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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 metabolic by-products: polychlorinated biphenyls (PCBs), chlordanes (CHLs), hydroxyl (OH-) and methylsulfonyl ($MeSO_2$-) PCBs, polybrominated biphenyls (PBBs), OH-PBBs, polybrominated diphenyl ether (PBDE) and hexabromocyclododecane (HBCD) flame retardants and OH- and methoxyl (MeO-) PBDEs, 2,2-dichloro-bis(4-chlorophenyl)ethene (*p,p′*-DDE), 3-$MeSO_2$-*p,p′*-DDE, pentachlorophenol (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 bears 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 ΣPCBs, *p,p′*-DDE, ΣCHLs, Σ$MeSO_2$-PCBs, 3-$MeSO_2$-*p,p′*-DDE, PCP, ΣPBDEs, total-(α)-HBCD, ΣOH-PBDEs, ΣMeO-PBDEs and ΣOH-PBBs 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 BMFs were ≤1 in the brain, with the exception of ΣOH-PBBs (mean BMF = 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 organohalogen 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.

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 (*Erignathus 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

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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 ($MeSO_2$-PCB) metabolites of PCBs, and DDT and chlordane metabolites have been reported (Gebbink et al., 2008a,b; Letcher et al., 2009; 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 organohalogens classified as legacy POPs (e.g., PCBs and several classes of OC pesticides) have 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). $MeSO_2$-PCBs and 3-$MeSO_2$-*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) (Sørmo 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 class.

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 K_{OW} 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 of 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 August 1999 to March 2000, 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 I_3 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 $MeSO_2$- 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. Briefly, around 0.5 g of ringed seal blubber tissue was spiked with internal standards, which included a $^{13}C_{12}$-labeled PCB congener mixture (6 congeners for PCBs and OCs), 3-$MeSO_2$-2-CH_3-2′,3′,4′,5,5′-pentaCB (for $MeSO_2$-PCBs/-*p,p′*-DDE), BDE30 and 71 (for PBDEs, MeO-PBDEs, total-(α)-HBCD and PBBs), a $^{13}C_{12}$-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 H_2SO_4. Neutral and phenolic POPs were extracted from the acid with hexanes.

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After dilution with H_2O, the $MeSO_2$-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 $MeSO_2$-PCBs/DDE and neutrals were recombined and cleaned on a Florisil column and the $MeSO_2$-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 congener- 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 at <−80 °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 $min^{-1}{\cdot}mg^{-1}$) of the former rate (1056 pmol $min^{-1}{\cdot}mg^{-1}$). Thus, the microsomes were still possessed high catalytic activity.

Procedures for oxidative metabolism *in vitro* PBDE metabolism (individual congeners) assay have been described elsewhere (McKinney 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, -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; McKinney 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 $Ratio_{CB153}$ for the samples (to which NADPH was added) was then compared to the $Ratio_{CB153}$ of the controls (to which NADPH was not added) (Eq. (2)).

$$Ratio_{CB153} = PeakArea(\mathrm{BDE}-\mathrm{X}) \div PeakArea(\mathrm{CB153}) \quad (1)$$

$$Fraction\,Remaining = Ratio_{CB153}(Sample) \div Ratio_{CB153}(Control) \quad (2)$$

2.4. Quality control and assurance

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 $MeSO_2$-PCBs, OH-PCB and OH-PBDE the MLOQs were around 0.05 ng/g wet wt for all the tissues. The MLOQs were based on a signal to noise (S/N) ratio of 10. The recoveries of the internal standards in the ringed seal blubber were on average 78 ± 18%. The sum (Σ) concentrations of PCBs and OC pesticides (i.e., CHLs and DDTs) were within 5% and 8% of the certified values of the NIST SRM 1945 (pilot whale blubber homogenate) (Schantz et al., 1995). Σ-PBDE was within 13% of the certified values of SRM 1945 (Stapleton et al., 2007).

2.5. Data analysis

Concentrations of all of the organohalogens are reported on a lipid weight (lw) basis and used to determine the BMFs of polar bear liver,

Table 1
The arithmetic mean (±SE) of concentrations of sum (Σ) of classes or individual neutral and phenolic organohalogen compounds in the blubber of male and female ringed seals ($n=15$) from East Greenland and collected in 2002.

Analyte	Mean ± SE (ng/g, lipid weight)	No. samples > MLOQ
Lipid (%)	98 ± 1	
ΣPCB[a]	686 ± 69	15
ΣOH-PCB[b]	n.d.	0
Σ$MeSO_2$-PCB[c]	36 ± 5	15
ΣCHL[d]	241 ± 40	15
p,p′-DDE	350 ± 69	15
3-$MeSO_2$-*p,p′*-DDE	1.5 ± 0.4	11
4-OH-HpCS	n.d.	0
PCP	1.0 ± 0.4	15
ΣPBDE[e]	149 ± 87	15
Total-(α)-HBCD	19 ± 2	15
BB-101	0.25 ± 0.12	9
ΣOH-PBDE[f]	0.7 ± 0.5	6
ΣMeO-PBDE[g]	4.6 ± 0.4	15
ΣOH-PBB[h]	0.5 ± 0.3	12

n.d. — not detected and less than the MLOQs. For PCBs, OC pesticides and PBDEs the MLOQs were about 0.1 ng/g lipid wt, and for $MeSO_2$-PCBs and phenolics about 0.05 ng/g lipid wt.

