Biotransformation of PCBs in Relation to Phase I and II Xenobiotic-Metabolizing Enzyme Activities in Ringed Seals (Phoca hispida) from Svalbard and the Baltic Sea

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · DECEMBER 2008

Impact Factor: 5.33 · DOI: 10.1021/es801682f · Source: PubMed

7 AUTHORS, INCLUDING:

Augustine Arukwe
Norwegian University of Science and Tech…
147 PUBLICATIONS **3,654** CITATIONS
SEE PROFILE

Bert Van Bavel
Norwegian Institute for Water Research
221 PUBLICATIONS **5,662** CITATIONS
SEE PROFILE

Nigel G Yoccoz
University of Tromsoe
303 PUBLICATIONS **11,568** CITATIONS
SEE PROFILE

Geir Wing Gabrielsen
Norwegian Polar Institute
260 PUBLICATIONS **8,214** CITATIONS
SEE PROFILE

Environ. Sci. Technol. **2008,** *42,* 8952–8958

Biotransformation of PCBs in Relation to Phase I and II Xenobiotic-Metabolizing Enzyme Activities in Ringed Seals (*Phoca hispida*) from Svalbard and the Baltic Sea

HELI ROUTTI,*,†,‡ ROBERT J. LETCHER,§ AUGUSTINE ARUKWE,|| BERT VAN BAVEL,⊥ NIGEL G. YOCCOZ,# SHAOGANG CHU,§ AND GEIR W. GABRIELSEN†

Norwegian Polar Institute, Polar Environmental Centre, 9296 Tromsø, Norway, Centre of Excellence in Evolutionary Genetics and Physiology, Department of Biology, University of Turku, 20014 Turku, Finland, Wildlife Toxicology and Disease Program, Wildlife and Landscape Science Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, Ontario, K1A 0H3, Canada, Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway, MTM Research Centre, Örebro University, 70182 Örebro, Sweden, and Department of Biology, University of Tromsø, 9037 Tromsø, Norway

Received July 7, 2008. Revised manuscript received September 13, 2008. Accepted September 24, 2008.

Polychlorinated biphenyls (PCBs) may induce activity of hepatic enzymes, mainly Phase I monooxygenases and conjugating Phase II enzymes, that catalyze the metabolism of PCBs leading to formation of metabolites and to potential adverse health effects. The present study investigates the concentration and pattern of PCBs, the induction of hepatic phase I and II enzymes, and the formation of hydroxy (OH) and methylsulfonyl (CH_3SO_2═$MeSO_2$) PCB metabolites in two ringed seal (*Phoca hispida*) populations, which are contrasted by the degree of contamination exposure, that is, highly contaminated Baltic Sea ($n = 31$) and less contaminated Svalbard ($n = 21$). Phase I enzymes were measured as ethoxyresorufin-*O*-deethylation (EROD), benzyloxyresorufin-*O*-dealkylation (BROD), methoxyresorufin-*O*-demethylation (MROD), and pentoxyresorufin-*O*-dealkylation (PROD) activities, and phase II enzymes were measured as uridine diphosphophate glucuronosyl transferase (UDPGT) and glutathione-*S*-transferase (GST). Geographical comparison, multivariate, and correlation analysis indicated that Σ-PCB had a positive impact on Phase I enzyme and GST activities leading to biotransformation of group III (vicinal *ortho-meta*-H atoms and ≤1 *ortho*-chlorine (Cl)) and IV PCBs (vicinal *meta-para*-H atoms and ≤2 *ortho*-Cl). The potential precursors for the main OH-PCBs detected in plasma in the Baltic seals were group III PCBs. $MeSO_2$−PCBs detected in liver were mainly products of group IV PCB metabolism. Both CYP1A- and CYP2B-like enzymes are suggested to be involved in the PCB biotransformation in ringed seals.

Introduction

Marine predators occupying some of the highest trophic levels in the marine food web, such as seals, are exposed to high levels of persistent organic pollutants through their diet. Persistent organic pollutants, mainly polychlorinated biphenyls (PCBs) have been detected at extremely high concentrations in the seals from industrialized areas, for example, the Baltic, compared to those from remote Arctic regions (*1, 2*). Adverse health effects have been associated with PCB loads in highly contaminated seal populations, for example, the Baltic seals, including reproduction impairment, pathological changes (*3, 4*), and endocrine disruption (*5, 6*), but they have not yet been linked to high contaminant exposures by any specific toxic mechanisms. The potential toxicity of two classes of PCB metabolites, namely hydroxylated (OH) and methylsulfonyl (CH_3SO_2═$MeSO_2$) PCBs, has been demonstrated by several studies conducted in vitro or in vivo on rodents (*7*). Therefore, knowledge of the biotransformation of PCBs is crucial for understanding the possible mechanisms of toxic effects.

The biotransformation process ultimately defines the levels and congener patterns of PCBs in biota (*7*). Other factors influencing the toxicokinetics and bioaccumulation of PCBs include dietary exposure, age, and gender (*8*). Biotransformation of PCBs is a complex process involving xenobiotic-metabolizing Phase I (cytochrome P450, i.e., CYP) and conjugating Phase II enzymes, which can lead to the formation and retention of OH− and $MeSO_2$−PCB metabolites (*7*).

In seals, initial pharmacokinetic models of PCB biotransformation have been based on relative comparisons of PCB patterns in diet and tissues of harbor and gray seals (*9*). These models suggest that the bioaccumulation pattern depends on the structural features of individual PCB congeners. The phenomenon has been hypothesized to be caused by CYP-mediated metabolism of PCBs. In seals, xenobiotic-metabolizing enzyme systems have been characterized using several techniques (*8, 10*−*13*). Induction of CYPs has been reported in seals exposed to high levels of contaminants (*5, 8*). The role of specific CYPs involved in the biotransformation of PCBs has been investigated by in vitro metabolism/inhibition assays using gray and harbor seal liver microsomes (*14, 15*).

Although several approaches have been used to investigate PCB biotransformation in seals, there is still a lack of knowledge concerning PCB biotransformation in marine mammals. Furthermore, with the exception of a few studies reporting PCB metabolites in seals (*7, 16, 17*), especially in recent times, OH−PCB residues in seal tissues are not well characterized even though their toxicological potential may be significant in wildlife. The objective of the present study is to investigate the PCB biotransformation in two contrasting ringed seal (*Phoca hispida*) populations: highly contaminated Baltic Sea and less contaminated Svalbard. Congener-specific PCB, OH−PCB, and $MeSO_2$−PCB patterns and concentrations were determined and the relationships were examined with the catalytic activities of Phase I and II enzymes.

* Corresponding author e-mail: heli.routti@npolar.no; phone: +47 77750663; fax: + 47 77750501.
† Polar Environmental Centre.
‡ University of Turku.
§ Carleton University.
|| Norwegian University of Science and Technology.
⊥ Örebro University.
University of Tromsø.

