

## ORGANOHALOGEN CONTAMINANTS AND METABOLITES IN BELUGA WHALE (*DELPHINAPTERUS LEUCAS*) LIVER FROM TWO CANADIAN POPULATIONS

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**Abstract**—Contaminants described as organochlorines (OCs; e.g., polychlorinated biphenyls [PCBs]) are present in tissues of marine mammals, including beluga whales (*Delphinapterus leucas*), but the complexity of contaminant exposure often is not fully known. The PCBs, OC pesticides, polybrominated diphenyl ether (PBDE) flame retardants, methylsulfonyl (MeSO<sub>2</sub>)- and hydroxy (OH)-PCB metabolites, and OH-PBDEs and methoxylated (MeO)-PBDEs were determined in the liver of beluga whales from two Canadian populations: the St. Lawrence Estuary (SLB; *n* = 6), and western Hudson Bay in the Canadian Arctic (CAB; *n* = 11). The ΣPCB, ΣDDT, and ΣPBDE concentrations were higher (*p* < 0.05) in SLB versus CAB. Of 18 detectable OH-PCBs in SLB (mainly 4-OH-CB107, 4-OH-CB112, and 4'-OH-CB120), only 4'-OH-CB120 was found in CAB. The ΣOH-PCB concentrations were less than 0.2% of the ΣPCBs in both populations but were higher (*p* < 0.05) in SLB (65 ± 22 ng/g lipid wt) than in CAB (3.1 ± 0.5 ng/g lipid wt). The ΣMeSO<sub>2</sub>-PCB concentrations were higher in SLB (3,801 ± 1,322 ng/g lipid wt) relative to CAB (77 ± 23 ng/g lipid wt) and were 11 and 4%, respectively, of the ΣPCB concentrations. Of the 15 OH-PBDEs, only four congeners were detectable, but not quantifiable (notably 2'-OH-BDE68 and 6-OH-BDE47), in animals from both populations. Of the 15 MeO-PBDEs, 4'-MeO-BDE17 and 6-MeO-BDE47 in the SLB (*n* = 2) and 2'-MeO-BDE68 and 6-MeO-BDE47 in the CAB (*n* = 2) had concentrations from 20 to 100 ng/g lipid weight. The OH-PBDEs and MeO-PBDEs most likely are of natural origin and accumulated in beluga whales, whereas the OH-PCBs and MeSO<sub>2</sub>-PCBs are metabolites derived from accumulated PCBs. Canadian beluga whale liver contains previously unidentified organohalogen contaminants and metabolites and, thus, a complexity of contaminant exposure that may be impacting the health of Canadian beluga whale populations.

**Keywords**—Canadian beluga whale Liver Polychlorinated biphenyls Polybrominated diphenyl ethers Hydroxyl- and methylsulfonyl-containing analogues

### INTRODUCTION

Marine mammals, such as beluga whales (*Delphinapterus leucas*), appear to be particularly vulnerable to the accumulation and potential effects of lipophilic organohalogen contaminants, including polychlorinated biphenyls (PCBs) and “new” or more recently detected classes of persistent contaminants, such as polybrominated diphenyl ether (PBDE) flame retardants [1,2]. Toothed marine mammal species often occupy high trophic levels in food webs, live for a relatively long time, store large amounts of fat (blubber), and have slow elimination capacities that lead to accumulation of organohalogen [3]. Several Canadian Arctic beluga whale (CAB) populations exist, as does one relatively isolated, threatened, and highly contaminated [4,5] population inhabiting the estuary of the St. Lawrence River (SLB). Although not generally observed in CAB, high occurrences of immunological and reproductive dysfunction in SLB have been strongly suggested to be associated with organochlorine (OC) contaminant exposures [6]. Martineau et al. [7] reported in 2002 that for SLB found dead, 27% of examined adults had cancers, and the major primary

causes of death were respiratory and gastrointestinal infections with metazoan parasites (22%), cancer (18%), as well as bacterial, viral, and protozoan infections (17%). The estimated annual rate of all cancer types was much higher than that reported for any other population of cetacean. High incidence of cancers observed in the SLB population probably is associated etiologically with mutagenic compounds (e.g., polycyclic aromatic hydrocarbons [PAHs]) activated by cytochrome P450 (CYP) enzyme-mediated metabolism [7]. Other population stress factors such as the genetic ramifications of in-breeding and ship collisions also could contribute to the high mortality of SLB.

Unlike PCBs and several OC pesticides, such as DDT, the use of PBDEs as commercial flame retardant additives is more recent, and concentrations in the tissue of biota continue to increase. As observed for PCBs, adipose PBDE concentrations are higher in SLB than in CAB [2]. Despite lower levels of PBDEs, and in contrast to decreasing levels of most PCBs and OCs in SLB and CAB, temporal studies have demonstrated that PBDE concentrations are on the rise in both populations [2,8,9]. Major environmentally relevant PBDE congeners are 2,2',4,4'-tetrabromo-DE (BDE47), 2,2',4,4',5-pentabromo-DE (BDE99), 2,2',4,4',6-pentabromo-DE (BDE100), 2,2',4,4',5,5'-hexabromo-DE (BDE153), and 2,2',4,4',5,6'-hexabromo-DE (BDE154). Although the fate and potential effects of

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PBDEs in marine mammals remain under-researched, recent studies in experimental animals have demonstrated that PBDE exposures are associated with some effects similar to those reported for PCBs, such as reproductive and liver dysfunction, endocrine system modulation, and thyroid-associated developmental neurotoxicity [10–12].

Biotransformation is involved in the toxicokinetics of lipophilic organohalogen, where conversion can lead to more hydrophilic and readily excretable forms or metabolism may generate retainable and/or persistent metabolites that may or may not be bioactivated from a toxicological or effects perspective. Biotransformation capacity is dependent on several factors, including species, age, and sex, and is an inducible process. The liver is a major site for the biotransformation of organohalogen or other persistent organics. The oxidative CYP subfamilies 1 to 4 are largely involved in xenobiotic metabolism (e.g., CYP1A, CYP2B, CYP3A, CYP2E, and epoxide hydrolase [EH]). Hydroxylated (OH) metabolic products of exogenous compounds are subsequently more susceptible to phase II, enzyme-mediated conjugation processes, including glucuronidation and sulfation.

For marine mammals, a classification scheme has been proposed to assess the capacity for CYP-mediated oxidative metabolism of PCBs based on congener-specific tissue residue analyses [13]. A more definitive and direct way of assessing metabolic capacity toward PCBs is via determination of retained tissue metabolite residues [14]. The OH-PCBs have been identified as an important class of PCB metabolites in biota and reported mainly in blood. The OH-PCBs form via CYP-mediated, direct insertion of the OH-group on a precursor PCB congener or via CYP-mediated arene epoxide formation, followed by either EH-mediated or spontaneous epoxide opening [15]. A few studies have suggested or shown that CYP2B is expressed either weakly or not at all in toothed whales, including beluga (*Delphinapterus leucas*) [16–18], which would influence OH-PCB metabolite formation, because animals that strongly express CYP2B-like enzymes generally have higher OH-PCB metabolite concentrations in blood (e.g., polar bear) [19]. Blood retention of OH-PCBs is a function of competition with thyroxine ( $T_4$ ) for binding to thyroid hormone transport proteins. Transthyretin (TTR) is the major thyroid hormone transport protein in humans and laboratory rodents and, presumably, in marine mammals. The OH-PCBs have been shown to disturb thyroid hormone and vitamin A levels in rats [20].

The OH-PCBs and other chlorinated phenolic contaminants (e.g., pentachlorophenol and 4-OH-heptachlorostyrene [4-OH-HpCS]) have been reported in the plasma of humans, fish, birds, and mammals [21–25]. Methylsulfonyl ( $MeSO_2$ )-PCB metabolites are formed via arene epoxide intermediates and are persistent contaminants in marine mammals as well as in humans [22,23,26,27]. Disruption of enzyme activity and endocrine function has been reported in rats experimentally exposed to  $MeSO_2$ -PCBs [28,29].

Methoxylated (MeO) and OH-PBDEs also have been detected mainly in the blood, and to a lesser extent in the fat, of fish, birds, and mammals from the Baltic Sea and Atlantic Ocean [30–32] as well as European arctic environments [33,34]. In addition, tetrabrominated-MeO-diphenyl ethers have been reported in marine mammals (whales, dolphins, dugong, and seals) from the Southern Hemisphere and at concentrations higher than the levels of PBDEs [35]. Current thinking indicates that MeO-PBDEs in wildlife mostly are a

consequence of accumulation via natural sources in marine environments (e.g., via formation in sponges and algae) [31,32,36], whereas OH-PBDEs can be of natural and/or of metabolic derivation from the enzyme-mediated degradation of precursor and accumulated PBDEs [37]. Metabolism of PBDEs has been reported in laboratory rats, where OH-PBDE metabolites were detected in animals dosed with BDE47, BDE99, BDE100, and BDE209 [37,38]. In North American aquatic systems, OH-PBDEs have been reported as residues in the plasma of Great Lakes fish [39] and Pacific coast killer whales (*Orcinus orca*) [40]. Estrogenic and thyroidogenic effects have been reported in laboratory rats experimentally exposed to OH-PBDEs [38,41]. For example, Malmberg [42] showed that the 3-OH-BDE47, 4-OH-BDE42, 4'-OH-BDE49, and 6-OH-BDE47 congeners had binding affinities to human TTR that were greater than those for  $T_4$ .

