# Methylsulfone-PCB and -DDE Metabolites in Polar Bears - Comparison to Parent Compounds in the Diet

Letcher, R.[A], Norstrom, R.[A,B], Bergman, Å[C], D. Muir.[D]
[A]Carleton University, Centre for Anal. & Environ. Chem., Ottawa, Ontario K1S 5B6.
[B]Environment Canada, Canadian Wildlife Service, Hull, Québec K1A 0H3, Canada
[C]Stockholm University, Environmental Chemistry, Stockholm S-106 91, Sweden.
[D]Department of Fisheries and Oceans, Winnipeg, Manitoba R3T 2N6, Canada

## Introduction

Marine mammals are at higher risk than most other species to halogenated xenobiotic toxicity because they are fatty, high trophic level animals that accumulate elevated concentrations. The polar bear (*Ursus maritimus*) lives in a relatively uncontaminated arctic environment, but exists almost entirely on a diet of marine mammals, mainly ringed seal (*Phoca hispida*). Polar bears are consequently exposed to relatively high levels of PCBs and other chlorinated organic compounds. PCBs and chlordane-related compounds have been found at mg/kg (ppm) levels in polar bear tissues throughout the Canadian Arctic[1]. The possible effect of these contaminants is unknown. It has usually been assumed that parent PCBs and other halogenated compounds are the immediate cause of toxic effects. However, evidence is accumulating that some of the effects in Baltic seals in the 1970s associated with PCB and DDT contamination were due to a sulfur-containing metabolites of these compounds, the methylsulfone adducts (MSFs)[2]. MSF-PCBs were originally identified in blubber of seals from the Baltic Sea, at levels approximately 10% of the total PCBs[3].

A preliminary study on the presence of MSF-PCBs and MSF-DDEs in fat and liver of polar bears and several other species was reported at Dioxin'91[4]. Fifteen MSFs and two MSF-DDEs were identified in liver and fat. In the present study, the structural relationships and concentrations of MSF-PCBs and MSF-DDE in polar bear liver and fat relative to parent PCBs in the bears and in ringed seal are examined.

## Methods

Liver (1 g) and fat (1 g) of 5 polar bears shot by aboriginal hunters in the western Hudson Bay area in 1985 were taken for analysis of PCBs, DDE and their methylsulfone metabolites. A sample of 5 ringed seal blubber samples from the Arctic was also analyzed for the PCB congeners which are the precursors to the methylsulfone metabolites identified in polar bears. PCBs and DDE were determined according to the methods outlined in Norstrom et al.[1]. For methylsulfone analysis, samples were ground with excess sodium sulfate and extracted in a column through a 3 cm plug of 33 % KOH on silica gel. After addition of 10 ng 3-MSF-4-Me-52'3'4'5'-PnCB as an internal standard, bulk lipids were removed by gel-permeation chromatography[5]. The sample was cleaned up by concentrated sulfuric acid partitioning and basic alumina chromatography[6], evaporated to 50 µl and analyzed by GC-ECD using a 30 m Restek-5 column (injection port temperature 290°, initial temperature 100°, 20°/min to 220°, 3°/min to 300°). Identities of most compounds were established by GC-MS using authentic standards[6]. However, some identities must be considered tentative because of potential coelution on the GC. In cases where we possessed only one of the 3- and 4-MSF-

PCB, the response factors were assumed to be the same for both isomers. The response factor for 3-MSF-DDE was used for 2-MSF-DDE. The response factor for 3- and 4-MSF-236-2'3'4'5'-HpCB was assumed to be the same as that of the highest hexachloro-MSF-PCB, 4-MSF-236-2'3'4'-HxCB. Until structures can be more definitively determined and authentic standards are available for all congeners, the results should be considered semi-quantitative.

## Results and Discussion

The identities and concentrations of the MSF-PCBs, -DDEs and their parent compounds are presented in Table 1. DDE and total PCB concentrations are also given for comparison. Identities and levels of total MSF-PCBs and were similar to those found previously, approximately 1.5 μg/g lipid in liver and 0.4 μg/g lipid in fat. The average ratio of s-MSF-PCBs in liver to fat was 3.6 ± 0.94. This ratio is about twice that of s-PCBs, 1.67 ± 0.78, and DDE, 2.11 ± 0.97, and intermediate to the liver/fat ratio of epoxide type compounds such as dieldrin and oxychlordane, which is generally greater than 10[1]. Relatively non-specific, selective retention of persistent polar compounds in liver may be related to the presence of lipid-containing stellate cells, which are specialized in vitamin A storage[7]. Although 2- and 3-MSF-DDE levels were high in liver, 0.3 μg/g lipid, they were undetectable in adipose tissue. High affinity of MSF-DDEs for binding in tissues such as adrenal cortex[8] may compete for storage in adipose tissue, causing this highly irregular tissue distribution.