10.1021/es801682f CCC: $40.75
Published on Web 10/31/2008

TABLE 1. Concentrations (Mean ± Standard Error, ng/g Wet Weight) of the Polychlorinated Biphenyls (PCBs) and PCB Metabolites in Ringed Seals from the Baltic and Svalbard[a]

	Svalbard			Baltic		
	n	mean ± SE	min−max	*n*	mean ± SE	min−max
age		10.6 ± 1.6	2−31		9.2 ± 0.93	2−20
sex (males/females)		14/7			19/13	
			liver			
lipid (%)	21	3.3 ± 0.11	2.8−4.9	31	3.8 ± 0.13	2.6−5.6
Σ_{43}-PCB	18	45 ± 9.4	9.0−130	31	863 ± 202	141−6472
Σ_{10}-$MeSO_2$−PCB	21		<0.3−4.8	27	22 ± 3.9	<0.3−96
			plasma			
lipid (%)	18	0.70 ± 0.04	0.42−1.1	30	0.67 ± 0.03	0.24−1.0
Σ_{43}-PCB	18	22 ± 14	2.6−260	30	57 ± 8.7	4.6−185
Σ_4-OH−PCB	19	0.37 ± 0.06	<0.02−1.2			
Σ_{17}-OH−PCB				30	13.8 ± 1.5	3.1−37

[a] Σ_{43}-PCB: CB-47/48, −52, −66, −74, −99/113, −101, −105, −110, −114/122, −118, −128, −138, −141, −153, −156, −157, −170/190, −172/192, −174, −177, −178, −180/193, −182/187, −183, −189, −194, −195, −196/203, −197, −199, −201/204, −202, −206, −209. Σ_4-OH−PCB: 4-OH−CB107/4′-OH−CB108, 4-OH−CB163, 3′-OH−CB184. Σ_{17}-OH−PCB: 4-OH−CB107/4′-OH−CB108, 3-OH−CB118, 4′-OH−CB120, 4-OH−CB134, 3′-OH−CB138, 4-OH−CB146, 4-OH−CB162, 4-OH−CB163, 4′-OH−CB172, 4′-OH−CB177, 4-OH−CB178, 4-OH−CB187, 4-OH−CB193, 4′-OH−CB199, 4′-OH−CB202, 4′-OH−CB208. Σ_{10}-$MeSO_2$−PCB: 4-$MeSO_2$−CB49, 4-$MeSO_2$−CB64, 4-$MeSO_2$−CB70, 4-$MeSO_2$−CB87, 3-$MeSO_2$−CB101, 4-$MeSO_2$−CB101, 4-$MeSO_2$−CB110, 4-$MeSO_2$−CB132, 3-$MeSO_2$−CB149, 4-$MeSO_2$−CB174.

Experimental Section

Sample Collection and Preparation. The Ministry of Forestry and Agriculture in Finland granted the Finnish Game and Fisheries Research Institute special permission to sample Baltic ringed seals in April 2002, 2006, and 2007 (65°10′N, 24°20′E). The seal samples from Svalbard, Norway (77°47′N to 78°23′N, 17°00′E) were obtained in May and June 2007. At Svalbard, the seals were sampled with special permission granted to the Norwegian Polar Institute by the Governor of Svalbard and during the local hunting season under local hunting law of Svalbard. All the samples were collected after the weaning period during the moulting season of the seals. The age of the seals was determined by counting annual layers from the thin transverse sections of the canine or molar tooth. Samples for chemical analysis were stored at −20 °C until analyzed. Samples for enzyme activity analysis were frozen in liquid nitrogen in the field and stored at −80 °C until analyzed.

Chemical Analysis. The method for analysis of PCBs in liver and plasma samples was based on previously described methods with some modifications (*18, 19*). Detailed information about congeners analyzed is given in Table 1. $MeSO_2$−PCBs in liver samples were analyzed according to previously described methods with some modifications (*20, 21*). The extraction and cleanup of plasma (or serum) for OH−PCBs is based on procedures described elsewhere with some modifications (*20, 22*). PCB metabolites analyzed are listed in Table S1, Supporting Information. All the methods are described in detail in Supporting Information.

Enzyme Assays. Phase I enzyme activities in liver microsomes were studied using ethoxyresorufin-*O*-deethylase (EROD), benzyloxyresorufin-*O*-dealkylase (BROD), methoxyresorufin-*O*-demethylase (MROD), and pentoxyresorufin-*O*-dealkylase (PROD) activity assays based on the end point method according to Burke et al. (*23*). In dogs, EROD and BROD are catalyzed by CYP1A1/2 (*24*) and CYP2B11 (*25*), respectively. In rodents, MROD and PROD have been used as model substrates for CYP1A2 (*26*) and CYP2B (*27*), respectively. Methods for anlaysis of actvities of Phase II enzymes, microsomal uridine-diphosphate glucuronosyltransferase (UDPGT), and cytosolic glutathione S-transferase (GST), were based on previously described methods (*28, 29*). The total amount of protein was determined with the method of Bradford (*30*), using bovine serum albumin as standard. Detailed information of preparation of microsomal and cytosolic fractions and enzyme assays are given in Supporting Information.

Data Analysis. Sum concentrations of PCBs, $MeSO_2$−PCBs and OH−PCBs include congeners/metabolites detected in 60% or more of the samples for the population. For these congeners/metabolites, the samples with concentrations below the minimum level of quantification (MLOQ) were replaced by randomly generated normally distributed data, assuming 1/2 of the detection limit as the mean, with 40% variation. Statistical analyses were carried using R, version 2.6.1 (The R Foundation for Statistical Computing, R Development Core Team). Possible impacts of geographical area on PCBs and their metabolites, and enzyme levels were investigated using linear models. All the continuous variables were ln-transformed. Parameter estimates (β) ± standard error (SE), *F*-statistics, residual degrees of freedom, and level of significance are given in the text for each model. Diagnostic plots of residuals were used to verify that the model assumptions were met (most importantly constant variance between residuals). One individual having extremely high concentration of plasma Σ-PCB was removed from the data for plasma Σ-PCB analysis. The removal of this individual did not result in substantial changes on relationships between plasma Σ-PCB and area. Hepatic lipid concentrations were more elevated in the high than in the low contaminated seal population ($\beta = 0.090 \pm 0.042$, $F_{1,47} = 4.50$, $p = 0.039$; Table 1). To avoid the confounding effect of lipid concentration with area, hepatic Σ-PCB concentrations were lipid normalized for the linear model. Possible impacts of lipid concentrations on hepatic wet weight Σ-PCB concentrations were tested separately for each population using linear models.