We recently characterized CYP1A, CYP2B, CYP3A, CYP2E, EH, and UDP-glucuronosyl transferase (UDPGT) cross-reactive proteins and catalytic activity in the liver of SLB and CAB [16]. In the present study, OH-PCBs, OH-PBDEs, MeO-PBDEs, PBDEs, PCBs,  $MeSO_2$ -PCBs, and OC pesticides were identified, quantitatively determined, and compared in liver tissues from SLB and CAB. Although several earlier studies have reported the occurrence of organohalogen contaminants in beluga whales, this is one of a few reports that have monitored and/or determined OH-PCBs, OH-PBDEs, and MeO-PBDEs in this species.

## MATERIALS AND METHODS

### Chemicals and standards

The PCB and OC pesticide standards were supplied by the Canadian Wildlife Service (Ottawa, ON, Canada). Standards for PBDE, MeO-PCB, MeO-PBDE, and  $MeSO_2$ -PCB analyses were kindly provided by Åke Bergman and Göran Marsh (Stockholm University, Sweden), with the exception of BDE28 and BDE49, which were purchased from Wellington Laboratories (Guelph, ON, Canada). The internal standards used were as follows: CB83 and CB122 for PCBs and OCs, BDE30 for PBDEs, 4-OH-2,3',5,5'-tetrachlorobiphenyl (4-OH-CB72) for OH-PCBs, 2'-OH-2,4,4'-tribromodiphenyl ether (2'-OH-BDE28) for OH-PBDEs, and 3- $CH_3SO_2$ -2- $CH_3$ -2',3',4',5,5'-pentachlorobiphenyl ( $MeSO_2$ -PCB-IS) for  $MeSO_2$ -PCBs. The PCB numbering is abbreviated using the notation of Ballschmiter and Zell [43], and PBDEs are numbered analogously. The PCB and PBDE metabolites are numbered by keeping the same Ballschmiter and Zell notation and adding the position of the OH- and/or  $MeSO_2$ -group as a prefix to the name [44]. The chromatographic supports used were as follows: Florisil® deactivated with 1.2%  $H_2O$  (Fisher Scientific, Ottawa, ON, Canada), basic alumina deactivated with 2.3%  $H_2O$  (Fisher Scientific), and silica acidified with 22%  $H_2SO_4$  (Aldrich, Milwaukee, WI, USA). All solvents and other reagents were of at least analytical-grade quality.

### Liver sampling

The liver tissues of stranded SLB ( $n = 6$ ) were opportunistically sampled from stranded and already dead animals collected in 2000 to 2003. The CAB ( $n = 11$ ) liver tissues were taken within 30 to 90 min of death from individuals killed as part of native subsistence hunts in 2002 and 2003 on the western shore of Hudson Bay (near Arviat, Nunavut, Canada). The SLB set was comprised of three males (two adults and

one juvenile) and three females (two juveniles and one neonate), whereas the CAB set was comprised of three males (two adults and one juvenile) and seven females (five adults, one juvenile, and one neonate). For CAB, age classification was based on the color change from gray to white at sexual maturity (approximately seven years of age for females and eight years of age for males). For SLB, dentine growth layers groups were counted on longitudinally cut teeth. The tissues were put into long-term storage at  $-20^{\circ}\text{C}$  until chemical analysis. Complete details of the beluga whales and tissue sampling have been described by McKinney et al. [16].

#### Organohalogen and metabolite extraction

Extraction of neutral, phenolic, and aryl sulfone organohalogens from beluga whale liver tissue was based on methods described elsewhere for blood, serum, or plasma with modifications [19,23,24,27,45,46]. Briefly, 0.5 to 2.0 g of liver were ground with five equivalents of sodium sulfate until homogeneous. All analytes were then extracted from the sample by column chromatography with 60 ml of 1:1 dichloromethane (DCM):hexanes. A 10% portion by volume of the lipid extract was taken for gravimetric determination of lipid. Phenolic analytes were isolated from the tissue, deprotonated, and extracted into an aqueous phase of 1 M KOH (in 1:1 ethanol: $\text{H}_2\text{O}$ ). After acidification, phenolics were back-extracted with 1:1 methyl-*tert*-butyl ether (MtBE):hexanes and derivatized to their methoxylated analogues by treatment with diazomethane. The MeO-derivatized fraction was cleaned up on an acid silica column (3 g) eluted with 50 ml of 15:85 DCM:hexanes.

The organic phase from the original extract (after one alcoholic KOH treatment) contained all neutral analytes. Analytes were separated on a column of 8 g of Florisil® in sequential fractions as follows: 38 ml of hexanes to elute PCBs and PBDEs (F1), 34 ml of 15:85 DCM:hexane to elute OCs (F2), 54 ml of 1:1 DCM:hexanes to elute heptachlor epoxide and dieldrin (F3), and 90 ml of 7% MeOH in DCM to elute MeSO<sub>2</sub>-PCBs (F4). The F4 fraction was subjected to further cleanup on a basic alumina column (3 g) eluted with 50 ml of 1:1 DCM:hexanes (the first 10 ml were discarded).

#### Quantification of contaminants and metabolites

The PCBs and OCs were separated and determined on a fused silica DB-5 column ((5% phenyl)methylpolysiloxane; length, 30 m; inner diameter, 250  $\mu\text{m}$ ; film thickness, 0.25  $\mu\text{m}$ ; J&W Scientific, Folsom, CA, USA) in an Agilent 6890 gas chromatography (GC) system (Palo Alto, CA, USA) equipped with a <sup>63</sup>Ni microelectron-capture detector ( $\mu\text{ECD}$ ) detector. An Agilent 7683 Series automatic liquid sampler and injector were used. The carrier gas was helium, and the makeup gas was 5% methane in argon. The GC ramping program was as follows: the oven temperature was held at  $90^{\circ}\text{C}$  for 1 min, increased to  $200^{\circ}\text{C}$  at  $15^{\circ}\text{C}/\text{min}$  and held for 2 min, then increased to  $280^{\circ}\text{C}$  at  $2.5^{\circ}\text{C}/\text{min}$  and held for 9 min for a total run time of 51.5 min. After PCB and OC determination in F1, F2, and F3, the three fractions were subsequently combined for determination of PBDEs and MeO-PBDEs. The PBDEs, OH-PCBs, and OH-PBDEs (derivatized to MeO-analogues), MeO-PBDEs (in the neutral fraction), and MeSO<sub>2</sub>-PCBs were determined on an Agilent 6890N GC system with an Agilent 5973N mass spectrometry (MS) detector in the electron-capture, negative-ionization (ECNI) mode using selected ion monitoring (SIM). An Agilent 7683 Series injector and autosampler were used as per the GC  $\mu\text{ECD}$ . Helium was used as the carrier

gas, and methane was used as the reagent gas. The GC ramping program for PBDE- and MeO-PBDE (F1/F2/F3)-containing fractions was as follows: temperature was held at  $80^{\circ}\text{C}$  for 2 min, then increased to  $290^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$  and held for 15 min for a total run time of 38 min. The MS-ECNI(SIM) was set up for isotopic Br anions (i.e.,  $m/z$  79 and 81 amu) [43]. For methylated OH-PCBs and other chlorinated phenolics, the initial oven temperature was held at  $80^{\circ}\text{C}$  for 1 min, followed by an increase in the rate to  $10^{\circ}\text{C}/\text{min}$  to  $250^{\circ}\text{C}$  and held for 5 min, then increased again by  $5^{\circ}\text{C}/\text{min}$  to  $300^{\circ}\text{C}$  and held for a further 5 min. The SIM monitoring of MeO-PCBs and other methylated, chlorinated phenolics was for  $[\text{M}]^{-}$ ,  $[\text{M}+2]^{-}$ , and  $[\text{M}-15]^{-}$  ( $[\text{M}-\text{CH}_3]^{-}$ ). Specifically, the ions monitored were as follows:  $m/z$  322 and 324 for 4-MeO-HpCS;  $m/z$  322, 324, and 307 for MeO-tetra-CBs;  $m/z$  356, 358, and 341 for MeO-penta-CBs;  $m/z$  390, 392, and 375 for MeO-hexa-CBs; and  $m/z$  424, 426, and 410 for MeO-hepta-CBs. Finally, for GC-MS(ECNI)(SIM) determination of MeSO<sub>2</sub>-PCBs (F4), the GC oven program initially held the temperature at  $100^{\circ}\text{C}$  for 3 min, then increased to  $220^{\circ}\text{C}$  at  $20^{\circ}\text{C}/\text{min}$  and held for 1 min, followed by an increase to  $280^{\circ}\text{C}$  at  $3^{\circ}\text{C}/\text{min}$  and held for 8 min. The total run time was 38 min. The MeSO<sub>2</sub>-PCB ions monitored were  $[\text{M}]^{-}$  and  $[\text{M}+2]^{-}$  for tetra-, penta-, hexa-, and heptachlorinated MeSO<sub>2</sub>-PCBs. All injections were made in the splitless mode using an injection volume of 1  $\mu\text{l}$ .

