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Biotransformation versus Bioaccumulation: Sources of Methyl Sulfone PCB and 4,4′-DDE Metabolites in the Polar Bear Food Chain

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In the polar bear food chain from the Canadian Arctic, methyl sulfone ($MeSO_2$-) PCBs and 4,4′-DDE were below detection in arctic cod (<0.01 ng/g, lipid wt). Ringed seal blubber contained 3-$MeSO_2$-4,4′-DDE (0.4 ng/g) and 14 3- and 4-$MeSO_2$-PCB isomer pairs (∑$MeSO_2$-PCB, ca. 13 ng/g) formed by the biotransformation of PCBs not chlorine substituted at the meta−para positions on one ring (*m,p*-PCBs). Bioaccumulation/formation efficiencies relative to CB153 (BFE-) from cod to seal were 0.001−0.086 for $MeSO_2$-PCBs and 0.004 for 3-$MeSO_2$-4,4′-DDE. Twelve $MeSO_2$-PCB isomer pairs (∑$MeSO_2$-PCBs, 432 ± 57 ng/g) and 3-$MeSO_2$-4,4′-DDE (2.0 ± 0.7 ng/g) were identified in polar bear fat; BFE′ values were 0.03−0.62 and 0.0001 for $MeSO_2$-PCBs and 3-$MeSO_2$-4,4′-DDE, respectively. Methyl sulfone formation is important but not the major route for *m,p*-PCB and 4,4′-DDE biotransformation in polar bear and ringed seal. Fifteen $MeSO_2$-PCB congeners in the bear are likely bioaccumulated from seal relative to the completely bioaccumulated 3-/4-$MeSO_2$-CB132. Strong evidence exists for the partial formation of seven $MeSO_2$-PCBs in the bear. We conclude that $MeSO_2$-PCBs have high biomagnification potential in food chains.

Introduction

The polar bear (*Ursus maritimus*) is the most carnivorous of the bears. It is at trophic level 5, the highest among arctic mammals, and adapted to an ice habitat hunting ringed seal (*Phoca hispida*), the most abundant resident species of seal in the Arctic/sub-Arctic (*1, 2*). Arctic cod (*Boreogadus saida*) is a main prey item of ringed seal along with amphipods. The Arctic cod−ringed seal−polar bear food chain is better defined than most food chains in temperate or tropical marine ecosystems, where species diversity is higher and predator−prey relationships are more complex. Polar bears maximize their caloric intake by preferentially eating blubber and skin and thus are exposed to a high intake of PCBs, 4,4′-DDT, and 4,4′-DDE (*3*). Polar bears are unique among wild mammals and birds studied to date in the low bioaccumulation factors (BFs) of normally recalcitrant PCBs and 4,4′-DDE, presumably because of biotransformation (*4*).

Changes in the PCB congener composition between prey and predator are controlled by the capacity of the predator to depurate an exogenous compound, with or without metabolism. Polar bear fat has been shown to store persistent methyl sulfonyl-containing metabolites of meta−para (*m,p*-) chlorine unsubstituted PCBs ($MeSO_2$-PCBs) at 0.1−0.6 mg/kg lipid levels, which is ca. 5% of the ∑PCB concentrations (*5*−*7*). A $MeSO_2$ metabolite of 4,4′-DDE, 3-$MeSO_2$-4,4′-DDE, was also found at concentrations in the 0.001−0.01 mg/kg range (*6*). $MeSO_2$-PCB levels of 1.9 ± 0.7 mg/kg lipid measured in liver are higher than in fat (*7*). $MeSO_2$-PCBs and -4,4′-DDEs persist in other seal species, including ringed seal from the Baltic Sea (*8*). However, there is no known metabolite data for Arctic ringed seal.

The phase I metabolism of *m,p*-PCBs to form epoxide intermediates is mediated via cytochrome P450 (CYP) 2B-type enzymes mainly in the endoplasmic reticulum of the liver (*9*). Phase II conjugation of 3,4-epoxide PCB intermediates with the nucleophilic and mainly cytosolic glutathione (GSH) occurs with mediation by glutathione-*S*-transferase (GST). Subsequently, phase III reactions occurring via the mercapturic acid pathway, and further transformations occurring as a result of enterohepatic recirculation generate $MeSO_2$ metabolites (*10*). Epoxide degradation can also occur competitively via a hydrolytic pathway mediated by microsomal epoxide hydrolase (EH). Persistent 3- and 4-$MeSO_2$-PCB and 2- and 3-$MeSO_2$-4,4′-DDE metabolites appear to be sufficiently lipophilic and resist further metabolic degradation since these compounds are found in the tissues of a variety of organisms, including humans (*5*−*8, 11, 12*). To our knowledge, bioaccumulation of $MeSO_2$-PCB or -4,4′-DDE metabolites has not been demonstrated within any food chain. The simplicity of the polar bear food chain lends itself to studying the bioaccumulation potential of $MeSO_2$-PCB and -DDE metabolites, relative to endogenous metabolic formation from PCBs and DDE. In the present study, Arctic cod, ringed seal blubber, and polar bear fat taken from individuals of the Canadian high Arctic were analyzed for PCB, 4,4′-DDT, 4,4′-DDE, and $MeSO_2$ metabolite compounds. The relative importance of sulfone formation versus bioaccumulation from the diet was assessed in this food chain.

