The Bioaccumulation and Metabolic Formation of Methyl Sulfone PCB and 4,4'-DDE Metabolites in the Polar Bear Food Chain

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1. Introduction

It has been well documented that chlorinated hydrocarbon contaminants (CHCs) bioaccumulate and biomagnify in Arctic biota [1]. However, the patterns of residual CHCs in fatty tissue have been shown to vary among Arctic species, including DDTs and PCBs [2]. Changes in the CHC patterns among Arctic species results from contaminant clearance which is largely a function of metabolism, and occurs mainly in the liver [2]. Polar bears in particular demonstrate a unique ability to metabolize normally recalcitrant PCB congeners and 4,4'-DDE, a metabolite of the insecticide 4,4'-DDT [3].

A lipophilic CHC metabolite may bioaccumulate in fatty tissue if certain criteria are met such as limited sites of metabolic attack on the molecule and the introduction of metabolically stable functional groups. Metabolites of CHCs that are known to bioaccumulate in food chains include the epoxide metabolite of *cis*- and *trans*-chlordane, oxychlordane, and 4,4'-DDE [1]. Moreover, 3- and 4-methyl sulfone ($MeSO_2$-) PCB and 2- and 3-$MeSO_2$-4,4'-DDE metabolites are lipophilic and resistant to further metabolic degradation since they have been shown to persist in the tissues of birds, fish, molluscs and mammals, including humans [4-8]. However, no studies have been reported that demonstrate that $MeSO_2$-PCB or -4,4'-DDE metabolites bioaccumulate within any food chain.

The glutathione (GSH) conjugation reaction with electrophilic compounds is a detoxification reaction common in the defence of mammals, fish, insects, higher plants, and microorganisms against oxidative stress. *Meta-para* PCB epoxides are electrophilic intermediates resulting from cytochrome P450 (CYP) 2B-type mediated biotransformation, and are subsequently degraded to $MeSO_2$-PCB metabolites via the mercapturic acid (MAP) pathway [9]. Nevertheless, the process of sulfone formation is one of several possible metabolic pathways by which PCBs can degrade, including the formation of hydroxylated metabolites [10].

In the present study, whole arctic cod, ringed seal blubber and polar bear fat are analysed for $MeSO_2$-PCBs and -4,4'-DDEs and their precursor PCB and 4,4'-DDE compounds, to determine if the sulfone metabolites bioaccumulate in the polar bear food chain. The relative contributions of metabolic formation, accumulation from the diet, and clearance, to the observed metabolite levels in each species of the food chain was evaluated. Further, the importance of *meta-para*/chlorine unsubstituted PCB and 4,4'-DDE biotransformation leading to sulfone metabolites relative to other metabolic pathways was assessed.

2. Methods

Six adipose tissues were obtained from adult male polar bears using a precise sampling protocol (Dr. Malcolm Ramsay , U. of Saskatchewan). The samples were obtained from freshly-killed individuals within three days of each other at the end of April, 1993 by Inuit hunters in the Resolute Bay area of the Canadian Arctic. Adult male polar bears were chosen to minimize the effect of sex and age on the food chain level comparisons. Blubber samples of eleven ringed seals (i.e., five males and six females) and two pools of nine arctic cod each, were also collected from the Resolute Bay area. Seal ages were unknown, except for two juvenile females and adult status for 3 males.

The tissues were spiked with a mixture of a fully ^{13}C-labeled standard mixture of tetra-, penta-, and hexa-ClBz, and CB-28, CB-52, CB-118, CB-153, CB-180 and CB-194. Just prior to GC/EI-MS analysis, CB-154 normalization standard was added. Tissues were also spiked with $MeSO_2$-IS (purchased from Dr. Åke Bergman, Wallenberg Laboratory, Stockholm University, Sweden). The extraction and sample clean-up procedure has been described in detail elsewhere [8]. The $MeSO_2$-PCB and -4,4'-DDE compound levels were determined using a GC/ECD or GC/ECNI-MS internal standard method based on the $MeSO_2$-IS, and their identities confirmed using GC/ECNI-MS. For the polar bear tissue fractions, the PCBs and 4,4'-DDE, were determined by a single fraction, single injection GC/MSD approach using a characterized polar bear adipose tissue extract as a secondary standard. The PCBs and 4,4'-DDE in ringed seal and arctic cod tissue samples were determined by a single fraction, double injection GC/EI-MS approach using two external standard mixtures. The PCB and 4,4'-DDE levels were recovery-corrected using the average recovery of the five higher chlorinated ^{13}C-labeled PCB standards (i.e., CB-52, CB-118, CB-153, CB-180 and CB-194). The $MeSO_2$-PCB chemical names were abbreviated and simplified (Figures 1) on the basis of the IUPAC derived numbering system of the parent PCBs [8].