All analytes under study were identified by comparison of GC retention and (when applicable) ECNI mass spectra to authentic standards. The  $\Sigma\text{PCB}$  is comprised of the following 47 individual and coeluting congeners, in order of elution (if detected) as shown in Figure 1. The  $\Sigma\text{DDT}$  consists of *p,p'*-DDT (1,1,1-trichloro-2,2-bis[4-chlorophenyl]ethane), 1,1-dichloro-2,2-bis[4-chlorophenyl]ethane (*p,p'*-DDD), and 1,1-dichloro-2,2-bis[4-chlorophenyl]ethene (*p,p'*-DDE). The  $\Sigma\text{HCH}$  is the sum of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -hexachlorocyclohexanes.  $\Sigma\text{Chlordane}$  ( $\Sigma\text{CHL}$ ) is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, and heptachlor epoxide. The  $\Sigma\text{Mirex}$  is the sum of Mirex and photo-Mirex. Hexachlorobenzene, octachlorostyrene, and dieldrin also were determined. The PCBs and OCs were then quantified by an external standard method.

For all other organohalogens and metabolites, quantification was performed using an internal standard method based on the relative response factor (RRF) of the ECNI response of an analyte versus the appropriate internal standard—for example, in the case of PBDEs, the ECNI RRF of each congener monitored versus BDE30 (internal standard). The  $\Sigma\text{PBDE}$  is the sum of the following nine congeners in the following order of elution (if detected): PBDE 28, 49, 47, 100, 99, 154, 153, 138, and 183. The  $\Sigma\text{OH-PCB}$  is the sum of the quantification of 13 congeners, if detected, as MeO-PCB analogues: 4'-OH-CB104, 4'-OH-CB121, 3'-OH-CB85, 4'-OH-CB120, 4-OH-CB112, 4-OH-CB107, 4-OH-CB165, 3'-OH-CB138, 4'-OH-CB130, 4-OH-CB187, 4'-OH-CB159, 3'-OH-CB180, and 4-OH-CB193. Depending on detectability in the sample, some as-yet-unidentified penta-, hexa-, and heptachlorinated OH-PCBs also were included, namely four OH-Cl<sub>5</sub>-CBs, four OH-Cl<sub>6</sub>-CBs, and one OH-Cl<sub>7</sub>-CB. Although these congeners could not be definitively identified by comparison to previous OH-PCB determinations in other animals (see, e.g., [19,24]), they were still quantified using the RRFs of standards in their appropriate chlorinated isomer groups. Fourteen OH-PBDE congeners were analyzed for (as MeO-PBDE analogues) 6'-OH-BDE17, 4'-OH-BDE17, 6'-OH-BDE49, 2'-OH-BDE68, 6-



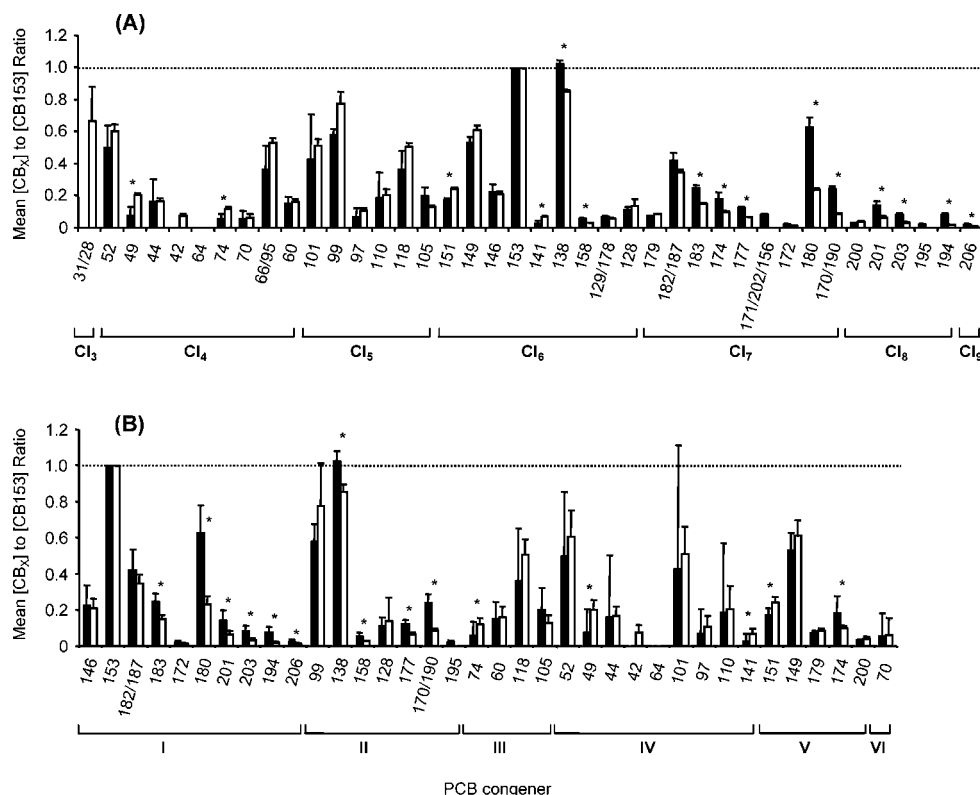


Fig. 1. The ratios of the arithmetic means of polychlorinated biphenyl (PCB) congener to CB153 concentrations in the livers of St. Lawrence (black bars;  $n = 6$ ) and Canadian Arctic (white bars;  $n = 11$ ) beluga whales (A) arranged by chlorine-substitution pattern and (B) arranged by metabolic classification [12]. Significant differences ( $p < 0.05$ ) between the two populations are marked with asterisks. Error bars represent the standard error of the mean.

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, and 6-OH-BDE137. For MeO-PBDEs in the neutral fraction, structural conformation of 2'-MeO-BDE68 and 6-MeO-BDE47 was confirmed by monitoring the nominal mass of isotopic  $[M]^-$  ( $m/z$  512) and  $[M+2]^-$  ( $m/z$  514) of the molecular ion cluster, and  $[M]^-$  ( $m/z$  434) and  $[M+2]^-$  ( $m/z$  436) of the  $[M-Br]^-$  fragment anion [46]. Quantification of detected MeO-PBDE or (methylated) OH-PBDE residues was accomplished using an internal standard method based on relative ECNI RRFs of  $^{79}\text{Br}$  plus  $^{81}\text{Br}$  anions of BDE30 and (methylated) 2'-OH-BDE28, respectively. For six MeSO<sub>2</sub>-PCB congeners, standards were not available (3-MeSO<sub>2</sub>-CB64, 3-MeSO<sub>2</sub>-CB91, 4-MeSO<sub>2</sub>-CB91, 3'-MeSO<sub>2</sub>-CB87, 3-MeSO<sub>2</sub>-CB149, 3'-MeSO<sub>2</sub>-CB141, and 4'-MeSO<sub>2</sub>-CB141). Their identities were confirmed by comparison to MeSO<sub>2</sub>-PCB patterns from previous studies employing GC-MS(ECNI) analysis [27], and the RRFs of standards in their appropriate chlorinated isomer groups were used to quantify these analytes. Thus, the  $\Sigma\text{MeSO}_2\text{-PCB}$  is the sum (if quantifiable) of the aforementioned congeners and the following 16 congeners in the mixed standard: 3-MeSO<sub>2</sub>-CB52, 3'-MeSO<sub>2</sub>-CB49, 4-MeSO<sub>2</sub>-CB52, 4'-MeSO<sub>2</sub>-CB49, 4-MeSO<sub>2</sub>-CB64, 3-MeSO<sub>2</sub>-CB70, 3'-MeSO<sub>2</sub>-CB101, 4-MeSO<sub>2</sub>-CB70, 4'-MeSO<sub>2</sub>-CB101, 3-MeSO<sub>2</sub>-CB110, 3-MeSO<sub>2</sub>-CB149, 4-MeSO<sub>2</sub>-CB110, 4'-MeSO<sub>2</sub>-CB87, 3'-MeSO<sub>2</sub>-CB132, 4'-MeSO<sub>2</sub>-CB132, and 4'-MeSO<sub>2</sub>-CB174.