**Table 1. Mean and standard deviation of levels (ng/g lipid) and ratios of methylsulfone (MSF) -PCBs and -DDE, and tris-(p-chlorophenyl)-methanol in polar bear liver and fat (n=5).**

| | Liver | | Fat | | |
|---|---|---|---|---|---|
| | mean | SD | mean | SD | |
| 3-MSF-25-2'4'-TeCB | 151 | 107 | 35 | 13 | ng/g |
| 4-MSF-25-2'4'-TeCB | 177 | 93 | 39 | 18 | |
| 3-MSF-25-2'4'5'-PnCB | 185 | 89 | 81 | 32 | |
| 4-MSF-25-2'4'5'-PnCB | 104 | 41 | 70 | 31 | |
| 4-MSF-236-3'4'-PnCB | 17 | 8 | 9 | 6 | |
| 3-MSF-25-2'3'4'-PnCB | 229 | 111 | 31 | 12 | |
| 4-MSF-25-2'3'4'-PnCB | 116 | 56 | 57 | 25 | |
| 4-MSF-25-2'3'5'6'-HxCB | 101 | 59 | 6 | 3 | |
| 4-MSF-236-2'4'5'-HxCB | 240 | 69 | 63 | 31 | |
| 3-MSF-236-2'3'4'-HxCB | 38 | 16 | 4 | 1 | |
| 4-MSF-236-2'3'4'-HxCB | 15 | 6 | 9 | 4 | |
| 3-MSF-25-2'3'4'5'-HxCB | 30 | 14 | 7 | 4 | |
| 4-MSF-25-2'3'4'5'-HxCB | 15 | 5 | 15 | 7 | |
| (3-MSF-236-2'3'4'5'-HpCB)[1] | 29 | 11 | <0.01 | <0.01 | |
| (4-MSF-236-2'3'4'5'-HpCB)[1] | 20 | 15 | <0.01 | <0.01 | |
| s-MSF-PCBs | 1467 | 628 | 424 | 174 | ng/g |
| s-PCBs | 16133 | 9854 | 11247 | 5518 | ng/g |
| Ratio s-MSF-PCB/s-PCBs | 0.1157 | 0.0738 | 0.0412 | 0.0112 | |
| 2-MSF-DDE | 152 | 91 | <0.01 | <0.01 | ng/g |
| 3-MSF-DDE | 152 | 59 | <0.01 | <0.01 | |
| s-MSF-DDEs | 303 | 143 | <0.01 | <0.01 | ng/g |
| DDE | *1621 | *690 | 646 | 115 | ng/g |
| Ratio s-MSF-DDEs/DDE | *0.1713 | *0.0318 | | | |
| tris-(p-Cl-phenyl)-MeOH | 666 | 232 | 0.01 | <0.01 | ng/g |

[1] Tentative chlorine substitution *n=4

In addition to MSF compounds, tris-(p-chlorophenyl)-methanol was present at approximately 0.7 μg/g lipid in liver, but, like MSF-DDE, was absent in fat. The presence and selective tissue distribution of this compound has already been documented, but the levels were about 10 times higher, 4-7 μg/g lipid[9], therefore recoveries may be relatively low in the MSF procedure.

Figure 1 shows the relative fractional composition of the MSF-PCBs in liver and fat. In the majority of cases, both 3- and 4-MSF compounds were present at about equal amounts, and the congeners were similarly distributed in the two tissues. Congener 4-MSF-25-2'3'5'6'-HxCB may actually be the missing 3-MSF-236-2'4'5'-HxCB, since they coelute. Only one heptachloro-MSF-PCB pair was found. They were tentatively identified as 3- and 4-MSF-236-2'3'4'5'-HpCB because the parent of this compound, PCB-174, is the only major heptachloro PCB in environmental samples which has a 25- or 236- substitution[10]. Levels of parent PCBs in polar bear tissues were only occasionally above the detection limit, and were at least 5-10 times lower than their MSF-metabolites. No MSF metabolites of major PCBs in polar bear tissues were detected. The ratio of s-MSF-PCBs to remaining unmetabolized s-PCB was 0.12 in liver and 0.04 in fat. This implies that the MSF-PCBs identified in polar bears have similar persistence to unmetabolized PCBs. All of the MSFs identified had either 25- or 236- chlorine substitution and 3- or 4-MSF substitution on one ring, indicating that a requirement for formation (or perhaps whole-body retention) of MSF metabolites is *meta-para* epoxidation. The absence of MSF adducts of other metabolizable PCBs may indicate that MSFs are not formed or that they are formed and further metabolized or easily excreted. PCB-52 (25-2'5'-TeCB) is major in seals but has two free *meta-para* positions and may form the bis-methylsulfone. The ratio of MSF-DDEs to DDE in liver was 0.17. This probably indicates that DDE is not as rapidly metabolized as the MSF-PCB precursors, but yields a fairly high percentage of MSF metabolites. The presence of the 2-MSF-DDE is unusual. Normally only 3-MSF-DDE is found[3,4].