[a] PCBs monitored: CB-28/31, -42, -44, -49, -52, -60, -64/71, -66/95, -70, -74, -84/101, -87, -97, -99, -105, -110, -118, -128, -129/178, -138, -141, -146, -149, -151, -153, -156/171/202, -158, -170/190, -172, -174, -177, -179, -180, -182/187, -183, -194, -195, -196/203, -200, -201 and -206.

[b] $MeSO_2$-PCBs monitored: 3′/4′-$MeSO_2$-CB49, 3/4-$MeSO_2$-CB52, 3/4-$MeSO_2$-CB64, 3/4-$MeSO_2$-CB70, 3′/4′-$MeSO_2$-CB87, 3/4-$MeSO_2$-CB91, 3′/4′-$MeSO_2$-CB101, 3/4-$MeSO_2$-CB110, 3′/4′-$MeSO_2$-CB132, 3′/4′-$MeSO_2$-CB141, 3/4-$MeSO_2$-CB149, 3/4-$MeSO_2$-CB174.

[c] OH-PCB monitored (determined as MeO-derivatives): 4′-OH-CB79, 4-OH-CB97, 4′-OH-CB101/4-OH-CB134, 4-OH-CB107/4′-OH-CB108, 2′-OH-CB114, 3-OH-CB118, 4′-OH-CB120, 4′-OH-CB127, 4′-OH-CB130, 3′-OH-CB138, 4-OH-CB146, 4′-OH-CB159, 4-OH-CB162, 4-OH-CB163, 4′-OH-CB172, 4′-OH-CB177, 4-OH-CB178, 3′-OH-CB180, 3′-OH-CB182, 3′-OH-CB183, 3′-OH-CB184, 4-OH-CB187, 4-OH-CB193, 4′-OH-CB199, 4′-OH-CB200, 4′-OH-CB201, 4′-OH-CB202, 4,4′-diOH-CB202, 3′-OH-CB203/4′-OH-CB198, 4′-OH-CB208.

[d] CHL monitored: oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, heptachlor-epoxide.

[e] PBDEs monitored: BDE-17, 28, -47, -66, -85, -99, -100, -138, -153, -154, -183, -190, -209.

[f] OH-PBDE monitored (determined as MeO-derivatives): 6′-OH-BDE17, 6′-OH-BDE17, 6′-OH-BDE49, 2′-OH-BDE68, 6-OH-BDE47, 3-OH-BDE47, 5-OH-BDE47, 4′-OH-BDE49, 4-OH-BDE42, 6-OH-BDE90, 6-OH-BDE99, 2-OH-BDE123, 6-OH-BDE85, 6-OH-BDE137.

[g] MeO-PBDE monitored: 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.

[h] OH-PBB monitored (determined as MeO-derivatives): one tri-brominated and two tetra-brominated OH-PBB.

brain and fat relative to ringed seal blubber. As we detailed previously (Gebbink et al., 2008a,b), while some of the POP levels were associated with lipid content (e.g., PCBs, PBDEs, CHLs, DDTs and $MeSO_2$-PCBs), others were not (i.e., all phenolic classes of chlorinated and brominated POPs). For the present study, the same was found for ringed seal blubber (Table 1). However, in the ringed seal blubber all of the phenolic classes of OHCs were either non-detectable (i.e., OH-PCBs and 4-OH-HpCS) or exceedingly low and <1.0 ng/g lw (i.e., OH-PBDEs and OH-PBBs) relative to the lipid associated OHC classes that were at concentrations that were at least 2 orders of magnitude higher concentrations in the ringed seal blubber (see Table 1). Thus, for the comparison of concentrations among OHC classes in ringed seal (blubber) or polar bear (tissues), and especially for the determination of BMFs, lipid normalization was warranted.

Prior to data handling, the BMFs (concentration in polar bear tissue/concentration in ringed seal blubber, ng/g lw) were $\log_{10}$-transformed for optimal normality of the data distribution according to a Shapiro–Wilk's test (Zar, 1994). Contaminant BMFs based on adipose, brain and liver of male and female bears relative to ringed seal blubber were compared. We chose not to test the significance of the difference in the mean BMFs. The factors contributing to a BMF represents the integration of complicated processes in both prey and predator. Therefore the true meaningfulness of POP BMF is not fully substantiated until all physiological and ecological factors are accounted for. The standard error of the BMFs were calculated using the SE of the mean concentration of the contaminants in the polar bear tissues and the ringed seal blubber. Significant depletion in the *in vitro* microsomal assay of the PBDE congeners and α-HBCD, relative to the negative controls, were determined 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 $\alpha = 0.05$.

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 organohalogens or sum of classes of organohalogens, the ΣPCB concentration was highest, followed by *p,p′*-DDE and ΣCHL in the ringed seal blubber (Table 1). Mean levels and patterns of *p,p′*-DDE, ΣPCB and ΣCHL were similar to those levels reported in East Greenland ringed seals sampled in 2001 (Vorkamp et al., 2004).