#### Quality control

Mean recoveries were  $99\% \pm 9\%$ ,  $84\% \pm 11\%$ ,  $76\% \pm 16\%$ ,  $61\% \pm 13\%$ , and  $101\% \pm 12\%$  for PCBs/OCs, PBDEs

and MeO-PBDEs, OH-PCBs, OH-PBDEs, and MeSO<sub>2</sub>-PCBs, respectively. The higher sulfone recoveries may be caused by matrix effects (residual lipids in the samples) on the fragmentation patterns by ECNI, because reanalysis by GC- $\mu$ ECD gave more consistent recoveries of  $68\% \pm 3\%$ . The method limits of quantifications (MLOQs) for PCBs/OCs, PBDEs, MeO-PBDEs, MeSO<sub>2</sub>-PCBs, OH-PCBs, and OH-PBDEs were all around 0.5 ng/g lipid weight ( $\sim 0.01$  ng/g wet wt) based on a signal to noise ratio (S/N) of 10.

The precision of the method was confirmed by intraday duplicate analysis of three individual liver samples. For PCBs, PBDEs and MeO-PBDEs, and MeSO<sub>2</sub>-PCBs, the internal standard concentrations were within 8, 8, and 10%, respectively, between duplicates. The intraday repeatability for OH-PCBs was higher; however, using the internal standard method of quantification, the variability in the congeners actually detected in the duplicated analyses was within 15%. With each batch of five beluga whale liver tissues analyzed, a method blank ( $n = 4$ ) also was run. In the case of BDE47, low levels were periodically detected in the blanks, and the area of this response in the blank was subtracted from peak responses in the samples.

#### Data analysis

All statistical analyses of variance in contaminant concentrations in livers of beluga whales from the two populations was carried out using Statistica (Ver 6.0; StatSoft, 2003; Tulsa, OK, USA) or Excel (Microsoft, Redwood City, WA, USA). All hypotheses tested for analysis of variance in analyte concentrations between the SLB and CAB used a two-tailed Student's  $t$  test, where the maximum probability of a type I error

Table 1. The arithmetic mean ( $\pm$  standard error [SE]) and range of concentrations (ng/g lipid wt) of classes of major neutral and phenolic organohalogen compounds in the liver of beluga whales from the St. Lawrence River Estuary and from western Hudson Bay (Arviat, Nunavut, Canada)<sup>a</sup>

Analyte	St. Lawrence River Estuary ( <i>n</i> = 6)		Western Hudson Bay ( <i>n</i> = 11)	
	Mean ( $\pm$ SE) (range)	No. samples > MLOQ <sup>b</sup>	Mean ( $\pm$ SE) (range)	No. samples > MLOQ
Lipid (%)	3.2 $\pm$ 0.6 (0.7–5.1)		3.4 $\pm$ 0.2 (2.0–4.9)	
$\Sigma_9$ PBDE <sup>c*</sup>	2,210 $\pm$ 656 (246–3,030)	6	53 $\pm$ 16 (2–183)	11
$\Sigma_5$ PBDE <sup>*</sup>	1,989 $\pm$ 433 (212–2,765)	6	53 $\pm$ 16 (2–183)	11
$\Sigma$ OH-PBDE	<0.5	0	<0.5	0
$\Sigma$ MeO-PBDE <sup>d</sup>	25/20	2	43/100	2
$\Sigma$ PCB <sup>c*</sup>	31,937 $\pm$ 6,498 (1,351–45,392)	6	1,737 $\pm$ 1,253 (322–4,369)	11
$\Sigma$ OH-PCB <sup>e*</sup>	65 $\pm$ 22 (<0.5–145)	5	3.1 $\pm$ 0.5 (<0.5–5)	10
$\Sigma$ MeSO <sub>2</sub> -PCB <sup>g*</sup>	3,801 $\pm$ 1,322 (18–8,629)	6	77 $\pm$ 23 (18–251)	11
$\Sigma$ DDT <sup>*</sup>	4,536 $\pm$ 1,152 (39–7,832)	6	284 $\pm$ 81 (41–913)	11
3-MeSO <sub>2</sub> - <i>p,p'</i> -DDE <sup>h</sup>	84 $\pm$ 44 (<0.5–256)	5	22 $\pm$ 10 (2–110)	11
OCS <sup>i</sup>	11 $\pm$ 4 (<0.5–23)	3	1.4 $\pm$ 0.3 (<0.5–3.5)	6
4-OH-HpCS <sup>j</sup>	1.5 $\pm$ 0.4 (<0.5–4)	4	0.5 $\pm$ 0.2 (<0.5–0.5)	2
$\Sigma$ HCH <sup>k</sup>	74 $\pm$ 18 (7–136)	6	45 $\pm$ 8 (12–97)	11
$\Sigma$ CHL <sup>l*</sup>	2,355 $\pm$ 551 (56–3,857)	6	808 $\pm$ 153 (181–1,556)	11
Heptachlor epoxide	193 $\pm$ 59 (2–339)	5	123 $\pm$ 25 (<0.5–251)	9
$\Sigma$ Mirex <sup>*</sup>	47 $\pm$ 10 (<0.5–77)	5	0.59 $\pm$ 0.04 (<0.5–0.7)	5
HCB <sup>m</sup>	121 $\pm$ 24 (22–174)	6	144 $\pm$ 30 (9–264)	11
Dieldrin <sup>*</sup>	757 $\pm$ 187 (34–1,222)	5	275 $\pm$ 49 (<0.5–500)	9

<sup>a</sup> The set of beluga from the St. Lawrence River Estuary (SLB) was comprised of three males (two adults and one juvenile) and three females (two juveniles and one neonate), whereas the set of beluga from the Canadian Arctic, western Hudson Bay (CAB), was comprised of three males (two adults and one juvenile) and seven females (five adults, one juvenile, and one neonate). Asterisks indicate significantly higher concentrations ( $p < 0.05$ ) in SLB versus CAB.

<sup>b</sup> MLOQ = method limit of quantification.

<sup>c</sup> PBDE = polybrominated diphenyl ether.

<sup>d</sup> Only two SLB and two CAB animals were analyzed for methoxylated-PBDEs (MeO-PBDEs) because of limited sample fraction.

<sup>e</sup> PCB = polychlorinated biphenyl.

<sup>f</sup> OH-PCB = hydroxylated-PCB.

<sup>g</sup> MeSO<sub>2</sub>-PCB = methylsulfonyl-PCB.

<sup>h</sup> *p,p'*-DDE = 1,1-dichloro-2,2-bis[4-chlorophenyl]ethene.

<sup>i</sup> OCS = octachlorostyrene.

<sup>j</sup> HpCS = heptachlorostyrene.

<sup>k</sup> HCH = hexachlorocyclohexane.

<sup>l</sup> CHL = chlordane.

<sup>m</sup> HCB = hexachlorobenzene.

was set to  $\alpha = 0.05$ . Therefore, statistical significance of variance was set at  $p \leq 0.05$ . Variables that did not approximate the normal distribution using the Shapiro–Wilk's *W* test ( $p < 0.01$  [47]) were log<sub>10</sub>-transformed to meet the assumptions of the statistical tests. Summary statistics for any analytes were computed only if at least 50% of the samples had concentrations above the MLOQ. Any sample concentrations within such a group below the MLOQ were assigned a random value between zero and the MLOQ. The variance caused by the effect of differences in individual extractable lipid content in liver tissue on OC, PCB, MeO-PBDE, PBDE, and MeSO<sub>2</sub>-PCB concentrations was minimized by using lipid-normalized concentrations (ng/g lipid wt). Age, sex, and sampling date were examined [16] as possible variables affecting organohalogen concentrations in animals from both beluga whale populations.

## RESULTS

### PCBs and PCB metabolites

Examination of the data showed no clear relationship between any organohalogen compound class and age, sex, or sampling date, with only two exceptions. First, a negative correlation ( $r^2 = 0.76$ ) between  $\Sigma$ PBDE concentration and age was found in SLB. Sex was not a factor in individual differences between  $\Sigma$ PBDE concentrations for either population. Second, a positive correlation was found for  $\Sigma$ MeSO<sub>2</sub>-PCB

and age ( $r^2 = 0.61$ ) in SLB. The number of samples obtained overall was small (SLB,  $n = 6$ ; CAB,  $n = 11$ ), the samples were heterogeneous (in terms of age and sex,) and no relationship was found based on subclassification (age, sex, and collection date) in general. Therefore, the results were evaluated only on a population basis (SLB vs CAB), as has been done previously with small data sets comprised of ethically and logistically difficult-to-obtain samples (see, e.g., [18,19]).

