Inter-Specific Variation in Polychlorinated Biphenyl (PCB) Methyl Sulphone ($MeSO_2$-CB) Metabolite Formation in Cetaceans - Preliminary Observations

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Introduction

PCB Methyl sulphones ($MeSO_2$-CB) are persistent metabolites of PCBs and can be found in cetacean blubber and liver[1]. $MeSO_2$-CBs can bind intracellular receptors in uterus and lung[2,3], potentially causing adverse effects on embryo implantation / viability during the early gestation period[4]. They have been reported in several cetacean species, including Beluga Whale (*Delphinapterus leucas*)[1,5], False Killer Whale (*Pseudorca crassidens*)[1] and the Blue Whale (*Balaenoptera novaeangelica*)[6].

$MeSO_2$-CBs are formed by glutathione attack of the PCB arene oxide intermediate formed by cyt. P450 metabolism, followed by degradation to a cystein conjugate, further metabolism by intestinal bacterial C-S-lyase, methylation and finally oxidation[1]. $MeSO_2$-CBs arise from PCB isomers with 2,5- or 2,3,6- chlorine substitution to form 3- and 4-$MeSO_2$-CBs[1]. The type and proportion of different $MeSO_2$-CBs isomers formed from PCBs are influenced by molecular structure (e.g. degree of chlorination of the other phenyl ring[7]) and the PCB bio-transformation capacity of the species[1]. Cetaceans and phocids are able to metabolise PCBs with *ortho-meta* H-atoms and a maximum of 1 *ortho*-Cl (PB-type), although cetacean metabolism of PCB with *meta-para* H-atoms is generally lower/absent in cetaceans[8,9,10] and may influence $MeSO_2$-CBs formation. In this study, inter-specific variation in $MeSO_2$-CB formation from PCB parent molecules is assessed for 6 cetacean species, by examining ratios of total PCB to $MeSO_2$-CB concentration and isomer patterns in blubber. Where available, data on enzymatic biotransformation activities for these species were used to substantiate interpretations of $MeSO_2$-CB formation capacity.

Materials and Methods

Blubber was sampled from 1 Irish Sea Harbour Porpoise (*Phocoena phocoena*), 1 Pilot Whale (*Globicephalus melas*), 1 White-Sided Dolphin (*Lagenorhyncus acutus*), 1 Common Dolphin (*Delphinus delphis*), 1 Risso's Dolphin (*Grampus griseus*) and 4 Aegan Sea (Crete) Striped Dolphins (*Stenella coerueoalba*). Published sample extraction and clean up methodology was used with minor modification[11]. Gas chromatography with ECD and MS detection was used to determine the concentrations of PCBs 118, 138, 153, 180 and 170 and $MeSO_2$-CBs 3-49, 4-49, 3-87, 4-87, 3-101, 4-101 according to published methodology[11,12].

Results and Discussion

$MeSO_2$-CB Burdens

$MeSO_2$-CB concentrations shown in Table 1 were similar to those reported in other studies, except for Harbour Porpoise where concentration was higher[1,6]. Cetacean $MeSO_2$-CB burdens

are generally lower than those reported in seals[12]. This may be due to differences in geographical origin/exposure and number of isomers quantified for total PCB-$MeSO_2$ determinations.

PCB:$MeSO_2$ -CB Ratios

Inter-specific variation in PCB:$MeSO_2$-CB ratios result from differences in enzymatic capacity, gut bacterial metabolism and excretion characteristics between species[7,13,14]. Total PCB and $MeSO_2$-CB lipid weight concentrations (sum of major isomers detected) were used to calculate PCB:$MeSO_2$-CB. Ratios for each species showed that blubber PCB levels were generally greater than $MeSO_2$-CB levels, possibly because many of the persistent PCBs detected lacked 2,5- or 2,3,6-Cl substitution required for $MeSO_2$-CB formation[1] (Tab 1).