Experimental Section

Tissue Sampling. Polar bear fat samples were collected using a precise sampling protocol from six adult male polar bears (7−13 years of age) to minimize the effect of sex and age on the contaminant levels (*13*). Subcutaneous adipose tissue samples were taken from the base of the tail of freshly killed polar bears as part of the controlled Inuit hunt in the Resolute Bay area of the Canadian Arctic. The samples were taken within 3 days of each other at the end of April 1993 and stored at −40 °C until further use. The blubber samples of 11 ringed seals (i.e., five males and six females) and two pools of nine whole arctic cod were also collected from the same area and at the same time as for polar bear and stored at −20

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TABLE 1. Total DDT, 4,4′-DDE, PCB, and $MeSO_2$-PCB and -4,4′-DDE Levels (ng/g Lipid wt) in the Polar Bear Food Chain from the Canadian High Arctic[a]

species (year)	tissue[b]	*N*	∑DDT[c]	4,4′-DDE	∑PCB[d]	∑$MeSO_2$-PCB[e]	3-$MeSO_2$-4,4′-DDE[e]	∑$MeSO_2$-PCB/∑PCB ratio[e]	3-$MeSO_2$-4,4′-DDE/4,4′-DDE ratio[e]
arctic cod (1993)	whole body pools[f]	2	25.9/34.0	9.3/8.1	71.8/33.0	< 0.01/< 0.01	< 0.01/< 0.01	N/A/N/A	N/A/N/A
ringed seal (1993)	blubber (male)	5	542 ± 182	350 ± 130	447 ± 92	13.4 ± 6.3	0.4 ± 0.2	0.029 ± 0.0081	0.0012 ± 0.0004
	blubber (female)	6	474 ± 88	322 ± 62	387 ± 73	12.3 ± 1.8	0.4 ± 0.1	0.034 ± 0.0082	0.0011 ± 0.0003
polar bear (1993)	fat	6	301 ± 32	268 ± 27	6207 ± 948	432 ± 57	2.0 ± 0.7	0.061 ± 0.006	0.008 ± 0.002

[a] The concentrations are the arithmetic mean ± SD. [b] The percent lipid was 6.5/7.0% in Arctic cod, 90.1 ± 6.3% in ringed seal blubber, and 65.0 ± 3.2% in polar bear fat (*17*). [c] The ∑DDT includes 4,4′-DDT, 4,4′-DDD, and 4,4′-DDE. [d] Fourty-seven and 53 PCB congeners were above the detection limit in whole Arctic cod and ringed seal blubber, respectively, and 22 PCB congeners in polar bear. [e] Using an ANOVA and a two-tailed *t*-test, there was no significant ($p > 0.05$) difference between the levels for ringed seal males and females. [f] There were two pools, each containing nine individual cod. Levels for both pools are shown.

°C or lower until further use. The ringed seals were adults (5−8 years of age), except for two juvenile females (<2 years of age). The Arctic cod pools were comprised of a random sampling of adult fish (ca. 3 years of age) caught by gill netting.

Chemicals and Standards. The $MeSO_2$-PCB chemical name (*11*) has been abbreviated and simplified from the IUPAC-derived numbering system of PCBs (Figure 1) (*14*). Authentic standards of 25 $MeSO_2$-PCB congeners and 3-$MeSO_2$-4,4′-DDE were used to identify and quantify metabolite levels *(11)*. The compound 3-$MeSO_2$-2-CH_3-2′,3′,4′,5,5′-Cl_5CB ($MeSO_2$-IS, 100 pg/μL) was used as the $MeSO_2$-PCB and -4,4′-DDE internal standard. An Aroclor 1:1:1 (A1242:A1254:A1260) secondary external standard mixture was prepared in toluene and used to quantify the PCBs in arctic cod and ringed seal. The quantitative composition of the PCB mixture (72 congeners) was determined using standard mixtures A−C and D of CLB-1 (National Research Council of Canada, 24 congeners), CB99 (Ultra Scientific), and determination by GC/FID (*11*).

Tissue Extraction, Contaminant Enrichment, and Chemical Analysis. Stable isotope labeled $^{13}C_{12}$ internal standards of CB28, CB52, CB118, CB153, CB180, and CB194 (CIL, Andover, MA) were spiked to the samples. CB154 is not found in Aroclor PCB technical mixtures and was therefore used as a performance standard. Polar bear fat samples of 1.0 g, ringed seal blubber samples of ca. 2.0 g, and whole pooled samples of Arctic cod (10 and 40 g) were extracted in anticipation of the native contaminant levels (*4, 15*). The PCB/DDT/$MeSO_2$ metabolite extraction, separation, and GC/ECNI-MS analysis of the $MeSO_2$-PCB/-DDE fraction has been described elsewhere (*11, 16*).

The PCB and DDTs in polar bear were quantified using GC/EI-MS and a secondary standard prepared from a cleaned-up polar bear fat extract and analyzed by GC/FID (*13*). Only 20 PCB congeners were found above the detection limit (0.05 ng/g lipid wt). The PCB/DDE fractions from seal blubber and cod were analyzed by a slightly modified approach from that of polar bears (*13, 17*). Separate injections using PCB and DDT external standard mixtures were used for quantitation. Coelution can occur for *m,p*-PCBs with other PCB congeners of the same isomer group, depending on the GC column used (*18*). In this study, coelution of *m,p*-PCB congeners occurred for CB28/CB31 and CB70/CB76. CB132 was well resolved from other congeners but has been shown to coelute with CB153 and CB105 (*18*). Of the 72 PCB congeners in the standard, 47 in cod and 53 in ringed seal were above the detection limit. PCB and $MeSO_2$-IS recoveries were >85% and ca. 90%, respectively. All concentrations were normalized to the extractable lipid. The PCB/DDT concentrations were recovery-corrected using the average recovery of the five higher chlorinated $^{13}C_{12}$-labeled PCB standards.