The mean organohalogen contaminant and metabolite concentrations generally were higher in the liver tissues of SLB than in those of CAB (Table 1). Although the concentration ranges for all contaminant classes were large, the mean  $\Sigma$ PBDE,  $\Sigma$ PCB,  $\Sigma$ OH-PCB, and  $\Sigma$ MeSO<sub>2</sub>-PCB concentrations were all significantly higher ( $p < 0.05$ ) in SLB samples than in CAB samples. The mean  $\Sigma$ PCB and  $\Sigma$ DDT concentrations were approximately 16-fold, the  $\Sigma$ OH-PCB 22-fold, and the  $\Sigma$ MeSO<sub>2</sub>-PCB 49-fold higher in SLB than in CAB. However, in both populations,  $\Sigma$ OH-PCB metabolites comprised less than 0.2% of  $\Sigma$ PCB. The mean  $\Sigma$ MeSO<sub>2</sub>-PCB concentration was 58- and 27-fold greater than  $\Sigma$ OH-PCB in SLB and CAB livers, respectively. In addition,  $\Sigma$ MeSO<sub>2</sub>-PCB comprised a larger contribution to the  $\Sigma$ PCB concentrations in SLB liver tissues (11%) than in CAB (4%) liver tissues.

The highest mean concentrations in SLB were as follows:  $\Sigma$ PCB >  $\Sigma$ DDT >  $\Sigma$ MeSO<sub>2</sub>-PCB. In CAB, the order was as

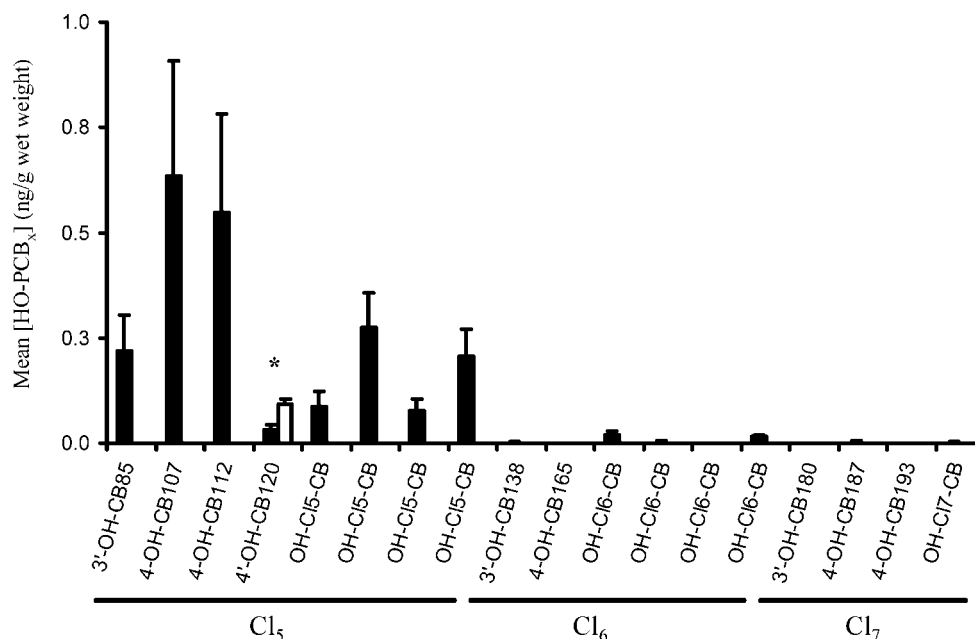


Fig. 2. The arithmetic mean concentrations of individual, hydroxylated-polychlorinated biphenyl (OH-PCB) congeners in the liver tissues of beluga whales from the St. Lawrence Estuary (black bars;  $n = 6$ ) and from the Canadian Arctic (white bars;  $n = 11$ ). The OH-PCBs were determined as methoxylated-PCB derivatives (see *Materials and Methods*). Significant differences ( $p < 0.05$ ) are marked with asterisks. Error bars represent the standard error of the mean.

follows:  $\Sigma\text{PCB} > \Sigma\text{CHL} > \Sigma\text{DDT}$ . Regardless of males and females, for the CAB,  $\Sigma\text{PCB}$  concentrations showed significant ( $p < 0.05$ ) correlations with concentrations of the highly recalcitrant CB153 ( $r^2 = 0.99$ ),  $\Sigma\text{PBDE}$  ( $r^2 = 0.60$ ), and  $\Sigma\text{MeSO}_2\text{-PCB}$  ( $r^2 = 0.73$ ), but not with  $\Sigma\text{OH-PCB}$  ( $r^2 = 0.10$ ). With a lower number of samples and five individuals exceeding  $\Sigma\text{PCB}$  concentrations of 30,000 ng/g lipid weight, it was not surprising that only weak relationships for  $\Sigma\text{PCB}$  to  $\Sigma\text{MeSO}_2\text{-PCB}$  and  $\Sigma\text{PBDE}$  concentrations were found for SLB ( $r^2 = 0.21$  and 0.36, respectively).

The PCB congeners in the tri- to nonachlorinated isomer classes were detected. Taking the ratio of individual congener concentrations to the concentration of the slowly metabolized CB153 showed differences in the PCB congener profiles in livers of individuals from the two populations. The CAB exhibited significantly ( $p < 0.05$ ) larger ratios for most tri- to hexachlorinated PCB congeners than SLB, whereas SLB showed significantly larger ratios for hepta- and octachlorinated PCB congeners (Fig. 1A). The PCB congeners also were grouped according to the metabolic classification scheme for marine mammals [13]. This classification divides congeners into five metabolic groups according to the number and positioning of chlorine atoms around the biphenyl ring. For instance, group III PCBs have only *ortho-meta* unsubstituted sites and one or less *ortho*-Cl atoms and, therefore, likely are metabolized by CYP1A-type enzymes. Group IV congeners have *meta-para* vicinal H-atom pairs and two or less *ortho*-Cl atoms and, therefore, are better substrates for CYP2B- or CYP3A-mediated metabolism. The SLB showed larger ratios to CB153 of the recalcitrant group I and II PCB congeners (Fig. 1B). However, SLB appeared to have depleted CB153 ratios of some group III and IV congeners relative to CAB liver tissues.

In SLB, 18 penta- to heptachlorinated OH-PCB congeners were found, although pentachlorinated congeners comprised more than 90% of the  $\Sigma\text{OH-PCB}$  (Fig. 2). The 4-OH-CB107

and 4-OH-CB112 were the dominant congeners detected in the St. Lawrence population. In CAB, only one OH-PCB congener, 4'-OH-CB120, was quantifiable. The concentration of 4'-OH-CB120 was significantly higher ( $p < 0.05$ ) in CAB relative to that in SLB. A probable metabolite of octachlorostyrene, 4-OH-HpCS, also was detected at low levels in the phenolic fraction from SLB and CAB liver tissues (Table 1).

Tetra- to hexachlorinated  $\text{MeSO}_2\text{-PCBs}$  were detected in both populations, but significant differences in the ratios of individual  $\text{MeSO}_2\text{-PCB}$  congeners to CB153 concentrations between the SLB and CAB were observed for a number of congeners (Fig. 3). The concentration ratios were higher for SLB and indicated different congener patterns between the two populations. The dominant congeners were 3'- and 4'- $\text{MeSO}_2\text{-CB101}$ , 3'- and 4'- $\text{MeSO}_2\text{-CB49}$ , and 3'- and 4'- $\text{MeSO}_2\text{-CB87}$  in beluga whale liver tissue from both populations. A methylsulfonated metabolite of DDE, 3- $\text{MeSO}_2\text{-}p,p'\text{-DDE}$ , also was found at higher, but insignificantly different ( $p < 0.05$ ), levels in SLB relative to those in CAB.