3. Results and Discussion

The biomagnification factors (BMFs) of the major CHC classes from cod to seal and seal to bear (Table 1) are indicative of the changes in the CHC composition. For example, the BMFs for Σ-DDT and 4,4'-DDE were less than one from seal to bear, likely a consequence of biotransformation. The high BMF for CB-153 from cod to seal and seal to bear was consistent with the resistance of CB-153 to metabolic degradation by either CYP1A or CYP2B-type enzymes [11].

Table 1. Biomagnification Factors (BMFs) for the Polar Bear Food Chain[†]

compound	cod to seal blubber	seal blubber to bear fat
Σ-HCH	1.4	1.6
Σ-ClBz	0.1	16.7
Σ-CHLOR	4.0	9.2
Σ-DDT[‡]	17.1	0.6
4,4'-DDE	38.5	0.8
Σ-PCB	7.8	15.1
Σ-$MeSO_2$-PCB	N/A	29.7
Σ-$MeSO_2$-4,4'-DDE	N/A	5.4
CB-153	26.6	47.3

[†] Ratio of tissue concentration at one food chain level to those at the next lowest level (lipid weight).
[‡] The sum of 4,4'-DDT, 4,4'-DDD and 4,4'-DDE.
N/A = not applicable.

Therefore, to better observe 4,4'-DDE and 4,4'-DDT metabolism, the concentrations were converted to ratios to CB-153 ($(R^{153})_{species}$) [3,11,12]. The BMF of 4,4'-DDE from cod to seal was about two times the BMF of Σ-DDT, probably due to metabolic conversion of 4,4'-DDT to 4,4'-DDE.

The ratios of DDTs to CB-153 were used to calculate the maximum ratio of 4,4'-DDE in seal by addition of the maximum metabolic conversion of 4,4'-DDT to 4,4'-DDE in seal (i.e., the decrease in the ratio of 4,4'-DDT from cod to seal) plus the accumulation of 4,4'-DDE from cod. The ratio of the maximum potential 4,4'-DDE ratio relative to the actual 4,4'-DDE ratio in seal was 1.18 suggesting that the higher BMF of 4,4'-DDE relative to S-DDT was due, in large part, to conversion of 4,4'-DDT to 4,4'-DDE in seal. However, the value greater than one suggested that 4,4'-DDT was metabolized to metabolites other than 4,4'-DDE and/or 4,4'-DDE was itself further metabolized.

Because there were no detectable $MeSO_2$-PCBs or -4,4'-DDE in arctic cod (i.e., < 0.05 ng/g, lipid weight), all of these compounds in ringed seal must have been formed by the seal itself from the precursor PCBs and 4,4'-DDE. Although the formation of $MeSO_2$-4,4'-DDE was indicated in seal, 4,4'-DDE metabolism by the MAP pathway did not appear to be large, since the level of Σ-$MeSO_2$-4,4'-DDE was only ca. 3 % of 4,4'-DDE in blubber. Similarly, in bear, Σ-$MeSO_2$-4,4'-DDE in bear was less than 1 % of 4,4'-DDE. The apparent BMFs of Σ-$MeSO_2$-4,4'-DDE for seal blubber to bear fat were in the same order as that of other CHCs (Table 1), suggesting that some of the metabolite may have been transferred from seal to bear. However, the BMF for 4,4'-DDE was less than one from seal to bear, indicating that some proportion of 4,4'-DDE may have been biotransformed to $MeSO_2$-4,4'-DDE in bear. Assuming all metabolism of 4,4'-DDE in bear led to $MeSO_2$-4,4'-DDE, the ratio of the maximum potential $MeSO_2$-4,4-DDE to CB-153 ratio in bear (i.e., the decrease in 4,4'-DDE ratio from seal to bear) to the actual $MeSO_2$-4,4-DDE to CB-153 ratio in bear was 7656. The ratio of ratios vastly exceeded unity, suggesting that metabolism of 4,4'-DDE in bear favoured a route other than $MeSO_2$-4,4'-DDE formation and/or $MeSO_2$-4,4'-DDE was cleared by non-metabolic pathways.