Of the OH-PCB congeners and 4-OH-HpCS 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 Sandau et al. (2000) who reported a mean ΣOH-PCB concentration ($n = 5$) of 0.081 ng/g ww and non-detectable 4-OH-HpCS (<0.05 ng/g lw) in the plasma of ringed seals from Kuujjuaq, Quebec, Canada (1999). Non-detectable OH-PCB and 4-OH-HpCS residues in the blubber of the present East Greenland ringed seals indicated that bioaccumulation of OH-PCB and 4-OH-HpCS from consumption of ringed seal blubber is most probably not a source of the levels in bears. The lack of OH-PCBs and 4-OH-HpCS in blubber suggested that ringed seals have a low capacity to biotransform PCBs 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-OH-HpCS 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 (Gebbink 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 (1.0 ± 0.4 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 (Renner, 1988).

In East Greenland ringed seal blubber, the mean $\Sigma MeSO_2$-PCB was similar in concentration (mean 36 ± 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-$MeSO_2$-*p,p′*-DDE, was detected in ringed seal blubber and found at levels that were comparable to 3-$MeSO_2$-*p,p′*-DDE concentrations in Canadian ringed seals (Table 1) (Letcher et al., 1998). The finding of $MeSO_2$-PCBs and 3-$MeSO_2$-*p,p′*-DDE in the ringed seal blubber indicates that biomagnification and biotransformation are both possible sources of these $MeSO_2$-metabolites in polar bears.

The mean ΣPBDE concentration (149 ± 87 ng/g lw; Table 1) in ringed seal blubber was comparable though somewhat higher than previous studies on East Greenland ringed seals collected in 2001 (Vorkamp et al., 2004). Also, the PBDE congener pattern in the present seal blubber showed that 55–85% of the ΣPBDE concentration was comprised of BDE-47 with much lesser proportions of BDE-99 (<15%), -100 (<10%) and -153 (<4%), This was comparable to the pattern found in East Greenland and Canadian ringed seal blubber (Ikonomou et al., 2002; Vorkamp et al., 2004). Relative to the present ringed seals, similar total-(α)-HBCD levels were found in Svalbard ringed seals captured in 2003 (Sørmo et al., 2006).

Of the congeners monitored (Table 1), only very low levels of one OH-PBDE congener (6-OH-BDE47: 0.7 ± 0.5 ng/g lw) and three MeO-PBDE congeners (6-MeO-BDE47, 2′-MeO-BDE68, and 6-MeO-BDE85; ΣMeO-PBDE: 4.6 ± 0.4 ng/g lw) 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 (Carté 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-BDE85 congener is also *ortho*-MeO-substituted and is likely also of natural origin, since all natural OH- and MeO-PBDEs identified to date have been *ortho*-OH/MeO-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-OH-HpCS in the present ringed seal blubber (this study) or low levels of mainly OH-*ortho*-substituted OH-PBDEs 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., 2000).

Three identified OH-PBB congeners, one tri-brominated and two tetra-brominated, were detected in the seal blubber (ΣOH-PBB = 0.5 ± 0.3 ng/g lw or ww; Table 1). We reported the same three OH-PBBs in the present polar bears, which were mainly in the fat (mean ΣOH-PBB of 14 ng/g ww) and brain (mean ΣOH-PBB of 10 ng/g ww) (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-PBDEs, the source of OH-PBBs is unclear, although they may have been formed by oxidative metabolism of PBBs 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 <1.0 ng/g ww, and a di-OH-PBB (2,2′-di-OH-BB80) was recently identified as a natural product in marine bacteria (Isnansetyo 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, $MeSO_2$-PCB and 3-$MeSO_2$-*p,p′*-DDE levels in East Greenland polar bears, and therefore in the present study tissue-specific BMFs for these contaminants were calculated separately for male and female polar bears. In general, the ringed seal to polar bear, mean BMFs for ΣPCBs, *p,p′*-DDE, ΣCHLs, $\Sigma MeSO_2$-PCBs, 3-$MeSO_2$-*p,p′*-DDE, PCP, ΣPBDEs, total-(α)-HBCD, ΣOH-PBDEs, ΣMeO-PBDEs and ΣOH-PBBs indicated that these organohalogens bioaccumulate, and in some cases the BMFs for bear adipose and liver were >1 and biomagnification was suggested (Figs. 1 and 2).

On a congener-specific basis and among tissues, CB-52, -95/-66, -101/-90, -177 and -178 had ringed seal to polar bear BMF close to or less than unity, CB-99, -138, -146, -153, -172, -183, -187, -196/-203 and -202/-171 had BMFs ranging from 2 to 10, and CB-180 and -194 had BMFs exceeding 25. The mean BMFs for ΣPCBs were higher in the adipose, brain and liver of male polar bears relative to mean BMFs in the same female tissues (Fig. 1). For all tissues where quantifiable levels were possible, the BMFs for $\Sigma MeSO_2$-PCB and 3-$MeSO_2$-*p,p′*-DDE in male polar bears were higher than for female polar bears. Lower BMFs for female bears is likely largely due to the ability of females to depurate contaminant loads via lactational transfer to their cubs (Polischuk et al., 2002).