#### PBDEs, OH-PBDEs, and MeO-PBDEs

The  $\Sigma_5\text{PBDE}$  and  $\Sigma_9\text{PBDE}$  concentrations were significantly higher ( $p < 0.05$ ) in the liver tissues of SLB than in CAB (Table 1). In juvenile SLB animals, these concentrations were an order of magnitude higher than those in the two adult SLB males, in comparison to the PCBs concentrations, for which no obvious differences were observed. Nonetheless,  $\Sigma_9\text{PBDE}$  levels were 14- and 2-fold lower than  $\Sigma\text{PCB}$  and  $\Sigma\text{DDT}$  in SLB, whereas they were 36- and 5-fold lower in CAB, respectively. The PBDE congener profiles showed similarities in the liver tissues of beluga whales from both populations, with BDE47, BDE99, and BDE100 concentrations comprising approximately 40, 20, and 20%, respectively, of the  $\Sigma_5\text{PBDE}$  in SLB and CAB (Fig. 4). The BDE153 and BDE154 congeners made up a significantly lower-percentage proportion of  $\Sigma_5\text{PBDE}$  in CAB versus SLB animals. The minor

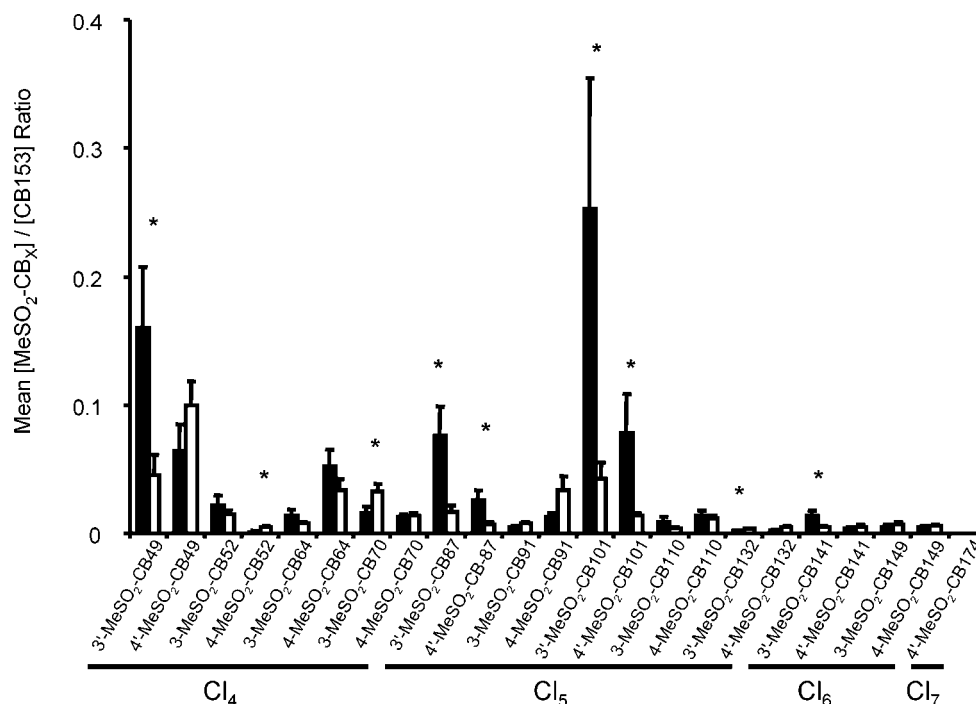


Fig. 3. The ratio of the arithmetic mean of the individual, methylsulfonyl (MeSO<sub>2</sub>)-polychlorinated biphenyl (PCB) congener to CB153 concentrations in the livers of beluga whales from the St. Lawrence (black bars;  $n = 6$ ) and the Canadian Arctic (white bars;  $n = 11$ ). Significant differences ( $p < 0.05$ ) between the two populations are marked with asterisks. Error bars denote the standard error of the mean.

congeners BDE28, BDE138, and BDE183 were detected only in SLB. The BDE49 congener was not detected in samples from either population. Other unidentified peaks with a <sup>79</sup>Br/<sup>81</sup>Br anion response also were detected in the neutral, PBDE-containing fractions in liver tissues of animals from both populations.

Although the levels of OH-PBDEs were less than the limit

of quantification ( $<0.01$  ng/g wet wt) in all samples from SLB and CAB, some congeners were nonetheless detectable ( $3 < \text{S/N} > 10$ ) in the phenolic fractions extracted from some liver samples of animals from both populations. Only two OH-PBDEs were detectable and could be identified, 2'-OH-BDE68 and 6-OH-BDE47 (Fig. 5). Of the 15 MeO-PBDEs monitored,

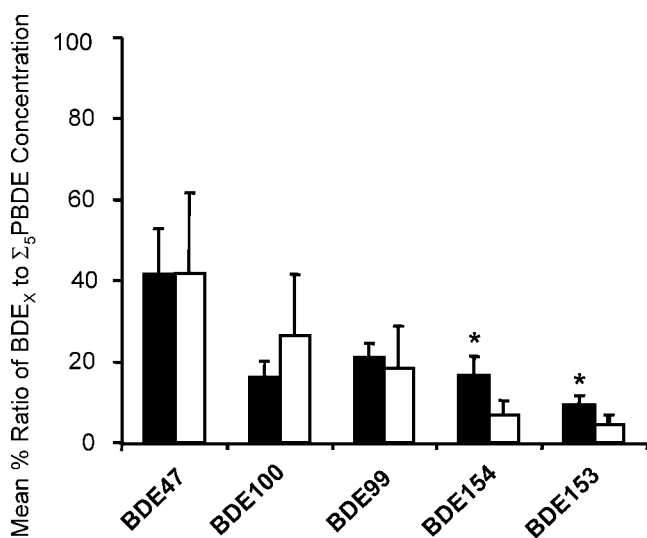


Fig. 4. The arithmetic mean percentage ratio of individual polybrominated diphenyl ether (PBDE) congeners (BDE47, BDE99, BDE100, BDE153, and BDE154) to the sum of the polybrominated diphenyl ether ( $\Sigma_5$ PBDE) congener concentrations in the liver tissues of St. Lawrence (black bars;  $n = 6$ ) and Canadian Arctic (white bars;  $n = 11$ ) beluga whales. The ratios are based on the five BDE congeners that were quantifiable in animals from both populations. Significant differences ( $p < 0.05$ ) between the two populations are marked with asterisks. Error bars represent the standard error of the mean.

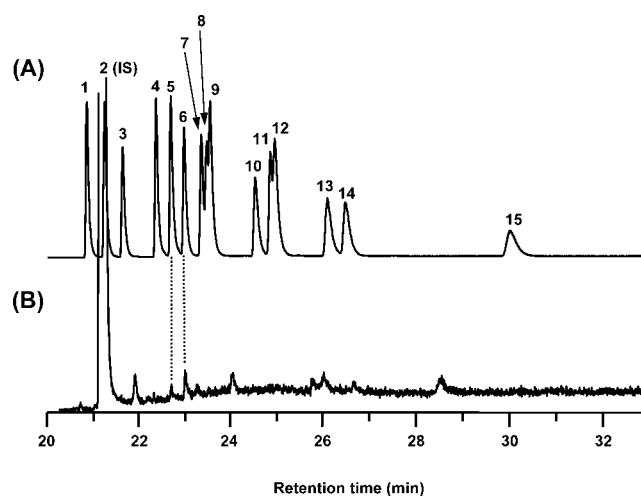


Fig. 5. Total ion mass chromatograms by gas chromatography—mass spectrometry (electron capture, negative ion; selected ion monitoring anions: <sup>79</sup>Br<sup>-</sup> and <sup>81</sup>Br<sup>-</sup>) of (A) methoxylated-polybrominated diphenyl ether (MeO-PBDE) external standard mixture and (B) the derivatized halogenated phenolic fraction extracted from the liver tissues of an adult female beluga whale from the Canadian Arctic. Peak identification is as follows: 1 = 6'-OH-BDE17; 2 = 2'-OH-BDE28 (internal standard [IS]); 3 = 4'-OH-BDE17; 4 = 6'-OH-BDE49; 5 = 2'-OH-BDE68; 6 = 6-OH-BDE47; 7 = 3-OH-BDE47; 8 = 5-OH-BDE47; 9 = 4'-OH-BDE49; 10 = 4-OH-BDE42; 11 = 6-OH-BDE90; 12 = 6-OH-BDE99; 13 = 2-OH-BDE123; 14 = 6-OH-BDE85; and 15 = 6-OH-BDE137.



4'-MeO-BDE17 and 6-MeO-BDE47 were quantifiable in the SLB, whereas only 2'-MeO-BDE68 and 6-MeO-BDE47 were quantifiable in the CAB (Table 1). For either the SLB or CAB, 6-MeO-BDE47 made up 60 to 80% of the  $\Sigma$ MeO-PBDE concentration.

### DISCUSSION

The toxicokinetics of anthropogenic organohalogenes have been understudied in marine mammals. Metabolic processes affect the toxicity and excretion of these contaminants. Previously, several phase I CYP monooxygenase and phase II conjugative biotransformation enzymes were characterized in the liver tissues of the present St. Lawrence and Canadian Arctic (western Hudson Bay) beluga whale samples [16]. These results were suggestive of a metabolic potential of these beluga whales toward organic contaminants such as PCBs and PBDEs.