To investigate PCB patterns, the congeners were divided into seven groups based on the number of chlorine (Cl) atoms (4−10 Cl-atoms) and into five groups based on the pattern of Cl-substitution as suggested by Boon et al. (*9*) (CB I: no vicinal hydrogen (H)-atoms (CB 153,178, 182/187, 183, 172/192, 180/193, 189, 202, 197, 196/203, 194, 199, 208, 207, 206, 209); CB II: vicinal H-atoms only in ortho and meta positions in combination with ≥2 *ortho*-Cl (CB 138, 128, 170/190, 177, 195); CB III: vicinal *ortho-meta*-H atoms and ≤1 *ortho*-Cl (CB 66, 74, 118, 114/122, 105, 156, 157); CB IV: vicinal *meta-para* H-atoms and ≤2 *ortho*-Cl (CB 52, 101, 110, 141); CB V: vicinal *meta-para* H-atoms and ≥3 *ortho*-Cl (CB 174)).

Multivariate descriptive methods, including principal component analysis (PCA) and principal component analysis

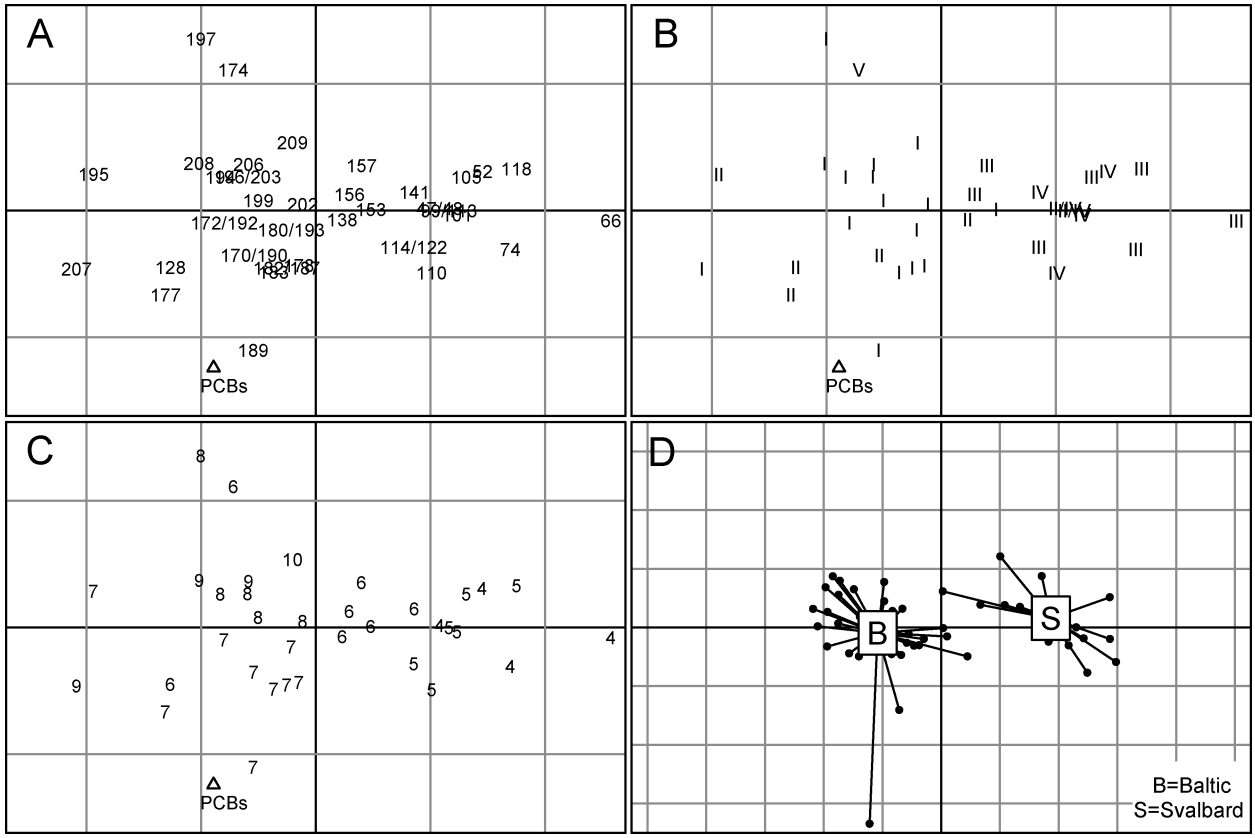

FIGURE 1. Ordination plots from PCA based on covariance matrix of log ratio PCBs in liver. PCBs are named by congener number (A), metabolic group (B), and number of Cl-atoms (C). Sample scores are grouped by geographical area (D). Σ_{43}-PCBs is shown as a supplementary variable. The 1st axis explains 51.9% of the variation, and 2nd axis explains 9.69%.

with instrumental variables, also named redundancy analysis (RDA), were used to investigate aspects regarding the PCB pattern (*31*). Because we dealt with proportions summing up to one, all the multivariate analysis were based on PCA derived from the covariance matrix of centered log-ratio of proportions ($\ln(CB_x/\Sigma\text{-PCB})$) (*32*). The pattern of hepatic PCB congeners was analyzed using PCA. Differences of PCB pattern in liver and plasma were investigated by PCA based on the difference of log-ratio PCBs in liver and log-ratio PCBs in plasma ($\ln(CB_x/\Sigma\text{-PCB})_{liver} - \ln(CB_x/\Sigma\text{-PCB})_{plasma}$). Congeners showing high uncertainty were not included in the PCA (CB 201/204 for PCA liver; CB 189, 195, 197 and 201/204 for PCA liver-plasma difference). Consequently, 0.9% (PCA liver) and 0.1% (PCA liver-plasma difference) of the data included in the PCAs were < MLOQ and replaced by random values as described above. The relationships between the hepatic PCB pattern and the explanatory variables EROD, PROD, and GST were studied using RDA. The RDA model was highly significant based on Monte-Carlo permutation test (1000 replicates, RV coefficient 0.247, $p < 0.001$). Correlations based on ln-transformed data are shown as Pearson correlation coefficients.

Results and Discussion

PCB Levels. Hepatic Σ-PCB concentrations were several times higher in the ringed seals from the Baltic compared to the seals from Svalbard ($\beta = 1.97 \pm 0.17$, $F_{1,47} = 137$, $p < 0.001$; Table 1). The main PCB congener CB153 measured as lipid weight in liver was 80% lower in ringed seals from the present study compared to the ringed seals sampled in the Baltic in 1997−98 (*2*) ($\beta = -1.59 \pm 0.18$, $F_{1,49} = 75.6$, $p < 0.001$). Although the seal samples from the earlier study and from the present study have been analyzed using different analytical methods, the result indicates that PCB levels in the Baltic are decreasing. Geographical difference of Σ-PCB concentration in plasma ($\beta = 1.28 \pm 0.18$, $F_{1,45} = 50.5.1$, $p < 0.001$) was smaller compared to liver Σ-PCB concentration. This can be explained by the high variation of plasma Σ-PCB; some individuals from Svalbard had surprisingly high circulating levels of Σ-PCB probably because of dietary intake preceding the sampling. The liver lipid content was higher in the seals from the Baltic than from Svalbard (see methods; Table 1), and it was positively related to hepatic Σ-PCB concentrations only in the Baltic population ($\beta = 2.03 \pm 0.65$, $F_{1,29} = 9.76$, $p = 0.004$). These results indicate that long-term contaminant exposure may lead to fatty liver in seals, as observed in laboratory rodents (*33*).