Assessment of Bioaccumulation and Biotransformation. The recalcitrant congeners CB153 (2,2′,4,4′,5,5′) and CB180 (2,2′,3,4,4′,5,5′) are resistant to biotransformation in many organisms (*4, 15*). They possess chlorine substitution patterns that are not favored by CYP1A- and CYP2B-type enzymes because of the lack of adjacent, chlorine-unsubstituted carbons (*19*). Analyte concentrations were normalized to CB153 (C′) to minimize the variation among individuals and species (*19, 20*). For example, $C'_x = C_x/C_{153}$ and $C'_{mx} = C_{mx}/C_{153}$, where *x* denotes the PCB congener and m*x* denotes the $MeSO_2$-PCB congener of PCB *x*.

Uptake from prey of most of the PCBs (except octachloro- and nonachloro-PCBs), 4,4′-DDE, 4,4′-DDT, and their $MeSO_2$ metabolites is likely to be similar for a given species because these compounds have log K_{ow} values of 4−7 (*21*). Log K_{ow} values for several trichloro- to heptachloro-$MeSO_2$-PCBs and 3-$MeSO_2$-4,4′-DDE were computer modeled (Advanced Chemistry Development (ACD) Labs, Toronto, Canada) and ranged from 3.82 to 6.51. Therefore, BF′ (i.e., $= C'_{predator}/C'_{prey}$) is a good measure of bioaccumulation. Assuming that CB153 represents the maximum bioaccumulation potential for slowly metabolized lipophilic compounds, BF′ for such compounds should be ≈1.0. A BF′ of <1.0 is indicative that some biotransformation is occurring. The fraction of compound metabolized at each step in the food chain can be estimated from BF′. Thus, the fraction of congener *x* taken up from the diet that is metabolized by ringed seal and polar bear, or the "metabolized fraction" is $MF'_{x(seal)} = (1\text{-}BF'_{x(seal)})$ and $MF'_{x(bear)} = (1\text{-}BF'_{x(bear)})$, respectively. Assuming that there is no direct bioaccumulation of metabolites from the diet, the maximum concentration of metabolites of *x* that can accumulate in seal and bear is $MF'_{x(seal)} \cdot C_{153(seal)}$ and $MF'_{x(bear)} \cdot C_{153(bear)}$, respectively.

Bioaccumulation of $MeSO_2$ metabolites (or any other compound) can be treated in a similar way to that of PCB congeners. However, formation of metabolite from biotransformation of precursor PCBs may also occur. $C'_{mx(seal)}/C'_{mx(cod)}$ and $C'_{mx(bear)}/C'_{mx(seal)}$, or the apparent bioaccumulation factors (AF′), represent a combination of bioaccumulation and metabolic formation and may be >1.0. The degree to which definitive conclusions can be made on the relative importance of these two processes from ratios of CB153-normalized concentrations depends on several factors. The following three general classes can be defined for bioaccumulation of $MeSO_2$-PCB metabolites from prey to predator and can be used for any metabolite with formation potential from remaining precursor compounds that bioaccumulate in a predator.

Class I. The precursor PCB congener is not present in the prey, but the metabolite is found in the prey and predator. Therefore, $C'_{mx(seal)}/C'_{mx(cod)}$ and $C'_{mx(bear)}/C'_{mx(seal)}$ would be the true bioaccumulation factors, BF'_{mx}, of the metabolite

relative to CB153 in seal and bear, respectively. If there are sufficient numbers of $MeSO_2$-PCBs for which this class applies, it may be possible to develop a general relationship for prediction of BF'_{mx} for those congeners that are metabolized slowly.

Class II. The metabolite is not present in the prey but is present in the predator. C'_{mx} in the predator is entirely due to metabolic formation from precursor PCB in the prey, and BF'_{mx} has no meaning. Thus, the *x* fraction metabolized to m*x* and retained in the predator is $MF'_{mx} = C'_{mx}/M'_x$.

Class III. Both metabolite and precursor PCB are found in the prey. If the apparent metabolite bioaccumulation factor, $AF' = C'_{mx(predator)}/C'_{mx(prey)}$, in the predator is higher than the BF'_{mx} predicted from class I, both bioaccumulation and metabolic formation are probably contributing to the observed metabolite levels in the predator, but bioaccumulation alone may be occurring up to $AF' = 1.0$. Assuming that BF'_{mx} can be estimated from class I rules, the fraction of C'_{mx} that was formed by metabolic conversion of precursor PCB is $(1- (BF'_{mx}/AF'_{mx}))$, and the fraction of *x* metabolized to m*x* and retained in the predator is $MF'_{mx} = (AF'_{mx} - BF'_{mx})/M'_x$. If the AF'_{mx} in the predator is significantly >1.0, metabolic formation in the predator is indicated. In this case, $(1-1/AF'_{mx})$ is the minimum m*x* fraction in the predator that was formed by precursor PCB metabolism in the predator, because the metabolite bioaccumulation potential is unlikely to be higher than that of CB153.