#### PCBs

Different congener profiles of parent PCBs between populations and species of marine mammals suggest variability in metabolic capacities and/or exposures. St. Lawrence beluga whales generally have higher levels of OC contaminants than CAB because of historical exposures [4,5]. Here, we show variation in the levels of PCB congeners between SLB and CAB, in agreement with a previous study quantifying PCBs in the livers of St. Lawrence and Hendrickson Island (Canadian Arctic) beluga whales [5], indicative of higher exposures to OCs in SLB. The SLB ( $n = 2$ ) and CAB ( $n = 2$ ) adult males that we sampled had an average liver  $\Sigma$ PCB concentration of 35,944 and 3,323 ng/g lipid weight, respectively, which is consistent with the concentrations of 53,884 and 4,894 ng/g lipid weight, respectively, found for beluga collected from the same regions 10 years earlier [5]. The  $\Sigma$ PCB concentrations in the present adult male SLB liver samples were somewhat lower than those found in blubber of dead males from this population between 1982 and 1994 [9] but somewhat higher than those found in biopsy specimens from live captured males collected from 1994 to 1998 [48]. However, the  $\Sigma$ PCB was not comprised of the same congener suite in the three separate studies. Greater representation of higher-chlorinated congeners in the  $\Sigma$ PCB concentrations indicated their persistent/accumulative nature in comparison to lower-chlorinated congeners in both populations.

After correction to the slowly metabolized CB153, different congener profiles were found in the liver tissues according to PCB metabolic class between SLB and CAB (Fig. 1B). Higher ratios of groups I and II PCBs in SLB versus CAB, however, likely are related to differences in exposure; higher-chlorinated compounds are less volatile and tend to be less represented in remote regions such as the Arctic. In contrast, depleted ratios of groups III and IV PCBs in SLB compared to CAB more likely may be caused by increased metabolism as a function of induced phase I (e.g., CYP1A) and, possibly, phase II biotransformation activities in more exposed populations [49]. It is not possible with the present data to distinguish between different patterns in source (diet) or difference in metabolic capabilities among animals.

#### PBDEs

To our knowledge, in addition to the present beluga whale study, the only other report of PBDEs in the liver of any other cetacean species was for harbor porpoise (*Phocoena pho-*

*coena*) from the North Sea [50]. The hepatic PBDE congener profiles based on congeners that were quantifiable in animals from both populations (Fig. 4) were similar in the SLB versus CAB animals (Fig. 4), with the exception of BDE153 and BDE154, which were significantly higher ( $p < 0.05$ ) in the SLB animals (Fig. 4). Regardless, the BDE47, BDE99, BDE100, BDE153, and BDE154 congener profile in liver was comparable to that reported for the blubber tissues of SLB, where BDE47, BDE99, and BDE100 accounted for more than 75% of the  $\Sigma$ PBDE concentration [8]. Comparison of PBDE levels in the present study to those found in previous studies must be interpreted with caution, because PBDE levels in the North American environment and biota are rapidly increasing [2]. Nonetheless, liver  $\Sigma$ PBDE concentrations (74 ng/g lipid, sampled in 2003) in western Hudson Bay male beluga whales in the present study were higher than those found in adipose tissues of male Cumberland Sound (eastern Canadian Arctic) beluga (16 ng/g lipid, sampled in 1997) and male Kugmallit Bay (western Canadian Arctic) beluga whales (17 ng/g lipid, sampled in 2001) [2]. This finding may be caused by temporal differences in PBDE concentrations or by the closer proximity of Hudson Bay to possible sources at lower latitudes. This finding is less likely to be caused by a preference of PBDEs for liver tissue, because we found lipid-corrected concentrations of  $\Sigma$ PBDE in the liver tissues of our two St. Lawrence adult male beluga whales (average, 389 ng/g lipid) sampled in 2001 similar to that reported by Lebeuf et al. [8] in the blubber tissues of five adult St. Lawrence beluga whales (average, 541 ng/g lipid) sampled in 1999. Boon et al. [50] also found no preference for PBDEs in liver versus blubber tissues of harbor seals (*Phoca vitulina*) and harbor porpoises.

In agreement with other biota studies [2], PBDE congener profiles in beluga whales exhibited large variation from the commercial formulations as well as from profiles in environmental matrices. Differences in PBDE congener profiles between species in the same food chain also have been reported [2,33,50], which is suggestive of PBDE biotransformation potential. Alternately, selective bioaccumulation based on the different physicochemical properties (e.g.,  $\log K_{ow}$ ) of the congeners also may be a factor. Although consistent with other studies (i.e., our finding that PBDE concentrations are still lower in beluga whales than PCBs), because PBDE levels increase in wildlife, the need for further research concerning PBDE metabolism is warranted. In fact, in a recent in vitro PBDE metabolism study with hepatic microsomes from CAB, we found that *ortho-meta* bromine-unsubstituted BDE15, BDE28, and BDE47 were significantly metabolized (depleted) by 100, 11, and 5%, respectively, whereas BDE49, BDE99, BDE100, BDE153, BDE154, and BDE183 showed no significant depletion within the 90-min time frame of the assay [51].

#### OH-PCBs, OH-PBDEs, and MeO-PBDEs

Both PCBs and PBDEs may be metabolically transformed with potential biological activity to halogenated phenolic compounds (HPCs) [14,37]. In the present study, we report on quantifiable concentrations of retained OH-PCB congeners and 4-OH-HpCS and just-detectable levels of OH-PBDEs in the liver of beluga whales from two Canadian populations. Most reports have focused on blood plasma for chlorinated HPCs. A number of OH-PCB congeners, 4-OH-HpCS, and thyroid hormone-like OH-PBDEs have been shown to have high competitive binding affinity for TTR [12,14,38].

To our knowledge, this is the first report on the monitoring



and/or detection of OH-PCBs or OH-PBDEs in a tissue other than blood in a marine mammal species. A single previous publication reported OH-PCBs in human liver [22]. The OH-PCBs, OH-PBDEs, and 4-OH-HpCS may be retained in the liver, in part, because of fatty acid conjugation and interaction with hepatic proteins/enzymes, for example. The pentachlorophenol and an OH-DDT were both shown to be conjugated by liver fatty acids in rats [52,53]. However, it is possible that the presence of HPCs in liver is caused by residual blood [54]. In addition to being protected by protein-binding interactions, the lifetime of OH-PCBs in the liver also may be related to an affinity for enzymes mediating conjugation processes. The OH-PCBs have demonstrated strong (inhibitory) interactions in vitro with phase II intestinal biotransformation enzymes in catfish (*Ictalurus punctatus*), particularly glucuronosyl transferases [55]. In addition, in vitro inhibition of thyroxine sulfation by OH-PCBs has been shown in rats [20].

We found elevated levels of OH-PCB metabolites in the livers of beluga whales inhabiting the estuary downstream of the contaminated St. Lawrence River in comparison to those from the relatively less contaminated western Hudson Bay region. It is unlikely that the OH-PCBs in beluga whale liver may be caused by food-chain bioaccumulation, although OH-PCBs have been reported in fish (major constituents of beluga whale diets) from marine (Baltic Sea) and freshwater (Detroit River, Great Lakes) systems [21,24]. Metabolism of the precursor PCB congeners is more than likely the mechanism for OH-PCBs in beluga whales, because a source of OH-PCBs for uptake by beluga from either population has yet to be identified. Differences in CYP monooxygenase and/or phase II conjugative biotransformation activities exist between animals from the two differentially exposed populations. This has been shown qualitatively by immunological assessment for the present beluga whales [16]. Gray (*Halichoerus grypus*) and ringed (*Phoca hispida baltica*) seals from the heavily polluted Baltic Sea exhibited hepatic CYP1A-mediated 7-ethoxyresorufin *O*-deethylase activities higher than those from reference sites, which were significantly correlated with liver PCB loads [49]. Evidence for differential metabolic capacity between the two Canadian beluga populations also comes from the fact that OH-PCBs and MeSO<sub>2</sub>-PCBs were at higher concentrations relative to PCBs in SLB relative to CAB: The  $\Sigma$ OH-PCB and  $\Sigma$ MeSO<sub>2</sub>-PCB levels were 22- and 49-fold higher in SLB than in CAB, whereas  $\Sigma$ PCB were only 16-fold higher in the former population than in the latter. The higher exposure of SLB to enzyme-inducing contaminants is consistent with higher PCB metabolite to PCB ratios.

