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Concentrations of chlorinated and brominated contaminants and their metabolites in serum of harbour seals and harbour porpoises

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ABSTRACT

Harbour seals (*Phoca vitulina*) and harbour porpoises (*Phocoena phocoena*) are top predators in the North Sea and consequently accumulate a variety of pollutants in their tissues. Concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and their hydroxylated metabolites (HO-PCBs and HO-PBDEs) were measured in serum of wild harbour seals ($n=47$) and captive harbour porpoises ($n=21$). Both species exhibit long life spans and do not have extreme situations, such as complete fasting during periods of lactation, in their annual cycles. For PCBs, concentrations in adult males were slightly higher than in juveniles and lowest in juvenile females. For PBDEs, juveniles have higher levels than adult males and females, probably as a consequence of lactational transfer. However, differences between these age–gender groups were not statistical significant, indicating that individual variation was limited within each species, even without knowing the feeding status of the animals. Body condition, particularly emaciation, has a major influence on the levels of chlorinated and brominated contaminants in serum. Profiles of PCBs were CB 153>CB 138>CB 187>CB 180 and CB 153>CB 138>CB 149>CB 187>CB 180 for harbour seals and porpoises respectively. For PBDEs, BDE 47 was the predominant congener followed by BDE 100 and 99 in both species. In harbour seals, concentrations of sum PCBs (median: 39,200 pg/ml) were more than 200 times higher than levels of sum PBDEs (median: 130 pg/ml) and almost 10 times higher than concentrations of sum HO-PCBs (4350 pg/ml). In harbour porpoises, concentrations of sum PCBs (median: 24,300 pg/ml) were about 20 times higher than concentrations of PBDEs (median: 1300 pg/ml). HO-PCBs were detected in only 4 harbour porpoises and this at very low concentrations. Naturally-produced MeO-PBDEs were only found in harbour porpoises at concentrations ranging from 120 to 810 pg/ml. HO-PBDEs were not found in any species. In general, harbour seals accumulate less compounds and have mostly lower concentrations than harbour porpoises possibly as a result of a better developed metabolism.

1. Introduction

The bioaccumulative potential and toxicity of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) as well as pesticides (hexachlorobenzene (HCB), dichloro-diphenyl-trichloroethane (DDT) and metabolites) in marine mammals have been the focus of numerous papers worldwide (Tanabe et al., 1994; Bruhn et al., 1995; Reijnders and Aguilar, 2002; Reijnders and Simmonds, 2003; Thron et al., 2004; Ross, 2006). These types of chlorinated and brominated contaminants have been associated with immunological, reproductive and mostly endocrine/cytotoxic (e.g. thyroid hormone action) effects in various marine mammal species and, due to their persistence in the environment, are still a threat to the health condition of aquatic organisms in general (Damstra et al., 2002; Beineke et al., 2005; Das et al., 2006; Bossart, 2007). Among these, PCBs and PBDEs are assumed to have comparable toxic action mechanisms since they have similar chemical properties (de Boer et al., 1998; Birnbaum and Staskal, 2004). Despite their ban in Europe (PCBs in 1970s, most PBDE congeners in 2004), both types of contaminants can still be found at all levels of the aquatic food chains (Ruus et al., 1999; Boon et al., 2002).

PCBs and PBDEs may undergo metabolic/enzymatic breakdown resulting in methylsulfone and hydroxylated PCB and PBDE metabolites

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(Letcher et al., 2000) or lower brominated PBDE congeners (Letcher et al., 2000; Birnbaum and Staskal, 2004). Debromination of PBDEs into lower brominated congeners has been shown to occur in a few terrestrial and aquatic organisms such as birds (Pirard and De Pauw, 2007; Van den Steen et al., 2007), rats (Huwe and Smith, 2007) and fish (Stapleton et al., 2004; Benedict et al., 2007). Although methylsulfone and hydroxylated metabolites are more polar and consequently easier to eliminate from the body than their parent compounds, considerable amounts of these metabolites are retained in the body of several species (Sandala et al., 2004; Gebbink et al., 2008; Jaspers et al., 2008). Hydroxylated metabolites can be formed by direct insertion of a HO-group or by formation of an arene oxide intermediate that rearranges to a HO-group. Both ways are possible, but the extent to which each pathway occurs is probably highly dependent of the species (Letcher et al., 2000). Effects of these metabolites are mostly related to disturbances of hormonal and endocrine systems as they can bind to and interact with several hormone receptors and transport proteins (Cheek et al., 1999; Birnbaum and Staskal, 2004; Shimokawa et al., 2006). As a result, toxic effects can have a great impact on the health condition of organisms in general. Hydroxylated metabolites are not particularly associated with lipids as can be seen for the parent compounds, but have a high affinity for plasma proteins. Therefore, they can primarily be found in blood (Gebbink et al., 2008).

