DETERMINATION OF POPS IN HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) STRANDED ON THE BELGIAN NORTH SEA COAST

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Introduction

Harbour porpoises (*Phocoena phocoena*) may be considered as good indicators of coastal pollution, because they stay in coastal waters and do not present large-scale migration. Thus, high concentrations of persistent organic pollutants (POPs) in the organism are expected from animals living in polluted seas, such as the North or Baltic Sea (1,2). The main threats to the harbour porpoise are accidental capture by fishing gear, declining fish stocks, high levels of contaminants, and other anthropogenic stress factors. Organohalogen contaminants (such as PCBs and DDTs) have been found at levels that may present risk to cetaceans (3,4). The relationship between organochlorines and reproductive, endocrine and immunological disorders has also been strongly suspected in marine mammals from highly contaminated areas (4). A less efficient metabolism of DDTs and *ortho*-substituted PCBs in cetaceans compared to seals and terrestrial mammals could also lead to greater biomagnification and possible adverse effects (5). In this context, the enantiomeric ratio of chiral PCBs in animals may give additional information on possible degradation pathways and may help in understanding the behavior of PCBs in food webs.

This study aims to evaluate the occurrence and trends of important organic contaminants, such as organochlorine pesticides, PCBs and PBDEs, in harbour porpoise, a representative top predator for the North Sea ecosystem. The enantiomeric occurrence of chiral PCBs (PCB 95, PCB 132 and PCB 149) was compared with the congener pattern of achiral PCBs.

Methods

Liver samples were obtained from 21 harbour porpoises stranded at the Belgian North Sea coast between 1997-2000 and were kept at –20 °C until analysis. The samples were analysed for 74 PCB congeners, HCH isomers (α-, ß-, and γ-), HCB, DDT and metabolites (6 *op*- and *pp*-isomers) and for 8 PBDE congeners (IUPAC no. 28, 47, 66, 71, 99, 100, 153 and 154). The method was previously described (6,7). Briefly, 2-5 g liver was ground with anhydrous Na_2SO_4 and, after the addition of internal standards, extracted with hexane:acetone (3:1) using an accelerated Soxhlet extractor for 2 h. After lipid determination, the extract was cleaned-up using acidified silica gel and eluted with hexane. Extracts were analysed by GC-ECD and GC/MS. For chiral analysis, extracts were further cleaned up by Florisil and fractionated by HPLC using a Cosmosil 5-PYE column. Heart cut technology was used in the process and 80 mL eluant was collected for each chiral congener. The GC separation was carried out on a Chirasil-Dex column. The enantiomeric ratio was defined as the proportion of peak area of the first to the second eluting atropisomer peak (E1/E2), regardless whether its optical rotation is known or not.

Results and discussion

The most abundant organochlorine pesticides were DDT and its metabolites, followed by HCB and HCHs (Table 1). HCB and HCH concentrations were relatively low, and g-HCH was the most abundant HCH isomer with an average contribution of 96 % to the total HCH concentration. p,p'-DDE was the

main contributor to the total DDT. Concentrations of p,p'-DDT and total DDT were lower than those previously reported from North or Baltic Sea porpoises (1).

PCBs were the most important contaminants in the harbour porpoises (Table 1). Similar with that

Table 1. Concentrations of selected POPs (µg/g lipid) measured in the liver of 21 harbour porpoises stranded at the Belgian North Sea coast.