PCB Biotransformation and PCB Metabolites. PCB biotransformation capacity and substrate selectivity depends on the Cl-substitution pattern of the molecule and it has been shown that there are wide interspecies and interpopulation variations. In seals, metabolism of CB III and IV and persistence of CB I and II have been suggested by bioaccumulation (*9, 34*) and in vitro studies (*15*). The results of the present study based on investigations on PCB pattern, xenobiotic-metabolizing enzyme activities and PCB metabolite formation in two contrasting ringed seal population further support these previous reports.

The results of PCA analysis of hepatic PCBs indicate that CB III and IV were relatively lower in the highly contaminated seals compared to the low contaminated animals, while the trend was opposite for CB I, II and V (Figure 1B). PCB pattern shifted more toward CB I, II, and V ($r = 0.67, 0.71$, $p < 0.001$ and $r = 0.31$, $p = 0.03$, respectively,) and less toward CB III and IV ($r = -0.86$ and -0.87, respectively, $p < 0.001$) with increasing hepatic Σ-PCB concentration. The observed difference in PCB pattern between the Baltic and Svalbard seals is more likely to be related to biotransformation than

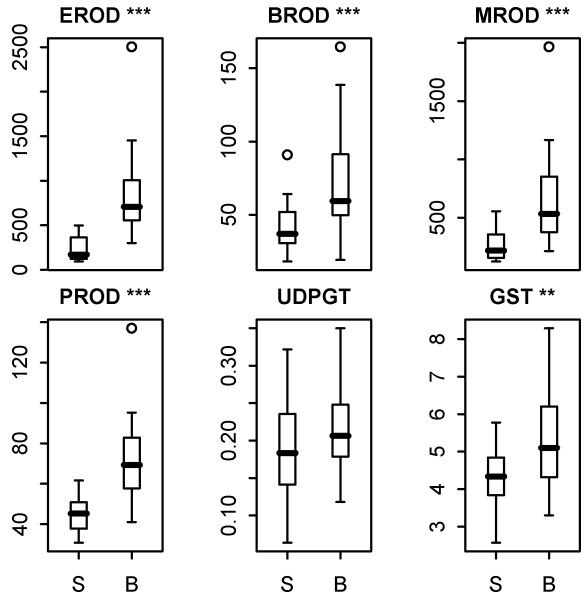

FIGURE 2. Hepatic Phase I and II enzyme activities in ringed seals from Svalbard (S) and the Baltic (B) (umol/min × g protein/ml for EROD, PROD, MROD, BROD, GST, and nmol/min × g protein/ml for UDPGT). Three asterisks (***) represents $p < 0.001$, while two asterisks (**) represents $p < 0.01$.

to the contaminant exposure, because the results of multivariate analysis carried only on the Baltic seals (PCA for liver and RDA) corresponded with the results carried on the two populations, although the dietary intake of PCBs may differ between the areas. Therefore, the relatively higher proportion of low-chlorinated PCBs in Svalbard ringed seals (Figure 1C) may not be purely related to dietary PCB exposure, although low-chlorinated compounds are more volatile and tend to be proportionally more represented in the remote regions. Recent study on Svalbard ringed seals (*35*) also indicates that lower-chlorinated PCBs tend to be relatively more represented in low-contaminated seals compared to the seals with higher-contaminant exposure.

Xenobiotic-metabolizing enzyme activities were higher in the Baltic seals compared to the seals from Svalbard (Figure 2 and Table S2, Supporting Information). EROD activity was negatively correlated to relative concentration of CB III ($r = 0.64$, $p < 0.001$) and IV ($r = 0.73$, $p < 0.001$) (Figure 3). The EROD activities were positively correlated to Σ-PCBs ($r = 0.76$, $p < 0.001$) and Σ-OH-PCBs ($r = 0.72$, $p < 0.001$) (Figure 3). The correlations between PROD, MROD, and BROD activities and PCBs were similar to the results obtained from EROD activity, although less pronounced. The results of the present study strengthen the hypothesis that xenobiotic metabolizing enzyme system is induced by contaminant exposure in ringed seals (*11*).

Concentrations of OH−PCBs were higher in the Baltic seals compared to the seals from Svalbard ($\beta = 2.60 \pm 0.15$, $F_{1,46} = 300$, $p < 0.001$). In the Baltic seals, more than a half of Σ-OH−PCB concentrations determined in plasma consisted of 4-OH−CB107/4′-OH−CB108 (Table S1, Supporting Information), which is also the major metabolite detected in the Baltic gray seal blood reported in 1994 (*36*). Potential precursors of this metabolite belong to CB III, including CB 118 and CB 105 (*7, 37, 38*). There are likely a greater number of OH−PCBs that are formed, but their short overall half-life may result to nonmeasurable OH−PCB residue level. In contrast to OH−PCBs, $MeSO_2$−PCB metabolites are derived from PCB of the same chlorine atom configuration. The $MeSO_2$−PCBs detected in the ringed seal liver were mainly formed from group IV and to lesser extent from group V PCBs (Table S1, Supporting Information), which was opposite

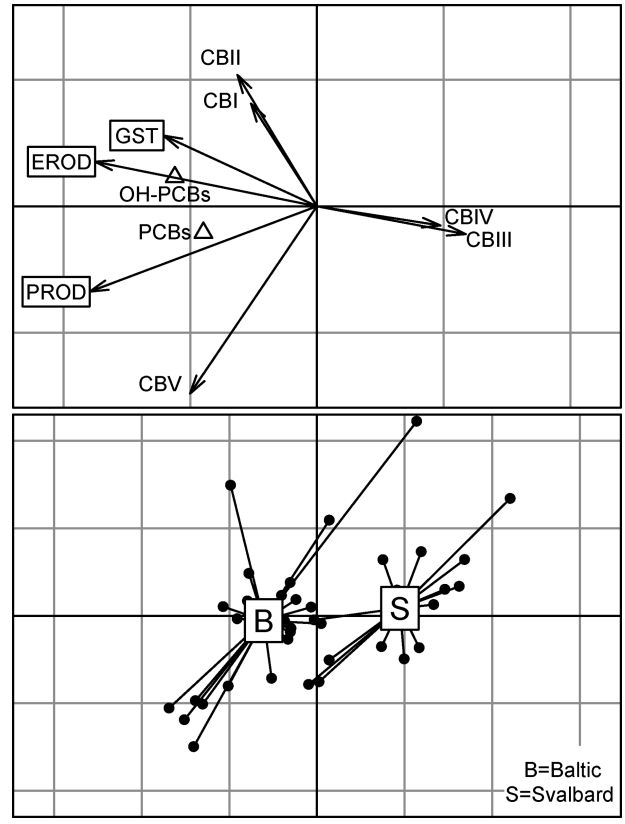

FIGURE 3. Ordination plots from RDA analysis based on covariance matrix of log-ratio PCB-groups in liver. The upper plot shows the relationships between response variables (CB I−V), and explanatory variables (EROD, PROD, and GST), and supplementary variables (plasma Σ-OH−PCB and liver Σ_{43}-PCBs). The plot below shows the sample scores. The 1st linear combination of the explanatory variables explains 35% of the response variables,and the 2nd explains 6.1%. The 1st axis explains 91% of the variation, and 2nd axis explains 8.8%.