Harbour seals (*Phoca vitulina*) and harbour porpoises (*Phocoena phocoena*) are common marine mammals in West-European waters (Burns, 2002; Hammond et al., 2002). They are known to accumulate high contaminant concentrations in their tissues because of their longer life spans and top-position in aquatic food chains (Shaw et al., 2005, 2007). Although seasonal changes in blubber thickness may occur, both species do not have extreme fasting periods in their annual cycles as both species continue eating during their reproductive and lactational periods (Kastelein et al., 1997; Burns, 2002; Lockyer, 2007). Weijs et al. (2009a) suggested a higher capacity in harbour seals for metabolizing PCBs and PBDEs compared to harbour porpoises. However, considering the assumed toxicity of the resulting metabolites (Meerts et al., 2000; Birnbaum and Staskal, 2004) and their presence in blood, concerns have been raised about the higher metabolic capacity of harbour seals in terms of their global health and the conservation of marine mammals on a longer term.

While extensive studies described PCBs and PBDEs in blubber and other tissues of caught or stranded marine mammals, fewer data were documented in blood of free-ranging seals and harbour porpoises (Bang et al., 2001; Sørmo et al., 2003; Sørmo, 2005). Levels of persistent organic pollutants (POPs) in blood depend not only on environmental contamination; but also numerous biotic factors are suspected to modulate concentrations: gender, diet, age, pregnancy, lactation and weaning (Debier et al., 2006). The objective of the present study was to investigate the occurrence and distribution of PCBs, PBDEs, their hydroxylated metabolites (HO-PCBs and HO-PBDEs), HCB and DDTs (*p,p′*-DDE, *p,p′*-DDT and *p,p′*-DDD) in blood of free-ranging harbour seals, harbour porpoises held in captivity and a stranded harbour porpoise in order to elucidate the metabolism of these compounds. Several factors including species, age class, gender and year of sampling were apprehended to get further understanding of PCB and PBDE kinetic in harbour seals and harbour porpoises.

2. Materials and methods

2.1. Samples, chemicals and target compounds

Serum samples of 21 harbour porpoises in captivity from 2006–2008 were provided by SOS Dolfijn, Dolfinarium Harderwijk (The Netherlands), and were taken for regular medical purposes from the tail fluke. Information about the medical situation of these animals at the time of sampling can be found in Table 1. Serum was isolated by centrifugation at 4000 rpm during 15 min (Hettich EBA-20) and kept at −20 °C until further analysis. A serum sample of an adult harbour porpoise, stranded on the North Sea coast in 2003 was also analyzed. Serum samples of free-ranging harbour seals were collected from 47 animals caught in the frame of monitoring programs for the health assessment organized on Helgoland and Lorenzenplate (North Sea, Germany) in 2006–2008 and in Rømø (Denmark) in 2008. Seals were physically restrained and blood was drawn from the extradural venous sinus into sterile evacuated blood collection tubes (serum tubes Monovette®, Germany) and kept at −20 °C. Serum was isolated by centrifugation at 1500 g during 20 min at 20 °C (Multifuge 3S-R, Kendro) (Hasselmeier et al., 2008).

In all samples, target compounds were PCBs (IUPAC-numbers: 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187,

Table 1
Medical information of the harbour porpoises, held in captivity during rehabilitation, at the time of sampling.

