Chlorinated Hydrocarbon Contaminants and Methyl Sulfone Metabolites in Harbour Porpoises (*Phocoena Phocoena*) in Swedish Waters

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Introduction

This study represents possibly the first analyses of aryl methyl sulfone metabolites in harbour porpoises *(Phocoena phocoena)*. These metabolites of PCBs where first detected in grey seal blubber (1). In later studies methyl sulfone metabolites have been found in several other mammals (2,3,4), and have been shown to be highly persistent contaminants.

PCB and DDE methyl sulfones are formed via glutathione attack on an arene oxide intermediate, and further metabolised via the mercapturic acid pathway in (5).

The capacity to metabolise PCBs is known to be low in small cetaceans in general compared to birds and terrestrial mammals (6). Although, this group of mammals seems to be able to metabolise some of the lower chlorinated biphenyls (6).

So far, only a limited selection of contaminants have been analysed in harbour porpoises from Swedish waters (7). Levels of PCBs in juvenile and adult male porpoises from Swedish waters were found to be similar to those found in juvenile and adult male seals from the same area (7,8). This is of serious concern, since contaminant related disorders have been documented in seals from the Baltic Sea (9).

In the present study levels of PCBs (se material and methods), S-DDTs (*op'*-DDT, *pp'*-DDT, *op'*-DDE, *pp'*-DDE, *op'*-DDD, *pp'*-DDD), aryl methyl sulfones of PCB ($MeSO_2$-PCBs) and aryl methyl sulfone of DDE ($MeSO_2$-DDE), were compared between different tissues (blubber, nuchal fat, liver, brain, muscle) of adult male harbour porpoises from the west coast of Sweden.

Material and methods

Samples: Blubber, nuchal fat, liver, muscle and brain tissue samples were collected from three male harbour porpoises killed incidentally in fishing nets in the west coast of Sweden (ICES rectangles 4456) 1996, and were stored frozen (-20 °C) until chemical analysis.

Blubber from five different positions from each animal were chosen to cover variations in sample area. (However, for $MeSO_2$ analysis the five blubber samples were pooled.)

Extraction: All blubber, nuchal fat and brain samples (10 g) were Soxhlet extracted wet with acetone/hexane (59:41), while the liver and muscle tissues (10 g) were cold extracted with acetone/hexane (10:25), hexane/diethyleter (9:1). After extraction the brain, liver and muscle tissues were liquid-liquid partitioned with phosphoric acid (0,9%)/NaCl (0,1M) solution according to Jenssen et al (10). The organic phase was then evaporated and the lipid weight determined gravimetrically.

Clean-up & enrichment: The clean-up was carried out by means of dialysis with semi-permeable membranes (SPMs) to reduce the bulk of the lipids (11). The dialysate fraction was introduced to a HR-GPC column (polystyrene-divinylbenzene polymer, 600x7.5 mm). The lipid and membrane polymer residues were reduced in this step.

Aliquots were taken for analyses of mono- to tetra-*ortho* CBs (bulk-PCBs), S-DDTs and $MeSO_2$-PCB/DDE. The $MeSO_2$-PCB/DDE aliquot was further cleaned-up by liquid-liquid partitioning with DMSO after the addition of an internal standard (i.s.) 3-$MeSO_2$-4-Me-5,2′,3′4′,5′-PCB. The bulk-PCB aliquot was eluted on a 100x10 mm multi-layer column column with 10% H_2O-, 33% KOH- and 40% H_2SO_4 SiO_2. A ^{13}C-labelled standard mixture including seven PCBs was added before elution.

HRGC/MRMS analysis: The samples were injected on a Fisons GC8000 with an on-column injector coupled to a Fisons MD800 mass selective detector. The identification and the quantification of $MeSO_2$-PCB/DDE was done using an external standard with 22 $MeSO_2$-PCB/DDE with different chlorination degrees. The $MeSO_2$-PCB/DDE standards were a kind of gift from Åke Bergman, Department of Environmental Chemistry, Wallenberg Laboratory, Stockholm University.

