SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 22, No. 11, pp. 2650–2658, 2003

Printed in the USA
0730-7268/04 $12.00 + .00

HYDROXYLATED AND METHYLSULFONE-CONTAINING METABOLITES OF POLYCHLORINATED BIPHENYLS IN THE PLASMA AND BLUBBER OF BOWHEAD WHALES (*BALAENA MYSTICETUS*)

PAUL F. HOEKSTRA,*†‡ ROBERT J. LETCHER,§ TODD M. O'HARA,‖ SEAN M. BACKUS,‡ KEITH R. SOLOMON,† and DEREK C.G. MUIR†‡

†University of Guelph, Department of Environmental Biology, Guelph, Ontario N1G 2W1, Canada
‡Environment Canada, National Water Research Institute, Burlington, Ontario L7R 4A6
§University of Windsor, Great Lakes Institute for Environmental Research, Windsor, Ontario N9B 3P4, Canada
‖Department of Wildlife Management, North Slope Borough, Barrow, Alaska 99723, USA

(*Received* 23 *December* 2002; *Accepted* 18 *March* 2003)

Abstract—Bowhead whale (*Balaena mysticetus*) blubber ($n = 20$) and plasma ($n = 19$) samples were collected during the 1997 to 2000 Inuit subsistence harvests in Barrow, Alaska, USA, to quantify the concentrations of methylsulfone ($MeSO_2$)-containing and hydroxylated (OH) polychlorinated biphenyl (PCB) metabolites in this cetacean. The distribution of $MeSO_2$-PCBs in blubber was dominated by 4-$MeSO_2$-substituted congeners, the most abundant being 4-$MeSO_2$-CB-70, 3′-$MeSO_2$-CB-132, and 4-$MeSO_2$-CB-64. Mean (±1 standard error) sum (Σ) $MeSO_2$-PCBs concentrations in blubber were low (6.23 ± 0.81 ng g^{-1} lipid normalized) compared to concentrations previously reported in other marine mammals. However, similar ratios of $MeSO_2$-PCB metabolites to parent PCB congeners among marine mammals suggest that cytochrome P450 2B-like biotransformation and other necessary enzyme-mediated processes and mechanisms that influence the formation and clearance of $MeSO_2$-PCBs exist in the bowhead whale. Pentachlorophenol was the most abundant halogenated phenolic compound quantified in bowhead plasma (1.55 ± 0.19 ng g^{-1} wet wt). Despite indirect evidence for arene epoxidation of the biphenyl moiety inferred from $MeSO_2$-PCB formation, ΣOH-PCB concentrations in bowhead plasma were low (1.52 ± 0.31 ng g^{-1} wet wt) compared to humans and marine mammals and were comprised of only two detectable OH-PCB congeners (4′-OH-CB-130 and 4-OH-CB-187). Further research is required to elucidate the toxicokinetics and distribution of OH-PCBs in this cetacean.

Keywords—Arctic Biotransformation Cetacean Polychlorinated biphenyl Pentachlorophenol

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a well-known class of semivolatile, persistent organochlorine contaminants (OCs) found in virtually every component of the biosphere because of both long-range transport by atmospheric and oceanic vectors and their environmental persistence [1]. The environmental distribution of PCBs is a function of their resistance to abiotic and biotic degradation and hydrophobicity, resulting in their bioaccumulation in the lipid reservoirs of biota. Numerous factors, such as diet, age, and gender, influence the toxicokinetics and accumulation of PCBs in biota [2]. Enzyme-mediated biotransformation is an important influence on PCB persistence, and its significance in PCB toxicokinetics is dependent on congener structure and the metabolic capacity of the organism [3,4]. Metabolism of xenobiotics (as a detoxification process) results in the formation of more polar metabolites and, thus, increases the likelihood for elimination from the organism.

Two classes of PCB metabolites have been determined as retained or persistent, namely hydroxylated (OH) and methylsulfone ($MeSO_2$)-containing PCBs, respectively. Persistent $MeSO_2$-PCBs have been found in the fat and liver tissues of several biota (summarized by Letcher et al. [5]), and formation is conditional on the capacity of an organism to form 3,4- and/or 2,3-arene epoxide PCB intermediates, which are intermediates of cytochrome P450 (CYP) isozyme products that are subsequently transformed to $MeSO_2$-containing analogues of the parent congeners, mainly by CYP2B-like mediated enzymatic processes [6]. The $MeSO_2$-PCBs are sufficiently lipophilic to accumulate in biota and have been linked to endocrine-related effects, including disruption of thyroid hormone homeostasis, cytotoxicity, and competitive binding with the glucocorticoid receptor [5]. Recently, antiestrogenic activity in vitro via a competitive interaction/mechanism with the estrogen receptor was demonstrated for several environmentally relevant $MeSO_2$-PCB congeners in several human and fish cell assays [7].

In contrast to $MeSO_2$-PCBs, OH-PCBs have been quantified in the blood of relatively few species, such as humans, seals, and polar bears from Sweden and/or Canada, and recently in various species of Detroit River fish [5,8–11]. The introduction of the OH group into the aromatic ring of the parent PCB congener can occur either by direct insertion in the *meta*-position or by arene epoxide formation and subsequent epoxide hydrolase-mediated ring opening with or without an intermolecular 1,2-shift of H and Cl atoms [12]. Whereas OH-PCBs are subject to further metabolism, their structural similarities to thyroxine result in high affinity and competitive interaction via noncovalent binding with thyroid hormone transport proteins [13], thereby impeding thyroid hormone and retinol transport and, possibly, impairing hormone metabolism and function [5].

Our current understanding of PCB biotransformation in some cetaceans has been derived from immunologic and cat-

* To whom correspondence may be addressed (paul.hoekstra@ec.gc.ca).

alytic characterization of CYP enzymes, congener-specific accumulation, and extrapolation from mammalian in vitro metabolism models using hepatic microsomes [14–19]. In general, cetaceans appear to have limited ability for metabolism of nonplanar contaminants via CYP2B-like enzymes, including parent PCB congeners of $MeSO_2$- and OH-PCB metabolites, compared to other marine mammals, such as pinnipeds and polar bears [16,20]. Studies of the concentration and formation of $MeSO_2$-PCB metabolites in cetaceans have been limited to a few individuals or species [21–25]; however, to our knowledge, the presence of OH-PCBs in any cetacean has not been documented.

The bowhead whale (*Balaena mysticetus*) is a large mysticete found in the arctic waters of the Bering-Chukchi-Beaufort Seas that primarily feeds on pelagic marine invertebrates [26]. The longevity of this species, its lipid-rich diet, and maintenance of lipid deposits results in the accumulation of lipophilic contaminants [27]. The comparison of PCB congener patterns between bowhead whales and prey suggests a structure-dependent capability of this species to eliminate PCBs [28]. The subsistence hunt of this species by northern Alaskan Inuit communities provides a valuable opportunity to study the accumulation and possible biotransformation of PCBs to potentially toxic and/or biologically active metabolites in apparently healthy cetacean specimens.

MATERIALS AND METHODS

Field sampling

Methodology for the collection of bowhead whale samples has been previously described [29,30]. Complete bowhead whale blubber cores ($n = 20$; 10 males and 10 females) and matching plasma samples ($n = 19$; 9 males and 10 females) were obtained from Inuit subsistence hunters in Barrow, Alaska, USA (71°17′N, 156°45′W) through the North Slope Borough Department of Wildlife Management and with approval from the Alaskan Eskimo Whaling Commission in 1997 through 2000. The Department of Wildlife Management personnel recorded the specimen information (i.e., body length, sex, etc.) for each whale landed. Age estimates for the bowhead whales sampled in the present study were not available. However, maturation classification of individual whales was estimated based on body lengths at sexual maturation and known age characteristics (length: female adults, >13.0–13.5 m; male adults, >12.5–13.0 m) [31].