Another useful parameter that can be calculated is the bioaccumulation/formation efficiency, $BFE'_{predator} = (C'_{mx})_{predator}/[(C'_{mx})_{prey} + (MF'_{mx})_{predator}]$. The maximum BFE′ is ca. 1.0, which is when the bioaccumulation efficiency of the metabolite and CB153 are the same and the PCB precursors from the prey are completely biotransformed to methyl sulfone metabolites in the predator. The situation is more complicated for $MeSO_2$-4,4′-DDE since 4,4′-DDE itself is a metabolite of 4,4′-DDT and may also be bioaccumulated or formed at each trophic level.

Results and Discussion

The aryl methyl sulfone fraction isolated from polar bear fat (Figure 1A) and ringed seal blubber (Figure 1B) contained structures of persistent $MeSO_2$-PCB congeners consistent with other biota (*5*−*8*, *11*). That is, the $MeSO_2$-PCBs are (i) 3- and 4-$MeSO_2$-substituted and occur in pairs derived from the same *m*,*p*-PCB precursor, (ii) trichloro- to heptachloro-substituted, and (iii) 2,5-dichloro- or 2,5,6-trichloro-substituted on the $MeSO_2$-containing phenyl ring. In polar bear, all congeners possess at least a 4′-chlorine on the non-$MeSO_2$-containing phenyl ring. The minimum requirement of 2,5-dichloro-substitution on persistent $MeSO_2$-PCB congeners may be due to the 3,4-epoxide stabilization and thus partial survival from EH-mediated hydrolysis. Either 4′- or 3′,5′-chlorine substitution is required in animals with high CYP2B-type activity (birds and terrestrial mammals (*19*, *22*)) to hinder a secondary epoxide formation at the 3′,4′-position of the $MeSO_2$-PCB. Applying these rules to the PCB commercial mixtures that were produced in the highest quantities (Aroclors 1242, 1254, and 1260 and their equivalents manufactured in Europe and Japan (*23*)), only 12−17 congeners are likely to be biotransformed into $MeSO_2$-PCBs. There are an additional maximum of seven PCBs that could form 3-/4-$MeSO_2$-PCBs if PCBs with 2,5-dichloro- or 2,5,6-trichloro- and not 4′-chlorine substitution are considered. The 14 3-/4-$MeSO_2$-PCB congener pairs identified so far in biota, including those reported here, likely represent the majority of the $MeSO_2$-PCB metabolites formed.

$MeSO_2$-PCB or -4,4′-DDE metabolites were below detection (<0.01 ng/g) in arctic cod (Table 1). Of the 28 $MeSO_2$-PCB congeners identified in seal blubber, the trichloro- to hexachloro-substituted congeners 4-$MeSO_2$-CB31, 3- and

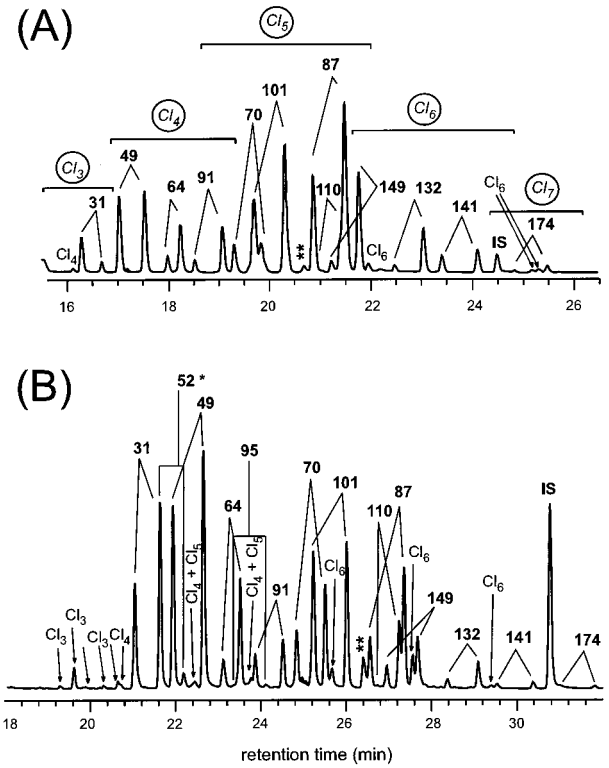

FIGURE 1. Chromatograms of the aryl methyl sulfone fraction obtained from (A) polar bear fat using GC/ECD and (B) ringed seal blubber using GC/ECNI-MS (SIM). With the exception of a few minor peaks, all peaks were $MeSO_2$-PCBs and -DDEs. The number labels refer to the 3- and 4-$MeSO_2$-PCB pairs. For example, 31 refers to 3- and 4-$MeSO_2$-CB31. The asterisk in panel B denotes that 3-$MeSO_2$-CB52 is a minor contributor with coeluting 4-$MeSO_2$-CB31. The Cl_x denotes a $MeSO_2$-PCB isomer identified by GC/ECNI-MS (TIC) (*16*). The double asterisk denotes the 3-$MeSO_2$-4,4′-DDE peak.