Results and discussion

Concentrations of $MeSO_2$-PCB and $MeSO_2$-DDE, ratios of S-$MeSO_2$-PCB/S-PCBs and $MeSO_2$-DDE/pp′-DDE are summarised in Table 1. $MeSO_2$-penta-CBs represent the highest concentrations of $MeSO_2$ in all tissues analysed (between 55-65 % of total $MeSO_2$-PCB), while $MeSO_2$-hexa-CBs are found in the lowest concentrations (11-18 % of total $MeSO_2$-PCB). This indicates that harbour porpoises have a relative low capacity to metabolise hexa-CBs compared to penta-CBs.

The relatively high S-$MeSO_2$-PCB/S-PCBs and $MeSO_2$-DDE/pp′-DDE ratios in brain, liver and muscle compared to blubber and nuchal fat are most likely explained by the high concentration of S-PCBs and S-DDTs in blubber and nuchal fat, since PCBs and DDE mainly accumulate in triglyceride rich tissues like blubber. $MeSO_2$ are also thought to be more selectively retained in tissues, specially in liver, due to specific protein binding (2, 4), which could also explain the relatively high ratios for liver, brain and muscle.

Concentrations of $MeSO_2$ congeners show a similar distribution pattern between tissues when comparing the three sampled porpoises. It is worth to notice that 3-$MeSO_2$-CB101 and 3-$MeSO_2$-DDE was found in all samples analysed and that 3-$MeSO_2$-CB101 was the most abundant. The significance of the concentrations of $MeSO_2$ in harbour porpoises is at present unknown. Ongoing studies on reproduction biology and histopathology will likely increase our understanding of the potential health affects of these compounds.

The concentrations (ng g^{-1} wet weight) of S-PCBs (including tetra-, penta-, hexa- and hepta-CBs) and S-DDTs found in the five different tissues analysed from the three male harbour

porpoises are summarised in Table 2. The highest concentration of S-PCBs (porpoise 1: 24-79 μg g^{-1}, porpoise 2: 19-48 μg g^{-1}, porpoise 3: 42-138 μg g^{-1}) and S-DDTs (porpoise 1: 5-68 μg g^{-1}, porpoise 2: 24-44 μg g^{-1}, porpoise 3: 43-127 μg g^{-1}) were found in blubber and nuchal fat. These concentrations are in the range were health risks to cetaceans can be expected (12). A comparison of the concentrations of PCBs and S-DDTs in the different tissues shows that these were similarly distributed in all the three animals. Hexa-CBs showed the highest concentration in all tissues analysed and constituted between 67-85 % of total PCBs, while tetra-CB and penta-CB were represented between 4-12 % each of total PCBs. These results strengthen the theory of low metabolism for hexa-CBs and higher for penta-CB.
These are preliminary results. The project is still ongoing and will finish within a two month period. Some further analyses will be done to confirm our results.