Compounds	No. samples	Mean* ± SD	Median	Range
		µg/g lipid		
HCB	21	0.7 ± 0.4	0.6	0.1- 5.7
Sum HCHs	21	0.2 ± 0.2	0.2	0.1 – 2.3
γ-HCH/ S HCHs	21	0.96 ± 0.08	1.00	0.71 – 1.00
P,p'-DDE	21	2.3 ± 1.5	1.9	0.2 – 24.4
P,p'-DDD	21	0.8 ± 0.6	0.6	0.1 – 14.6
P,p'-DDT	21	0.2 ± 0.3	0.1	nd – 1.9
Sum DDTs	21	3.4 ± 2.3	2.6	0.3 – 44.3
P,p'-DDE/ S DDTs	21	0.69 ± 0.06	0.70	0.55 – 0.77
CB 153	21	8.5 ± 7.1	7.1	0.3 – 98.8
Tri-CB	21	0.1 ± 0.1	0.1	nd – 0.4
Tetra-CB	21	1.0 ± 0.6	0.9	0.2 – 16.5
Penta-CB	21	4.0 ± 2.6	3.8	0.5 – 37.1
Hexa-CB	21	20.7 ± 15.4	18.4	0.9 – 241.6
Hepta-CB	21	9.2 ± 7.5	7.4	0.3 – 99.5
Octa-CB	21	1.2 ± 1.2	1.0	nd – 8.6
Nona-CB	21	0.2 ± 0.2	0.1	nd – 0.8
Deca-CB	21	0.1 ± 0.1	0.1	nd – 0.3
Sum 74 PCBs	21	36.4 ± 26.4	33.8	1.9 – 404.5
Sum 7 ICES PCBs**	21	17.8 ± 13.7	15.2	0.8 – 198.4
BDE 47	20	1.06 ± 0.86	0.90	0.19 – 2.83
Sum 8 BDEs	20	2.29 ± 1.79	2.18	0.41 – 5.81

nd – not detected;* - two extremely high for DDTs and PCBs were not included
** - IUPAC no. 28, 52, 101, 118, 153, 138, 180

found in another study (8), the major contributing PCB congeners were 153 (24 %), followed by 138 (13 %), 149 (8.1 %), 187 (7.4 %), 180 (6.8 %) and 99 (4.2 %). The hexa-CB congeners dominated the profile (58 %), followed by hepta-CBs (26 %) and penta-CBs (11 %). PCB concentrations found in the porpoises stranded on the Belgian North Sea coast are in agreement with concentrations previously reported in literature (1,2,9).

Higher concentrations of organochlorine compounds were found in porpoises stranded on the Belgian/Dutch coast of the North Sea in comparison with the English coast (9). There are reasons why this might be, for instance the discharges from the Rhine, Meuse and Scheldt rivers or the coastal currents from the French to Dutch coast. Thus, concentration gradients of organochlorine pollutants are expected for the North Sea, with the highest values in the Southern part.

Two liver samples had abnormal high PCB values (359 and 404 µg/g lipid). This is probably not due to the analytical procedure since other parameters, such as PBDEs, HCHs, HCB and % lipid were

all within the range of other samples, but might be due to local pollution and discharges or to a particularly bad health condition of the animal (4). Due to infectious diseases, animals can loose weight rapidly, leading to a thinning of the blubber layer (in parallel with a redistribution of contaminants from the blubber) and thus to an increased concentration of organic pollutants in the blubber. However, these values are at the lower end of the concentrations (up to 3000 µg/g lipid) observed in marine mammals stranded during the 1988 and 1990 epizootic episodes (3).

PBDEs were found in relatively high concentrations (mean 2.3 µg/g lipid) (Table 1). In contrast with PCB values, there were no extremely high values for PBDEs and the range was relatively small (one order of magnitude). These values are in accordance with the levels (mean: 2.4, range: 0.1 – 7.7 µg/g lipid) measured in 60 harbour porpoises stranded on the English coast of North Sea and on the Welsh coast (9). As found in other studies (9), the principal contributor to the sum of BDEs was BDE 47. Other important PBDE congeners routinely detected in marine biota were BDE 99, 100, 154 and 153.

Median concentrations of PCBs, DDTs and PBDEs were higher in the adult group (n=8) than in the juveniles (n=13). For HCB and HCHs, no difference was observed between the age groups. Concentrations of PCBs, DDTs, PBDEs and HCB were significantly higher in males (n=15) than in females (n=6), probably due to a loss of POP load of females through gestation and lactation. Furthermore it was observed that the increase in PCB body burden for males with age is faster than for females.