Samples were transported to the National Water Research Institute (Environment Canada, Burlington, ON) with United States (U.S.) export (00US022083/9 and 02US694250/9) and Canadian import (CA02CWIM0052) permits under the Convention on International Trade in Endangered Species and the U.S. Marine Mammal Protection Act (Permit 789-1399; http://www.highnorth.no/Library/Trade/GATT_WTO/us-ma-ma.htm). Blubber samples were homogenized and transferred to precleaned glass containers. Whole blood was collected directly into sterile polypropylene plastic screw-top vials (Wheaton Scientific, Millville, NJ, USA) and centrifuged, and the plasma was subsampled. All blubber and plasma samples were stored at −20°C before extraction.

Extraction and quantification of PCBs and $MeSO_2$-containing metabolites

The techniques employed for the extraction and analysis of PCBs, $MeSO_2$-PCBs, and other OCs in bowhead whale blubber have been described elsewhere [30,32,33]. In brief, bowhead whale blubber samples were homogenized with sodium sulfate and spiked with surrogate recovery standards, CB-30 (2,4,6-trichlorobiphenyl), CB-204 (2,2′,3,4,4′,5,6,6′-octachlorobiphenyl), and 3-$MeSO_2$-2-CH_3-2′,3′,4′,5,5′-pentachlorobiphenyl ($MeSO_2$-ISTD) to monitor analyte recovery. Samples were extracted using *n*-hexane and dichloromethane (DCM), with lipids and other biogenic materials removed via gel permeation chromatography. The lipid content was determined gravimetrically. Each sample was concentrated and eluted through a 33% potassium hydroxide (KOH)/silica gel column (1.5 g) with 50% DCM:50% *n*-hexane by volume (v/v) and then concentrated. Each sample was transferred to a Florisil (8 g, 1.2% H_2O deactivated) column (U.S. Silica, Berkeley Springs, WV, USA) and collected in two fractions. The first fraction (F1) was eluted with 75 ml of DCM:*n*-hexane and contained PCBs and other OCs. A second fraction (F2), containing $MeSO_2$-PCBs, was collected with 80 ml of 7% methanol:93% DCM (v/v). The F2 was passed through a basic alumina column (3.0 g, 2.3% H_2O deactivated) to remove possible coeluting artifacts and concentrated to 100 μl in 2,2,4-trimethylpentane (iso-octane).

The F1 eluant (collected above) was chromatographed on 100% activated silica gel into two fractions. The first, containing parent PCB congeners, was collected using 65 ml of 100% *n*-hexane. The second fraction, containing OC pesticides and by-products, was collected with 95 ml of 50% DCM:50% *n*-hexane (v/v). Each eluant was fortified in iso-octane and concentrated to 100 μl. The 2,3,4,4′,5,6-hexachlorobiphenyl, CB-166, was added as a performance standard.

The sum (Σ) concentrations of parent PCBs (ΣPCB) and other OCs (technical chlordane components and recalcitrant metabolites [ΣCHLOR], DDT-related compounds [ΣDDT], hexachlorocyclohexane isomers [ΣHCH], and chlorinated benzenes [ΣClBz]) in bowhead whale blubber were previously reported [30]. Analysis was performed using a Hewlett-Packard (Wilmington, DE, USA) 5890 gas chromatograph (GC) with a ^{63}Ni-electron capture detector (ECD) [34]. Compound separation was completed using a DB-5 column (60-m length × 0.25-mm internal diameter [i.d.] × 0.25-μm internal film thickness [d.f.]; J&W Scientific, Folsom, CA, USA) with H_2 carrier gas, and quantification was performed using a series of multiple external standards that were analyzed after every 10 samples. A standard reference material (SRM1945 adult female pilot whale [*Globicephala melaena*] blubber homogenate) from the National Institute of Standards and Technology (Gaithersburg, MD, USA) was used to confirm the precision of the analytical method. Analyte concentrations were within 15% of the certified values. The mean recovery (±1 standard error [SE]) of CB-30 and CB-204 internal standards was 76% ± 3.2% and 86% ± 2.1%, respectively, and concentrations were adjusted accordingly.

The quantification of $MeSO_2$-PCB congeners (see Table 1) was performed by GC-ECD using previously established methodology [5,32,33]. The nomenclature of $MeSO_2$-PCBs has been abbreviated and simplified based on the systematic numbering technique applied to PCB congeners [5,35]. Authentic standards of $MeSO_2$-PCBs were used for the quantification of methylsulfone metabolite concentrations, and method detection limits (MDLs) were established as 0.001 ng g^{-1} (wet wt). The mean recovery (±1 SE) of the $MeSO_2$-ISTD was 90% ± 3.9% in all samples, and concentrations were subsequently

corrected. The SRM1945 was extracted and analyzed concurrently to ascertain the reproducibility of $MeSO_2$-PCB data.

Quantification of OH-PCBs and other OCs

Bowhead whale plasma samples (2–9 g) were extracted and analyzed for several PCB congeners (see Table 1), OH-PCB metabolites, pentachlorophenol (PCP), and other OC compounds. The total lipid content of plasma samples was determined colorimetrically [11,36]. Plasma samples were spiked with 4′-OH-CB-159 (2,3,3′,4,5,5′-hexachloro-4′-biphenyl), CB-30, and CB-204 internal standards and extracted using established liquid:liquid extraction techniques [8,9,11]. In brief, plasma proteins were denatured with 2-propanol and HCl and were extracted with 50% methyl *t*-butyl ether:50% *n*-hexane (v/v). Samples were concentrated and partitioned with KOH (0.5 M). The neutral and basic compounds (e.g., parent PCBs and other OCs) were separated, eluted through an acidified silica gel column (22% H_2SO_4, 3 g) to remove lipids, and fractionated using activated silica gel as described above. Analysis of PCBs and other OCs was performed by GC-ECD [30].

The partitioned phenolic compounds were acidified, back-extracted with methyl *t*-butyl ether:*n*-hexane, and derivatized to the methoxylated analogues (MeO-PCBs) using diazomethane before analysis via GC equipped with a mass selective detector in electron capture-negative ionization mode [8,9]. Compound separation was completed using a HP5-MS column (30-m length × 0.25-mm i.d. × 0.25-μm d.f.; Agilent Technologies, Wilmington, DE, USA) with He carrier gas. Quantification of derivatized phenolic compounds was carried out using an authentic standard mixture of 30 halogenated phenolic compounds (HPCs), including methylated OH-PCBs, PCP, and 4-hydroxyheptachlorostyrene. The MDLs for all HPCs analyzed varied from 0.1 to 0.01 ng g^{-1} (wet wt).

To maintain consistency with other investigations, the nomenclature of OH-PCB and MeO-PCBs was based on the chlorine substitution of the biphenyl moiety and the OH or MeO functional groups numbered thereafter [5]. Mean recoveries (±1 SE) of the MeO-PCB and neutral PCB internal standards were 75% ± 8.1% and 85% ± 3.5%, respectively, and concentrations were adjusted accordingly.

Data analysis

Statistical analyses were performed using Systat®, Version 8.0 (SPSS, Chicago, IL, USA). All hypotheses tested were two-tailed, and the maximum probability of a type I error (α) was set at 0.05. Initial analysis showed that $MeSO_2$-PCB concentrations in blubber were significantly correlated with lipid content ($p < 0.05$ for all comparisons, data not shown). As a result, statistical comparisons of PCB and $MeSO_2$-PCB concentrations (Table 1) were performed using lipid-normalized (l.w.) concentrations to reduce the variability of lipid content on analyte concentrations [37].

The pattern of parent PCB congeners and $MeSO_2$-PCB metabolites in the bowhead and pilot whale blubber were examined by determining the ratio of individual congener concentrations relative to CB-153. Because of a lack of vicinal H atoms on both biphenyl moieties, 2,2′,4,4′,5,5′-Cl-substituted hexachlorobiphenyl CB-153 is slowly biotransformed or eliminated in cetaceans compared to other PCB congeners [38]. Each parent PCB congener of $MeSO_2$-PCB metabolites was assigned to a specific structural group (see Fig. 1): PCBs with vicinal *meta-para* H-atoms and two or fewer *ortho* chlorines (group IV), congeners with *meta-para* H atoms with three or more *ortho* chlorines (group V), and congeners with *meta-para* or *ortho-meta* H atoms and one *ortho* Cl atom (group VI) [21,38].