Using PCB:$MeSO_2$-CB to estimate $MeSO_2$-CB formation capacity, Harbour Porpoise had the highest capacity (PCB burdens were only 10 times greater than $MeSO_2$-CB burdens), White-Sided Dolphin and Pilot Whale had an intermediate capacity and Risso's, Striped and Common Dolphins had the lowest (PCB burdens up to 100 times greater than $MeSO_2$-CB burdens) (Tab 1). PCB:$MeSO_2$-CB were not directly comparable with ratios from other studies presented in Table 1, due to the low number of samples and differences in isomers selected to quantify total PCB-$MeSO_2$ in this study. However, blubber PCB:$MeSO_2$-CBs for Pilot Whale and White-Sided Dolphin were similar to that reported in Beluga Whale[1]. Interestingly, the Harbour Porpoise ratio was in the range of those reported for Harbour and Grey Seals[12]. Unlike other cetaceans, Harbour porpoises possess some CYP2B activity in the range of Harbour and Grey Seals[9,10]. It is possible that [PB]-type PCBs and 3-$MeSO_2$-CBs induced CYP2B activity in the Harbour Porpoise[13,14] which increased metabolism and resulted in the low PCB:$MeSO_2$-CB ratio.

PCB:$MeSO_2$-CB for Striped Dolphin suggested a low capacity for PCB biotransformation and may be partly, due to low CYP1A-dependent mixed function oxidase (MFO) activity (EROD & B*a*POH) reported in this species[15], below that reported for Arctic Beluga Whales[16] despite variation in exposure (Tab 1). Ratios for Risso's and Common Dolphin were similar to Striped Dolphin, suggesting they may also have low enzyme activity, but MFO data for these species are needed to support this. The ratio for White-Sided Dolphin was similar to that reported in Beluga Whale suggesting similar capacities for MFO metabolism of PCB. Finally, PCB:$MeSO_2$-CB observed for Pilot Whale indicated a greater capacity for PCB biotransformation than Common, Risso's and Striped Dolphins, although reported MFO activities from N. Pacific Pilot Whales, are lower than would be expected (Tab 1). This may be due to differential enzyme induction in whales from different geographical areas (as seen in arctic polar bears (*Ursinus maritimus*)[17] or other intra-specific factors (e.g. sex and age).

Isomer Composition

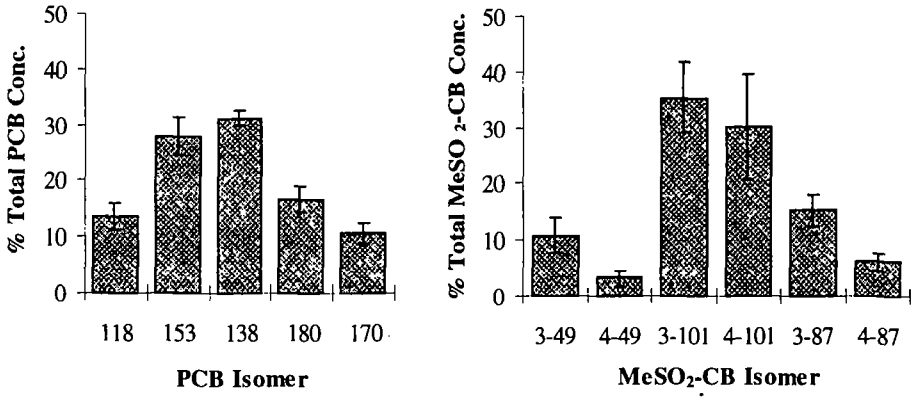
The major PCBs detected were 118, 138, 153, 170 and 180, persistent in cetaceans due to low activity / absence of CYP2B enzymes required for their metabolism[8,9] (Fig 1a). PCB isomer compositions in all 6 species were similar to those reported in cetaceans by other studies[9,18,19]. The major $MeSO_2$-CBs isomers detected were 3-49, 4-49, 3-101, 4-101, 3-87 and 4-87, the parent PCB isomers possessing 2,5-chlorine substitution needed for 3 or 4 epoxidation (sulphone insertion) of the phenyl ring (Fig 1b). In general, blubber $MeSO_2$-CB compositions were similar to those reported in other piscivorous marine mammals with the proportion of Cl_5-$MeSO_2$-CBs exceeding Cl_3- and Cl_4-$MeSO_2$-CBs[1,11].