The mean BMF of ΣPCBs in polar bear adipose in the present study was found to be comparable to the BMF of ΣPCBs of ringed seal to polar bears from the Canadian high Arctic (Letcher et al., 1998; Muir et al., 1988). In the present study, the ringed seal to polar bear ΣPCB mean BMF in the liver was higher than in any other tissue (Fig. 1), which may be possibly explained by specific protein binding in the liver. The ΣPCB mean BMF in the adipose was likewise higher than for the brain tissue. With the exception of the female brain, the mean BMFs for ΣPCB in male and female polar bear tissues were all >1. Even though biotransformation of PCBs takes place in the polar bear, the BMF values strongly suggests that the rate of biotransformation to OH-PCBs/$MeSO_2$-PCBs was much lower than the rate of PCB uptake through the seal blubber diet.

Among the OCs, in addition to *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT, only the CHLs could be detected in the ringed seal blubber. For the present East Greenland bears, the $MeSO_2$-PCB and 3-$MeSO_2$-*p,p′*-DDE, mean BMFs of >1 (Fig. 1) for the applicable tissues is consistent with this previous finding, and indicated that in bears the rate of dietary accumulation and metabolic formation of $MeSO_2$-PCBs was higher than the rate of elimination (Letcher et al., 1998).

For polar bear brain, all CHLs had BMFs ≪ 1 and demonstrating the effectiveness of the blood-brain barrier, but could also be a function of the lipid composition of the brain and the central nervous system (Gebbink et al., 2008b). For bear liver and adipose, BMFs for *cis*- and *trans*-nonachlor were <1, BMFs for *cis*- and *trans*-chlordane were >14 (adipose) and >5 (liver), and BMFs for heptachlor epoxide and oxychlordane were ~6 (adipose) and >20 (liver), which indicated the capacity of polar bears to metabolize chlordanes and nonachlors as has been demonstrated in previous studies on circumpolar polar bears (Muir et al., 1988; Norstrom et al., 1998; Verreault et al., 2005a).

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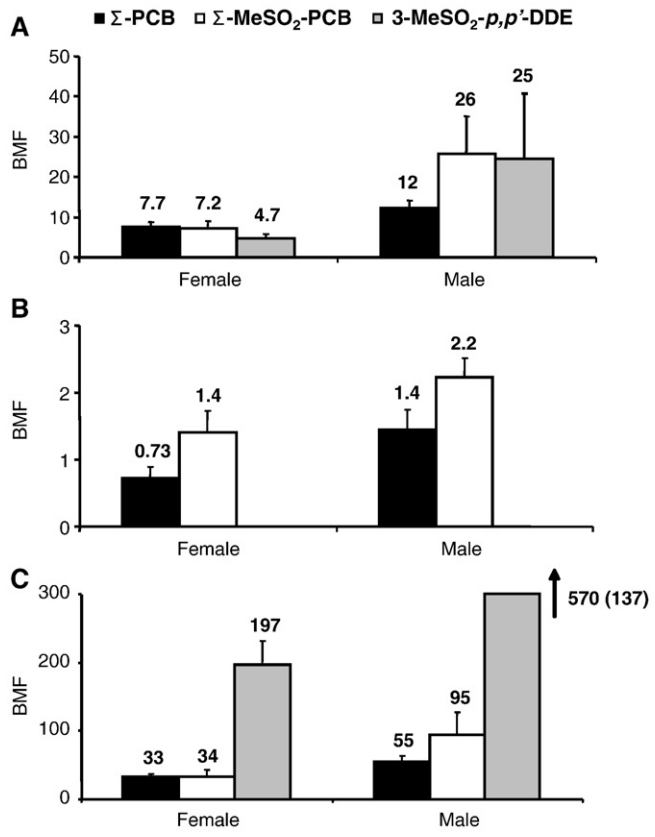

Fig. 1. Biomagnification factors (±SE) of ΣPCB, ΣMeSO$_2$-PCB and 3-MeSO$_2$-*p,p'*-DDE from ringed seals (6 females and 9 males; mean age = 5.4 years) to male (n = 10; mean age = 10.6) and female (n = 10; mean age = 14.5) polar bear (A) adipose, (B) brain and (C) liver from East Greenland. 3-MeSO$_2$-*p,p'*-DDE was not detected in the brain.

The mean BMFs for ΣCHL, *p,p'*-DDE, PCP, ΣPBDE, total(α)HBCD, BB-101, ΣOH-PBDE, ΣMeO-PBDE, ΣOH-PBB, showed no apparent difference between male and female polar bear tissues, and thus were reported together (Fig. 2). PCP bioaccumulated from the ringed seal trophic level to the polar bear level for all tissues, with BMF in the liver tissue being higher than in the other tissues (Fig. 2). High affinity to TTR binding and/or other proteins in the liver are factors that may have contributed to the high liver BMF of PCP. Although there has not been any evidence that marine mammals are capable of metabolizing HCB, several other species (rats, mice and fish) were shown to biotransform PCP (Renner, 1988).

