PCBs, DDTs AND METHYLSULFONE PCB AND 4,4´-DDE METABOLITES IN CETACEANS FROM THE ATLANTIC OCEAN

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Introduction

Polychlorinated biphenyls (PCBs) and 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (4,4´-DDT) and its degradation product 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (4,4´-DDE) are chlorinated hydrocarbon contaminants (CHCs) that are present globally in marine mammals. In cetaceans such as St. Lawrence beluga whale, high levels of PCBs and DDTs have been implicated as contributing factors in the incidence of tumors, adrenal lesions, immunosuppression and reproductive impairment [1]. An important element of PCB and DDT toxicokinetics involves biotransformation mediated by cytochrome P450 (CYP) enzymes. The metabolic capacity of cetaceans towards PCBs and DDTs is generally low compared to terrestrial mammals, birds and seals [2]. From immunochemical, catalytic and PCB residue studies, cetaceans appear to possess a particularly low capacity to metabolize PCBs with no chlorine atoms in *meta-para* positions [3]. Rodent-like CYP2B or possibly CYP2C enzyme mediation is possibly operative in any biotransformation of *meta-para* PCBs.
Metabolic capacity towards *meta-para* PCBs has recently been demonstrated for a number of cetacean species by the formation of persistent methyl sulfone ($MeSO_2$-) -PCB and -4,4´-DDE metabolites. For example, Canadian beluga whale [4,5], and harbour porpoise from Irish and Swedish marine environments [6,7] contain persistent $MeSO_2$-PCBs and -DDEs. The mechanism of $MeSO_2$-PCB and -DDE formation has been described elsewhere [8]. As in other wildlife species and humans [8], the persistent $MeSO_2$-PCBs that dominate in cetaceans possess trichloro- to heptachloro-substitution, 3- and 4-$MeSO_2$-substitution, and 2,5-dichloro- or 2,5,6-trichloro-substitution on the $MeSO_2$-containing phenyl ring. $MeSO_2$-PCBs lacking adjacent, chlorine unsubstituted carbons, and/or > five chlorines are generally more resistant to further biotransformation. $MeSO_2$-PCBs and -DDEs have also demonstrated protein binding and specific tissue localization in liver, lung and kidney in a number of wildlife species [8]. Specific retention in liver has been observed in Swedish harbour porpoise [7] and St. Lawrence beluga whale [5]. The present study aims to broaden the limited knowledge of $MeSO_2$-PCB and -DDE metabolites in cetaceans [9]. PCBs, 4,4´-DDT, 4,4´-DDE, $MeSO_2$-PCBs and 3-$MeSO_2$-4,4´-DDE were determined in the tissues of four cetacean species originating from the Atlantic Ocean (Table 1).

Materials and Methods

Samples were obtained from one individual of each of the four cetacean species (adults). All the animals were stranded and deceased at the time of sampling (between 1991 and 1997). Blubber samples were collected from striped dolphin, pygmy sperm whale and finback whale found on Rhode Island (U.S.A.). Blubber and liver from sperm whale (age 20-25 years) were collected from an individual found at Rømø, Denmark (Table 1). The samples were stored at -20 °C. Approximately 2.0 gram of tissue were extracted and two contaminant fractions were isolated; one containing PCBs/DDTs and the other containing the aryl methylsulfones. The procedure of Letcher *et al.* [10]. was used with minor modifications [9]. Gas chromatography/electron impact mass spectrometry (GC/MS) or GC/MS with electron capture negative ionization (single ion monitoring) was used to determine the concentrations of seventy-one PCBs, 4,4´-DDT, 4,4´-DDE, twenty-two $MeSO_2$-PCBs and 3-$MeSO_2$-4,4´-DDE. Authentic standards were used in the quantification (courtesy of Prof. Dr. Å. Bergman. Stockholm University, Sweden). Quantification was based on a $MeSO_2$-internal standard ($MeSO_2$-IS, 3-$MeSO_2$-2-Me-2´,3´,4´,5,5´-pentachlorobiphenyl) approach.

