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# PCB Biotransformation and Methyl Sulfone PCB Metabolites in Beluga Whale (*Delphinapterus leucas*) From the St. Lawrence River Estuary and Western Hudson Bay

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## Introduction

The St. Lawrence population of beluga whale has been subjected to human exploitation which have considerably reduced the number of individuals from historic times [1]. Exposure to organochlorine (OC) pollutants such as PCBs and various chlorinated pesticides may be impacting the present survival of the St. Lawrence beluga population. Residual OC levels in beluga have been positively correlated with a number of physiological abnormalities, including reproductive impairment, and disease complexes, which have likely contributed to the increased mortality and subsequent population decline [2].

The OC levels in the blubber of beluga carcasses from the St. Lawrence are considerably higher than in Arctic animals. Differences in OC level profiles in these two population groups are distinguished largely by loadings of DDTs and hexachloro- to nonachloro-PCBs [3]. Further comparison of the two populations revealed substantially different PCB congener patterns, which may be due to different levels of induction of cytochrome P450 (CYP450)-mediated biotransformation of PCBs, or to differences in the pattern of PCB exposure. Persistent methyl sulfonyl ($MeSO_2$-) containing metabolites of PCBs have been found in several mammalian species, including the carcass of one 25-year beluga female from the St. Lawrence river [4]. The presence of these metabolites is suggestive of CYP2B-type enzyme activity.

Blubber biopsies from seventeen individually identified, live St. Lawrence and five western Hudson Bay beluga from a previous study [5] were chemically analyzed to compare the

PCB biotransformation and $MeSO_2$-PCB metabolite profiles between an Arctic and St. Lawrence population of belugas. The difference in biotransformation capacities between the two populations determines the residual PCB and $MeSO_2$-PCB levels, and possibly the impact of these two classes of OCs with respect to their toxicological effects in beluga whale.

## Experimental Methods

Adipose biopsies of 0.020 to 0.200 grams were taken from seventeen male, free-ranging St. Lawrence beluga. The age of the individually identified whales were estimated from a long term re-sighting database (R. Michaud, personal communication) to be a minimum of 11 to 18 years (for one animal, 22 years of age). The sampling procedure has yet to be published. Briefly, a stainless steel hollow dart (22 cm long and 6.4 mm in diameter) was fired from a crossbow to biopsy free-ranging beluga. The darts were chemically washed and sterilised prior to use. Samples removed from the dart were sliced transversely between the dermis and hypodermis to obtain the inner portion. For five individually identified male (specific ages are presently unknown) western Hudson Bay samples, biopsies were taken with a standard biopsy punch. The OC levels in these samples have been previously reported [5]. All biopsies were placed in a chemically rinsed vial and stored at -20°C until further use. All the samples were from adult males to minimize the effect of age on contaminant levels. Although unusual for male cetaceans [3], a recent analysis of the carcasses of male beluga form the St. Lawrence found no significant correlation between s-PCB levels and age. The chemical analysis methodologies used for PCB determination (Freshwater Institute, Winnipeg) in biopsies are described in Muir *et al.* [6] and Letcher *et al.* [7]. A total number of seventy-four PCB congeners were quantified. The $MeSO_2$-PCB metabolites were isolated and quantified (NWRC, Hull) using the method of Letcher *et al.* [7]. The $MeSO_2$-PCB standards were purchased from Prof. Dr. Åke Bergman (Stockholm University, Sweden).The IUPAC numbering system was used to denote the chlorine substitution (in parentheses, Figure1) on the PCB and $MeSO_2$-PCB congeners.

## Results and Discussion

The arithmetic mean s-PCB levels for male St. Lawrence beluga were ca. 2.6-fold higher than the Arctic male animals (Table 1). However, the range of s-PCB levels for the St. Lawrence animals were substantially larger than the Arctic population, possibly due to variations in the diet among the St. Lawrence individuals and age-related effects. Further, the Arctic values were based on only five individual males. Mean s-PCB levels in blubber from the St. Lawrence beluga carcasses, collected in the same time period as the present individuals, were shown to be ca. 25-fold higher than randomly sampled western Hudson Bay beluga [3]. The higher s-PCB levels in the St. Lawrence animals relative to the Arctic animals are due to geographical and dietary factors. Local sources of PCBs from the Great Lakes and lower St. Lawrence river have been shown to be more important than atmospheric sources for St. Lawrence beluga, whereas atmospheric sources dominate in Arctic animals [8].