The influence of whale body length, sex, parent PCB concentrations, and all first-order effects on $MeSO_2$-PCB concentrations in bowhead whale blubber were examined by a general linear model (GLM) to test the hypothesis that male and female bowhead whales have different PCB metabolite concentrations and profiles. A second GLM was also used to investigate influence of ΣPCB on $MeSO_2$-PCB concentration. Whale length and all PCB data were $\log_{10}$ transformed to reduce the skewness and kurtosis of the raw data and to meet the assumptions for the GLM. All interaction terms were found to be nonsignificant according to type III sums of squares, and the GLM was reduced to

$$\log_{10}[MeSO_2\text{-PCB} - x] = \mu + \log_{10}(\text{body length}) + \text{sex} + \log_{10}[\Sigma\text{PCB or precursor PCB}] + \epsilon \quad (1)$$

where μ is a constant and ϵ is the random error component. A simplified GLM was also used to investigate the effect of age (i.e., length cohort) and sex on the ratio of $MeSO_2$-PCB congeners to CB-153 concentrations.

The concentrations of OCs, including OH-PCBs and PCP, are reported in Table 2. Summary statistics were computed only when more than 50% of samples contained detectable concentrations of neutral OCs and HPCs. Values below the established MDL were assigned a random number less than one-half the MDL. Concentrations of OH-PCBs, PCP, parent PCBs, and other OCs were not correlated with plasma lipid content ($p > 0.22$ for all comparisons) and are reported on a wet weight basis. The influence of gender and body length on ΣOC group and individual concentrations in plasma was investigated using a GLM. Plasma OC concentrations and relative proportions were compared to previously published values in bowhead blubber and liver using Model-I, one-way analysis of variance to test the hypothesis that relative proportions of OCs would be equal among these compartments. All a posteriori comparisons among OC groups were explored using Scheffé's test.

RESULTS AND DISCUSSION

$MeSO_2$-PCB metabolites in blubber

The bowhead whale is a baleen whale that primarily feeds on marine macroinvertebrates, such as zooplankton [26]. As a result, this species is exposed to, and subsequently accumulates, lower concentrations of persistent OCs than other cetaceans. The mean (±1 SE) ΣPCB concentrations in the bowhead whale blubber (451 ± 70 ng g^{-1} l.w.) (Table 1) were lower than those found in balaenopterid cetaceans, such as the North Pacific and North Atlantic minke whales (*Balaenoptera acutorostrata*) [39,40]. The ΣPCB in beluga whale blubber (*Delphinapterus leucas*) from the Alaskan Arctic was significantly greater (mean, 3,305 ng g^{-1} l.w.) than in bowhead whales, likely because of the relatively higher trophic status of this odontocete and increased dietary exposure via biomagnification within the marine food web [41]. Reported mean concentrations of ΣPCB in the Northwest Atlantic right whale were approximately 12 times greater (mean, 5,700 ng g^{-1} l.w.) in comparison to concentrations in bowhead whale blubber

Table 1. Mean, lipid-normalized (l.w.) concentrations (±1 standard error) of methylsulfone ($MeSO_2$)-containing polychlorinated biphenyls (PCBs) and ratio to parent congener in bowhead whale and pilot whale (Standard Reference Material [SRM] 1945) blubber[a]

		Bowhead whale (n = 20)		Pilot whale (n = 1)[a]	
Congener	Cl substitution[b]	ng g^{-1} l.w.	$MeSO_2$-x/PCB-x	ng g^{-1} l.w.	$MeSO_2$-x/PCB-x
3′-$MeSO_2$-CB-49	2,2′,4,5′	0.12 ± 0.04	0.011	0.46 ± 0.04	0.024
4′-$MeSO_2$-CB-49		0.46 ± 0.14	0.044	0.12 ± 0.01	0.006
3-$MeSO_2$-CB-52	2,2′,5,5′	0.37 ± 0.06	0.043	0.12 ± 0.01	0.002
4-$MeSO_2$-CB-52		0.59 ± 0.07	0.069	0.17 ± 0.02	0.003
3-$MeSO_2$-CB-64	2,4′,5,6	NQ[c]	—	0.25 ± 0.02	0.318
4-$MeSO_2$-CB-64		0.59 ± 0.07	0.223	0.56 ± 0.04	0.662
3-$MeSO_2$-CB-70	2,3′,4′,5	0.46 ± 0.06	0.134	0.58 ± 0.02	0.053
4-$MeSO_2$-CB-70		0.96 ± 0.15	0.282	0.84 ± 0.06	0.077
4′-$MeSO_2$-CB-87	2,2′,3,4,5′	0.30 ± 0.08	0.061	0.31 ± 0.03	0.012
3′-$MeSO_2$-CB-101	2,2′,4,5,5′	0.40 ± 0.06	0.027	0.33 ± 0.02	0.004
4′-$MeSO_2$-CB-101		0.19 ± 0.03	0.013	0.55 ± 0.02	0.007
3-$MeSO_2$-CB-110/ 3′-$MeSO_2$-CB-87[d]	2,3′,4′,5,6/ 2,2′,3,4,5′	0.15 ± 0.04	0.007	0.17 ± 0.02	0.003
4-$MeSO_2$-CB-110		0.29 ± 0.03	0.042	0.35 ± 0.03	0.009
3′-$MeSO_2$-CB-132	2,2′,3,4,5′,6′	0.68 ± 0.17	0.167	0.44 ± 0.06	0.040
4′-$MeSO_2$-CB-132		0.51 ± 0.14	0.126	0.21 ± 0.02	0.019
3′-$MeSO_2$-CB-141	2,2′,3,4,5,5′	NQ	—	0.13 ± 0.06	0.009
4′-$MeSO_2$-CB-141		NQ	—	0.13 ± 0.02	0.009
3-$MeSO_2$-CB-149	2,2′,4′,5,5′,6	0.45 ± 0.17	0.028	0.32 ± 0.01	0.004
4-$MeSO_2$-CB-149		NQ	—	0.33 ± 0.01	0.004
4′-$MeSO_2$-CB-174	2,2′,3,4,5,5′,6′	0.12 ± 0.03	0.022	0.15 ± 0.02	0.006
Σ$MeSO_2$-PCBs		6.23 ± 0.81	0.076	6.47 ± 0.22	0.018
ΣParent PCBs		81.4 ± 13.1		367 ± 16	
ΣPCB[e]		451 ± 70		1,947 ± 73[f]	

[a] SRM1945 values represent the mean concentration of three pseudoreplicates.
[b] Based on systematic number of PCB congeners.
[c] NQ = not quantified (signal:noise ratio, <5).
[d] Two coeluting compounds.
[e] ΣPCB = sum of polychlorinated biphenyls (including parent PCBs to $MeSO_2$-PCB metabolites): PCBs = 4/10, 7/9, 6, 8/5, 19, 12/13, 18, 15/17, 24/27, 16, 32, 54/29, 26, 25, 50, 28, 31, 33/21/53, 51, 22, 45, 46, 52, 49, 43, 47/48, 44, 59, 42, 64, 41/71, 40, 100, 63, 74, 76/98, 70, 95/66, 91, 55, 56/60, 92/84, 101, 99, 119, 83, 97, 87, 81, 85, 136, 110, 82, 151, 135, 144, 107/147, 149/133, 118, 114, 143, 141, 145, 153, 132, 105, 141/179, 137, 176/130, 163, 138, 158, 129/178, 175, 187, 182, 183, 128, 167, 185, 174, 177, 171, 156, 202/173, 172, 197, 180, 193, 191, 199, 170/190, 198, 201, 196/203, 189, 206, 195, 207, 194, 205, 208, and 209.
[f] Certified value for ΣPCB (based on 27 congeners) in SRM1945 is approximately 1730 ng g^{-1} (l.w.).