As with ringed seal blubber (Table 1), the PBDE congener pattern in the bear tissues was dominated by BDE-47, but with comparable levels of BDE-153 and/or BDE-154 in adipose and liver. On a congener-specific basis and among tissues, BDE-154 had a BMF of 4 and 13 in liver and adipose, respectively. BMFs for BDE-47, -99, -100 and -153 were all less than one in adipose and liver, and in the brain the BMFs were ≪1 for all PBDE congeners. As a consequence, the BMF for ΣPBDE only exceeded unity in polar bear liver (Fig. 2), suggesting preferential storage in liver relative to adipose and brain. This ΣPBDE BMF was also comparable the value of 0.3 previously reported for male polar bears from Svalbard (Sørmo et al., 2006). The BMFs for BDE-47, -99, -100, -153 and -154 were close to or <1 as reported for East Greenland bears (Muir et al., 2006), but has been based on data for ringed seal blubber from another study (Vorkamp et al., 2004). Any differences in the PBDE BMFs for East Greenland bears reported in Muir et al. (2006) are likely related to differences in the age, season, diet and/or differences in collection years between the ringed seals and the polar bears.

OH-PBDEs only accumulated in the adipose and appeared not to biomagnify (mean adipose BMF of 1.3 ± 1.0; Fig. 2). This may be related to a preference for OH-PBDE retention in blood rather than in the adipose, liver or brain tissues, similar to OH-PCBs (Sandala et al., 2004). However, we previously showed that formation of oxidative metabolism and/or selective retention is not nearly as pronounced for PBDEs as for PCBs, given the much lower ratio of OH-PBDE/PBDE in polar bear blood than the ratio of OH-PCB/PCB (Gebbink et al., 2008b). A lower ratio OH-PBDE/PBDE relative to OH-PCB/PCB ratio was also observed in Norwegian polar bears (Verreault et al., 2005b). The only OH-PBDE congener found in the bears, 6-OH-BDE-47, was also (the only congener) found in ringed seal blubber, whereas we did not detect any OH-PCBs in the ringed seal blubber. This OH-PBDE congener has also been identified as a natural compound that is produced by marine sponges (Carté and Faulkner, 1981), and was found to have bioaccumulative properties (Marsh et al., 2004). Taken together, these results suggest that this OH-PBDE in bear adipose tissue is likely more of a consequence of accumulation from seals rather than from PBDE biotransformation in the bears.

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 monoxygenase catalytic activity (Letcher et al., 1996). The positive control, BDE-15 was significantly (p = 0.00006) depleted by 41% during the assay. Of the environmental relevant PBDE congeners, only BDE-154 was significantly (p = 0.01) 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., 2009a,b), 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 I 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 thus 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-BDE47 and 2′-MeO-BDE68.

The BMF for total-(α)-HBCD, which only accumulated in the polar bear adipose tissue, was found to be 1.7 ± 0.6. Sørmo et al. (2006) found a lower but comparable BMF from Svalbard ringed seal blubber to polar bear adipose (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 biotransformation. The *in vitro* depletion of 24% for α-HBCD was significant (p = 0.02) in the microsomal assay (Fig. 3). Again, no oxidative metabolites were detected *in situ*. Rats exposed to the HBCD technical mixture were shown to have significant CYP2B induction (as measured by 7-pentoxyresorufin-*O*-dependentylase activity; Germer et al., 2006). Thus, 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 that the present, free-ranging East Greenland polar bears were exposed to and accumulated substantial levels of HBCD.

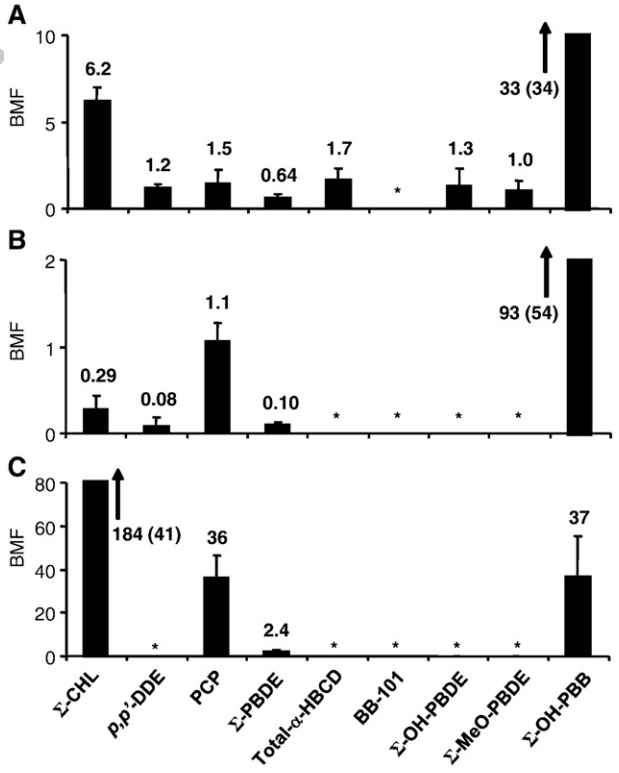

Fig. 2. Biomagnification factors (±SE) of the sum of classes of neutral and phenolic organohalogen compounds from ringed seals to polar bear (A) adipose, (B) brain and (C) liver from East Greenland (n = 20). *Compounds were not detected in the polar bear tissue.