The majority of the ratios of PCBs to CB-153 values decreased going from cod to seal blubber to polar bear fat, revealing an increasingly simplified PCB pattern, especially for PCBs with *meta-para* adjacent carbons (not shown). Overall, there was little difference in the PCB pattern in cod relative to the Aroclor standard (1242:1254:1260, 1:1:1) indicating minimal metabolic biotransformation activity. Fish have little or no CYP1A- and CYP2B-type metabolic activity towards PCBs relative to higher trophic level species [13]. Marine mammals have been shown to have a lower capacity than terrestrial mammals to metabolize PCBs with *meta-para* adjacent carbons, probably mediated by CYP2B-type enzymes [12]. PCB congeners possessing *meta-para* adjacent carbons were present in seal blubber but notably absent in polar bear, indicating a high degree of CYP2B-type activity in bear. These PCBs included CB-31, CB-49, CB-52, CB-64, CB-70, CB-91, CB-95, CB-101, CB-110, CB-141, CB-132, and CB-174, all of which were present in the form of $MeSO_2$-PCB metabolites in seal and bear (except 3-$MeSO_2$-CB52 and 3- and 4-$MeSO_2$-CB95). The rapid metabolism of PCBs with *meta-para* adjacent carbons in polar bear could be due to a combination of greater CYP2B-type induction as a consequence of high CHC exposure in the seal blubber diet, the induction of different CYP2B-type enzymes, or a higher metabolic capacity of CYP2B-type enzymes.

Since most $MeSO_2$-forming PCB congeners with *meta-para* adjacent carbons were detected in seal, only a proportion were converted to 3- and 4-$MeSO_2$-PCBs and other metabolites by the seal. The only exception was CB-132, which was below the detection limit in seal. Polar bear liver and fat tissue contained $MeSO_2$-PCBs, but no detectable levels of precursor PCBs (< 0.05 ng/g, lipid weight), with the exception of traces of CB-149. Assuming that all the precursor PCBs accumulated from seal were converted to $MeSO_2$-PCBs in bear, and that all $MeSO_2$-PCBs were not further metabolized and retained in bear, we can predict the maximum $MeSO_2$-PCB metabolite concentration in bear, using the sum of the ratios of the sum of 3- and 4-$MeSO_2$-PCB pairs, $(R^{153})_{seal}$, and their precursor PCBs, $(R^{153})_{seal(potential)}$, to CB-153 in seal. The $(R^{153})_{seal(potential)}$ portion of the maximum potential $(R^{153})_{bear}$ (Figure 1) suggested a high potential for $MeSO_2$-PCB formation in bear, particularly PCB congeners such as CB-52, CB-101

and CB-149. The actual $(R^{153})_{bear}$ values for each $MeSO_2$-PCB pair in bear fat were consistently less than maximum potential $(R^{153})_{bear}$, especially for the higher chlorinated $MeSO_2$-PCBs.