to the results reported previously for Baltic gray seal liver (*16*). This indicates that the species may have different PCB biotransformation capacity. The levels of PCB metabolites observed in the seals from the present study are suggested to be related to biotransformation rather than to dietary exposure. First, fish have very poor capacity to metabolize PCBs to OH−PCBs (*39*). Second, the main PCB congeners (CB 101, 110, 118,105, 149, 153, 138) show similar bioaccumulation pattern to the Baltic herring (*40*), which is one of the main dietary species of Baltic ringed seal (*41*). Third, although $MeSO_2$−PCBs have been detected in fourhorn sculpins (*42*), the bioaccumulation of them into the ringed seals is assumed to be minor as fourhorn sculpin is a minor species in the diet composition of the Baltic ringed seal (*41*).

The difference in the PCB pattern in liver and plasma also supports the hypothesis that CB III and IV are metabolized in seals. In the low-contaminated ringed seals, CB III and IV were relatively higher in the liver as compared to the plasma (Figure 4). This may be caused by differences in lipid composition between liver and plasma and in the lipophilicity of the individual congeners. Matrice-specific lipid composition and compound-specific octanol−water partitioning cefficient have been suggested to result to selective retention of contaminants in blubber and plasma in harp seals (*43*). In contrast, all CB groups showed similar pattern in liver and plasma in the Baltic seals. This may reflect that the more highly enzyme induced Baltic seals are degrading CB III and IV faster (in liver) when compared to Svalbard seals.

Role of Enzymes in PCB Biotransformation. Biotransformation of PCBs to $MeSO_2$− and OH−PCBs is a complex

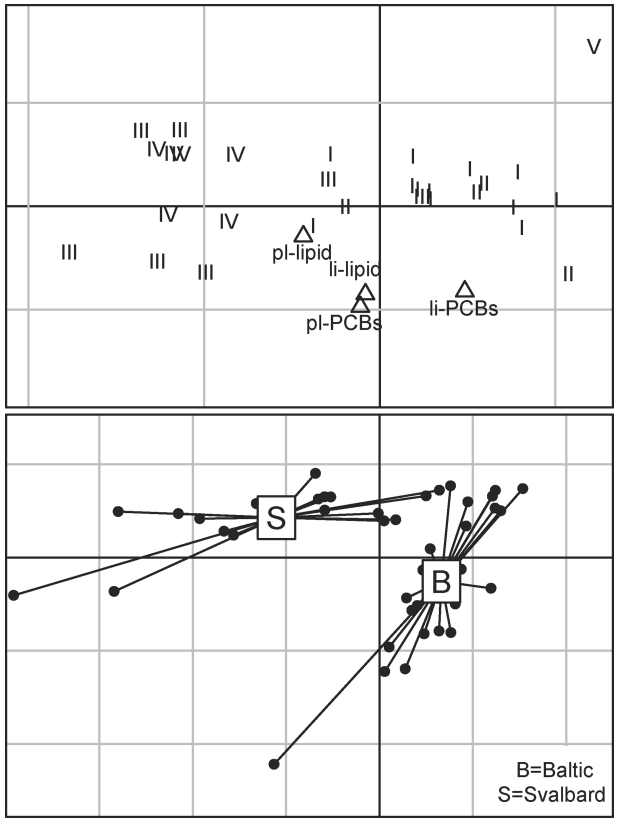

FIGURE 4. Ordination plots from PCA based on covariance matrix of the difference of log ratio PCBs in liver and log ratio PCBs in plasma. Sample scores are grouped by geographical area. Σ_{43}-PCB and lipid % for liver (li-) and plasma (pl-) are shown as supplementary variables. The 1st axis explains 47.6% of the variation, and the 2nd axis explains 14.3%.

mechanism involving several Phase I and II enzymes (*7*). In short, an initial step of the oxidative metabolism of PCBs is mediated by CYP enzymes. For OH−PCBs that have been reported (mainly in the blood) of wildlife, the OH group is directly inserted in para position. Alternatively, arene oxide is formed in the meta-para position or in some species in the ortho-meta position. Arene oxide intermediates can be further metabolized to either OH−PCBs by epoxide hydroxylase (*37*) or to $MeSO_2$−PCBs involving, for example, GST. OH−PCBs can be further metabolized by UDPGT, which is involved in the conjugation of the glucuronosyl group from glucuronic acid to OH−PCBs.

Group III PCBs having one or no *ortho*-Cl atom acquire easily a planar configuration and are thus preferred substrates to CYP1A-like enzymes in seals (*44*). Nonplanar PCBs with vicinal H atoms at the *meta-para*-carbons are preferred substrates of CYP2B isoenzymes, while CYP3A show relatively little substrate specificity (*45*). The negative relationship between phase I enzyme activities and proportions of group III and IV congeners (Figure 3) suggest that CYP enzymes are capable of metabolizing both planar and nonplanar PCBs in ringed seals. Ninety-seven percent of the $MeSO_2$−PCBs formed in ringed seals were metabolites of nonplanar PCBs (Table S1, Supporting Information), having two or three *ortho*-Cl and vicinal *meta-para*-H atoms (CB IV and V). Interestingly, there are contradictory findings about the presence of CYP2B in seals. Western blotting studies using polyclonal (*46*) and monoclonal (*10, 14*) antibodies and in vitro metabolism/inhibition study of PCBs (*15*) suggest that CYP2B-like enzymes are present in harbor, hooded, and gray seals. Studies comparing PCB congener pattern of tissue residues between species and food chains have also suggested the CYP2B-like enzyme to be present in seals (*9*). Although PROD is a model substrate for CYP2B in rats (*27*), inhibition, and mono- and polyclonal antibody studies suggest that PROD activity is CYP1A-mediated in ringed and gray seals (*11*). A study based on mRNA analysis indicate that CYP2B is absent in ringed seals, although in the same study Western blotting suggested the presence of CYP2B-like enzymes (*10*). CYP3A-like enzymes have been suggested to play a role in the CB IV-metabolism in gray seals (*15*). However, CYP3A activity has been suggested to be suppressed by CYP1A activity in ringed seals (*12, 34*). Elevated CYP3A activity in the Svalbard ringed seals compared to the seals from the Baltic (*12*) could explain the formation of 3′OH−CB184 (parent CB from groups I and V) only in the Svalbard seals (Table S1, Supporting Information).