[17]. Differences in OC concentrations among these cetaceans likely result from the proximity of habitat to local sources of contamination as well as prey selection.

The biotransformation of PCB congeners in groups IV to VI, including metabolism of parent congeners of $MeSO_2$-PCB metabolites (Fig. 1), by marine mammals is likely mediated via CYP enzymes with CYP2B-like activity [21,38]. Attempts have been made to immunologically characterize and measure activities using catalytic bioassays of hepatic CYP isozymes in cetaceans [15,16,19]; however, these properties have not been characterized in the bowhead whale. Nonetheless, biotransformation of PCBs, including parent congeners to $MeSO_2$-metabolites, has been inferred from congener profiles relative to PCB concentrations in prey, which are low relative to those of fish-eating birds and other marine mammals [28,38,42]. Whereas CYP2B-like gene expression and subsequent isozyme activity in bowhead whales appear to be limited, as-yet-uncharacterized CYP isoenzyme(s) with sufficient catalytic activity may be present and capable of mediating the biotransformation of PCB congeners according to various structural classifications [14,15,38,43].

The mean (±1 SE) concentration of Σ$MeSO_2$-PCBs in bowhead whale blubber (6.23 ± 0.81 ng g^{-1} l.w.) (Table 1) was lower than previous measurements in other cetaceans from different marine environments. Total $MeSO_2$-PCBs in a single blue whale (*Balaenoptera musculus*) from Japan was 10 ng g^{-1} (l.w.), although individual congeners were not quantified [23]. The sum concentrations of six $MeSO_2$-PCB congeners in single blubber samples from a Risso's dolphin (*Grampus griseus*), common dolphin (*Delphinus delphis*), pilot whale, and Atlantic white-sided dolphin (*Lagenorhynchus acutus*) from the Irish Sea ranged between 30 and 580 ng g^{-1} (l.w.) [22]. The mean concentration of Σ$MeSO_2$-PCB in male beluga whales from the western Hudson Bay (n = 7) and the St. Lawrence River estuary (Canada) (n = 30) were 159 and 230 ng g^{-1} (l.w.), respectively [21]. These interspecies differences in $MeSO_2$-PCB concentrations among cetaceans likely result from different dietary exposures to parent PCBs and/or the capacity for CYP-mediated enzymatic biotransformation of parent PCBs and $MeSO_2$-PCBs. However, interspecies comparisons must be treated with caution because of the lack of replicates for several species and differences in analytical techniques.

The formation of $MeSO_2$-PCB has been reported in deep-water sculpin (*Myoxocephalus thompsoni*) [44]. We hypothesize that zooplankton and other fish species likely have limited CYP2B-like activity (e.g., [45]) to biotransform PCBs to persistent metabolites, such as $MeSO_2$-PCBs, but the possible contribution of these compounds from dietary sources compared to the proportion derived from cetacean-based CYP-mediated biotransformation cannot be adequately assessed by the present study.

The Σ$MeSO_2$-PCB concentrations in the blubber of a stranded adult female pilot whale from the eastern U.S. sea-

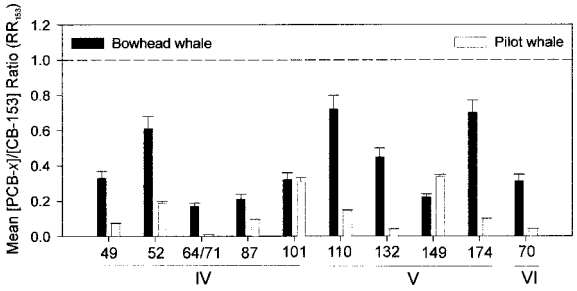

Fig. 1. Mean (+1 standard error) ratio of individual *meta-para* Cl-unsubstituted polychlorinated biphenyls (PCBs) to PCB-153 concentrations (RR_{153}) in blubber of bowhead whales (n = 20) and a pilot whale (Standard Reference Material 1945, n = 1; three pseudoreplicates). The PCB congeners arranged by structure-metabolism classification: group IV, one or no *ortho* chlorine; group V, three or more *ortho* chlorines; and group VI, same as group IV plus *ortho-meta* vicinal hydrogen atoms. Dashed line represents unity (PCB-153 = PCB-x = 1).

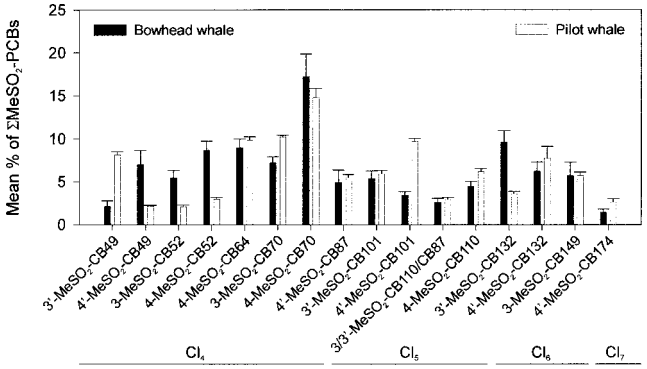

Fig. 2. Mean (+1 standard error) percentage of methylsulfone ($MeSO_2$)-containing polychlorinated biphenyl (PCB) congener concentration to sum (Σ) $MeSO_2$-PCB concentration in blubber of bowhead whale (n = 20; black bars) and a pilot whale (n = 1; three pseudoreplicates; white bars).

board (SRM1945) quantified in the present study (6.47 ng g^{-1} l.w.) was lower than previously reported concentrations in this species (210 ng g^{-1} l.w.) from the Irish Sea [22]. Whereas the lack of replicates invalidates any statistical comparison to the bowhead whale or other cetaceans, these data provide, to our knowledge, the first report of $MeSO_2$-PCB concentrations in a commercially available reference material that can be employed for future quality-control/assurance programs.

The mean ratio of $MeSO_2$-PCBs to parent PCB concentrations in bowhead whales (0.10 ± 0.02) was similar to that in other cetaceans and marine mammals. The mean ratio of $MeSO_2$-PCBs to the sum concentration of precursor PCB congeners in ringed seal (*Phoca hispida*) blubber from the Canadian Arctic was approximately 0.034 [33], whereas the congener-specific ratios in beluga whales from western Hudson Bay ranged between 0.05 and 0.14 [21]. Despite exposure to high concentrations of CYP enzyme-inducing PCB and OCs relative to prey and the presence of immunologically and catalytically determined rat-like CYP2B1 activity, the mean $\Sigma MeSO_2$-PCB to ΣPCB ratio in polar bear (*Ursus maritimus*) adipose tissue was approximately 0.06 [33]. Pinnipeds, odontocetes, and polar bears are regarded as possessing greater CYP2B biotransformation capacity compared to mysticetes [4,19,38,46]. However, the similar ratios suggest that common CYP2B-like metabolic capacities or related mechanisms that influence the formation and clearance of $MeSO_2$-PCBs exist among these species.

The mean, congener-specific $MeSO_2$-PCB concentrations quantified in bowhead whale blubber were less than 1 ng g^{-1} (l.w.) for all compounds. The most abundant congeners, ranked in order from highest to lowest, were 4-$MeSO_2$-CB-70, 3′-$MeSO_2$-CB-132, 4-$MeSO_2$-CB-52, 4-$MeSO_2$-CB-64, and 4′-$MeSO_2$-CB-132 and comprised approximately 50% of the $\Sigma MeSO_2$-PCBs (Fig. 2 and Table 1). The concentrations of $\Sigma MeSO_2$-PCBs and individual $MeSO_2$-PCB congeners in bowhead whale blubber were not significantly influenced by the concentration of parent PCB congeners (GLM, $p > 0.10$ for all comparisons). However, the 3- and 4-$MeSO_2$-CB-49, -52, -70, -101, -132, -149, and -174 metabolites and $\Sigma MeSO_2$-PCB concentrations were correlated with increasing ΣPCB concentrations (Fig. 3) in both male and female bowhead whales (GLM, $p < 0.03$ for all comparisons). These data suggest that $MeSO_2$-PCB formation increases with PCB exposure or that these metabolites are recalcitrant to further metabolism or excretion and, thus, will accumulate with time in the bowhead whale.