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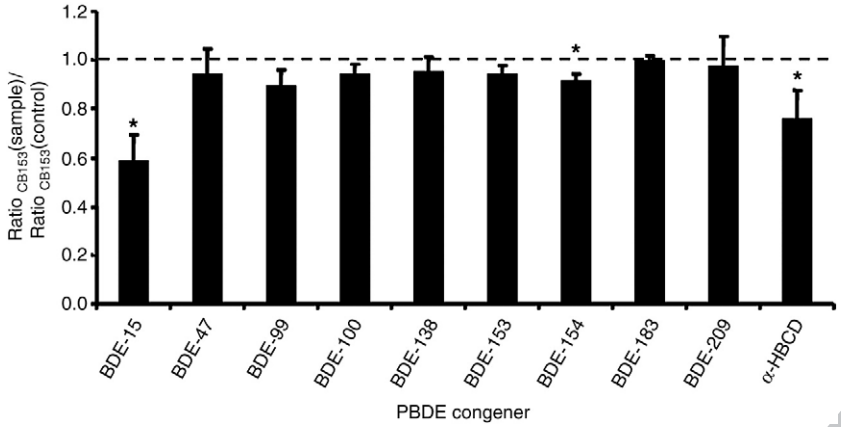

Fig. 3. Fraction of the PBDE congener concentration (±SD) remaining after a 90 min. *in vitro* assay ($n=3$ replicates) using hepatic microsomes from polar bear. The dotted line signifies no depletion. Significant depletion ($p<0.05$) is marked by an asterisk.

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 (Gebbink 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 a 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 tetra-brominated OH-PBBs. Besides being formed metabolically, OH-PBBs can be naturally produced. For instance, 2,2′-diOH-BB80 was identified in the *Pseudoalteromonas phenolica* sponge (Isnansetyo and Kamei, 2003). As has been observed previously with 2,2′-diOH-BB80 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-PCBs have demonstrated endocrine disrupting activity in laboratory mammals and humans and/or in *in vitro* assays (Hamers et al., 2006; Letcher et al., 2000; Ucán-Marín 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), 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., 2006a
Montie et al., 2009
Zar, 1984

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References

Bentzen TW, Follmann EH, Amstrup SC, York GS, Moller MJ, O'Hara TM. Variation in diet of southern Beaufort Sea polar bears inferred from stable isotope analysis. Can J Zool 2007;85:596–608.

Bentzen TW, Muir DCG, Amstrup SC, O'Hara TM. Organohalogen concentrations in blood and adipose tissue of Southern Beaufort Sea polar bears. Sci Total Environ 2008;406:352–67.

Braune BM, Outridge PM, Fisk AT, Muir DCG, Helm PA, Hobbs K, et al. Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: an overview of spatial and temporal trends. Sci Total Environ 2005;351–352:4-56.

Carté B, Faulkner DJ. Polybrominated diphenyl ethers from *Dysodea herbacea*, *Dysidea chlorea* and *Phyllospongia foliascens*. Tetrahedron 1981;37:2335–9.

Derocher AE, Wiig Ø, Andersen M. Diet composition of polar bears in Svalbard and the western Barents Sea. Polar Biol 2002;25:448–52.

Dietz R, Riget FF, Sonne C, Muir DCG, Backus SM, Born EW, et al. Seasonal trends and bioaccumulation of polybrominated diphenyl ethers in free-ranging East Greenland polar bears (*Ursus maritimus*). Environ Pollut 2006;146:166–73.

Fisk AT, de Wit CA, Wayland M, Kuzyk ZZ, Burgess N, Letcher RJ, et al. An assessment of the toxicological significance of anthropogenic contaminants in Canadian arctic wildlife. Sci Total Environ 2005;351–352:57–93.

Fu X, Schmitz FJ. New brominated diphenyl ether from an unidentified species of *Dysidea* sponge. ^{13}C NMR data for some brominated diphenyl ethers. J Natural Prod 1996;59:1102–3.

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Gebbink WA, Sonne C, Dietz R, Kirkegaard M, Riget FF, Born EW, et al. Tissue-specific congener composition of organohalogen and metabolite contaminants in east Greenland polar bears (*Ursus maritimus*). Environ Pollut 2008a;152:621–9.

Gebbink WA, Sonne-Hansen C, Dietz R, Kirkegaard M, Riget FF, Born EW, et al. Target tissue selectivity and burdens of diverse classes of brominated and chlorinated contaminants compounds in Polar Bears (*Ursus maritimus*) from East Greenland. Environ Sci Technol 2008b;42:752–9.

Germer S, Piersma AH, van der Ven L, Kamyschnikow A, Frey Y, Schmitz H-J, et al. Subacute effects of the brominated flame retardants hexabromocyclododecane and tetrabromobisphenol A on hepatic cytochrome P450 levels in rats. Toxicol 2006;218:229–36.

Grahl-Nielsen O, Andersen M, Derocher AE, Lydersen C, Wiig Ø, Kovacs KM. Fatty acid composition of the adipose tissue of polar bears and of their prey: ringed seals, bearded seals and harp seals. Mar Ecol Prog Ser 2003;265:275–82.

Hakk H, Larsen G, Klasson-Wehler E. Tissue distribution, excretion and metabolism of 2, 2′, 4, 4′, 5-pentabromodiphenyl ether (BDE-99) in male Sprague–Dawley rat. Xenobiotica 2002;32:369–82.

Hamers T, Kamstra JH, Sonneveld E, Murk AJ, Kester MHA, Andersson PL, et al. *In vitro* profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol Sci 2006;92:157–73.