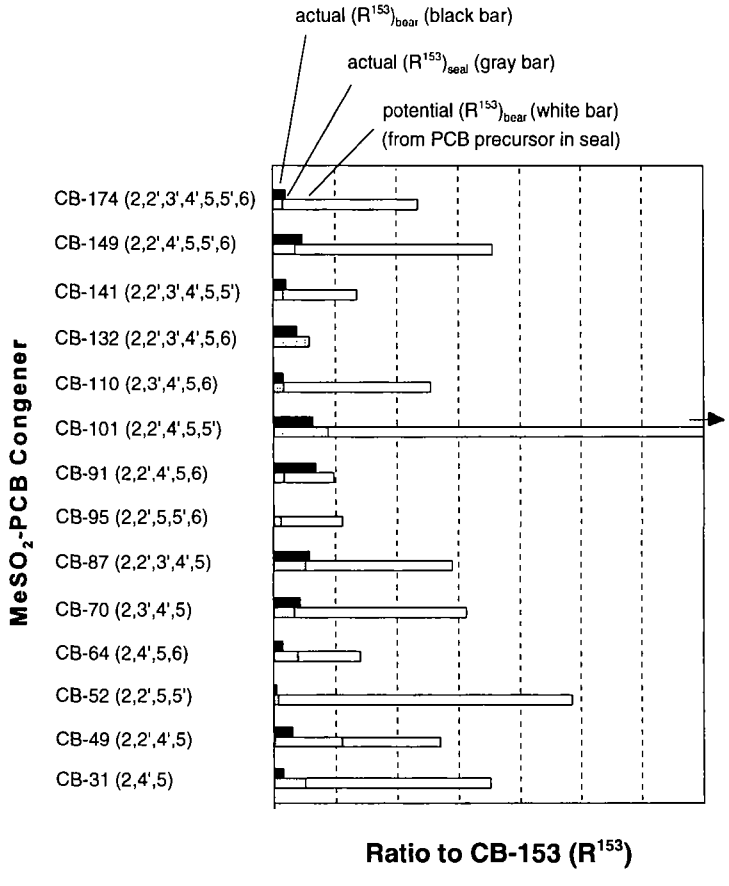

Figure 1. The maximum potential concentration ratios of the sum of the 3- and 4-$MeSO_2$-PCB congener pairs to CB-153 in polar bear fat (i.e., from the PCB precursor in seal), compared to the actual metabolite pair to CB-153 ratio in bear (i.e., $(R^{153})_{bear}$) and seal (i.e., $(R^{153})_{seal}$). The positions of chlorine substitution are denoted for each metabolite.

The results in Figure 1 suggest that the clearance of PCBs with *meta-para* adjacent carbons in bear mainly occur via an alternate metabolic pathway to $MeSO_2$-PCB formation. This appeared to be more true for the higher chlorinated PCBs, as the discrepancy between the maximum potential $(R^{153})_{bear}$ and the actual $(R^{153})_{bear}$ was larger. For some $MeSO_2$-PCB congener pairs, most notably the tetrachlorinated $MeSO_2$-PCBs, the actual $(R^{153})_{bear}$ was less than the actual $(R^{153})_{seal}$, indicating that bioaccumulation

alone could explain the levels in bears. Secondary metabolism of 3- and 4-$MeSO_2$-CB95 and -CB52 in bear was indicated since levels were below detection in bear (i.e., except for traces of 4-$MeSO_2$-CB52 in bear liver), and several *bis*-$MeSO_2$-PCBs were detected in bear liver. These sulfone metabolites possess a second *meta-para* chlorine unsubstitution on the other biphenyl ring. In other cases the actual $(R^{153})_{bear}$ was greater than the actual $(R^{153})_{seal}$, (e.g., $MeSO_2$-CB87, -CB91 and -CB149) indicating that metabolic formation in the bear was probable.

4. Conclusions

We estimate that ca. 10 % of the metabolism of PCBs capable of forming bioaccumulating $MeSO_2$-PCBs proceeds by this metabolic pathway in arctic ringed seal. In polar bear, metabolism of PCBs with *meta-para* adjacent carbons is complete, however, the proportion that froms $MeSO_2$-PCB metabolites also appears to be small. It can be concluded that at least one congener, $MeSO_2$-CB132, is bioaccumulated entirely from ringed seal by polar bears, and has a BMF approximately one half that of CB-153, indicating slow depuration from the bear. It is probable that a number of other congeners are also bioaccumulated mainly from ringed seal. It can also be concluded that formation and/or slow clearance of $MeSO_2$-CB70, -CB91, -CB149 and -CB141 occurs in the bear. In no case, except $MeSO_2$-CB132, did the actual $MeSO_2$-PCB level exceed the maximum potential level due to metabolic formation from precursor PCBs in seals, indicating that metabolic pathways other than the MAP were usually favoured. The MAP pathway appears highly favoured for CB-70 and CB-91. Only a small proportion of 4,4'-DDE metabolism favoured $MeSO_2$-metabolite in seal and bear. It is clear that $MeSO_2$-PCB uptake from seal is an important source of $MeSO_2$-PCBs in bear. Further, it appears that selective bioaccumulation, formation and retention of $MeSO_2$-PCBs all influence the resulting $MeSO_2$-PCBs congener levels and patterns in both seal and bear.

4. References

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