4-$MeSO_2$-CB49, 4-$MeSO_2$-CB64, 4-$MeSO_2$-CB87, 3- and 4-$MeSO_2$-CB101, and 4-$MeSO_2$-CB149 accounted for ca. 65% of the $\sum MeSO_2$-PCBs (Figure 1B, Table 1) with levels ca. >1 ng/g but <2 ng/g (*17*, *24*). Twelve unidentified minor trichloro- to hexachloro-$MeSO_2$-PCB isomers were also detected. These $MeSO_2$-PCB metabolites may be from PCB congeners with 2,5-dichloro- or 2,5,6-trichloro-substitution but without 4′-chlorine substitution (e.g., CB24, CB44, CB53, CB59, CB135, and CB136). These PCBs contribute in the range of ca. 0.1−4.0% to the composition of Aroclors 1242, 1254, and 1260 (*23*). In Baltic grey seal blubber (*8*), ca. 80% of the $\sum MeSO_2$-PCB was dominated by 3- and 4-$MeSO_2$-CB87, -CB101, and -CB149, and $\sum MeSO_2$-PCB levels were about 2 orders of magnitude higher than Canadian arctic ringed seals (Table 1). Until the present study, the identity of 4-$MeSO_2$-CB31 and 3-$MeSO_2$-CB95 had not been confirmed in biota (*17*).

The $\sum MeSO_2$-PCB to $\sum$PCB ratio for ringed seal was relatively invariant (Table 1), whereas the ratio has been shown to vary widely from ca. 0.01 to ca. 0.15 among Baltic seals (*8*). Along with differences in the bioavailable PCB patterns from the diet, greater CYP2B-type enzyme induction by appropriate contaminants in Baltic seals may partly explain the difference in the $MeSO_2$-PCB patterns between the Baltic and the Canadian Arctic seals populations. For example, $\sum$PCB levels in Baltic seals ranged from 15 to 2100 μg/g, whereas a range of 0.4−1.0 μg/g exists in Canadian arctic ringed seals (*3*, *4*, *8*, *15*).

Twenty-four $MeSO_2$-PCB congeners were quantified, and only four unidentified congeners were detected in polar bear fat (Figure 1A). The 3- and 4-$MeSO_2$-CB87 and -CB101 and

TABLE 2. Apparent Bioaccumulation Factors (AFs) for the Polar Bear Food Chain[a]

compound	seal blubber/cod	bear fat/seal blubber
∑DDT	17.1	0.6
4,4′-DDT	5.3	0.2
4,4′-DDE	38.5	0.8
∑PCB	7.8	15.1
CB153	26.6	47.3
∑$MeSO_2$-PCB	N/A	27.8 (148)[b]
3-$MeSO_2$-4,4′-DDE	N/A	5.4 (512)[b]

[a] The ratios represents the tissue concentration at one food chain level to the next lowest level (on a lipid weight basis). In the case of CB153, AF = BF, the true bioaccumulation factor. [b] The number in parentheses is the ratio of seal blubber to polar bear liver (*7*).

TABLE 3. Bioaccumulation/Formation Efficiency (BFE′) of 4,4′-DDE and $MeSO_2$-4,4′-DDE in the Polar Bear Food Chain from the Canadian High Arctic

	cod to seal blubber[b]	seal blubber to bear fat[b,c]
BFE'_{DDE}[a]	0.59 ± 0.22	0.012 ± 0.007 (0.008)
BFE'_{mDDE}[a,d]	0.004 ± 0.001	0.00010 ± 0.00007 (0.005)

[a] The BFE′ is based on ratios to CB153 (see the Experimental Section). [b] The BFE′ are the arithmetic mean values ± SD. [c] The number in parentheses is the BFE′ of seal blubber to bear liver. The amount of $MeSO_2$-4,4′-DDE in bear liver was ca. 40% relative to the total amount in bear fat (*17*). [d] Only 3-$MeSO_2$-4,4′-DDE was detected in ringed seal and polar bear fat, whereas 2-$MeSO_2$-4,4′-DDE was also detected in polar bear liver (*17*).

4-$MeSO_2$-CB149 congeners accounted for ca. 53% of the ∑$MeSO_2$-PCBs (Table 1), similar to previous findings (*5*, *6*, *11*). The 3-$MeSO_2$-4,4′-DDE level in bear fat was only marginally higher than in ringed seal. The majority of the individual $MeSO_2$-PCB levels were >10 ng/g, but not exceeding ca. 65 ng/g, and were significantly higher than the 0.02−1.62 ng/g range found in ringed seal blubber (*17*, *24*). The $MeSO_2$-PCB metabolite patterns in seal blubber (Figure 1B) and bear fat (Figure 1A) were similar in composition; however, there were fewer dominant $MeSO_2$-PCBs in polar bear. The ∑$MeSO_2$-PCB to ∑PCB ratio in polar bear was only 2-fold higher than ringed seal, despite a 32-fold higher level of ∑$MeSO_2$-PCBs (Table 1), and the absence of *m*,*p*-PCB precursors. The ∑$MeSO_2$-PCBs in polar bear liver (*7*) were 4−8-fold higher than in fat. In other biota, $MeSO_2$-PCB and -4,4′-DDE metabolites are known to be preferentially stored in the liver relative to adipose tissue (*5*, *11*, *17*).