Ikonomou MG, Rayne S, Addison RF. Exponential increases of the brominated flame retardants, polybrominated diphenyl ethers, in the Canadian Arctic from 1981 to 2000. Environ Sci Technol 2002;36:1886–92.

Isnansetyo A, Kamei Y. MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseodoalteromonas phenolica* sp. nov. O-BC30^{T}, against methicillin-resistant *Staphylococcus aureus*. Antimicro Agents Chemo 2003;47:480–8.

Kohli J, Wyndham C, Smylie M, Safe S. Metabolism of bromobiphenyls. Biochem Pharmacol 1978;27:1245–9.

Koss G, Weider T, Seubert S, Seubert A. 2, 2′, 4, 4′, 5, 5′-Hexabromobiphenyl: its toxicokinetics, biotransformation and porphyrinogenic action in rats. Fd Chem Toxicol 1994;32:605–10.

Kucklick JR, Struntz WDJ, Becker PR, York GW, O'Hara TM, Bohonowych JE. Persistent organochlorine pollutants in ringed seals and polar bears collected from northern Alaska. Sci Total Environ 2002;287:45–59.

Letcher RJ, Norstrom RJ, Lin S, Ramsay MA, Bandiera SM. Immunoquantitation and microsomal monooxygenase activities of hepatic cytochromes P4501A and chlorinated hydrocarbon contaminant levels in Polar bear (*Ursus maritimus*). Toxicol Appl Pharmacol 1996;137:127–40.

Letcher RJ, Norstrom RJ, Muir DCG. Biotransformation versus bioaccumulation: sources of methyl sulfone PCB and 4, 4′-DDE metabolites in the polar bear food chain. Environ Sci Technol 1998;32:1656–61.

Letcher RJ, Klasson-Wehler E, Bergman Å. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. In: Paasivirta J, editor. The handbook of environmental chemistry — new types of persistent halogenated compounds, vol. 3. Heidelberg, Germany: Springer-Verlag; 2000. p. 315–59. Part K.

Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jørgensen EH, Sonne C, et al. Exposure and effects assessment of persistent organic pollutants in Arctic wildlife and fish. Sci Total Environ 2009 Submitted May 2009.

Li H, Boon JP, Lewis WE, van den Berg M, Nyman M, Letcher RJ. Hepatic microsomal cytochrome P450 enzyme activity in relation to *in vitro* metabolism/inhibition of polychlorinated biphenyls and testosterone in Baltic grey seal (*Halichoerus grypus*). Environ Toxicol Chem 2003;22:636–44.

MacDonald RW, Bewers JM. Contaminants in the arctic marine environment: priorities for protection. Ices J Mar Sci 1996;53:537–63.

Malmberg T. Identification and characterization of hydroxylated PCB and PBDE metabolites in blood. PhD Thesis. 2004. Stockholm University, Stockholm, Sweden.

Malmvärn A, Marsh G, Kautsky L, Athanasiadou M, Bergman Å, Asplund L. Hydroxylated and methoxylated brominated diphenyl ethers in the red algae *Ceramium tenuicorne* and blue mussels from the Baltic Sea. Environ Sci Technol 2005;39:2990–7.

Marsh G, Athanasiadou M, Bergman Å, Asplund L. Identification of hydroxylated and methoxylated polybrominated diphenyl ethers in Baltic Sea salmon (*Salmo salar*) blood. Environ Sci Technol 2004;38:10–8.

Marsh G, Athanasiadou M, Athanassiadis I, Sandholm A. Identification of hydroxylated metabolites in 2, 2′, 4, 4′-tetrabromodiphenyl ether exposed rats. Chemosphere 2006;63:690–7.

McKinney MA, De Guise S, Martineau D, Béland P, Lebeuf M, Letcher RJ. Organohalogen contaminants and metabolites in beluga whale (*Delphinapterus leucas*) liver from two Canadian populations. Environ Toxicol Chem 2006a;25:1246–57.

McKinney MA, De Guise S, Martineau D, Béland P, Arukwe A, Letcher RJ. Biotransformation of polybrominated diphenyl ethers and polychlorinated biphenyls in beluga whale (*Delphinapterus leucas*) and rat mammalian model using an *in vitro* hepatic microsomal assay. Aquat Toxicol 2006b;77:87–97.

McKinney MA, Peacock E, Letcher RJ. Sea ice-associated diet change increases the levels of chlorinated and brominated contaminants in polar bears. Environ Sci Technol 2009;43:4334–9.

Montie EW, Hahn ME, Reddy C, Gebbink WA, Touhey KE, Letcher RJ. Organohalogen contaminants and metabolites in cerebrospinal fluid and cerebellum gray matter in short-beaked common dolphins and Atlantic white-sided dolphins from the western North Atlantic. Environ Pollut 2009;157:2345–58.

Muir DCG, Norstrom RJ, Simon M. Organochlorine contaminants in arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordane-related compounds. Environ Sci Technol 1988;22:1071–9.

Muir DCG, Backus SM, Derocher AE, Dietz R, Evans T, Gabrielsen GW, et al. Brominated flame retardants in polar bears (*Ursus maritimus*) from Alaska, the Canadian Arctic, Greenland, and Svalbard. Environ Sci Technol 2006;40:449–55.