Code	Days in rehabilitation	Gender	Estimated age at time of sampling (years)	Length (cm)	Weight (kg)	Condition at time of sampling
P1	3247	M	9	132.5	40.25	Healthy
P2	1946	M	6	120	29.55	Slightly anaemic due to blood loss associated with a urogenital lesion/ inflammation
P3	571	F	2	140	35.8	Healthy
P4	117	M	1	116.5	27.8	Healthy
P5	0	M	Adult	145	44	
P6	5	M	Adult	149	42.3	Sample on day of death, very severe inflammatory reaction probably due to pneumonia
P7	434	F	2	126	38.05	Anaemic
P8	37	F	1	113	26.46	Healthy, on antibiotics after recent stranding
P9	0	M	Adult	142	41.3	Sample shortly after stranding, inflammatory reaction in blood
P10	128	F	1	122	29.9	Healthy (animal at the end of treatment with antibiotics)
P11	42	M	1	110–114	22.85	Severe anaemia and inflammation
P12	9	F	1	118	19.6	Pneumonia, sepsis and gastric impaction, emaciation
P13	7	F	Adult	146	48.8	Anaemic and pregnant. Animal dies a month later due to acute hepatic lipidosis
P14	30	M	1	108–112.5	22.25	Severe anaemia, antiparasitic treatment
P15	174	F	2	136	34	Healthy (animal at the end of treatment)
P16	355	F	3	133–134.5	39.04	Healthy
P17	34	M	2	117–123	26.15	Laryngitis
P18	183	F	1	105	29.24	Anaemic due to lungworm infection (at time of sampling only on antibiotics after lungworm treatment)
P19	1	M	2	116	29.55	Healthy
P20	77	F	1	108.5	26.95	Chronic hepatitis of unclear significance
P21	32	F	2	116	27.2	Healthy

194, 199), PBDEs (IUPAC-numbers: 28, 47, 99, 100, 153, 154), HCB, and pesticides (*p,p′*-DDE, *p,p′*-DDT and *p,p′*-DDD). The following 22 HO-PCB congeners were investigated: 3-HO-CBs (numbers 118, 153, 138 and 180), 4-HO-CBs (numbers 79, 120, 107, 146, 127, 130, 163, 187, 162, 202, 177, 172, 193, 198, 199 and 208), and 4,4′-diHO-CB 202. The following 8 HO-PBDEs were also targeted: 2′-HO-BDE 68, 3-HO-BDE 47, 5-HO-BDE 47, 6-HO-BDE 47, 4-HO-BDE 42, 4′-HO-BDE 49, 6-HO-BDE 99 and 4-HO-BDE 90. Standards were from Accustandard (HO-PBDEs) or from Wellington Laboratories (HO-PCBs).

2.2. Sample preparation

The method for serum analysis was adapted from the methods described by Covaci and Voorspoels (2005) for the determination of neutral compounds in serum and by Weiss et al. (2006) for the determination of phenolic compounds. An accurate volume of serum (typically 1.5 ml) was spiked with internal standards (CB 143 and BDE 77 for neutrals and 4′-HO-CB 159 for phenolics), diluted 1:1 with Milli Q water, mixed with formic acid, sonicated for 20 min and extracted using solid-phase extraction (SPE) cartridges (6 ml/500 mg Oasis HLB, Waters). Elution was done by 10 ml of MeOH:DCM (1:1, *v/v*). After evaporation to near dryness, the analytes were reconstituted in 500 µl hexane:DCM (1:1, *v/v*) and fractionated on silica SPE cartridges (3 ml/500 mg, Varian). A first fraction containing PCBs and PBDEs was eluted with 5 ml hexane, while the phenolic compounds were eluted with 6 ml MeOH:DCM (1:1, *v/v*). Both fractions were evaporated to dryness.

The first fraction (neutrals) was cleaned-up on 500 mg acid silica (44%, *w/w*) and the analytes were eluted with 8 ml hexane:DCM (1:1, *v/v*). The cleaned extract was evaporated to dryness under a gentle nitrogen stream and reconstituted in 100 µl iso-octane. The second fraction (phenolics) was derivatized for 30 min with diazomethane when MeO-PCBs and MeO-PBDEs were formed. After solvent evaporation, the dried residue was reconstituted in 200 µl DCM and further cleaned-up on 500 mg acid silica (25%, *w/w*). Methoxylated compounds were eluted with 10 ml hexane:DCM (1:1, *v/v*), the extract was evaporated to dryness under a gentle nitrogen stream and reconstituted in 100 µl iso-octane.