Sources of $MeSO_2$-PCBs and -4,4′-DDE in the Arctic Food Chain. The phase I metabolism of PCBs in mammals is dependent on the induction and activity of CYP2B-, CYP1A1-, and possibly CYP3A-type isozymes, all of which vary among species (*19*, *22*, *25*). The activities of phase II and III enzymatic pathways leading to $MeSO_2$ metabolites are also species variable.

In comparison to recalcitrant CB153 (*19*), the AFs for ∑DDT, 4,4′-DDT and 4,4′-DDE were >1 from cod to seal, indicating high bioaccumulation potential in seal (Table 2). However, significant metabolism in bear was indicated by values <1 from seal to bear (*4*), whereas the AF for 3-$MeSO_2$-4,4′-DDE was ca. 7 times higher than that of 4,4′-DDE. The AF for ∑$MeSO_2$-PCB from seal to bear was approximately half that of CB153. These results indicated a high bioaccumulation potential for the $MeSO_2$ metabolites in bear. The lack of detectable 3-$MeSO_2$-4,4′-DDE in Arctic cod indicated total formation in seal from 4,4′-DDE. However, other dietary sources for seal were not taken into account. Ringed seals may periodically feed heavily on invertebrates (*2*) but are an unlikely source of $MeSO_2$-DDE or -PCBs since CYP-mediated metabolic capacities in invertebrates are known to be very low and substantially lower than in most fish species (*26*).

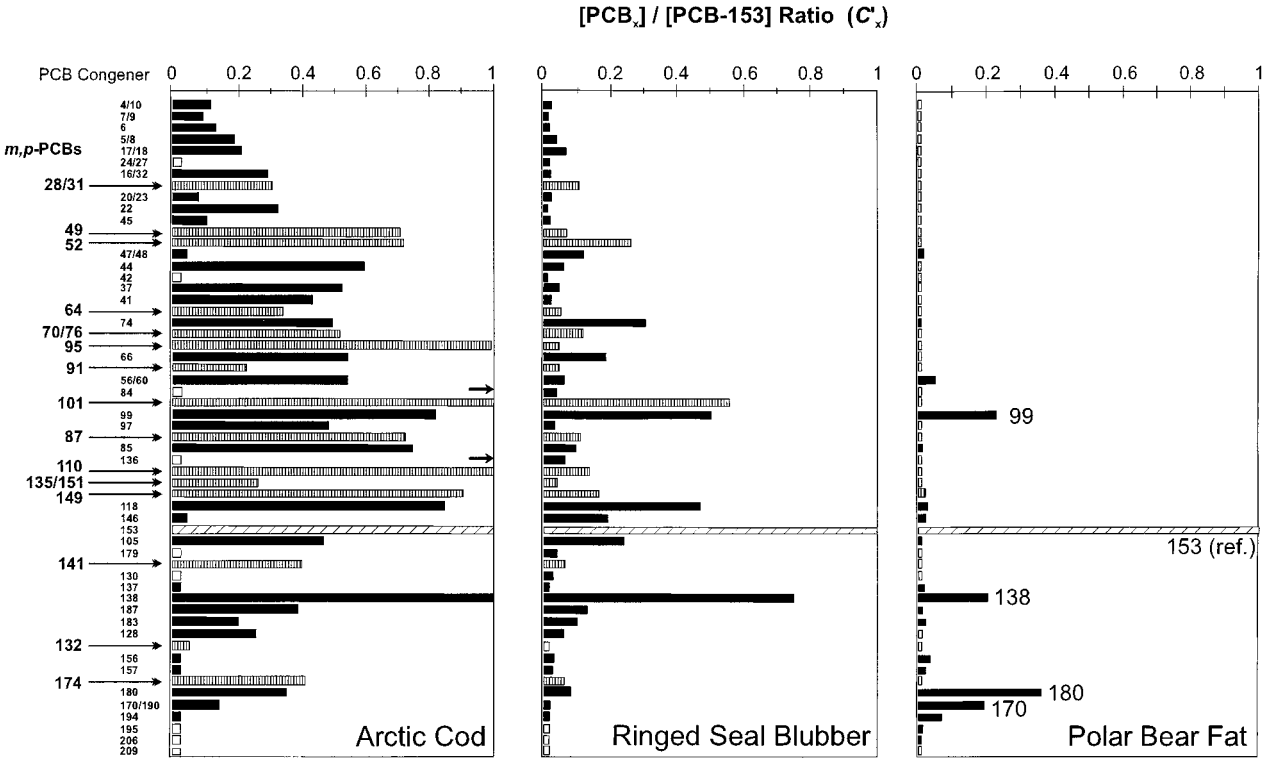

FIGURE 2. Ratios of PCB congener to CB153 concentrations (C'_x) in arctic cod, ringed seal blubber, and polar bear fat from the Resolute Bay area of the Canadian high Arctic. The clear boxes on the baseline denote congeners that were below detection. Lined bars and bold numbers indicate the PCB congeners that form persistent $MeSO_2$-PCB metabolites. The CB153 bar is crosshatched for reference.