Norstrom RJ, Belikov SE, Born EW, Garner GW, Malone B, Olpinski S, et al. Chlorinated hydrocarbon contaminants in polar bears from Eastern Russia, North America, Greenland, and Svalbard: biomonitoring of Arctic pollution. Arch Environ Contam Toxicol 1998;35:354–67.

Polischuk SC, Norstrom RJ, Ramsay MA. Body burdens and tissue concentrations of organochlorines in polar bears (*Ursus maritimus*) vary during seasonal fasts. Environ Pollut 2002;118:29–39.

Renner G. Hexachlorobenzene and its metabolism. Toxicol Environ Chem 1988;18:51–78.

Routti H, Letcher RJ, Arukwe A, van Bavel B, Yoccoz NG, Chu SG, et al. Biotransformation and metabolites of PCBs in relation to phase I and II xenobiotic-metabolizing enzyme activities in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Environ Sci Technol 2008;42:8952–8.

Sandala GM, Sonne-Hansen C, Dietz R, Muir DCG, Valters K, Bennett ER, et al. Hydroxylated and methyl sulfone PCB metabolites in adipose and whole blood of polar bear (*Ursus maritimus*) from East Greenland. Sci Total Environ 2004;331:125–41.

Sandau CD, Meerts IATM, Letcher RJ, McAlees AJ, Chittim B, Brouwer A, et al. Identification of 4-hydroxyheptachlorostyrene in polar bear plasma and its binding affinity to transthyretin: a metabolite of octachlorostyrene. Environ Sci Technol 2000;34:3871–7.

Schantz MM, Koster BJ, Oakley LM, Schiller SB, Wise SA. Certification of polychlorinated biphenyl congeners and chlorinated pesticides in a whale blubber standard reference material. Anal Chem 1995;67:901–10.

Smith TG, Sjare B. Predation of beluga and narwhals by polar bears in nearshore areas of the Canadian High Arctic. Arctic 1990;43:99-102.

Sonne C, Leifsson PS, Dietz R, Born EW, Letcher RJ, Hyldstrup L, et al. Xenoendocrine pollutants may reduce size of sexual organs in East Greenland polar bears (*Ursus maritimus*). Environ Sci Technol 2006a;40:5668–74.

Sonne C, Dietz R, Leifsson PS, Born EW, Kirkegaard M, Letcher RJ, et al. Are organohalogen contaminants a co-factor in the development of renal lesions in East Greenland polar bears (*Ursus maritimus*)? Environ Toxicol Chem 2006b;25:1551–7.

Sørmo EG, Salmer MP, Jenssen BM, Hop H, Bæk K, Kovacs KM, et al. Biomagnification of brominated flame retardants in the polar bear food chain in Svalbard, Norway. Environ Toxicol Chem 2006;25:2502–11.

Stapleton HM, Keller JM, Schantz MM, Kucklick JR, Leigh SD, Wise SA. Determination of polybrominated diphenyl ethers in environmental standard reference materials. Anal Bioanal Chem 2007;387:2365–79.

Teuten EL, Xu L, Reddy CM. Two abundant bioaccumulated halogenated compounds are natural products. Science 2005;307:917–20.

Thiemann GW, Iverson SJ, Stirling I. Polar bear diets and Arctic marine food webs: insights from fatty acid analysis. Ecol Monogr 2008;78:591–613.

Ucán-Marín F, Arukwe A, Mortensen A, Gabrielsen GW, Fox GA, Letcher RJ. Recombinant transthyretin purification and competitive binding with organohalogen compounds in two gull species (*Larus argentatus* and *Larus hyperboreus*). Toxicol Sci 2009;107(2):440–50.

Verreault J, Muir DCG, Norstrom RJ, Stirling I, Fisk AT, et al. Chlorinated hydrocarbon contaminants and metabolites in polar bear (*Ursus maritimus*) from Alaska, Canada, East Greenland, and Svalbard: 1996–2002. Sci Total Environ 2005a;351–352:369–90.

Verreault J, Gabrielsen GW, Chu SG, Muir DCG, Andersen M, Hamaed A, et al. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: glaucous gulls and polar bears. Environ Sci Technol 2005b;39:6021–8.

Verreault J, Maisonneuve F, Dietz R, Sonne C, Letcher RJ. Comparative hepatic activities of major xenobiotic-metabolizing enzymes and circulating polychlorinated biphenyls and polybrominated diphenyl ethers and hydroxylated analogues in cohorts of captive Greenland sledge dogs. Environ Toxicol Chem 2009a;28:162–72.

Verreault J, Letcher RJ, Sonne C, Dietz R. *In vitro* metabolism of polychlorinated biphenyls and activity of xenobiotic-metabolizing enzymes in dietary-exposed sledge dogs in Greenland and model beagle dogs. Comp Biochem Physiol Part C 2009b;150:91-100.

Vorkamp K, Christensen JH, Riget FF. Polybrominated diphenyl ethers and organochlorine compounds in biota from the marine environment of East Greenland. Sci Total Environ 2004;331:143–55.

Zar JH. Biostatistical analysis. 2nd ed. New Jersey, USA: Prentice-Hall, Inc; 1984.

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