Although 18 OH-PCB congeners were found in SLB, only 4'-OH-CB120 was detectable in CAB. The concentrations of 4'-OH-CB120 were weakly and positively correlated with both CYP2B/3A-type activity (testosterone-to-androstenedione conversion,  $r^2 = 0.48$ ,  $p = 0.03$ ) and UDPGT activity (1-naphthol glucuronidation,  $r^2 = 0.41$ ,  $p = 0.05$ ) activities in catalytically viable microsomes from these same CAB, as reported previously [16]. However, androstenedione, UDPGT, and 6 $\beta$ -testosterone hydroxylase activities also covaried significantly. In rats, 4'-OH-CB120 and 4-OH-CB107 are metabolically formed from CB118 [56]. The CB118 congener was one of the predominant PCB congeners in both the SLB and CAB hepatic tissues that we studied and, thus, could be a precursor of these retained metabolites. The 4-OH-CB107 congener, which also can be formed from CB105 in rat [56], was one of the major congeners in SLB, but it was not detected in

CAB. Closer examination of the structures of the OH-PCBs identified revealed that roughly half (4-OH-CB165, 3'-OH-CB180, 4-OH-CB187, and 4-OH-CB193) come from parent PCBs with no *ortho-meta* or *meta-para* vicinal hydrogen-atom pairs (Fig. 2) and could form from PCBs via direct OH insertion or via arene epoxides and a 1,2-shift mechanism. Although group I PCBs (Fig. 1B) are resistant to degradation and, thus, have a slow rate of metabolism, biotransformation to OH-PCB metabolites still occurs. The OH-PCB congeners derived from metabolically resistant group I PCB congeners also have been found in human liver [22], human plasma and gray seal blood [54], polar bear [19] and bowhead whale plasma [23], and various fish species [24]. Nonetheless, lower levels of OH-PCBs from group I PCB congeners were found in the beluga whales relative to the other quantifiable OH-PCBs. These other OH-PCBs must be derived from precursor PCB congeners with two or fewer *ortho*-chlorines and *ortho-meta* unsubstituted sites and some with *meta-para* vicinal H-atom pairs as well (groups II, III, or IV). However, none of the retained OH-PCBs could have come from parent PCBs with only *meta-para* unsubstituted sites on the biphenyl ring. It has been proposed that OH-PCB formation from *meta-para* chlorine-unsubstituted PCBs via the arene epoxide intermediate must compete with phase II conjugation of the epoxide with glutathione and subsequent formation of MeSO<sub>2</sub>-PCB metabolites [14].

Whereas  $\Sigma$ OH-PCB comprised around 1 to 10% of  $\Sigma$ PCB in human liver tissue [22] and represented an even greater proportion in blood tissues of other mammals [19,23,54],  $\Sigma$ OH-PCB to  $\Sigma$ PCB concentration ratios in the present beluga liver tissues were less than 0.2%. This suggests lower metabolic capacity of beluga whales to form and retain OH-PCBs from PCB precursors and/or greater capacity to deplete OH-PCBs by phase II conjugation processes. Similar factors may be influential in the just detectable OH-PBDE residues.

The MeO-/OH-pairs, 6-MeO-/OH-BDE-47 and 2'-MeO-/OH-BDE68, were determined in the liver of the present beluga whale (Table 1). To our knowledge, the only other report on MeO-PBDEs in a cetacean species is that of Wolkers et al. [33], who reported three unidentified MeO-tetrabromo-DEs ( $n = 4$ ; concentration range, 12–80 ng/g lipid wt) in the blubber of beluga whales from Svalbard (collected in 1998). The OH-PBDEs and MeO-PBDEs also recently were reported in the blood of Svalbard glaucous gulls and polar bears [46] as well as Baltic salmon [57]. Recent evidence strengthens the hypothesis that 6-MeO-/OH-BDE-47 and 2'-MeO-/OH-BDE68 are of natural origin and accumulated in the food webs of higher vertebrates [32]. It seems apparent that OH-/MeO-PBDEs of natural origin have the MeO- or OH-group in the *ortho* position relative of the diphenyl ether bond and have been identified in, for example, marine sponges, ascidians, and algae [36]. Teuten et al. [32], who isolated 6-MeO-BDE47 and 2'-MeO-BDE68 from the blubber of North Atlantic True's beaked whales (*Mesoplodon mirus*), have demonstrated natural origins using radiocarbon (<sup>14</sup>C) analyses. The OH-PBDEs with an OH-group in the *meta* or *para* position were neither detectable nor quantifiable in SLB or CAB liver, which might suggest that metabolism of PBDE to OH-PBDE does not occur in these beluga. Similar to OH-PCB formation and retention [14], it may be that *meta* or *para*, OH-substituted PBDEs may be preferential to blood (because the presence of high concentrations of thyroid hormone transport proteins). It has been argued that certain OH-PBDEs with an OH-group in the *meta*

or *para* position may be derived metabolically from PBDEs via CYP enzyme-mediated biotransformation [31,57].

#### Methyl sulfone PCBs and DDEs

In the only other study examining MeSO<sub>2</sub>-PCBs and OH-PCBs in liver tissue, Meironyté Guvenius et al. [22] reported that in Swedish humans, the  $\Sigma$ MeSO<sub>2</sub>-PCB and  $\Sigma$ OH-PCB concentrations were similar. In contrast, we found that  $\Sigma$ MeSO<sub>2</sub>-PCB concentrations were much higher in beluga liver than were  $\Sigma$ OH-PCB concentrations. However, MeSO<sub>2</sub>-PCBs comprised a comparable proportion of the liver PCB concentrations in beluga whale (4 and 11% in CAB and SLB, respectively) as in humans (2–17% [22]).

Liver  $\Sigma$ MeSO<sub>2</sub>-PCB concentrations were significantly higher (~50-fold) in SLB compared to those in CAB, and we similarly found higher levels of PCBs in individuals from the former population. Letcher et al. [27] found comparable levels of MeSO<sub>2</sub>-PCBs in the adipose tissues of SLB and CAB in a previous study, but those authors also found similar levels of PCBs between beluga whales from the two populations. Although  $\Sigma$ DDT concentrations were significantly elevated in SLB in comparison to CAB liver tissues, the DDT metabolite 3-MeSO<sub>2</sub>-*p,p'*-DDE was not found at significantly different concentrations between the two populations. Letcher et al. [27] previously found significantly higher levels of 3-MeSO<sub>2</sub>-*p,p'*-DDE in biopsied SLB adipose tissues than in biopsied CAB adipose tissues. These discrepancies between our results and those of Letcher et al. [27] may be caused, in part, by the different sampling techniques. In the present study, SLB liver tissues were mainly taken from dead, stranded animals. In Letcher et al. [27], adipose tissues were taken via biopsy from live animals. As mentioned previously, Hobbs et al. [48] found that the levels of highly chlorinated PCBs were lower in biopsied animals than were those previously found for dead animals.

It has been demonstrated in seals that (lipid-normalized) MeSO<sub>2</sub>-PCB concentrations were higher in liver than in blubber, muscle, or brain [26]. A preference of MeSO<sub>2</sub>-PCBs for liver tissues is explained by specific protein binding [14]. Elevated MeSO<sub>2</sub>-PCB concentrations in SLB versus CAB liver may be related to differences in exposures to parent PCBs or metabolites or, possibly, to elevated metabolic activities in the highly exposed SLB population. The MeSO<sub>2</sub>-PCBs are CYP2B1/2 inducers in rats [28], and CYP2B-type isozymes are ostensibly the CYPs involved in mediating the metabolism of *meta-para* unsubstituted PCBs, which are the precursors to MeSO<sub>2</sub>-PCBs [14].

#### CONCLUSION

The present study provides evidence in support of the congener-specific metabolism of PCBs to retained and/or persistent and potentially toxic metabolites in beluga whales. However, relatively low residue levels of OH-PCBs were found in the beluga whales of the present study, which is consistent with the low metabolic capacity of cetaceans toward higher-chlorinated PCB congeners. Compared to OH-PCBs, higher concentrations of MeSO<sub>2</sub>-PCBs were found in the livers, especially the SLB livers. The parent PCB congener profiles and elevated OH-PCB and MeSO<sub>2</sub>-PCB metabolite concentrations suggest a greater and, likely, exposure-influenced capacity of the highly contaminated SLB to metabolize such contaminants in comparison to CAB. We also report, to our knowledge for the first time in the tissue of an Arctic mammal from the

western hemisphere, on OH-PBDEs and MeO-PBDEs, which most likely are of natural origin and accumulated in the SLB and CAB food webs. Given that all these novel contaminants—MeSO<sub>2</sub>-PCBs, 3-MeSO<sub>2</sub>-*p,p'*-DDE, OH-PCBs, OH-PBDEs, and 4-OH-HpCS—have demonstrated endocrine-disrupting potential as well as other toxicities, this complexity of contaminant exposure may be impacting the health of Canadian beluga whale populations. However, our findings suggest that the potential exposure risks to beluga whales, either from the SLB or CAB populations, because of OH-PCB exposure is less than, for example, that to Arctic populations of high-trophic-level mammals (polar bears and seals) and birds (glaucous gulls), in which much higher OH-PCB concentrations and OH-PCB to PCB concentration ratios have been reported in blood [14,19,25].

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