TABLE 4. Bioaccumulation/Formation Efficiency (BFE′) of $MeSO_2$-PCBs in the Polar Bear Food Chain from the Canadian High Arctic[a]

$MeSO_2$-PCB congener[b]	BFE′ $_{mx}$ arctic cod to ringed seal[c]	BFE′ $_{mx}$ ringed seal to polar bear[d]
CB174	0.006 ± 0.004	0.019 ± 0.014*
CB149	0.019 ± 0.009	0.131 ± 0.051*
CB141	0.008 ± 0.004	0.091 ± 0.120*
CB132	0.036 ± 0.027	0.617 ± 0.105*
CB110	0.007 ± 0.004	0.026 ± 0.012*
CB101	0.029 ± 0.024	0.051 ± 0.010
CB91	0.045 ± 0.024	0.244 ± 0.088*
CB95	0.001 ± 0.001	nd (in bear)
CB87	0.035 ± 0.017	0.231 ± 0.103*
CB70	0.031 ± 0.021	0.073 ± 0.028
CB64	0.042 ± 0.030	0.120 ± 0.100
CB52	0.002 ± 0.002	nd (in bear)
CB49	0.075 ± 0.036	0.097 ± 0.041
CB31	0.086 ± 0.048	0.117 ± 0.057

[a] The BFE′ $_{mx}$ are the arithmetic mean values ± SD. Using an ANOVA with a two tailed *t*-test, the asterisk indicates a significantly higher ($p < 0.05$) BFE′ $_{mx}$ for seal to bear than for cod to seal. [b] The sum of each 3- and 4-$MeSO_2$-PCB pair. [c] In seal, MF′ $_{mx}$ = AF′ $_{mx}$ (see Experimental Section) since there is no accumulation of $MeSO_2$-PCBs from cod to seal. [d] Polar bear fat is considered the primary $MeSO_2$-PCB storage tissue. Individual $MeSO_2$-PCB levels in bear liver were less than ca. 5% relative to the total amount in bear fat (*17*).

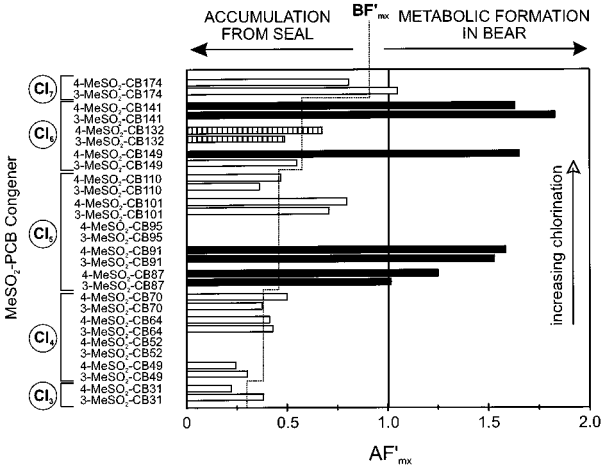

FIGURE 3. Apparent bioaccumulation factors (AF′$_{mx}$ = C′$_{mx(bear)}$ to C′$_{mx(seal)}$ ratio) for individual $MeSO_2$-PCBs in the fat of polar bear from the Resolute Bay region of the Canadian high Arctic. The expected mean BF′$_{mx}$ values are represented by the stepped, dashed line for the bioaccumulation-only class I (empirical linear regression model, BF′$_{mx}$ = 0.148 (number of Cl in $MeSO_2$-PCB) − 0.161, r^2 = 0.601, $p < 0.002$), for each Cl_x $MeSO_2$-PCB isomer group (white bars). The AF′$_{mx}$ fraction above the solid line is the minimum amount of $MeSO_2$-PCB formed in bear. The 3- and 4-$MeSO_2$-CB132 in bear are completely accumulated from seal (lined bars).

The relatively high BFE′$_{DDE}$ from cod to seal (Table 3) assumes that 100% of 4,4′-DDE in cod relative to CB153 is accumulated in seal. Therefore, 0.42 (70%) of the BFE′$_{DDE}$ can be explained by 4,4′-DDE accumulation in seal. The remaining 0.17 of the BFE′$_{DDE}$ is due to 4,4′-DDE formation in seal from 4,4′-DDT accumulated from cod. This represents a formation efficiency of 4,4′-DDE in seal from 4,4′-DDT in cod of 41% (1-BFE′$_{DDE}$ = 0.41). A proportion of 4,4′-DDE in seal arises from 4,4′-DDT metabolism in seal, and thus interpretation of the BFE′$_{mDDE}$ as the proportion of 4,4′-DDE metabolism is difficult. Nevertheless, the low BFE′$_{mDDE}$ for cod to seal suggested that $MeSO_2$-4,4′-DDE formation in seal is not an important metabolic route for 4,4′-DDE. The <0.1 BFE′$_{DDE}$ value for seal blubber to bear liver and fat reflected the high capacity of polar bear to metabolize both 4,4′-DDT and 4,4′-DDE. An even lower BFE′$_{mDDE}$ (i.e., ≤0.01) for both bear fat and liver suggested, as with seal, that $MeSO_2$-4,4′-DDE formation in bear is not the predominant metabolic pathway for 4,4′-DDE. Most of the primary metabolism of 4,4′-DDE in seal and bear may be ortho-meta ring hydroxylation or attack on the ethylene functional group. Ringed seal and polar bear may also further metabolize $MeSO_2$-4,4′-DDE and/or sequester it in tissues such as the adrenal cortex (*5*).