2.3. Analysis

For the analysis of methoxylated derivatives and of PBDEs, a GC–MS operated in electron capture negative ionisation (ECNI) mode was equipped with a 30 m × 0.25 mm × 0.25 µm DB-5 capillary column (J&W Scientific). The ion source temperature was 170 °C. The MS was used in the SIM mode with two ions monitored for each MeO-PCB congener in specific windows, while ions $m/z = 79$ and 81 were monitored for MeO-PBDEs and for PBDEs during the entire run. Two µl of the extract was injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 ml/min).

For the PCB, HCB and DDT (*p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD) analysis, a GC–MS operated in electron impact ionisation (EI) mode was equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE). The ion source temperature was 230 °C. The MS was used in the SIM mode with two ions monitored for each PCB homologue group in specific windows. Two µl of the extract was injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 ml/min).

2.4. Quality Assurance/Quality Control (QA/QC)

Multi-level calibration curves ($r^2 > 0.999$) in the linear response interval of the detector were created for the quantification. QC was performed by regular analyses of procedural blanks, by random injection of standards, spiked samples and solvent blanks. The Quality Control scheme is also assessed through regular participation to interlaboratory comparison exercises organized by AMAP (POPs in serum). Obtained values were deviating no more than 20% from the consensus values. The mean recovery of internal standard 4′-HO-CB 159 in serum was 96 ± 2%. Recoveries assessed through spiking experiments at 25 and 125 pg/ml ranged between 90 and 93% with precision (RSD) <2%. For analytes detected in the procedural blanks, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank. For analytes that were not detected in the procedural blanks (all HO-PCBs and HO-PBDEs), LOQs were calculated for S/N = 10.

2.5. Statistical analysis

Statistical analyses were conducted using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). Data were log-transformed and a value 1/2 LOQ was used for concentrations below LOQ. Outliers were

Table 2
Medians and range (minimum–maximum) in pg/ml of all compounds measured in the present study in serum of harbour seals and harbour porpoises.

	Harbour seals	Harbour porpoises
N	47	19
CB 52	180 (<40–689)	759 (471–6040)
CB 74	89 (<40–333)	332 (123–711)
CB 95	47 (<30–178)	889 (535–6330)
CB 99	1970 (456–8490)	1030 (503–9360)
CB 101	523 (173–1960)	1280 (606–2890)
CB 105	60 (<30–160)	199 (119–564)
CB 110	<30 (<30–111)	144 (<30–288)
CB 118	250 (73–689)	1200 (786–2900)
CB 128	1170 (256–5020)	610 (254–1510)
CB 138	7670 (1700–34,200)	3390 (1770–25,300)
CB 149	830 (327–3870)	2960 (1670–17,600)
CB 153	16,000 (2930–79,800)	6880 (3330–51,300)
CB 156	175 (<20–622)	<20
CB 170	1230 (200–8110)	428 (175–2690)
CB 180	3890 (626–22,400)	1400 (703–8190)
CB 183	720 (127–3890)	399 (206–2520)
CB 187	4030 (1047–19,000)	1820 (889–12,200)
CB 194	307 (67–2310)	173 (93–754)
CB 199	353 (60–2380)	266 (126–1110)
BDE 28	<10	14 (<10–33)
BDE 47	59 (11–348)	668 (271–1670)
BDE 99	<10 (<10–35)	155 (29–352)
BDE 100	57 (11–315)	334 (116–710)
BDE 153	<10 (<10–57)	31 (10–413)
BDE 154	<10 (<10–21)	78 (16–766)
4-HO-CB 79	147 (<20–467)	<20
4-HO-CB 107	1840 (301–6440)	<20 (<20–28)
3-HO-CB 118	<20	<20
4-HO-CB 120	69 (<20–241)	<20
4-HO-CB 127	<15 (<15–15)	<15
4-HO-CB 130	66 (<15–474)	<15 (<15–19)
3-HO-CB 138	117 (16–529)	<15
4-HO-CB 146	491 (108–2340)	<15
3-HO-CB 153	24 (<15–101)	<15
4-HO-CB 162	657 (191–1940)	<10
4-HO-CB 163	169 (25–985)	<10
4-HO-CB 172	22 (<10–136)	<10
4-HO-CB 177	151 (31–697)	<10
3-HO-CB 180	<10 (<10–32)	<10
4-HO-CB 187	253 (96–870)	<10
4-HO-CB 193	16 (<10–56)	<10
4-HO-CB 198	12 (<10–56)	<10
4-HO-CB 199	<10 (<10–28)	<10
4-HO-CB 202	257 (87–695)	<10
4-diHO-CB 202	<10 (<10–11)	<10
4-HO-CB 208	64 (23–200)	<10
HCB	<20	641 (343–1650)
p,p′-DDE	2750 (722–8440)	3860 (1590–15,600)
p,p′-DDD	<50 (<50–107)	636 (269–3320)
p,p′-DDT	213 (<50–678)	510 (197–2330)
6-MeO-BDE 47	<10	195 (100–732)
2′-MeO-BDE 68	<10	34 (10–95)