**Table 1. Concentrations of s-PCBs, s-$MeSO_2$-PCBs (ng/g, lipid wt.) and s-PCB to s-$MeSO_2$-PCB Ratios in the Blubber Biopsies of Live St. Lawrence and Western Hudson Bay Male Beluga.**

| | Western Hudson Bay [1] | St. Lawrence River [2] |
|---|---|---|
| age range (years) [3] | adult males | 11 - 18 |
| s-PCBs | | |
| range | 3923 - 10896 | 202 - 86327 |
| mean ± SD | 6837 ± 2878 | 17897 ± 21313 |
| median | 6353 | 10218 |
| s-$MeSO_2$-PCBs | | |
| range | 50.2 - 252 | 22.3 - 902 |
| mean ± SD | 142 ± 48 | 279 ± 267 |
| median | 166 | 190 |
| s-$MeSO_2$-PCB/s-PCB Ratios (%) | | |
| range | 1.9 - 9.3 | 2.2 - 5.8 |
| mean ± SD | 7.5 ± 5.4 | 2.8 ± 0.9 |
| median | 2.8 | 2.0 |

1 Five individual whales. See Experimental Methods Section for the range of ages.
2 Seventeen individual whales. See Experimental Methods Section for the range of ages.
3 The estimated minimum age of one individual St. Lawrence male was 22 years; however, neither the s-PCB or s-$MeSO_2$-PCB levels were outlying values.

PCB to PCB-153 concentration ratios were generated (Figure 1a) for representative PCB congeners of each of the five structural groups, with respect to metabolism, as described by Boon *et al.* [9]: I) a lack of vicinal H atoms, II) with one vicinal H in *ortho-meta* positions and ≥ two *ortho* Cl, III) same as (II) but with one *ortho* Cl , IV) vicinal H in the *meta-para* positions and having two *ortho* Cl, and V) same as (IV) but with ≥ three *ortho* Cl. The difference in ratios for the PCB-118 (group III) was similar for the two population groups, despite higher s-PCB levels in the St. Lawrence group. This is unusual since, 1) odontocetes are known to have highly inducible CYP1A-type activity [10], 2) pentachloro-PCB-118 is a *ortho-meta* chlorine-unsubstituted, *mono-ortho* PCB and therefore a potential substrate for CYP1A-type isozymes, and 3) Muir *et al.* [3] found that the relative PCB-118/PCB-153 ratios (beluga ratio ÷ prey ratio) were lower for carcasses of more highly OC exposed St. Lawrence beluga relative to eastern Hudson Bay animals. However, Norstrom *et al.* [11] observed similar profiles of PCDD/Fs and *non-ortho* PCBs in Arctic and St. Lawrence beluga, despite the large differences in exposure to these compounds, suggesting similar CYP1A-type activity in both populations. Further, the PCB-126 to PCB-153 concentration ratio in St. Lawrence and Baffin Island (Canadian Arctic)