Σ-PCBs were positively correlated to GST activity ($r = 0.54$, $p < 0.001$), which further led to a decreased concentration of CB IV ($r = -0.48$, $p < 0.001$) (Figure 3). This suggests that contaminant-induced GST leads to increased metabolism of PCB congeners that are precursors to $MeSO_2$−PCBs. Congeners of CB IV, such as CB101 and 110, are well-known precursors of 3- and 4-$MeSO_2$-substituted PCBs of the same chlorination pattern (*7*). In contrast, similar levels of UDPGT in the high- and low-contaminated seals (Figure 2 and Table S2, Supporting Information) suggest that the sensitivity of UDPGT induction response is low to contaminant exposure to the present suite of compounds/congeners. It may be postulated that the OH−PCBs identified thus far in plasma of ringed seals are poor substrates or inducers for ringed seal liver microsome UDPGT isoforms. However, it is more likely that the lack of OH−PCB relationship to UDPGT is caused by the fate of OH−PCB being governed by more competitive biochemical processes such as protein binding and conjugation via other Phase II pathways, such as via sulfatase mediaton. These results are consistent with recent findings in Greenland sledge dogs (*47*).

Species Comparison and Implications of PCB Biotransformation. Biotransformation of PCBs has been studied in several marine mammal and bird species. Given that OH−PCBs in free-ranging mammals have been shown to be rather metabolic than dietary origin (*17*), the ratios between summed PCB metabolites, and summed parent PCB concentrations in a given tissue provides an indication about the metabolic PCB capacity of the species. In the present study, the ratio of Σ-OH−PCB/Σ-PCB concentrations in plasma indicates that highly contaminated ringed seals are more efficient to biotransform PCBs to OH-metabolites than low contaminated ringed seals (Σ-OH−PCB/Σ_{43}-PCB 0.42 in the Baltic and 0.02 in Svalbard). However, the Baltic ringed seals are less efficient to metabolize $MeSO_2$−PCBs (Σ_{10}-$MeSO_2$−PCB/Σ_{43}-PCB 0.03) compared to Baltic gray seals (Σ-$MeSO_2$/Σ-PCB 0.91−1.1) (*7*). Also induction of CYP1A is more pronounced in gray seals than in ringed seals from the Baltic (*11*). Although seals have been suggested to be have better PCB biotransformation capacity than cetaceans (*9*), the $MeSO_2$−PCB formation was lower in the Baltic ringed seals than in the highly contaminated belugas (Σ_{16}-$MeSO_2$−PCB/Σ_{36}-PCB 0.12) (*21*). Interestingly, the capacity to form OH−PCBs was similar in the high contaminated ringed seals and low contaminated bowhead whales (Σ_2-OH−PCB/Σ_{102}-PCB 0.55) (*48*). Comparison between highly contaminated marine mammals and birds suggests that PCB biotransformation capacity or metabolite retention is poor in ringed seals to compared to polar bears (Σ_{33}-OH−PCB/Σ_{51}-PCB 25.5) (*20*) but efficient in comparison to glaucous gulls (Σ_{26}-OH−PCB/Σ_{45}-PCB 0.03) (*22*). The species difference was similar for the $MeSO_2$−PCB formation capacity in liver (Baltic ringed seal: Σ_{10}-$MeSO_2$−PCB/Σ_{43}-PCB 0.03; Polar bear Σ_{24}-$MeSO_2$−PCB/Σ_{51}-PCB 0.07; Glaucous gull Σ_{12}-$MeSO_2$−PCB/Σ_{45}-PCB 0.001). Metabolite formation of PCBs differs also qualitatively between species. In polar bears and

glaucous gulls, majority of OH−PCBs are formed from CB I and II (*20, 49, 50*), while in ringed seals and cetaceans, the parent compounds for the main OH−CBs are CB III congeners (this study, refs *21, and 48)*.

PCB metabolites have been related to several endocrine disrupting effects in experimental animals and in humans (*7*). The main OH−PCBs in ringed seals are para-substituded hydroxy metabolites. These metabolites show high similarity to thyroid hormones, which are natural substrates to transthyretin (TTR). The affinities of the para-substituted OH−PCBs to TTR are up to 10 times higher than that of thyroxine (*51*). TTR forms a carrier protein complex with retinol-binding protein (*52*). Decreased thyroid hormone and vitamin A levels observed in PCB-exposed rodents have been associated to high levels 4′-OH−CB79 formed (*53*). OH−PCBs may disturb thyroid hormone homeostasis also by several other mechanisms (*7, 54*). In the ringed seals, disturbed thyroid hormone and vitamin A homeostasis have been related to high PCB exposure, but the mechanisms are still unclear (*5, 6*). Therefore, further research is needed to investigate the potential role of PCB metabolites in endocrine disruption in ringed seals.

Acknowledgments

We acknowledge E. Helle, M. Kunnasranta, M. Nyman and Finnish Game and Fisheries Research Institute for the sampling in the Baltic Sea and J. Ikonen, Ø. Overrein, T. Sandal and H. Wolkers for their help in sampling in Svalbard. We thank H. Björnhoft for the assistance in PCB analysis. We are grateful to M. Nyman for commenting on the manuscript. This study was financed by Nordic Council of Ministers, Kone Foundation, Research Council of Norway, Norwegian Polar Institute, and the Biological Interactions Graduate School.

Supporting Information Available

Methods for analyzing PCBs, PCB metabolites and enzyme activities and tables showing the PCB metabolites and results of linear models for enzyme activities. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Jensen, S.; Johnels, A. G.; Olsson, M.; Otterlind, G. DDT and PCB in marine animals from Swedish waters. *Nature* **1969**, *224*, 247–250.

(2) Nyman, M.; Koistinen, J.; Fant, M. L.; Vartiainen, T.; Helle, E. Current levels of DDT, PCB, and trace elements in the Baltic ringed seals (*Phoca hispida baltica*) and grey seals (*Halichoerus grypus*). *Environ. Pollut.* **2002**, *119*, 399–412.

(3) Helle, E.; Olsson, M.; Jensen, S. PCB levels correlated with pathological changes in seal uteri. *Ambio* **1976**, *5*, 261–263.

(4) Bergman, A.; Olsson, M. Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: Is environmental pollution the cause of a widely distributed disease syndrome? *Finn. Game Res.* **1985**, *44*, 47–62.

(5) Nyman, M.; Bergknut, M.; Fant, M. L.; Raunio, H.; Jestoi, M.; Bengs, C.; Murk, A.; Koistinen, J.; Backman, C.; Pelkonen, O.; Tysklind, M.; Hirvi, T.; Helle, E. Contaminant exposure and effects in Baltic ringed and grey seals as assessed by biomarkers. *Mar. Environ. Res.* **2003**, *55*, 73–99.

(6) Routti, H.; Nyman, M.; Jenssen, B. M.; Bäckman, C.; Koistinen, J.; Gabrielsen, G. W. Bone-related effects of contaminants in seals may be associated with vitamin D and thyroid hormones. *Environ. Toxicol. Chem.* **2008**, *27*, 873–880.