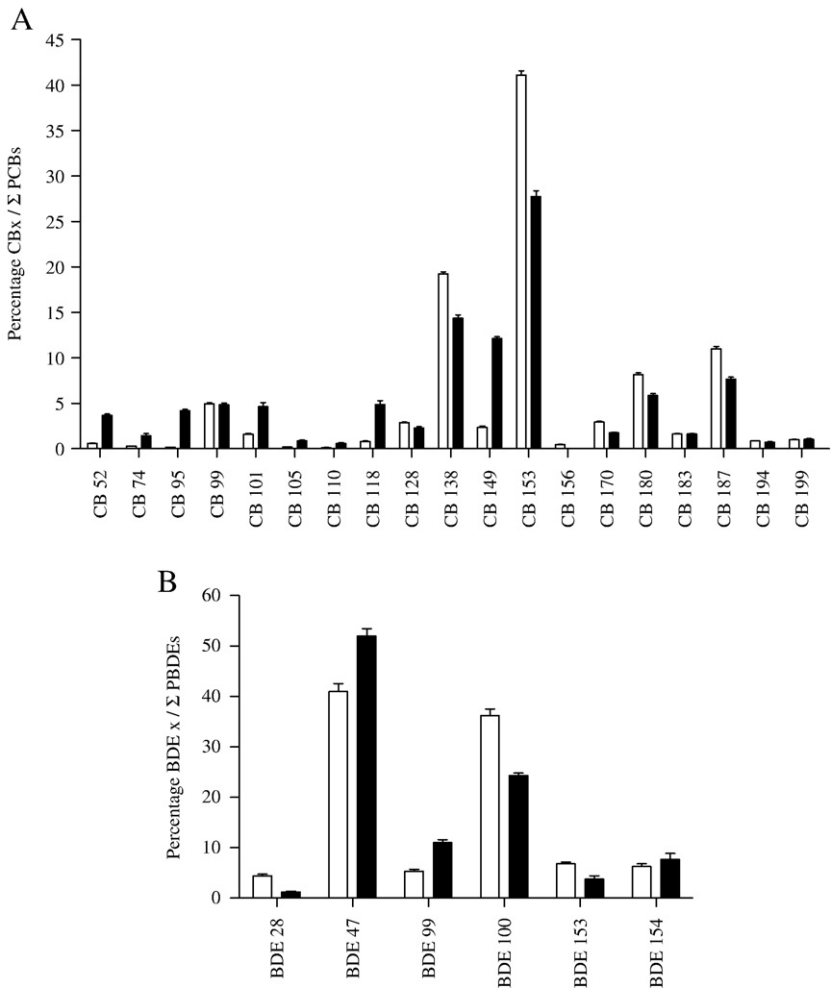

Fig. 1. Mean percentages of individual PCB and PBDE congeners in the sum of PCBs (A) and PBDEs (B) in serum of harbour seals (white bars; $n=47$) and harbour porpoises (black bars; $n=19$). Error bars represent standard errors (SE).

detected using boxplots and were removed for further statistical analysis. The influence of age, gender, location (only for harbour seals) and year of sampling was investigated with a two-way ANOVA test followed by a Tukey's post hoc test. Age (juvenile—J and Adult—A) and gender (Male—M and Female—F) were used as fixed variables, location (Germany—G and Denmark—D) and year of sampling (2006, 2007 and 2008) as random variables. The level of statistical significance was defined at $p<0.05$.