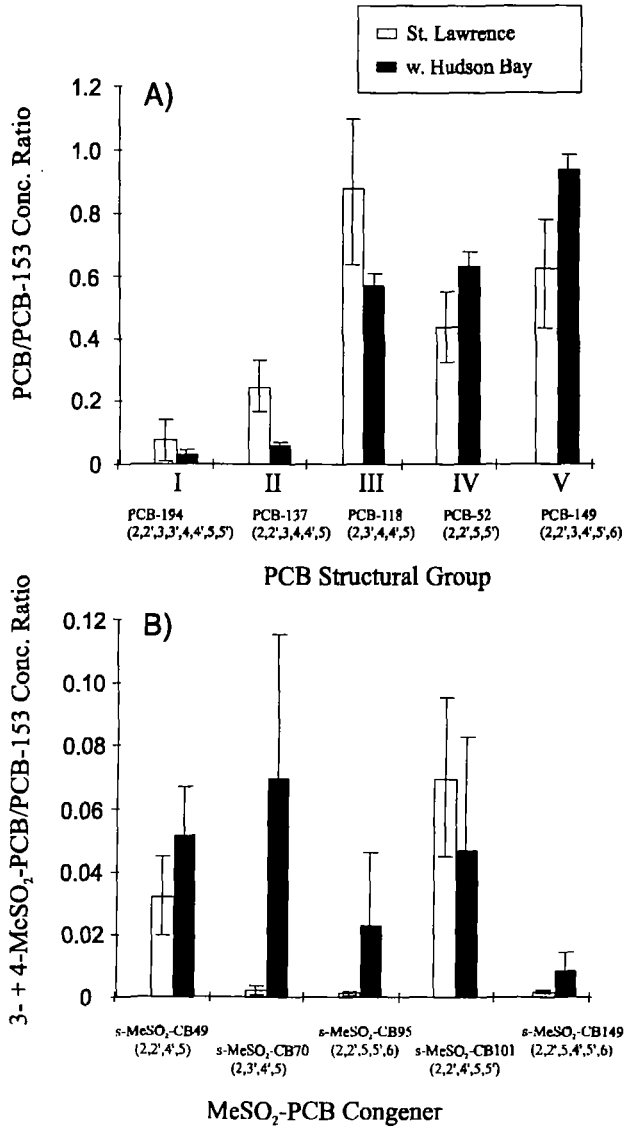


**Figure 1.** A) The arithmetic mean PCB to PCB-153 concentration ratios for representative PCB congeners of the five structural groups with respect to metabolism [9], and B) the arithmetic mean 3- + 4-$MeSO_2$-PCB to PCB-153 concentration ratios for the dominant $MeSO_2$-PCB metabolites, in adult male beluga from the St. Lawrence river and western Hudson Bay. The ±SD (standard deviation) is denoted by the error bars. See Experimental Section for the number of individuals in each data set.

beluga had similar values of 0.0006 and 0.0008, respectively. White *et al.* [12] found that hepatic CYP1A content and activity in Canadian Arctic beluga were highly correlated with the concentrations in blubber of *non-ortho* and *mono-ortho* PCB congeners, compounds that induce CYP1A in other mammals. Differences in exposure may also account for the different PCB-118 to PCB-153 ratios in the two populations. The diets regimes differ between St. Lawrence and Arctic animals, and it has been shown that PCB exposure in St. Lawrence beluga is mostly from hexachloro- to nonachloro-PCB congeners (i.e., PCB-118 is pentachloro-substituted) relative to the Arctic group [3]. The ratios (Figure 1) for *meta-para* chlorine unsubstituted PCB-149 (group V) and PCB-52 (group IV), which are CYP2B-type substrates and form persistent $MeSO_2$-PCB metabolites, were slightly lower for the St. Lawrence group. Odontocetes are known to have low metabolic activity towards CYP2B-type substrates. PCB-194 (group I) and PCB-137 (group II) ratios were relatively unchanged, indicating that exposure to these compounds, and the biotransformation capacity toward *meta-para* PCB congeners with ≥ three *ortho* chlorines, was similar in the two populations.

The mean s-$MeSO_2$-PCB level in the St. Lawrence male group (ca. 0.3 μg/g, lipid wt.) was similar to the concentrations in blubber found previously in a lone St. Lawrence beluga female (0.4 μg/g, lipid wt.) [4]. As was found for s-PCBs, there were ca. 2-fold higher s-$MeSO_2$-PCB levels in the beluga males of the St. Lawrence relative to the Arctic population (Table 1). The s-$MeSO_2$-PCB to s-PCB ratio was ca. 2.6-fold higher in the Arctic group. The greater ratio likely reflects the greater Arctic input, relative to the St. Lawrence, of tetrachloro- and pentachloro-PCBs, which contain more PCB precursors to the $MeSO_2$-PCBs. The 3- + 4-$MeSO_2$-PCB to PCB-153 ratios (Figure 1b) of the dominant $MeSO_2$-PCB metabolites in blubber also appear to reflect the input of precursor PCBs. Tetrachloro- and pentachloro-$MeSO_2$-PCBs are more significant in the Arctic animals, whereas hexachloro-$MeSO_2$-PCBs appear to be favoured in the St. Lawrence beluga. However, these ratios do not take into consideration differences in the diet of the two populations. The variation in $MeSO_2$-PCB clearance rates among the two populations may also be a factor. For example, secondary CYP2B-type metabolism of 3- and 4-$MeSO_2$-CB95 can occur since these metabolites contain a second *meta-para*, chlorine-unsubstituted position. $MeSO_2$-CB95 may be metabolized by CYP1A, since it has 3,4-chlorine substitution on one phenyl ring. The low ratio of these two metabolites in the St. Lawrence group may indicate higher CYP1A- and CYP2B-type activity relative to the western Hudson Bay animals.

## Conclusions

Based on PCB and $MeSO_2$-PCB ratios to PCB-153, the results provide conflicting evidence of differences in enzyme activity in live adult male beluga from the western Hudson Bay and St. Lawrence populations. Analysis of the diet of the two populations is probably required to answer this question in the absence of actual enzyme activity measurements. The $MeSO_2$-PCB metabolites constitute a larger proportion of the s-PCBs in the Arctic group, and have a different profile, presumably due to greater exposure to less chlorinated PCBs which are precursors to the metabolites, but possibly due to differences in further metabolism and excretion of some $MeSO_2$-PCBs. S-$MeSO_2$-PCB levels are higher in the St. Lawrence group. These results

suggest that potential $MeSO_2$-PCB-mediated toxicities in beluga may differ in the two population groups.

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