It was suggested in other studies (1,2) that, in harbour porpoises the PCB-TEQ values are much higher than PCDD/PCDF-TEQ values (1.1 to 3.6 pg/g lipid). The TEQ values of mono-ortho PCBs (MO-PCBs) calculated in range between 110 and 166 pg/g lipid and were similar with values obtained from specimens caught in the Black Sea (10) or Mediterranean Sea (11). Recently, Jimenez et al (11) have shown that for other marine mammals (such as pilot whale and some dolphin species), the contribution of mono-ortho PCBs to the total TEQs was between 60 and 99 %, while the contribution of PCDD/PCDFs was almost negligible.

A significant difference in ERs of chiral PCBs is observed with age in this study, though the number of samples analyzed and the age range are limited. All samples of adult porpoises (Table 2) revealed nonracemic ERs for chiral PCBs (PCB 95, 132 and 149) with different enrichments. Enrichments of the second eluted atropisomer of PCB 95 and PCB 149 together with an enrichment of the first eluted atropisomer of PCB 132 were found in all adult porpoises. Contrarily, racemic or nearly racemic ERs were found in three out of four juvenile animals. The another juvenile animal (sample no. 19) had a weight of 27 kg comparable with the body weight of the adults. The other juveniles had body weights ranging from 7 to 16 kg. The enantiomeric ratios in sample no. 19 were also similar to those of adult animals.

The ERs of individual chiral PCBs were different from each other in individual samples and are significant correlated. Assuming that on Chirasil-Dex column the elution order of PCB 132 atropisomers is reversed to that of PCB 95 and PCB 149 atropisomers, a good correlation could be found between $1/ER_{PCB132}$ and ER_{PCB95} ($r = 0.887$), and between $1/ER_{PCB132}$ and ER_{PCB149} ($r = 0.909$). Thus, it can be concluded that porpoises have similar enantioselective degradation and bioaccumulation for these three chiral PCBs. Being consistent fish consumers, porpoises may provide a more suitable model for a fish-based diet than do other omnivorous mammals.

It was speculated that the value of ER will mostly depend on exposure time, but not on the degree of contamination. Indeed, no relationship could be found between ERs of chiral PCBs and total PCBs in studied samples. Thus, van Scheppingen (2) suggested that the amount of the most persistent congener, PCB 153 can be related to the feeding pattern of the male porpoises. In contrast to PCB 153, the congener PCB 101 seems to be relatively easy metabolized by porpoises. Thus, it could be shown that, in porpoises, the ratios between PCB 153/PCB 101 ($R_{PCB153/PCB101}$) have a similar trend with the enantiomeric ratios of chiral PCBs. The relationships (r) between $R_{PCB153/PCB101}$ and ER of chiral PCBs were 0.746, 0.720 for PCB 95, PCB 149, respectively and 0.793 for 1/ER of PCB 132. For enantiomers with similar volatility and partitioning properties, ERs are subject only to biological removal

Table 2. Enantiomeric ratios (area first peak/area second peak) in 11 porpoise liver samples.

Sample No.	Age	Sex	Length (cm)	Weight (kg)	ERs PCB 95	PCB 149	PCB 132
5	a	m	151	39	1.31	1.30	0.57
6	a	m	144	33	2.40	1.63	0.45
7	a	f	134	30	1.38	1.28	0.59
10	j	m	103	16	0.99	1.00	0.97
12	j	m	80	7	1.12	1.19	0.94
13	j	m	99	16	1.11	0.96	0.99
15	a	m	142	41	2.54	1.81	0.47
17	a	m	144	43	2.30	1.57	0.44
18	a	m	149	37	1.94	1.40	0.54
19	j	m	103	27	2.22	1.55	0.54
21	a	m	150	39	2.31	1.31	0.59

mechanism processes, while physical/chemical properties do not affect them. Therefore, ER may be a better biomarker for biological metabolism than the congener pattern.

In conclusion, juvenile porpoises are at risk due to high transfer of contaminants from the mother and to differences between PCB congener profiles seen in juveniles and adults. PCBs have a much higher contribution to TEQ toxicity than PCDD/PCDFs, as the harbour porpoises seem to have a decreased capacity of metabolising PCB congeners.

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