Interactions between congener-specific and $\Sigma MeSO_2$-PCB concentrations and whale gender and body length were not observed (GLM, $p > 0.15$). It was previously observed that PCB concentrations were significantly lower in mature female bowhead whale (i.e., females >13 m in length) compared to male bowheads from the same length cohort [30], likely because of the movement of lipophilic contaminants to offspring during lactation and gestation [27]. However, the relatively small sample size for each length cohort limited the statistical power for comparison of $MeSO_2$-PCB concentrations by gender.

It should be noted that the predominant $MeSO_2$-PCBs in bowhead whale blubber were dissimilar to those found in most other cetaceans (Fig. 2). In general, 3′- and 4′-$MeSO_2$-CB-101 and 3′-$MeSO_2$-CB-87 were the most abundant congeners found in common dolphin, Risso's dolphin, white-sided dolphin, pilot whale, and harbor porpoise from the Irish Sea [22]. In male beluga whales from the western Hudson Bay, 3-$MeSO_2$-CB-52, 3′-$MeSO_2$-CB-49, and 4-$MeSO_2$-CB-70 were the most abundant congeners, whereas 3′- and 4′-$MeSO_2$-CB-49, -87, and -101 comprised approximately 85% of the $\Sigma MeSO_2$-PCBs quantified in male belugas from the St. Lawrence River estuary [21]. Variability of $MeSO_2$-PCB profile may be caused by differences in PCB exposure and subsequent induction of CYP enzymes that are appropriate catalysts for parent PCB metabolism, age, physiological status, and other factors influencing the PCB metabolism and retention of recalcitrant metabolites.

The relative proportion of $MeSO_2$-PCB congeners in bow-

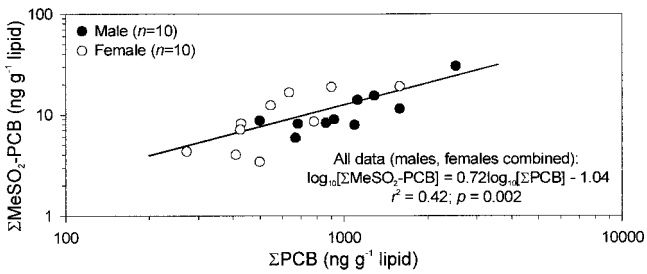

Fig. 3. The Model-1, first-order linear regression between $\log_{10}$-transformed sum (Σ) polychlorinated biphenyl (PCB) and Σmethylsulfone ($MeSO_2$)-PCB concentrations (ng g^{-1} lipid) in blubber from male (n = 10) and female (n = 10) bowhead whales.

head whale blubber (Fig. 2) is comparable to that in beluga whales from the western Hudson Bay, but the ratios of 3-$MeSO_2$- and 4-$MeSO_2$-CB-52, -64, and -70 metabolites were reduced (or nondetectable) in beluga whale blubber from the St. Lawrence River estuary stock. Letcher et al. [21] hypothesized that secondary biotransformation of $MeSO_2$-PCBs, particularly those with vicinal H atoms in the *ortho-meta* or *meta-para* positions on the biphenyl moiety, in this beluga population is mediated via the increased induction of CYP1A and/or CYP2B-like pathways relative to the less contaminated western Hudson Bay stock [21]. Overall, the data imply that CYP-related isozyme activity necessary for secondary $MeSO_2$-PCB biotransformation in the bowhead whale is low or negligible and is not induced at current exposure concentrations.

The greater abundance of 3- versus 4-$MeSO_2$-PCBs in other cetaceans [5] was not observed in bowhead whale blubber. The percentage of 4-substituted $MeSO_2$-PCBs to $\Sigma MeSO_2$-PCB metabolites in odontocetes ranged from 25 to 38% [21,22,24], compared to approximately 60% in bowhead whale blubber. As well, the percentage of 4-$MeSO_2$-CB-52 to the sum of 3- and 4-$MeSO_2$-CB-52 concentrations in rat liver microsomes was 88%, compared to 38% in mice [47]. The relative abundance of 3- and 4-$MeSO_2$-PCB congener pairs could depend on selective formation, accumulation, and/or secondary biotransformation, clearance, and tissue- and protein-specific association of the $MeSO_2$-PCB metabolites. In humans, for instance, a greater proportion of 3-$MeSO_2$-PCBs (90% of $\Sigma MeSO_2$-PCBs) was found in liver, whereas 4-substituted $MeSO_2$-PCB metabolites (≈60%) were selectively accumulated in adipose tissue [48].

Neutral and OH-PCBs and phenolic OCs

The rank order of ΣOC in bowhead whale plasma was ΣPCB > ΣCHLOR > ΣHCH ≥ ΣClBz > ΣDDT (Table 2). In general, the relative abundance of ΣOC in bowhead plasma reflects a combination of the overall proportions observed in liver and blubber (Fig. 4). No effects of gender and body length on $\log_{10}$-transformed, wet weight OC concentrations in plasma were observed ($p > 0.28$ for all comparisons). The ΣPCB, ΣDDT, and ΣCHLOR concentrations in blubber generally increased with age in male and subadult female whales (as interpreted by body length) [30]. This interaction likely reflects the time-integrated accumulation of recalcitrant lipophilic contaminants in blubber, whereas the relative abundance of OCs in plasma represents the culmination of recent dietary exposure, biotransformation, and possible remobilization into the circulatory system.

In addition, differences in lipid content and composition may influence OC concentrations and patterns. Lipids in cetacean blubber are predominantly triglycerides, whereas blood contains relatively more polar lipids (e.g., phospholipids), which may result in distinct partitioning of OCs [49]. As well, recent feeding or reproductive activity dramatically influences lipid dynamics in whole blood, including plasma [27], thereby affecting the relative distribution of OCs compared to other tissues.

The collection of blood samples from marine mammals for OC analysis provides valuable information for health assessment of species for which tissue samples are difficult to obtain [50]. However, our study only quantified OCs in plasma from landed whales and did not address the possible contaminant burden in other hematologic compartments that may serve as important reservoirs for OCs in blood. For example, Boon et al. [51] found that 66% of ΣPCB in whole blood was associated with erythrocytes, either by sorption to the cellular membrane or by hemoglobin. As a result, a significant proportion of OCs present in the circulatory system may have been omitted from the analysis of bowhead whale plasma alone, and a more detailed research design is required to better describe circulating PCBs and metabolites in this cetacean. The evaluation of OCs in bowhead plasma is further complicated by the fact that contaminant concentrations in cetaceans are typically reported

Table 2. Mean (±1 standard error [SE]) concentrations and range (ng g^{-1} wet wt) of major phenolic and neutral persistent organochlorine analytes (OCs) and sum (Σ) of group concentrations in bowhead whale plasma samples (n = 19)

Analyte	Mean (±1 SE)	Data range	No. of samples > MDL[a]
Density (g ml^{-1})	0.91 ± 0.04	0.62–1.11	—
% Lipid	1.67 ± 0.12	1.20–3.50	—
Phenolic compounds			
Pentachlorophenol	1.55 ± 0.19	0.16–3.48	19
4′-OH-CB-130	0.94 ± 0.27	0.32–3.29	10
4-OH-CB-187	0.39 ± 0.15	0.31–2.39	4
ΣOH-PCBs	1.52 ± 0.31	0.53–3.80	10
ΣOH-OCs	3.16 ± 0.53	0.47–9.66	19
Neutral compounds			
Hexachlorobenzene	0.14 ± 0.02	0.27–2.04	17
ΣClBz[b]	1.35 ± 0.44	0.33–7.20	19
Oxychlordane	0.60 ± 0.09	0.13–1.51	17
ΣCHLOR[c]	1.72 ± 0.22	0.52–3.70	19
p,p′-DDE	0.36 ± 0.04	0.11–1.89	18
ΣDDT[d]	1.16 ± 0.13	0.14–2.36	19
β-HCH	0.51 ± 0.09	0.26–1.75	16
ΣHCH[e]	1.38 ± 0.10	0.47–1.78	18
CB-153	0.46 ± 0.11	0.10–0.82	15
ΣPCB[f]	2.78 ± 0.54	1.09–9.37	19

[a] MDL = method detection limit.
[b] ΣClBz = sum of chlorinated benzenes (ClBz); 1,2-diClBz, 1,2,3-triClBz, 1,2,4-triClBz, 1,3,5-triClBz, pentaClBz, and hexachlorobenzene.
[c] ΣCHLOR = sum of chlordane components and related metabolites (*cis*-chlordane, *trans*-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor, and heptachlor *exo*-epoxide).
[d] ΣDDT = dichlorodiphenyltrichloroethane-related compounds (*o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, and *p,p′*-DDT).
[e] ΣHCH = sum of hexachlorocyclohexane isomers; α-HCH, β-HCH, and γ-HCH.
[f] ΣPCP = sum of 102 congeners and coelutions (see Table 1 for details).