3. Results

Median values and ranges (minimum and maximum) of all compounds measured in this study in serum of harbour seals and harbour porpoises are presented in Table 2.

3.1. Levels and profiles in harbour seals

BDE 28 and HCB were not detected in any investigated sample, while *p,p′*-DDD and congeners CB 110, BDE 99, BDE 154 and BDE 153 were found in less than 50% of all samples.

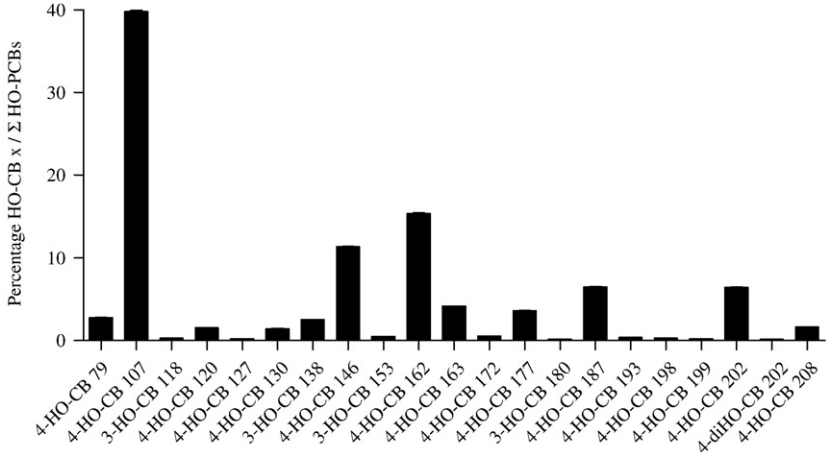

Fig. 2. Mean percentages of HO-PCB congeners in sum of HO-PCBs in serum of harbour seals. Error bars represent standard errors (SE).

HO-compounds	PCB precursors	
	direct insertion	NIH-shift
4-HO-CB107	CB 107	**CB 118, 105**
3-HO-CB118	**CB 118**	CB 107,126
4-HO-CB120	CB 120	**CB 118**
4-HO-CB130	CB 130	**CB 128, 138**
3-HO-CB138	**CB 138**	CB 130, 157
4-HO-CB146	CB 146	**CB 138, 153**
3-HO-CB153	**CB 153**	CB 146, **128**
4-HO-CB172	CB 172	**CB 170, 180**
3-HO-CB180	**CB 180**	CB 172
4-HO-CB187	**CB 187**	**CB 183**
4-HO-CB-199	**CB 199**	CB 204
4-HO-CB202	CB 202	**CB 199**
4,4'-diHO-CB202	CB 202 (*)	**CB 199**

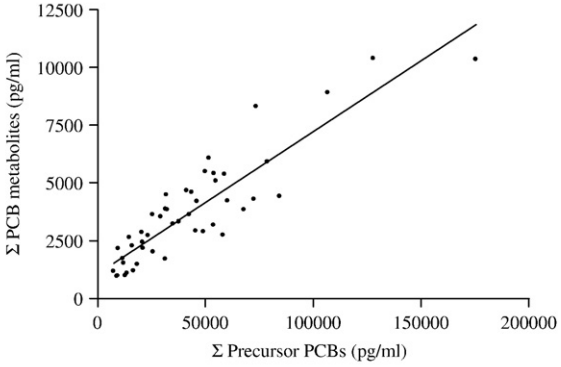

Fig. 3. Relationship between HO-PCB metabolites and their possible precursor congeners in serum of harbour seals ($r^2 = 0.80$; $p < 0.0001$). Only congeners in bold were measured in the present study and were therefore included in the calculations. The table was made according to Jaspers et al. (2008). (*) Double insertion.