Blubber 4:3-$MeSO_2$-CB ratios showed that 3-$MeSO_2$-CBs were more abundant than 4-$MeSO_2$-CBs in all species except Risso's Dolphin (Tab 1). This may partly be due to higher metabolism of PCBs by CYP1A enzymes in cetaceans, which produce 3-epoxide intermediates for 3-$MeSO_2$-CBs formation[1]. Variation in the metabolism and clearance of 3- and 4-$MeSO_2$-CBs

Table 1 Blubber PCB & $MeSO_2$-CB Concentrations and Hepatic CYP1A-dependent MFO Activity in Cetaceans & Phocids

Species	ΣPCB* μg g^{-1} ±	Σ$MeSO_2$* μg g^{-1} ±	PCB:$MeSO_2$	4:3-$MeSO_2$	EROD pmol prod./min/mg	B*a*POH
Risso's Dolphin	7.34	0.07	1 : 105	3 : 1	n/d	n/d
Striped Dolphin	21.52 ± 2.76	0.20 ± 0.03	1 : 108	1 : 2	191[15]	7.2
Common Dolphin	2.80	0.03	1 : 93	1 : 2	n/d	n/d
Beluga Whale	-	-	1 : 53[1]	1 : 2.4[1]	291[16]	191
White-Sided Dolphin	15.48	0.31	1 : 50	1 : 3	n/d	n/d
Pilot Whale	10.25	0.21	1 : 49	1 : 3	42[15]	7.8
Harbour Porpoise	6.19	0.58	1 : 11	1 : 1.6	2460[9]	n/d
Blue Whale	-	-	1 : 2[6]	n/a	n/d	n/d
Harbour Seal	-	-	[a]1 : 10-58[11]	n/a	[c]2800[19]	10783
Grey Seal	-	-	[b]1 : 4-32[11]	1 : 1.4[1]	[c]2600[20]	n/d

*Lipid weight concentration, EROD = ethoxy resorufin-*O*-deethylase, B*a*POH = benzo-*a*-pyrene hydroxylase, s.e. = Standard error, n/d = No data, n/a = Not available, [b]Range for 12 seals, [a]Range for 11 seals, [c]Average for adult female seals.



Figures 1a and 1b Mean Percentage Contribution (± std. error) to Total Blubber PCB and $MeSO_2$-CB Concentrations of Major Isomers Detected for the 6 Species of Cetacean Studied.

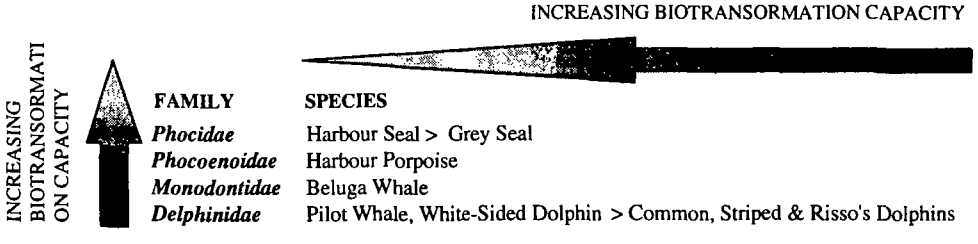


Figure 2 Proposed Phylogenetic Variation in PCB Biotransformation Capacity

also influences 4:3-$MeSO_2$-CB ratio. In rats for example, 3-$MeSO_2$-CBs are stronger inducers of hepatic CYP2B activity than 4-$MeSO_2$-CBs[7,13,14].

Phylogenetic Variation Based on the limited data available, inter-specific variation in PCB biotransformation capacity may also be related to phylogenetic origin. Observed PCB:$MeSO_2$-CB ratios and reported CYP1A enzyme activities (EROD & B*a*POH) were similar for species of the same family with Phocoenidae (Harbour Porpoise) having the highest capacity for PCB biotransformation and PCB-$MeSO_2$ formation and Delphinidae the lowest (Pilot Whale, White-Sided Dolphin > Common, Striped and Risso's Dolphins) (Fig 2). However, this requires confirmation from a larger data set of animals group by age and sex, with comparable contaminant exposure.

Conclusion This is the first study to report $MeSO_2$-CB concentrations in cetaceans from European waters and highlights the widespread occurrence of these contaminants in cetaceans. Some of the levels of $MeSO_2$-CBs reported in this study are among the highest reported in cetaceans. At present the $MeSO_2$-CB burdens required to induce measurable health effects on cetaceans, are not known. The PCB:$MeSO_2$-CB ratios and isomer compositions observed in this study suggest that PCB biotransformation capacity can vary widely between species and phylogenetically. Although, burdens of $MeSO_2$-CB are significantly lower than PCBs, their potential to interact with intracellular proteins[2,3] suggests that these contaminants are of toxicological significance that warrants further investigation.

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