Results and Discussion

The variations in Σ-PCB, 4,4´-DDT and 4,4´-DDE concentrations (Table 1 and 2) in the blubber of all the cetaceans are a combined function of species, sex, age, geographical location and diet. Diet was likely a major influence, which was exemplified by the three orders of magnitude higher Σ-PCB concentration in the female striped dolphin versus female finback whale. Sperm whale prey mostly on squid, and occasionally on demersal fish in deep water of the Atlantic and the Mediterranean. Striped dolphin inhabit warmer and deeper waters, and feed on fish. Pygmy sperm whale also prefer warmer Atlantic waters, and eat fish, squid, crabs and krill. The northwestern population of finback whale remain in the Continental Shelf area eating a variety of spawning or post-spawning fish, and also supplement their diet with krill or copepods. Biotransformation capacity also affects the PCBs and 4,4´-DDE toxicokinetics in these cetaceans. Persistent $MeSO_2$-PCBs and 3-$MeSO_2$-4,4´-DDE were found in all four species (Table 1 and 2). It is unlikely that the sulfones in the cetaceans were accumulated through the diet. Generally, fish and amphipods appear to possess an insignificant capacity to form sulfone metabolites [8].
From the concentrations in blubber, the $MeSO_2$-PCB forming and clearance capacity of the cetaceans appeared to be proportional to the levels of PCBs (Table 1). The relatively large variance in the Σ-PCB and Σ-$MeSO_2$-PCB concentrations was in contrast to the small range of Σ-$MeSO_2$-PCB to Σ-PCB ratios (0.006 to 0.02). The PCB patterns in blubber were similar among the cetaceans species (not shown). However, a large portion of the Σ-$MeSO_2$-PCB concentration (ca. 44%) in the blubber of striped dolphin, pygmy sperm whale and finback whale was from tetrachloro-$MeSO_2$-PCB congeners. In the blubber of sperm whale, 3´- and 4´-$MeSO_2$-CB49, -CB87 and -CB101 were dominant compared to the much lesser 3- and 4-$MeSO_2$-CB70, 3-$MeSO_2$-CB52, 4-$MeSO_2$-CB64 and 4´-$MeSO_2$-CB95 (not shown). Σ-$MeSO_2$-PCB in the blubber of western Hudson Bay beluga whale was also found to have a higher proportion of trichloro- and tetrachloro-$MeSO_2$-PCBs, whereas the more highly induced St. Lawrence beluga possessed a simplified $MeSO_2$-PCB pattern much like the present sperm whale [4].

The range of 4,4´-DDT concentrations in the blubber of the sperm whale was > 1000-fold higher than in the other cetaceans (Table 2). The 4,4´-DDE concentration in sperm whale was 3 to 4 times

higher than the 4,4′-DDT concentrations, whereas the 4,4′-DDE concentrations were at least three orders of magnitude higher than the 4,4′-DDT concentrations in the remaining animals. Regardless, the 3-$MeSO_2$-4,4′-DDE to 4,4′-DDE ratios were very small among the cetaceans, and ranged from 0.0009 to 0.005. This indicated a large capacity to metabolize 4,4′-DDT to 4,4′-DDE, but a small capacity to form 3-$MeSO_2$-4,4′-DDE from 4,4′-DDE.

Table 1. Concentrations of Total (Σ-) PCB, Σ-$MeSO_2$-PCB, and Metabolite to Parent Compounds Ratios in Blubber and Liver of Several Cetaceans Species (ng/g, lipid weight basis).

Family Species (Sex)	Tissue	Σ-PCB[a]	Σ-$MeSO_2$-PCB[a]	Σ-$MeSO_2$-PCB /Σ-PCB
Physeteridae				
Sperm whale (M) (*Physeter marcocephalus*)	Blubber	8834	69	0.008
	Liver	7978	1060	0.1
Pygmy sperm whale (M) (*Kogia breviceps*)	Blubber	4108	26	0.006
Delphinidae				
Striped dolphin (F) (*Stenella coeruleoalba*)	Blubber	67940	412	0.006
Balaenopteridae				
Finback whale (F) (*Balaenoptera physalus*)	Blubber	405	7	0.02

[a] Seventy-one individual PCB congeners comprised the Σ-PCB, including the $MeSO_2$-PCB precursors CB-31, -49, -52, -64, -70, -87, -95, -101, -110, -141, -149 and –174. [b] Twenty-two individual $MeSO_2$-PCB congeners comprised the Σ-$MeSO_2$-PCB (3′- and 4′-$MeSO_2$-CB49, -CB87, -CB101, -CB132, -CB141 and –CB174, 3- and 4- $MeSO_2$-CB52, -CB70, -CB110 and –CB149, 4- $MeSO_2$-CB64 and 4′- $MeSO_2$-CB95).

Table 2. Concentrations of 4,4′-DDE, 3-$MeSO_2$-4,4′-DDE, and Metabolite to Parent Compounds Ratios in Blubber and Liver of Several Cetaceans Species (ng/g, lipid weight basis).

