri-brominated and two tetra-brominated, were detected in the seal blubber (ΣOH-PBB = 0.3 ± 0.3 ng/g lw or ww; Table 1). We reported the same three OH-PBB in the present polar bears, which were mainly in the fat (mean 45.2 ± 17.8 ng/g lw) (Sandau et al., 2000; Gebbink et al., 2008a), but with virtually the entire body burden residing in the fat (Gebbink et al., 2008b). This would suggest that OH-PBBs in bears are accumulated from the ringed seal blubber diet. Like OH-PCBs, the source of OH-PBBs is unclear, although they may have been formed by oxidative metabolism of PBDBs in the present ringed seals and/or may have been accumulated from natural marine products. The latter is likely since the concentration of BB-101 was 10 ng/g ww, and a di-OH-PBB (2,2-di-OH-BB88) was recently identified as a natural product in marine bacteria (Iinonieto and Kamei, 2003).

3.2. Organohalogen bioaccumulation and biomagnification in polar bear tissues

Gebbink et al. (2008b) showed that there were sex-specific differences in PCB, MeSO2-PCBs, MeSO2-p,p′-DDE levels in East Greenland polar bears, and therefore the results of the present study tissue-specific for these brominated congeners were calculated separately for male and female polar bears. In general, the ringed seal to polar bear bioaccumulation and biomagnification (BMFs) for these contaminants were calculated using a Student’s t-test. A similar t-test was used to assess the significance of the difference in mean POP concentrations between male and female ringed seals. The significance level was set to α = 0.05.
We further assessed the capacity of polar bears to oxidatively metabolize PBDE congeners using an in vitro microsomal assay (Fig. 3), which was optimized for CYP monooxygenase catalytic activity (Letcher et al., 1996). The positive control, BDE-15 was significantly (p = 0.00006) depleted by 41% during the assay. Of the environmentally relevant PBDE congeners, only BDE-154 was significantly (p = 0.001) depleted by 9%. We did not detect phenolic metabolites for either of these biotransformed congeners (BDE-15 and BDE-154). The BDE-154 depletion results suggested that the low ringed seal blubber to polar bear adipose BMF of BDE-154 we reported earlier (Verreault et al., 2005a,b) was similar to, as well as by Muir et al. (2006) for circumpolar bears, is at least in part due to the ability of polar bears to biotransformation this congener. However, lack of significant depletion of the other congeners suggests that at least from a Phase 1 CYP monooxygenase perspective, polar bears have a low capacity to metabolize environmentally relevant PBDEs to OH-PBDE metabolites. In studies with laboratory rats, oxidative metabolites of PBDE congeners, such as 3-OH-BDE47, were formed metabolically after dietary exposure of PBDEs (Marsh et al., 2006; Hakk et al., 2002).

The BMFs for MeO-PBDEs indicate that they bioaccumulated and were not biomagnified from ringed seal blubber to polar bear adipose tissue (Fig. 2). Similar to OH-PBDEs, sources of the MeO-PBDEs may be through food chain bioaccumulation or methylated metabolite formation from OH-PBDEs. Two of the three MeO-PBDEs that were detected in the polar bear tissues were also found in the seal blubber; in this case, the most likely source is bioaccumulation. The other congener (6-MeO-BDE17) was not detected in the ringed seal; in this case, metabolic formation may be the likely source. Several congeners have been identified as natural products from algae or sponges (Malmvärn et al., 2005; Teuten et al., 2005). Identified natural products were 6-MeO-BDE17 and 7-MeO-BDE8.

