Distribution of polychlorinated naphthalenes (PCNs) and non-*ortho* chlorinated biphenyls (PPCBs) in harbour porpoises (*Phocoena phocoena*) tissues

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Introduction

Polychlorinated biphenyls (PCBs) and naphthalenes (PCNs) have been used as technical mixtures for industrial purposes. PCBs and PCNs are persistent and lipophilic compounds, therefore they have a potential to accumulate in biological material. They are found in a wide range of environmental matrices including marine mammals[1]. Non-*ortho* PCBs (PPCBs); i.e. PCBs that lack chlorine substitution in the ortho positions of the biphenyl rings are known to have structures similar to toxic polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs)[2, 3]. They have shown to elicit a number of toxic and other biological effects including induction of hepatic drug-metabolising enzymes such as aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD). This has also been shown to be caused by some PCN congeners[4, 5](HxCNs; 63, 66, 67, 69 and HpCN 73; the congener numbering is according to Weidmann & Ballschmiter[14]).
Small cetaceans have a limited capacity to metabolise PCBs accumulated in their bodies[6] which could make them susceptible to adverse reproductive and immunological effects. Few studies have focused on planar organochlorines such as PCNs and PPCBs in harbour porpoises. This study presents the levels of three toxic PPCB (CBs # 77, 126 & 169) and PCN congeners (tetra-hepta chlorinated) and the distribution of these congeners in blubber, nuchal fat, muscle, liver, kidney and brain tissues of three adult male harbour porpoises (*Phocoena phocoena*) from the west coast of Sweden.

Material and Methods

Samples
Blubber, nuchal fat, lever, muscle, brain and kidney tissue samples were collected from three male harbour porpoises (1-3) killed incidentally in fishing nets on the west coast of Sweden in 1996. The samples were stored frozen (-20 °C) until chemical analysis.

Extraction
All blubber, nuchal fat and brain samples were Soxhlet extracted with acetone/hexane (59:41). The brain extracts were partitioned with phosphoric acid/NaCl (0.1M/0.9%) solution according to

Jenssen et al.[7] followed by determination of lipid weight. The blubber and nuchal fat tissues were volume reduced without partitioning with the above mentioned solution and the lipid weight determined gravimetrically.
The liver, muscle and kidney tissues were cold extracted completely according to the method described by Jenssen et al.[7]
Before extraction of ^{13}C-labelled PPCB standard mixture consisting of IUPAC TCB# 77, PnCB #126 & HxCB #169 was added.

Clean-up
The clean-up was carried out by means of dialysis with semi-permeable membranes (SPMs) to reduce the bulk of the lipids[8]. The dialysis fraction was introduced to a HR-GPC column to reduce the residual content of lipids and membrane polymer material.
Aliquots were taken for mono-tetra-*ortho* CB (BPCBs) and methyl sulfones of PCBs and DDE. The largest aliquot was eluted on a 100x10 mm SiO_2 column with three different silica layers; neutral (10% H_2O), basic (33% KOH) and acidic (40% H_2SO_4). The target compounds were eluted with 50 mL of hexane[9].
The fraction was introduced to two serially connected 2-(1-pyrenyl)ethyldimethylsilylated silica (PYE) columns to separate the PPCBs, PCNs from the BPCBs[10]. The flow rate was 0.7 and 1.5 mL/min in the forward and reversed direction respectively. The PPCBs and PCNs were backflushed from the columns with DCM. The column temperature was kept at 0 °C in the forward elution and then raised to 30 °C in the back elution[11]. The planar fraction was cleaned on a small SiO_2 column prior to GC/MS analysis.

GC/MS analysis
The samples were then injected on a Fisons GC 8000 with an on-column injector coupled to a Fisons MD 800 mass selective detector. A 30 m x 0.25 mm i.d. GC column (PTE 5, Supelco) was used. EI was used at 70 eV and the detection was carried out in SIM mode. Two most abundant isotopic ions from each chlorination degree (tetra through octa for PCNs and tetra through hexa for PPCBs) were monitored. The identification of PCNs was based on literature data[10] and on a Halowax 1014 mixture.

Results and Discussion

The results from GC/MS analysis of the three animals are presented in Table 1. The levels are based on wet weight basis. Given also is the lipid content in the different tissues studied.
There were chromatographic disturbances in some of the peaks detected in the GC/MS analysis of these samples. Some of these disturbances could be traced to coeluting peaks from BPCBs present in this fraction. However, we present some of the quantifiable peaks detected.
High concentrations of the quantified Σ-PCNs (sum of the detectable PCNs) were found in the blubber and nuchal fat of all three harbour porpoises. However, the highest concentrations of Σ-PCNs were found in the liver of porpoise 2 (0.92 ng/g ww). The levels of Σ-PCNs in the muscle, kidney and brain seem to be similar in porpoises 2 and 3 while they were 1.3-1.6 times higher in porpoise 1.