Species (Sex)	Tissue	4,4′-DDT	4,4′-DDE	3-$MeSO_2$-4,4′-DDE	3-$MeSO_2$-4,4′-DDE/ 4,4′-DDE
Sperm whale (M)	Blubber	1494	6098	0.11	0.005
	Liver	174	8302	5.9	0.00002
Pygmy sperm whale (M)	Blubber	1	1908	12	0.001
Striped dolphin (F)	Blubber	6	18120	17	0.002
Finback whale (F)	Blubber	1	163	0.16	0.0009

Similar ratios were reported for Canadian beluga whale from either western Hudson Bay or the St. Lawrence [4]. The ratios for Swedish harbour porpoise were considerably higher (0.01 to 0.03) [7] than reported for any of the present cetaceans. For the sperm whale, the difference in the Σ-PCB

and 4,4´-DDE concentrations was relatively small between the liver and blubber as compared to the sulfones (Table 1 and 2). The Σ-$MeSO_2$-PCB and 3-$MeSO_2$-4,4´-DDE concentrations and the to Σ-PCB and 3-$MeSO_2$-4,4´-DDE to 4,4´-DDE ratios were > 15 times higher in the liver relative to blubber. As in other wildlife species and humans [8], in the sperm whale the $MeSO_2$-PCBs and 3-$MeSO_2$-4,4´-DDE clearly showed a distinct preference for localization in the liver, rather than in the blubber.

$MeSO_2$-PCB and -DDEs have been reported to be in the blubber of a few odontocete (toothed) species of cetacean from the Baltic Sea, Canadian Arctic, Irish Sea, Aegean Sea, Japanese marine environment and the St. Lawrence river [4,5,6,7,8]. The present study extends the knowledge of persistent sulfones to several new species from two cetacean families (sperm whale, pygmy sperm whale and finback whale). It was not surprising that sulfones could be found in the odontocetes. However, the even lower expectation for CYP2B-like activity in baleen whales (mysticetes) appears to be underestimated [3], given our finding of sulfones in the blubber of Atlantic finback whale. The Σ-$MeSO_2$-PCB level was 40 times higher then in Japanese blue whale (10 ng/g, lipid weight basis), the only other baleen species where sulfones have been reported [8]. Despite the lack of catalytic or immunochemical evidence of CYP2B1/2 in cetaceans, the *meta-para* PCB substrates of these enzymes are still metabolized, although more slowly than seals [2,8,9]. Moreover, the formation $MeSO_2$-PCBs and -DDEs appears to be a common phenomena in cetaceans based on existing literature. The implications of exposure to sulfones clearly need to be included in the risk assessment of PCB and DDT exposure in cetaceans.

References

1. DeGuise S, Martineau D, Béland P and Fournier M; *Environ. Health Perspect.* **1995**, 103, 73
2. Boon J.P., Van Arnhem E., Jansen S., Kannan N., Petrick G., Schulz D., Duinker J.C., Reijnders P.J.H. and Goksøyr A. p. 119-160, in *Persistent Pollutants in Marine Ecosystems*, Eds. C.Walker and D.Livingston, Pergamon Press, **1992**.
3. Tanabe S, Watanabe S, Kan H and Tatsukawa R; *Mar. Mam. Sci.* **1988**, 4, 103
4. Letcher RJ, Norstom RJ, Muir DCG, Sandau CD, Koczanski K, Michaud R, DeGuise S and Béland P; *Environ Toxicol Chem* **1999**, submitted
5. Bergman Å, Norstrom RJ, Haraguchi K, Kuroki H and Béland P: *Environ. Toxicol Chem* **1994**, 13, 121
6. Troisi GM, Haraguchi K, Simmonds MP and Mason CF; *Arch. Environ. Contam. Toxicol.* **1998**, 35, 121
7. Karlson K, Ishaq R, Zebühr Y, Breggren P and Broman D; *Organohalogen Compounds* **1998**, 39, 277
8. Letcher R.J., Klasson-Wehler E. and Bergman Å. in *Anthropogenic Compounds: New Types of Persistent Halogenated Compounds (The Handbook of Environmental Chemistry)*; Eds. J. Paasivirta and O. Hutzinger, Springer-Verlag, **1999**; in press.
9. Hansler RJ, Montie E, Norstrom RJ, Moisey J, Boon JP, Van Den Berg M, Seinen W and Letcher RJ; in preparation for *Arch. Environ. Contam. Toxicol.*
10. Letcher RJ, Norstrom RJ and Bergman, Å; *Anal. Chem.* **1995**, 67, 4155