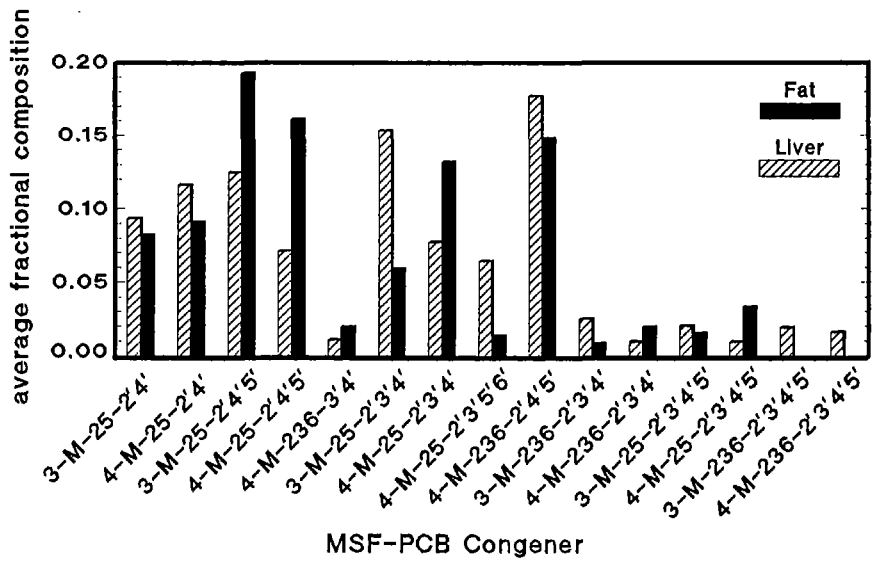


**Figure 1. Average Fractional makeup of MSF-PCB congeners in polar bear fat and liver.**

Figure 2 shows the fractional composition of MSF-PCBs in bear tissues relative to the that of the parent PCBs (excluding all other congeners) in seals. The identity of the parent PCB is indicated under the bars. The height of the bars indicates the comparative importance of MSF-PCB metabolites in polar bears normalized to the pattern of parent compound to which the bears are exposed in their diet. It appears that PCB-49 (25-2'4'-TeCB) and PCB-87 (25-2'3'4'-PnCB) result in a higher proportion of MSF metabolites than the other PCB congeners, assuming that the MSF-PCBs are not themselves accumulated from the diet. Further studies will address the possibility of bioaccumulation of MSF-PCBs and -DDEs from the diet.

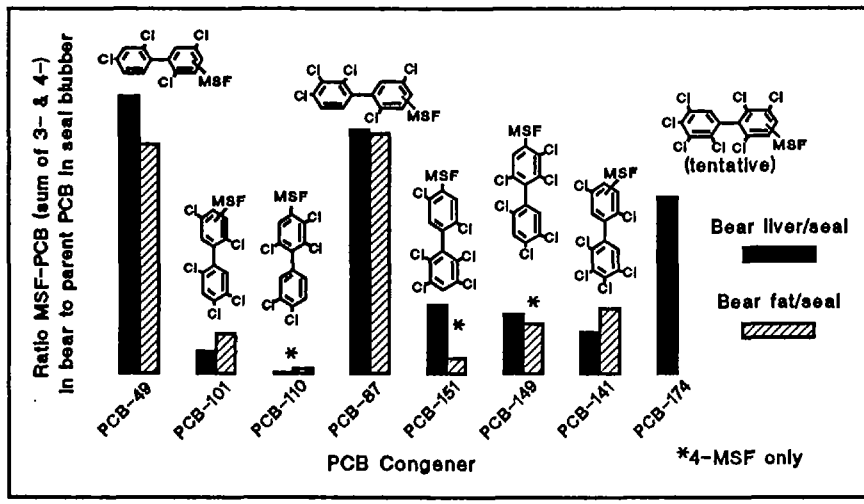


**Figure 2. Comparative fractional makeup of MSF-PCBs in polar bear fat and liver relative to that of the parent PCBs of these metabolites in ringed seal prey.**

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