The toxic HxCN congeners 66/67 (coelute on the column used in this study) were of highest abundance in the blubber, nuchal fat and liver tissues of all three porpoises. These two congeners are known to possess high bioaccumulation potential in the liver of exposed rats[12, 13]. Further, the liver in porpoise 2 seems to have the somewhat higher levels of HxCNs 66/67 and HpCN 73. This could be due to difference in exposure between these three specimen. The HpCN 73 is detected in all tissues accept in muscle (porpoise 1-3) and brain (porpoise 2 & 3), while the HpCN 74 was not detected in any of the samples. This suggests a stronger bioaccumulating potential of HpCN 73 than of 74 in harbour porpoise, especially in the liver as reported for rats in previous studies[12, 13].

The highest levels of TCNs were found in blubber of all three animals (0.10-0.14 ng/g ww) and the lowest were detected in the brains of all animals (0.022-0.035 ng/g ww).

The only quantifiable PeCNs were 52/60 (coelute on the used column). It was found mainly in blubber and nuchal fat.

The PPCBs analysed were CBs # 77, 126 and 169. There were chromatographic interferences, mainly in the tetra and penta trace. These interferences arrise possibly due to the presence of residual BPCBs. However, HxCB 169 may reflect the general distribution of the PPCB in the three harbour porpoises. HxCB 169 was found in highest amounts in the blubber and nuchal fat tissues (0.011-0.019 ng/g ww).

Concludingly, PCNs and PPCBs were found in the blubber, nuchal fat, liver, muscle, kidney and brain of three harbour porpoises collected from the west coast of Sweden. HxCNs 66/67 and HpCN 73 are found in highest levels in the blubber, nuchal fat and liver tissues. This support the data on the selective retention of the above mentioned congeners in exposed rats and call for further and more specific separation of coeluting congeners of toxic CNs (66 and 67). Complementary analysis will be done in the coming months to resolve some of the uncertainties in other detected and unquantifiable peaks.

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Table 1. Concentrations (pg/g ww) of polychlorinated naphthalene congeners† (PCNs) and non-*ortho* chlorinated biphenyls (PPCBs) in tissues of three male adult harbour porpoises collected from the west coast of Sweden.

Specimen	% lipids	TCNs						PnCNs	HxCN	HpCNs		Σ-PCNs	TCB	PnCB	HxCB
Tissue		42	3/34/3	28/43	29	39/35	38	52/60	66/67	73	74		77	126	169
Porpoise 1															
Blubber	92	26	29	11	4.0	23	12	13	180	14	n.d.	418	138	n.q.	16
Nuchal fat	89	13	17	6.3	n.q.	12	6.3	16	275	27	n.d.	429	n.q.	n.q.	11
Muscle	1.8	15	25	11	2.3	16	7.6	2.4	3.8	n.d.	n.d.	161	8.7	n.q.	0.67
Liver	4.7	6.0	27	12	2.6	20	9.4	2.2	221	46	n.d.	423	21	6.9	1.4
Kidney	3.1	13	27	12	2.0	20	8.8	1.4	8.2	1.3	n.d.	177	8.8	1.3	0.72
Brain	3.5	13	8.2	4.0	2.6	7.4	n.q.	2.9	2.9	0.30	n.d.	76	4.0	n.q.	0.40
Porpoise 2															
Blubber	92	22	32	12	3.4	22	11	16	343	26	n.d.	591	157	n.q.	17
Nuchal fat	86	16	17	6.1	3.2	9.3	n.q.	21	526	53	n.d.	703	n.q.	n.q.	19
Muscle	1.9	6.0	23	9.0	n.q.	14	6.1	n.q.	4.6	n.d.	n.d.	120	8.2	n.q.	0.59
Liver	4.8	3.8	21	9.4	2.0	16	7.3	1.6	673	131	n.d.	923	16	5.5	1.6
Kidney	3.4	2.0	14	8.7	n.q.	16	7.4	0.90	13	1.9	n.d	111	9.0	1.3	0.39
Brain	10	3.2	6.5	3.0	1.1	5.9	2.2	2.0	4.7	n.d.	n.d.	51	2.4	n.q	0.30
Porpoise 3															
Blubber	94	24	36	13	12	40	17	14	238	23	n.d.	560	182	n.q.	16
Nuchal fat	85	17	17	n.q.	8.8	15	n.q.	15	397	48	n.d.	577	n.q.	n.q.	17
Muscle	1.5	7.3	20	8.5	1.7	14	6.2	1.7	4.4	n.d.	n.d.	120	8.0	n.q.	0.48
Liver	4.5	5.6	13	6.4	1.4	9.8	4.6	n.q.	132	26	n.d.	240	9.0	3.8	0.67
Kidney	2.9	5.0	24	9.3	1.8	15	6.5	1.5	7.5	1.1	n.d.	133	8.8	2.0	0.39
Brain	8.3	4.4	6.8	3.3	2.7	6.5	3.0	n.q	5.0	n.d.	n.d.	58	n.q	n.q	0.47

n.q.= not quantified due to chromatographic interference.
n.d.=not detected in sample.
*=sum of the quantified congeners.
†=congener numbering according to Weidmann & Ballschmiter[14].