	Lipid (%)	3-52	4-52	3-49	4-49	3-64	4-64	3-91	4-91	3-70	4-70	3-87	3-101	3-110	3-132	4-132	3-141	4-141	3-149	3-DDE
Porpoise 1																				
Blubber	93	2.7	0.5	12	6.6	1.1	nd	3.3	nd	nd	0.5	10	35	1.1	1.3	2.6	2.3	0.8	4.7	6.8
Nuchal fat	89	2.4	nd	11	5.4	1.1	nd	3.1	nd	nd	nd	8.2	29	1.1	1.0	2.3	1.5	1.3	4.1	4.8
Liver	4.7	0.8	nd	3.6	1.5	0.4	0 8	0.5	nd	0.1	nd	3.2	8.0	0.5	0.3	0.5	0.4	nd	1.0	2.0
Muscle	1.8	nd	nd	1.0	0.5	0.2	0.2	0.2	0.7	nd	nd	0.9	2.7	0.2	nd	0.2	nd	nd	0.5	0.6
Brain	3.5	nd	nd	nd	0.8	0.2	*	0.2	0.4	nd	0.2	2.1	3.0	0.5	nd	0.2	0.7	nd	0.4	1.5
Porpoise 2																				
Blubber	91	nd	nd	13	7.3	1.9	nd	nd	46	nd	nd	13	24	2.7	1.5	4.5	2.7	nd	3.5	1.9
Nuchal fat	86	1.9	nd	7.7	4.4	0.9	nd	nd	nd	nd	nd	5.7	13	1.3	nd	2.3	nd	nd	2.5	0.9
Liver	4.8	nd	nd	2.0	0.9	0.3	0.5	0.4	nd	nd	nd	2.0	3.1	0.5	0.1	0.3	0.2	0.2	0.5	0.3
Muscle	1.9	nd	nd	0.8	0 3	0.2	nd	0.1	0.8	nd	nd	0.4	1.4	0.1	0.1	0.3	0.4	0.2	0.2	0.2
Brain	9.8	nd	nd	nd	0.3	0.1	0.1	0.0	0.3	nd	nd	0.6	0.7	0.3	nd	0.1	0.1	nd	0.1	0.2
Porpoise 3																				
Blubber	92	3.5	0.9	20	13	1.8	nd	6.2	nd	nd	nd	17	52	3.6	3.5	7.9	3.8	1.8	13	4.0
Nuchal fat	85	1.6	nd	7.5	5.4	0.6	nd	1.8	nd	nd	nd	8.2	23	0.8	nd	nd	nd	nd	10	2.3
Liver	4.5	1.1	nd	5.4	3.1	0.6	0.9	1.2	nd	0.2	nd	5.6	13	0.4	0.6	1.1	nd	nd	2.4	1.2
Muscle	1.5	nd	nd	0.3	0.2	0.04	nd	0.1	nd	nd	nd	0.2	0.7	0.1	nd	nd	nd	nd	0.2	0.1
Brain	8.3	nd	nd	nd	1.1	0.2	0.3	0.2	0.8	nd	nd	2.4	4.0	0.2	0.1	0.5	0.2	nd	*	0.8

	$S\text{-}MeSO_2$-PCBs	$S\text{-}MeSO_2$-PCBs/ S-PCBs (%)	3-DDE/ pp-DDE (%)
Porpoise 1			
Blubber	85	0.1	0.03
Nuchal fat	72	0.1	0.02
Liver	22	13	5.0
Muscle	7.3	50	15
Brain	8.6	25	24
Porpoise 2			
Blubber	120	0.3	0.01
Nuchal fat	40	0.1	0.004
Liver	11	14	1.2
Muscle	5.3	42	3.6
Brain	2.7	3.9	0.7
Porpoise 3			
Blubber	148	0.1	0.01
Nuchal fat	59	0.1	0.01
Liver	35	14	2.0
Muscle	1.8	7.9	2.4
Brain	10	5.7	2.3

Table1. Lipid concentration (%) and concentrations (ng g^{-1} wet weight) of 18 $MeSO_2$-CBs, total $MeSO_2$-PCB and $MeSO_2$-DDE in five different tissues from three male harbour porpoises. Ratios of $MeSO_2$-PCB/S-PCBs and $MeSO_2$-DDE/pp'-DDE are also shown.
n.d.= non detected
* = uncertain identification.

Table 2. Concentrations of seven CB congeners, S-PCBs, *op*-DDT, *pp*-DDT, *op*-DDE, *pp*-DDE, *op*-DDD, *pp*-DDD and S-DDTs are shown in five blubber samples, one nuchal fat sample (μg g^{-1} wet weight) and three other tissue samples (ng g^{-1} wet weight) in three male harbour porpoises from the west coast of Sweden. Lipid concentration (%) are also shown.
n.d. = non detected