The BMF for total-(α)-HBCD, which only accumulated in the polar bear adipose tissue, was found to be 1.7 ± 0.6 (Sermo et al., 2006) found a lower but comparable BMF from Svalbard ringed seal blubber to polar bear adipose tissue (0.6 ± 0.7). The BMF of total-(α)-HBCD in the present study suggested that the rate of accumulation was slightly higher than the rate of bioaccumulation. The in vitro depletion of 24% for α-HBCD was significant (p = 0.02) in the microsomal assay (Fig. 3). No other oxidative metabolites were detected in vitro. Rats exposed to the HBCD technical mixture were shown to have significant CYP2B induction (as measured by 7-pentoxyresorufin-O-depentylation activity; Germer et al., 2006). This biotransformation of α-HBCD via CYP2B enzymes could explain depletion of α-HBCD in the assay, noting that polar bears have relatively high liver CYP2B content (Letcher et al., 1996). Since the ringed seal blubber to polar bear adipose BMF for total-(α)-HBCD suggested some biomagnification (Fig. 2A), and metabolism in the 90 min in vitro assay showing rapid depletion (Fig. 3), this may suggest that the present, free-ranging East Greenland polar bears were exposed to and accumulated substantial levels of HBCD.
BB-101 was detected in ringed seal blubber but not in any of the polar bear tissues. This finding suggested that elimination/biotransformation of BB-101 in polar bears is more substantial than uptake. Possible metabolites of BB-101, the recently detected OH-PBBs were found in all of the polar bear tissues we studied (Gebbie et al., 2008a,b). The mean BMFs were ≫ 1 for all of the polar bear tissues with the highest observed for the brain. There was no significant difference between the BMFs among the tissues. OH-PBBs have been shown to be formed metabolically in dogs, rabbits and rats after dietary exposure of mono-brominated-PBB and hexa-brominated-PBBs (Kohli et al., 1978; Koss et al., 1994). In these studies, besides direct insertion of an OH-group, debromination followed by hydroxylation also occurred resulting in lower brominated OH-PBB metabolites. Debromination of BB-101 (i.e., penta-brominated) and subsequent hydroxylation by the polar bear may have resulted in the observed tri- and tetrabrominated OH-PBBs. Besides being formed metabolically, OH-PBBs can be naturally produced. For instance, 2,2′-diOH-BB80 was identified in the Pseudoalteromonas phenolis sponge (Iwanszeto and Kamel, 2001). As has been observed previously with 2,2′-diOH-DBB80 and the OH-PBDEs and MeO-PBDEs of natural origin, the substitution of the hydroxyl-group is on the ortho position would seem to favour the ringed seal to polar bear accumulation of OH-PBBs in the present bears.

3.3. Exposure implications for polar bears

There was a large variability in the ringed seal blubber and polar bear levels and BMFs among the differing organohalogen classes. We found large variations in BMFs among the halogenated phenolics, i.e., OH-PCBs, 4-Oh-HpCs, OH-PBBs and OH-PBDEs, even though there are similarities in their physico-chemical properties. Unlike the brominated phenolics, the chlorinated phenolics were not detected in the ringed seal blubber and thus likely entirely formed in the polar bears. Gebbink et al. (2008b) demonstrated that there is also a much greater preferential retention of OH-PCB and 4-Oh-HpCS metabolites in polar bear blood compared to OH-PBDEs. Our results indicate that despite apparently minor structural differences (ether linkage, OH- and halogen-substitution patterns), the sources and fate of these halogenated phenolics in polar bears are highly contrasting.

Our results show the increasing complexity of bioaccumulated and in some cases biomagnified, chlorinated and brominated contaminants and/or metabolites may be a contributing stress factor in the health of East Greenland polar bears. For instance, PCB, OC and/or PBDE concentrations have shown significant correlations with changes in immune, endocrine, reproductive and organ histopathological biomarkers in polar bears (Fisk et al., 2005; Letcher et al., 2009). Also, a number of PCB congeners, several OCs and especially halogenated phenolics such as OH-PBDEs have demonstrated endocrine disrupting activity in laboratory mammals and humans and/or in in vitro assays (Hamers et al., 2006; Letcher et al., 2009; Ulén-Marlin et al., 2009). However, there are other physiological and environmental factors that must be considered with respect to the variability and influence on contaminants exposure such differences in the age, diet (as a function of e.g., changes in sea ice), age and year and period of sample collection (Letcher et al., 2009). In this study, and based on supporting studies, we assumed that ringed seal blubber is highly representative of the diet of East Greenland polar bears. Thus, the calculated POP BMFs did not account for other dietary exposure contributions. For example, we recently reported for western Hudson Bay bears that in years spanning 1991 to 2007, seal dietary shifts occurred with a relative decrease in the proportion of bearded seals consumed and increases in the proportion of harbour and harp seals consumed in years with a longer period of open water (increasingly earlier ice break-up date) (McKinney et al., 2009). This shift in feeding ecology in polar bears from the western Hudson Bay sub-population was a dietary factor the accelerated or changed the rate of increase in the concentrations of several chlorinated and brominated contaminants including PCBs, OCs and PBDEs.

4. Uncited references

McKinney et al., 2009a
Montie et al., 2009
Zar, 1984

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References

Gebbink et al. (2008b)