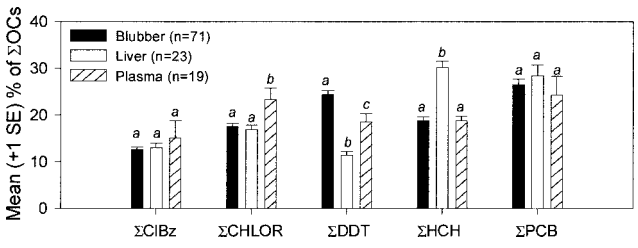

Fig. 4. The mean (+1 standard error [SE]) proportion (%) of sum (Σ) group concentrations of the total organochlorine contaminants (OCs) quantified in bowhead whale blubber, liver ([30]), and plasma (present study; Table 2). Columns with the same lettering (a, b, c, or d) were not statistically different from each other (analysis of variance, Scheffé's test, α = 0.05). CHLOR = chlordane; ClBz = chlorinated benzene; HCH = hexachlorocyclohexane; PCB = polychlorinated biphenyl.

in other compartments, such as complete blubber cores or biopsy specimens, whole blood (or erythrocytes), liver, and muscle (e.g., [17,27,30,53,54]).

Few HPCs were present in bowhead whale plasma at concentrations above the established MDL (Table 2). The most abundant HPC was PCP, which was quantified in all plasma samples extracted. Plasma concentrations of PCP in the bowhead whale were greater than mean concentrations in polar bear and ringed seal plasma (0.210 ± 0.097 and 0.237 ± 0.061 ng g^{-1} wet wt, respectively) from the western Canadian Arctic [9]. Pentachlorophenol is a fungicide/insecticide used as a wood preservative, but it is also a possible metabolite of hexachlorobenzene [55], an abundant environmental contaminant in the Arctic [1]. It is assumed that the PCP concentrations in arctic biota result from the biotransformation of hexachlorobenzene, because PCP does not readily bioaccumulate [56] and has limited potential for long-range transport due to rapid photolysis and reaction with photochemically produced -OH radicals [57]. The relatively high proportion of PCP in bowhead whale plasma may also be the direct result of the biotransformation of pentachloroanisole, an abundant OC contaminant found in arctic air [1]. The metabolism of pentachloroanisole to PCP has been found in other mammalian systems [58,59], but this pathway has not been quantified in any cetacean.

The concentrations of ΣOH-PCBs in bowhead plasma were mainly composed of 4′-OH-2,2′,3,3′,4,5′-hexachlorobiphenyl (4′-OH-CB-130), which was measurable in approximately 50% of the samples analyzed. The only other OH-PCB quantified at concentrations above the MDL was 4-OH-2,2′,3,4′,5,5′,6-heptachlorobiphenyl (4-OH-CB-187). However, this compound was only observed in a very limited number of samples (4 of 19). No other OH-PCB congener was detectable above the MDL established in the present study.

Published data regarding OH-PCBs in wildlife are limited. Concentrations in the bowhead whale plasma were relatively low (Table 2) compared to mean (±1 SE) plasma concentrations in other wildlife [5], including polar bears from the Canadian Arctic (92.6 ± 21.4 ng g^{-1} wet wt) [9]. The ΣOH-PCB concentrations in ringed seal plasma from the Canadian Arctic (0.081 ± 0.019 ng g^{-1} wet wt) were lower than those in the bowhead whale [9] and were mainly comprised of 4-OH-CB-187 (~23% of ΣOH-PCBs; three of five samples analyzed) and two coeluting OH-PCBs, 4-OH-CB-107/4′-OH-CB-108 (~9% of ΣOH-PCBs) (R.J. Norstrom and C.D. Sandau, Environment Canada, Ottawa, ON, personal communication).

The presence of $MeSO_2$-PCBs provides indirect evidence for the formation of arene oxide intermediates of PCB congeners, even for highly chlorinated congeners, via Phase I activity, which would suggest that OH-PCBs should be produced in the bowhead whale. However, the lack of significant OH-PCB concentrations in plasma may have been the result of various factors. For example, previous experiments have demonstrated that OH-PCBs have high binding affinities to transthyretin but not toward thyroxin-binding globulin or other thyroid hormone transport proteins [60]. Because the macromolecular content of mysticete blood is not well characterized, possible binding with other proteins, cellular components, or other macromolecules may influence the concentrations of OH-PCBs and other OCs in the circulatory system. The Phase II biotransformation of OH-PCBs via conjugation reactions with glucuronic acid or sulfate increases the water solubility of these metabolites and facilitates biliary excretion and/or enterohepatic recycling [61,62]. However, these pathways for contaminant detoxification in cetaceans have not been assessed. In addition, changes in intravascular blood composition [29] and drug redistribution occur postmortem [63] and may affect the distribution of OH-PCBs in vivo.

Information pertaining to the formation and relative abundance of $MeSO_2$- and OH-substituted PCB metabolites is necessary for understanding the toxicodynamics of PCBs in cetaceans and provides valuable data for the health assessment of this species. Contaminant profiles suggest that CYP activity in the bowhead whale is low. However, PCB metabolite data suggest that sufficient CYP2B-like isozyme activity exists in the bowhead whale for the generation of arene epoxide intermediates and subsequent $MeSO_2$-PCB formation. The presence of $MeSO_2$-PCBs suggests that bowhead whales should have the capacity to form OH-PCB, but the lack of significant concentrations of the latter type of PCB metabolite implies that other factors may influence the accumulation of HPCs in this cetacean. Further research is warranted to adequately address the impact of these (and potentially other) variables on the distribution of OH-PCBs in cetaceans. Additional work is also needed to properly investigate the circulating loads of OCs in cetacean blood, because unlike blubber, such circulating contaminants are readily available to target organs or sites and, thus, are potentially more toxicologically relevant.

Acknowledgement—The Co-operative Institute for Arctic Research (University of Alaska Fairbanks [UAF], Fairbanks, AK, USA) provided financial support for this project. Partial funding support for the present study was from the Natural Science and Engineering Research Council of Canada and the Canada Research Chairs Program (to R.J. Letcher). The authors are very grateful for the provision of samples by the subsistence Inuit hunters. Benny Akootckok, C.D.N. Brower, H. Brower, Jr., L.A. Dehn, J.C. George, C. Rosa, R. Suydam, V. Woshner, G. Sheffield, and many others assisted with sample collection. Technical support was provided by G. Sandala, E. Bennett (University of Windsor, ON, Canada), and C. Darling (University of Guelph, ON, Canada). The help of L. Dehn and C. Rosa (UAF) and of M. Gosho (National Oceanic and Atmospheric Administration, Seattle, WA, USA) in acquiring the necessary permits and sample transport was indispensable. This project would not have been possible without the support of the Department of Wildlife Management, including T. Albert, D. Norton, L. Dela Rosa, A. Brower, D. Vinas, and C. Willetto.