	Lipids (%)	#52	#101	#118	#105	#153	#138	#180	S-PCBs	op-DDT	pp-DDT	op-DDE	pp-DDE	op-DDD	pp-DDD	S-DDTs
Porpoise 1																
Blubber I	92	3.5	1.4	1.3	0.2	31	28	6.8	72	1.7	14	0.1	24	2.4	18	60
Blubber II	95	3.7	1.4	1.3	0.2	28	26	6.0	67	1.8	14	0.2	26	2.5	18	62
Blubber III	93	1.1	0.4	1.3	0.3	6.8	11	2.8	24	0.1	2.8	0.0	0.9	0.1	1.4	5.3
Blubber IV	88	4.6	1.4	0.2	0.2	30	29	6.6	72	2.3	17	0.2	28	2.5	18	68
Blubber V	95	4.7	1.7	1.3	0.3	35	29	7.1	79	1.8	13	0.2	27	2.7	19	64
Nuchal fat	89	5.7	1.9	1.7	0.1	26	25	4.3	64	1.7	10	0.2	24	2.6	16	55
Liver	4.7	8.7	4.4	4.4	0.9	71	55	19	163	3.2	nd	nd	41	8.8	36	89
Muscle	1.8	0.8	0.4	0.3	nd	5.8	5.1	1.5	14	nd	0.4	nd	4.1	0.8	3.2	8.5
Brain	3.5	1.3	0.8	0.8	0.3	17	10	4.7	35	0.2	nd	nd	6.3	1.1	4.8	12
Porpoise 2																
Blubber I	92	3.2	2.2	2.5	0.4	16	17	3.8	44	0.7	9.4	0.1	22	1.2	11	45
Blubber II	94	2.4	1.8	2.0	0.4	13	13	3.4	36	0.6	7.6	0.1	17	0.7	8.8	35
Blubber III	95	2.9	2.1	2.6	2.6	13	15	3.6	42	0.7	8.8	0.1	23	0.9	10	43
Blubber IV	79	1.4	1.0	1.0	0.2	6.2	7.4	1.8	19	0.4	5.7	0.0	11	0.6	6.6	25
Blubber V	95	2.5	1.8	2.1	0.2	14	13	3.4	37	0.6	7.1	0.2	18	1.1	9.0	36
Nuchal fat	86	2.9	2.5	2.7	0.5	19	16	4.1	48	0.6	7.0	0.1	21	0.9	8.4	38
Liver	4.8	4.9	3.7	4.7	0.5	29	26	8.1	76	nd	nd	nd	28	3.5	23	55
Muscle	1.9	0.7	0.7	0.6	0.1	4.5	3.9	1.4	12	nd	nd	nd	4.5	0.5	3.5	8
Brain	9.8	3.9	3.5	3.3	0.4	25	23	8.3	68	0.6	nd	nd	23	1.8	14	39
Porpoise 3																
Blubber I	94	5.4	1.5	1.6	0.2	51	44	10	114	2.2	26	0.2	36	3.9	29	98
Blubber II	93	5.4	1.4	0.9	0.2	47	40	8.9	104	2.3	25	0.2	32	3.6	28	91
Blubber III	93	2.9	2.1	2.6	2.6	13	15	3.6	42	0.7	8.8	0.1	23	0.9	10	43
Blubber IV	85	6.5	2.0	1.4	0.3	57	50	10	127	3.5	34	0.3	45	4.8	39	127
Blubber V	95	7.5	2.0	2.1	0.2	63	53	11	138	3.5	30	0.2	45	5.0	36	119
Nuchal fat	85	6.9	2.2	1.3	0.3	52	41	8.1	112	2.1	15	0.2	34	3.8	27	82
Liver	4.5	12	3.7	3.7	0.5	114	86	19	240	nd	nd	0.2	58	16	68	142
Muscle	1.5	1.0	0.3	0.3	0.1	11	7.8	2.0	22	nd	0.3	nd	5.0	1.4	6.0	13
Brain	8.3	7.7	2.6	2.5	0.5	87	63	14	177	1.2	4.2	nd	37	7.1	31	80

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