(7) Letcher, R. J.; Klasson-Wehler, E.; Bergman, Å Methyl Sulfone and Hydroxylated Metabolites of Polychlorinated Biphenyls. In *The Handbook of Environmental Chemistry*; Paasivirta, J., Ed.; Springer-Verlag: Berlin, 2000.

(8) Boon, J. P.; van Arnhem, E.; Jansen, S.; Kannan, K.; Petrick, G.; Schulz, D.; Duinker, J. C.; Reijnders, P. J. H.; Goksoyr, A. The Toxicokinetics of PCBs in Marine Mammals with Special Reference to Possible Interactions of Individual Congeners with the Cytochrome P450-Dependent Monoxygenase System—An Overview. In *Persistent Pollutants in Marine Ecosystems*; Walker, C. H., Livingstone, D. R., Agren, E., Eds.; Pergamon Press: Oxford, U.K., 1992.

(9) Boon, J. P.; van der Meer, J.; Allchin, C. R.; Law, R. J.; Klungsøyr, J.; Leonards, P. E. G.; Spliid, H.; Storr-Hansen, E.; Mckenzie, C.; Wells, D. E. Concentration-dependent changes of PCB patterns in fish-eating mammals: Structural evidence for induction of cytochrome P450. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 298–311.

(10) Wolkers, J.; Witkamp, R. F.; Nijmeijer, S. M.; Burkow, I. C.; de Groene, E. M.; Lydersen, C.; Dahle, S.; Monshouwer, M. Phase I and phase 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.* **1998**, *44*, 103–115.

(11) Nyman, M.; Raunio, H.; Pelkonen, O. Expression and inducibility of members in the cytochrome P4501 (CYP1) family in ringed and grey seals from polluted and less polluted waters. *Environ. Toxicol. Pharmacol.* **2000**, *8*, 217–225.

(12) Nyman, M.; Raunio, H.; Taavitsainen, P.; Pelkonen, O. Characterization of xenobiotic-metabolizing cytochrome P450 (CYP) forms in ringed and grey seals from the Baltic Sea and reference sites. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2001**, *128*, 99–112.

(13) Tilley, R. E.; Kemp, G. D.; Teramitsu, I.; Hall, A. J. Isolation of two cytochrome P450 cDNAs, CYP1A1 and CYP1A2, from harp seal (*Phoca groenlandica*) and grey seal (*Halichoerus grypus*). *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2002**, *132*, 181–191.

(14) van Hezik, C. M.; Letcher, R. J.; de Geus, H. J.; Wester, P. G.; Goksøyr, A.; Lewis, W. E.; Boon, J. P. Indications for the involvement of a CYP3A-like iso-enzyme in the metabolism of chlorobornane (Toxaphene) congeners in seals from inhibition studies with liver microsomes. *Aquat. Toxicol.* **2001**, *51*, 319–333.

(15) Li, H.; Boon, J. P.; Lewis, W. E.; van den Berg, M.; Nyman, M.; Letcher, R. J. Hepatic microsomal cytochrome P450 enzyme activity in relation to in vitro metabolism/inhibition of polychlorinated biphenyls and testosterone in Baltic grey seal (*Halichoerus grypus*). *Environ. Toxicol. Chem.* **2003**, *22*, 636–644.

(16) Larsson, C.; Norström, K.; Athanansiadis, I.; Bignert, A.; König; W, A.; Bergman, Å. Enantiomeric specificity of methylsulfonyl-PCBs and distribution of bis(4-chlorophenyl) sulfone, PCB, and DDE methyl sulfones in grey seal tissues. *Environ. Sci. Technol.* **2004**, *38*, 4950–4955.

(17) Verreault, J.; Dietz, R.; Sonne, C.; Gebbink, W. A.; Shahmiri, S.; Letcher, R. J. Comparative fate of organohalogen contaminants in two top carnivores in Greenland: Captive sledge dogs and wild polar bears. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2008**, *147*, 306–315.

(18) Van Bavel, B.; Dahl, P.; Karlsson, L.; Hardenc, L.; Rappe, C.; Lindström, G. Supercritical fluid extraction of PCBs from human adipose tissue for HRGC/LRMS analysis. *Chemosphere* **1995**, *30*, 1229–1236.

(19) van Bavel, B.; Mattias, M.; Karlsson, L.; Lindström, G. Development of a solid phase carbon trap for simultaneous determination of PCDDs, PCDFs, PCBs, and pesticides in environmental samples using SFE-LC. *Anal. Chem.* **1996**, *68*, 1279–1283.

(20) Gebbink, W. A.; Sonne, C.; Dietz, R.; Kirkegaard, M.; Riget, F. F.; Born, E.; Muir, D. C. G.; Letcher, R. Tissue-specific congener composition of organohalogen and metabolite contaminants in East Greenland polar bears (*Ursus maritimus*). *Environ. Pollut.* **2008**, *152*, 621–629.

(21) McKinney, M. A.; De Guise, S.; Martineau, D.; Beland, P.; Lebeuf, M.; Letcher, R. Organohalogen contaminants and metabolites in beluga whale (*Delphinapterus leucas*) liver from two Canadian populations. *Environ. Toxicol. Chem.* **2006**, *25*, 1246–1257.

(22) Verreault, J.; Shahmiri, S.; Gabrielsen, G. W.; Letcher, R. J. Organohalogen and metabolically-derived contaminants and associations with whole body constituents in Norwegian Arctic glaucous gulls. *Environ. Int.* **2007**, *33*, 823–830.

(23) Burke, M. D.; Mayer, R. T. Ethoxyresorufin—Direct fluorometric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholnathrene. *Drug Metab. Dispos.* **1974**, *2*, 583–588.

(24) Jayyosi, Z.; Muc, M.; Erick, J.; Thomas, P. E.; Kelley, M. Catalytic and immunochemical characterization of cytochrome P450 isozyme induction in dog liver. *Fundam. Appl. Toxicol.* **1996**, *31*, 95–102.