REFERENCES

1. De Wit CA, Fisk AT, Hobbs KE, Muir DCG, Kallenborn R, Krahn MM, Norstrom RJ, Skaare J. 2003. Persistent organochlorine pollutants. In de Wit CA, Fisk AT, Hobbs KE, Muir DCG, eds, *Arctic Monitoring and Assessment Program (AMAP) II*. Arctic Monitoring and Assessment Program, Oslo, Norway (in press).
2. O'Hara TM, O'Shea TJ. 2001. Toxicology. In Dierauf LA, Gulland FMD, eds, *CRC Handbook of Marine Mammal Medicine*. CRC, Boca Raton, FL, USA, pp 471–520.
3. Boon JP, van Arnhem E, Hansen S, Kannan N, Petrick G, Schulz D, Duinker JC, Reijnders PJH, Goksøyr A. 1992. The toxicokinetics of PCBs in marine mammals with special reference to possible interactions of individual congeners with the cytochrome P450-dependent monooxygenase system: An overview. In Walker CH, Livingstone DR, eds, *Persistent Pollutants in Marine Ecosystems*. Pergamon, Oxford, UK, pp 119–159.
4. Letcher RJ, Norstrom RJ, Lin S, Ramsay MA, Bandiera SM. 1996. Immunoquantification and microsomal monooxygenase activities of hepatic cytochromes P4501A and P4502B and chlorinated hydrocarbon contaminant levels in polar bear (*Ursus maritimus*). *Toxicol Appl Pharmacol* 137:127–140.
5. Letcher RJ, Klasson-Wehler E, Bergman Å. 2000. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. In Paasivirta J, ed, *New Types of Persistent Halogenated Compounds*. Springer-Verlag, New York, NY, USA, pp 315–360.

6. Lewis DFV. 1998. The CYP2 family: Models, mutants and interactions. *Xenobiotica* 28:617–661.
7. Letcher RJ, Lemmen JG, van der Burg B, Brouwer A, Bergman Å, Giesy JP, van den Berg M. 2002. In vitro antiestrogenic effects of aryl methyl sulfone metabolites of polychlorinated biphenyls and 2,2-bis(4-chlorophenyl)-1,1-dichloroethene on 17-estradiol-induced gene expression in several bioassay systems. *Toxicol Sci* 69:362–372.
8. Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. 2000. Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian Inuit. *Environ Health Perspect* 108:611–616.
9. Sandau CD, Meerts IATM, Letcher RJ, McAlees AJ, Chittim B, Brouwer A, Norstrom RJ. 2000. Identification of 4-hydroxyheptachlorostyrene in polar bear plasma and its binding affinity to transthyretin: A metabolite of octachlorostyrene? *Environ Sci Technol* 34:3871–3877.
10. Bergman Å, Klasson-Wehler E, Kuroki H. 1994. Selective retention of hydroxylated PCB metabolites in blood. *Environ Health Perspect* 102:464–469.
11. Li H, Drouillard KD, Bennett E, Haffner GD, Letcher RJ. 2003. Plasma-associated halogenated phenolic contaminants in benthic and pelagic fish species from the Detroit River. *Environ Sci Technol* 37:832–839.
12. Jerina DM, Daly JW. 1974. Arene oxides: A new aspect of drug metabolism. *Science* 185:573–582.
13. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman Å, Visser TJ. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. *Toxicol Ind Health* 14:59–84.
14. Boon JP, Oostingh I, van der Meer J, Hillebrand TJ. 1994. A model for the bioaccumulation of chlorobiphenyl congeners in marine mammals. *Eur J Pharmacol* 270:237–251.
15. Boon JP, Lewis WE, Goksøyr A. 2001. Immunochemical and catalytical characterization of hepatic microsomal cytochrome P450 in the sperm whale (*Physeter macrocephalus*). *Aquat Toxicol* 52:297–309.
16. Watanabe S, Shimada T, Nakamura S, Nishiyama N, Yamashita N, Tanabe S, Tatsukawa R. 1989. Specific profile of liver microsomal cytochrome P450 in dolphin and whales. *Mar Environ Res* 27:51–65.
17. Weisbrod AV, Shea D, Moore MJ, Stegeman JJ. 2000. Organochlorine exposure and bioaccumulation in the endangered Northwest Atlantic right whale (*Eubalaena glacialis*) population. *Environ Toxicol Chem* 19:654–666.
18. White RD, Hahn ME, Lockhart WL, Stegeman JJ. 1994. Catalytic and immunochemical characterization of hepatic microsomal cytochromes P450 in beluga whale (*Delphinapterus leucas*). *Toxicol Appl Pharmacol* 126:45–57.
19. White RD, Shea D, Schlezinger JJ, Hahn ME, Stegeman JJ. 2000. In vitro metabolism of polychlorinated biphenyl congeners by beluga whale (*Delphinapterus leucas*) and pilot whale (*Globicephala melas*) and relationship to cytochrome P450 expression. *Comp Biochem Physiol C Comp Pharmacol Toxicol* 126:267–284.
20. Goksøyr A. 1995. Cytochrome P450 in marine mammals: Isozyme forms, catalytic functions, and physiological regulations. In Blix AS, Walloe L, Ultang O, eds, *Whales, Seals, Fish and Man.* Elsevier Science, Amsterdam, The Netherlands, pp 629–639.
21. Letcher RJ, Norstrom RJ, Muir DCG, Sandau CD, Koczanski K, Michaud R, De Guise S, Béland P. 2000. Methylsulfone polychlorinated biphenyl and 2,2-bis(chlorophenyl)-1,1-dichloroethylene metabolites in beluga whale (*Delphinapterus leucas*) from the St. Lawrence River estuary and western Hudson Bay, Canada. *Environ Toxicol Chem* 19:1378–1388.
22. Troisi GM, Haraguchi K, Simmonds MP, Mason CF. 1998. Methyl sulfone metabolites of polychlorinated biphenyls (PCBs) in cetaceans from the Irish and the Aegean Seas. *Arch Environ Contam Toxicol* 35:121–128.
23. Haraguchi K, Kuroki H, Masuda Y. 1989. Occurrence and distribution of chlorinated aromatic methylsulfones and sulfoxides in biological samples. *Chemosphere* 19:487–492.
24. Bergman Å, Norstrom RJ, Haraguchi K, Kuroki H, Béland P. 1994. PCB and DDE methyl sulfones in mammals from Canada and Sweden. *Environ Toxicol Chem* 13:121–128.
25. Karlson K, Ishaq R, Becker G, Bergren P, Broman D, Colmsjö A. 2000. PCBs, DDTs and methyl sulfone metabolites in various tissues of harbor porpoises from Swedish waters. *Environ Pollut* 110:29–46.
26. Lowry LF. 1993. Foods and feeding ecology. In Burns JJ, Montague JJ, Cowles CJ, eds, *The Bowhead Whale.* Special Publication 2. Society for Marine Mammalogy, Lawrence, KS, USA, pp 201–238.
27. O'Shea TJ. 1999. Environmental contaminants and marine mammals. In Reynolds JE, Rommel SA, eds, *Biology of Marine Mammals.* Smithsonian Institution Press, Washington, DC, USA, pp 485–536.
28. Hoekstra PF, Wong CS, O'Hara TM, Solomon KR, Mabury SA, Muir DCG. 2002. Enantiomer-specific accumulation of PCB atropisomers in the bowhead whale (*Balaena mysticetus*). *Environ Sci Technol* 36:1419–1425.
29. Heidel JR, Philo LM, Albert TF, Andreasen CB, Stang BV. 1996. Serum chemistry of bowhead whales (*Balaena mysticetus*). *J Wildl Dis* 32:75–79.
30. Hoekstra PF, O'Hara TM, Pallant SJ, Solomon KR, Muir DCG. 2002. Bioaccumulation of organochlorine contaminants in bowhead whales (*Balaena mysticetus*) from Barrow, Alaska. *Arch Environ Contam Toxicol* 42:497–507.
31. O'Hara TM, George JC, Tarpley RJ, Burek K, Suydam RS. 2002. Sexual maturation in male bowhead whales (*Balaena mysticetus*) of the Bering-Chukchi-Beaufort Seas stock. *Journal of Cetacean Research and Management* 4:143–148.
32. Letcher RJ, Norstrom RJ. 1995. An integrated analytical method for determination of polychlorinated aryl methyl sulfone metabolites and polychlorinated hydrocarbon contaminants in biological matrices. *Anal Chem* 67:4155–4163.
33. Letcher RJ, Norstrom RJ, Muir DCG. 1998. Biotransformation versus bioaccumulation: Sources of methyl sulfone PCB and 4,4′-DDE metabolites in the polar bear food chain. *Environ Sci Technol* 32:1656–1661.
34. Hoekstra PF, O'Hara TM, Teixeira C, Backus S, Fisk AT, Muir DCG. 2002. Spatial trends and bioaccumulation of organochlorine pollutants in marine zooplankton from the Alaskan and western Canadian Arctic. *Environ Sci Technol* 21:575–583.
35. Guitart R, Puig P, Gomez-Catalan J. 1993. Requirement for a standardized nomenclature criterium for PCBs: Computer-assisted assignment of correct congener denomination and numbering. *Chemosphere* 27:1451–1459.
36. Frings CS, Fendley TW, Dunn RT, Queen CA. 1972. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. *Clin Chem* 18:673–674.
37. Hebert CE, Keenleyside KA. 1995. To normalize or not to normalize? Fat is the question. *Environ Sci Technol* 14:801–807.
38. Boon JP, van der Meer J, Allchin CR, Law RJ, Klungoyr J, Leonards PEG, Spliid H, Storr-Hansen E, Mckenzie C, Wells DE. 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: Structural evidence for induction of cytochrome P450. *Arch Environ Contam Toxicol* 33:298–311.
39. Aono S, Tanabe S, Fujise Y, Kato H, Tatsukawa R. 1997. Persistent organochlorines in minke whale (*Balaenoptera acutorostrata*) and their prey species from the Antarctic and the North Pacific. *Environ Pollut* 98:81–89.
40. Hobbs KE, Muir DCG, Born EW, Dietz R, Haug T, Metcalfe TL, Metcalfe C, Øien N. 2003. Levels and patterns of persistent organochlorines in minke whale (*Balaenoptera acutorostrata*) stocks from the North Atlantic and European Arctic. *Environ Pollut* 121:239–252.
41. Hoekstra PF, Dehn LA, George JC, Solomon KR, Muir DCG, O'Hara TM. 2002. Trophic ecology of bowhead whales (*Balaena mysticetus*) compared with that of other arctic marine biota as interpreted from carbon-, nitrogen-, and sulfur-isotope signatures. *Can J Zool* 80:223–231.
42. Boon JP, Eijgenraam F, Everaarts JM, Duinker JC. 1989. A structure-activity relationship (SAR) approach towards metabolism of PCBs in marine animals from different trophic levels. *Mar Environ Res* 27:159–176.
43. Li H, Boon JP, Lewis WE, van den Berg M, Nyman M, Letcher RJ. 2003. Hepatic microsomal cytochrome P450 enzyme activity in relation to in vitro metabolism/inhibition of polychlorinated biphenyls and testosterone in Baltic gray seal (*Halichoerus grypus*). *Environ Toxicol Chem* 22:636–644.
44. Stapleton HM, Letcher RJ, Baker JE. 2001. Metabolism of PCBs