On a PCB congener-specific basis, the majority of the C′$_x$ values decreased going from cod to seal blubber to polar bear fat, revealing an increasingly simplified pattern, especially for *m*,*p*-PCBs (Figure 2). There was little difference in the C′$_x$ values in cod relative to the Aroclor standard (1:1:1, A1242:A1254:A1260, not shown), indicating low activity of the CYP enzyme system characteristic of fish relative to mammals and birds (*19*, *26*). Like other seal species, and to a lesser extent cetaceans, Arctic ringed seals (Figure 2) had a lower CYP2B-type capacity, which is necessary to metabolize *m*,*p*-PCBs, relative to the terrestrial polar bear. The MF′$_{x(seal)}$ of *m*,*p*-PCBs (not shown) indicated that ca. 75−95% of the *m*,*p*-PCBs were metabolized by ringed seal. CB132 was below detection and was therefore completely metabolized in seal, consistent with observations in eastern Hudson Bay ringed seals (*18*). The *m*,*p*-PCBs were below detection in polar bear, except for traces of CB149 (*4*).

The variable BFE′$_{mx}$ values in seal (Table 4) indicated that $MeSO_2$-PCB formation represented <10% of the biotransformation of *m*,*p*-PCBs in seals, assuming that $MeSO_2$-PCBs are cleared at a similarly slow rate as CB153. Thus, all the $MeSO_2$-PCBs in seal are class II since $MeSO_2$-PCBs were below detection in cod. The BFE′$_{mx}$ for polar bear tended to be higher than in seal. At one extreme, the BFE′$_{m132}$ of 3- plus 4-$MeSO_2$-CB132, the metabolite pair for which only bioaccumulation in bear was possible, was highest. In contrast, there were nondetectable 3- and 4-$MeSO_2$-PCBs of CB52 and CB95, and thus no BFE′$_{mx}$ for bear. $MeSO_2$-CB52 and -CB95 possess a second meta−para chlorine-unsubstituted position on the non-$MeSO_2$-substituted phenyl ring and are further metabolized by CYP2B-type mediation in bears. Bis(sulfones) with the same degree of chlorination were detected in polar bear liver by GC/ECNI-MS (TIC) (not shown). Therefore, the combined efficiency of bioaccumulation plus formation in the polar bear is variable among congeners.

An alternative approach to compare the ratio of CB153-normalized $MeSO_2$-PCB concentrations in bear/seal was to generate the AF′$_{mx}$ values (Figure 3) to define the relative importance of $MeSO_2$-PCB bioaccumulation and formation in bear. A similar approach has been applied elsewhere to evaluate the biotransformation of PCBs in fish-eating predators (*19*, *20*). However, the complexity of factors affecting the $MeSO_2$-PCB bioaccumulative behavior is greater than that of PCBs.

Most $MeSO_2$-PCBs in polar bears probably fall into class III, that is, bioaccumulation directly from seal occurs to some extent (or solely), but there is a variable contribution from formation in the bear. The 3- and 4-$MeSO_2$-CB132 are the lone metabolites that can be confirmed to be in class I, and AF′$_{m132}$ is a true BF′$_{m132}$ (Figure 3) and AF′$_{m132}$ = BFE′$_{m132}$ (Table 4). Thus, values of AF′$_{mx}$ around 0.6 define the expected BF′$_{mx}$ for readily bioaccumulated $MeSO_2$-PCBs. Seventeen $MeSO_2$-PCBs, including 3- and 4-$MeSO_2$-CB132 had AF′$_{mx}$ values in the 0−1.0 range that appeared to increase with the degree of chlorination and fit into a linear empirical model for BF′$_{mx}$:

$$BF'_{mx} = 0.148 \text{ (number of Cl in } MeSO_2\text{-PCB)} - 0.161 \quad (r^2 = 0.601,\ p < 0.002) \quad (1)$$

This is a plausible bioaccumulation-only model (class I) because it fits two-thirds of the congeners, including 3- and 4-$MeSO_2$-CB132 (Figure 3). For class III (i.e., $AF'_{mx} > 1.0$) 3 and 4-$MeSO_2$-CB87, -CB91 and -CB141, and 4-$MeSO_2$-CB149, the AF'_{mx} were greater than predicted by the model. At least that portion of AF'_{mx} that falls above 1.0 must be formed by precursor *m*,*p*-PCB biotransformation in bear, since it is unlikely that biomagnification is greater than CB153. With respect to $MeSO_2$-PCB pairs, the 4-$MeSO_2$-CB149 had an AF'_{mx} 3 times that of 3-$MeSO_2$-CB149 with an AF'_{mx} close to that for bioaccumulation only. Along with other congeners (e.g., $MeSO_2$-CB64 and -CB87), a similar 3-/4-$MeSO_2$-CB149 accumulation asymmetry was observed in seal (Figure 1B), which is indicative of selective excretion of 3-$MeSO_2$−CB149 or formation of 4-$MeSO_2$-CB149.

In conclusion, the presence of $MeSO_2$-PCBs and DDEs in biota was previously thought to be due solely to metabolite formation in each species. However, we have shown that the factors contributing to the levels of persistent congeners are clearly complex. Bioaccumulation may account for the majority of the $MeSO_2$-PCBs found in polar bear, since only seven of 24 identified congeners showed clear indications of being partly formed in bear. The implications of these findings are considerable for PCB and DDE toxicological assessments. Where mammals are part of the food chain, bioaccumulation of persistent $MeSO_2$ metabolites as well as PCBs and DDE may occur.

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