(25) Klekotka, P. A.; Halpert, C. R. Benzyloxyresorufin as a specific substrate for the major phenobarbital-inducible dog liver

cytochrome P450 (P4502B11). *Drug Metab. Dispos.* **1995**, *23*, 1434–1434.
(26) Nerurkar, P. V.; Park, S. S.; Thomas, P. E.; Nims, R. W.; Lubet, R. A. Methoxyresorufin and benzyloxyresorufin: Substrates preferentially metabolized by cytochromes P4501A2 and 2B, respectively, in the rat and mouse. *Biochem. Pharmacol.* **1993**, *46*, 933–943.
(27) Burke, M. D.; Thompson, S.; Elcombe, C. R.; Halpert, J.; Haaparanta, T.; Mayer, R. T. Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Pharmacol.* **1985**, *34*, 3337–3345.
(28) Andersson, T.; Pesonen, M.; Johansson, C. Differential induction of cytochrome P-450-dependent monooxygenase, epoxide hydrolase, glutathione transferase and UDP glucuronosyl transferase activities in the liver of the rainbow trout by beta -naphthoflavone or Clophen A50. *Biochem. Pharmacol.* **1985**, *34*, 3309–3314.
(29) Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139.
(30) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(31) Rao, C. R. The use and interpretation of principal component analysis in applied research. *Sankhya* **1964**, *26*, 329–358.
(32) Aitchison, J.; Greenacre, M. Biplots of compositional data. *J. R. Stat. Soc. B* **2002**, *51*, 375–392.
(33) Chu, I.; Poon, R.; Yagminas, A.; Lecavalier, P.; Håkansson, H.; Valli, V. E.; Kennedy, S. W.; Bergman, Å; Seegal, R. F.; Feeley, M. Subchronic toxicity of PCB 105 (2,3,3′,4,4′-pentachlorobiphenyl) in rats. *J Appl. Toxicol.* **2000**, *18*, 285–292.
(34) Wolkers, J.; Burkow, I. C.; Lydersen, C.; Dahle, S.; Monshouwer, M.; Witkamp, R. F. Congener specific PCB and polychlorinated camphene (toxaphene) levels in Svalbard ringed seals (*Phoca hispida*) in relation to sex, age, condition and cytochrome P450 enzyme activity. *Sci. Total Environ.* **1998**, *216*, 1–11.
(35) Wolkers, H.; Krafft, B. A.; van Bavel, B.; Helgason, L. B.; Lydersen, C.; Kovacs, K. M. Biomarker Responses and Decreasing Contaminant Levels in Ringed Seals (*Pusa hispida*) from Svalbard, Norway. *J. Toxicol. Environ. Health A* **2008**, *71*, 1009–1018.
(36) Bergman, Å; Klasson-Wehler, E.; Kuroki, H. Selective retention of hydroxylated PCB metabolites in blood. *Environ. Health Perspect.* **1994**, *102*, 464–469.
(37) Kawano, M.; Hasegawa, J.; Enomoto, T.; Onishi, H.; Nishiso, Y.; Matsuda, M.; Wakimoto, T. Hydroxylated polychlorinated biphenyls (OH-PCBs): Recent advances in wildlife contamination study. *Environ. Sci.* **2005**, *12*, 315–324.
(38) Malmberg, T.; Hoogstraate, J.; Bergman, A.; Wehler, E. K. Pharmacokinetics of two major hydroxylated polychlorinated biphenyl metabolites with specific retention in rat blood. *Xenobiotica* **2004**, *34*, 581–589.
(39) Murk, A.; Morse, D.; Boon, J. P.; Brouwer, A. In vitro metabolism of 3,3′,4,4′-tetrachlorobiphenyl in relation to ethoxyresorufin-*O*-deethylase activity in liver microsomes of some wildlife species and rat. *Eur. J. Pharmacol. Environ. Toxicol. Pharmacol.* **1994**, *270*, 253–261.
(40) Burreau, S.; Zebühr, Y.; Broman, D.; Ishaq, R. Biomagnification of PBDEs and PCBs in food webs from the Baltic Sea and the northern Atlantic Ocean. *Sci. Total Environ.* **2006**, *366*, 659–672.
(41) Tormosov, D. D.; Rezvov, G. V. Information on the distribution, number and feeding habits of ringed and grey seals in the Gulfs of Finland and Riga in the Baltic Sea. *Finn. Game Res.* **1978**, *37*, 14–21.
(42) Bright, D. A.; Grundy, S. L.; Reimer, K. J. Differential bioaccumulation of non-ortho-substituted and other PCB congeners in coastal Arctic invertebrates and fish. *Environ. Sci. Technol.* **1995**, *29*, 2504–2512.
(43) Lydersen, C.; Wolkers, H.; Severinsen, T.; Kleivane, L.; Nordøy, E. S.; Skaare, J. U. Blood is a poor substrate for monitoring pollution burdens in phocid seals. *Sci. Total Environ.* **2002**, *292*, 193–203.
(44) Boon, J. P. Immunochemical and catalytic characterization of hepatic microsomal cytochrome P450 in the sperm whale (*Physeter macrocephalus*). *Aquat. Toxicol.* **2001**, *52*, 297–309.
(45) Lewis, D. F.; Eddershaw, P. J.; Dickins, M.; Tarbit, M. H.; Goldfarb, P. S. Structural determinants of cytochrome P450 substrate specificity, binding affinity and catalytic rate. *Chem.−Biol. Interact.* **1998**, *115*, 175–199.
(46) Goksoyr, A.; Beyer, J.; Larsen, H. E. Cytochrome P450 in seals: Monooxygenase activities, immunochemical cross-reactions and response to phenobarbital treatment. *Mar. Environ. Res.* **1992**, *34*, 113–116.
(47) Verreault, J.; Maisonneuve, F.; Dietz, R.; Sonne, D.; Letcher, R. J. Comparative hepatic activity of xenobiotic-metabolizing enzymes and concentrations of organohalogens and their hydroxylate analogues in captive Greenland sledge dogs. *Environ. Toxicol. Chem.* **2008**, in press.
(48) Hoekstra, P. F.; Letcher, R. J.; O'Hara, T. M.; Backus, S. M.; Solomon, K. R.; Muir, D. C. G. Hydroxylated and methylsulfone-containing metabolites of polychlorinated biphenyls in the plasma and blubber of bowhead whales (*Balaena mysticetus*). *Environ. Toxicol. Chem.* **2003**, *22*, 2650–2658.
(49) Sandala, G. M.; Sonne-Hansen, C.; Dietz, R.; Muir, D. C. G.; Valters, K.; Bennet, E. R.; Born, E. W.; Letcher, R. J. Hydroxylated and methyl sulfone PCB metabolites in adipose and whole blood of polar bear (*Ursus maritimus*) from East Greenland. *Sci. Total Environ.* **2004**, *331*, 125–141.
(50) Verreault, J.; Letcher, R. J.; Muir, D. C.; Chu, S.; Gebbink, W. A.; Gabrielsen, G. W. New organochlorine contaminants and metabolites in plasma and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. *Environ. Toxicol. Chem.* **2005**, *24*, 2486–2499.
(51) Lans, M. C.; Klasson-Wehler, E.; Willemsen, M.; Meussen, E.; Safe, S.; Brouwer, A. Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. *Chem.−Biol. Interact.* **1993**, *88*, 7–21.
(52) McNabb, A. *Thyroid Hormones*; Prentice Hall: Englewood Cliffs, NJ, 1992.
(53) Brouwer, A.; van den Berg, K. J. Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. *Toxicol. Appl. Pharmacol.* **1986**, *85*, 301–12.
(54) Boas, M.; Feldt-Rasmussen, U.; Skakkebaek, N. E.; Main, K. M. Environmental chemicals and thyroid function. *Eur. J. Endocrinol.* **2006**, *154*, 599–611.

ES801682F