by the deepwater sculpin (*Myoxocephalus thompsoni*). *Environ Sci Technol* 35:4747–4752.
45. Iwata H, Yoshinari K, Negishi M, Stegeman JJ. 2002. Species-specific responses of constitutively active receptor (CAR)-CYP2B coupling: Lack of CYP2B inducer-responsive nuclear translocation of CAR in marine teleost, scup (*Stenotomus chrysops*). *Comp Biochem Physiol C* 131:501–510.
46. Wolker J, Witkamp RF, Nijmeijer SM, Burkow IC, de Groene EM, Lydersen C, Dahle S, Monshouwer M. 1998. Phase I and II enzyme activities in ringed seals (*Phoca hispida*): Characterization of hepatic cytochrome P450 by activity patterns, inhibition studies, mRNA analyses, and Western blotting. *Aquat Toxicol* 44: 103–115.
47. Preston BD, Miller JA, Miller EC. 1984. Non-arene oxide aromatic ring hydroxylation of 2,2′,5,5′-tetrachlorobiphenyl as the major metabolic pathway catalyzed by phenobarbital-induced rat liver microsomes. *J Biol Chem* 258:8304–8311.
48. Guvenius DM, Hassanzadeh P, Bergman Å, Norén K. 2002. Metabolites of polychlorinated biphenyls in human liver and adipose tissue. *Environ Toxicol Chem* 21:2264–2269.
49. Tanabe S, Tatsukawa R, Maruyama K, Miyazaki N, Fujiyama T. 1982. Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin (*Stenella coeruleoalba*). *Agric Biol Chem* 45:2569–2578.
50. Jenssen BM, Skaare JU, Ekker M, Vongraven D, Silverstone M. 1994. Blood sampling as a non-destructive method for monitoring levels and effects of organochlorines (PCB and DDT) in seals. *Chemosphere* 28:3–10.
51. Boon JP, Reijnders PJH, Dols J, Wensvoort P, Hillebrand MTJ. 1987. The kinetics of individual polychlorinated biphenyl congeners in female harbor seals (*Phoca vitulina*), with evidence for structure-related metabolism. *Aquat Toxicol* 10:307–324.
52. Tampal N, Myers S, Pereg D, Robertson LW. 2001. Interactions of 4-chlorobiphenyl and 3,3′,4,4′-tetrachlorobiphenyl with hemoglobin. In Robertson LW, Hansen LG, eds, *PCBs: Recent Advances in Environmental Toxicology and Health Effects.* University Press of Kentucky, Lexington, KY, USA, pp 391–393.
53. Bruhn R, Kannan N, Petrick G, Schulz-Bull DE, Duinker JC. 1995. CB pattern in the harbor porpoise: Bioaccumulation, metabolism and evidence of cytochrome P450 IIB activity. *Chemosphere* 31:3721–3732.
54. Reddy ML, Reif JS, Bachand A, Ridgway SH. 2001. Opportunities for using Navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction. *Sci Total Environ* 274:171–182.
55. Van Raaij JA, Frijters CM, van den Berg KJ. 1993. Hexachlorobenzene-induced hypothyroidism. Involvement of different mechanisms by parent compound and metabolite. *Biochem Pharmacol* 46:1385–1391.
56. Braun WH, Young JD, Blau GE, Gehring PJ. 1977. The pharmacokinetics and metabolism of pentachlorophenol in rats. *Toxicol Appl Pharmacol* 41:395–406.
57. Chi J, Huang G-L. 2002. Modeling air-water exchange process of pentachlorophenol in the aquatic environment. *Water Qual Res J Can* 37:445–458.
58. Ikeda GJ, Sapienza PP. 1995. Distribution, metabolism and excretion of pentachloroanisole in the beagle dog and miniature pig. *Food Chem Toxicol* 33:409–421.
59. Ikeda GJ, Sapienza PP, Warr PI. 1994. Disposition and metabolism of radiolabeled pentachloroanisole in rats and rabbits. *Food Chem Toxicol* 32:1137–1146.
60. Lans MC, Spiertz C, Brouwer A, Koeman JH. 1994. Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. *Eur J Pharmacol* 270:129–136.
61. Bakke JE, Bergman AL, Larsen GL. 1982. Metabolism of 2,4′,5-trichlorobiphenyl by the mercapturic acid pathway. *Science* 217: 645–647.
62. Bergman Å, Larsen GL, Bakke JE. 1982. Biliary secretion, retention and excretion of five ^{14}C-labeled polychlorinated biphenyls in the rat. *Chemosphere* 11:249–253.
63. Hilberg T, Rogde S, Mrland J. 1999. Postmortem drug redistribution—Human cases related to results in experimental animals. *J Forensic Sci* 44:3–9.