In general, values of sum PCBs were more than 200 times higher compared to concentrations of sum PBDEs and about 15 times higher than concentrations of the sum DDTs (sum of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD). Values ranged from 9 180 pg/ml to 194 000 pg/ml for sum PCBs, 772 pg/ml to 9140 pg/ml for the sum DDTs and 37 pg/ml to 726 pg/ml for sum PBDEs. For PCBs, CB 153 was the predominant congener in all samples, followed by respectively CB 138, CB 187 and CB 180 (Fig. 1A). *p,p′*-DDE was the most dominant pesticide, followed by *p,p′*-DDT and *p,p′*-DDD. For PBDEs, BDE 47 and BDE 100 were the most dominant congeners. Other congeners, such as BDE 99, BDE 153 and BDE 154 (Fig. 1B), were of reduced importance. HCB was not detected in any serum sample in the present study.

No HO-PBDEs were found in any serum sample of harbour seals in the present study. Some HO-PCBs (3-HO-CB 118 and 4-HO-CB 127) were not found in any sample, while 4-HO-CB 199, 3-HO-CB 180 and 4,4′-diHO-CB 202 were detected in less than 50% of all samples. The highest concentrations were found for 4-HO-CB 107, a lower chlorinated compound, while higher chlorinated compounds showed lower values (Fig. 2). In all individuals, 4-HO-CB 107 was followed by 4-HO-CB 162 and 4-HO-CB 146.

When divided into 4 groups (AM—adult males, JM—juvenile males, JF—juvenile females and AF—adult females), distribution patterns of sum PBCs and sum HO-PCBs were AM>JM, JF>AF. Patterns of sum PBDEs and sum DDTs were JM, JF>AM>AF suggesting that AM have better developed metabolic capacities than juveniles. Unfortunately, differences between the age–gender groups were too small to be statistically significant (all $p > 0.05$). Location and year of sampling were not important for sum PCBs and sum DDTs, while only the year of sampling had a minor effect on concentrations of sum HO-PCBs and of sum PBDEs. For these latter two, the highest concentrations of sum HO-PCBs and sum PBDEs were found in 2006 followed by 2008 and 2007, respectively. However, these differences were considered to be a consequence of the different sample sizes ($n = 21$, 10 and 16 for 2006, 2007 and 2008 respectively).

In general, levels of sum PCBs were approximately 11 times higher than levels of their metabolites (ratio ΣHO-PCBs/ΣPCBs = 0.086). In this study, a good correlation ($r^2 = 0.80$; $p < 0.0001$) was found between the sum of HO-PCBs and their possible precursor congeners (Fig. 3).

3.2. Levels and profiles in harbour porpoises

CB 156 was found in less than 50% of all samples. The levels of sum PCBs (range: 13,300–148,300 pg/ml) were higher than sum DDTs (range: 2150–20,900 pg/ml) followed by sum PBDEs (range: 495–2900 pg/ml) and HCB (range: 343–1650 pg/ml). In general, PCB and PBDE profiles were: CB 153>CB 138>CB 149>CB 187>CB 180 for PCBs and BDE 47>BDE 100>BDE 99>BDE 154>BDE 153 for PBDEs (Fig. 1A and B). *p,p′*-DDE was the most dominant compound among the DDTs, followed by *p,p′*-DDD and *p,p′*-DDT.

Only 4-HO-CB 107 and 4-HO-CB 130 could be measured in serum samples of harbour porpoises at very low levels and in a limited number of samples (4 and 1 sample, respectively). No other HO-PCBs or HO-PBDEs were found in any serum sample of harbour porpoises in the present study.

The only AF harbour porpoise analyzed in this study was pregnant at the time of sampling (animal P13, Table 1) and had very low concentrations of sum PCBs, sum PBDEs, HCB and sum DDTs compared to all other individuals. Also, there was an outlier in the JF-group with concentrations 10–20 times higher than the average of the JF-group (animal P12, Table 1). Both samples were excluded from statistical analysis and further calculations (see further). In all other samples (AM, JM and JF), no significant effects of age, gender or time of sampling were found on concentrations of sum PCBs, sum PBDEs, sum DDTs and HCB. For all these compounds, AM had higher concentrations (although not significant) compared to the juveniles.

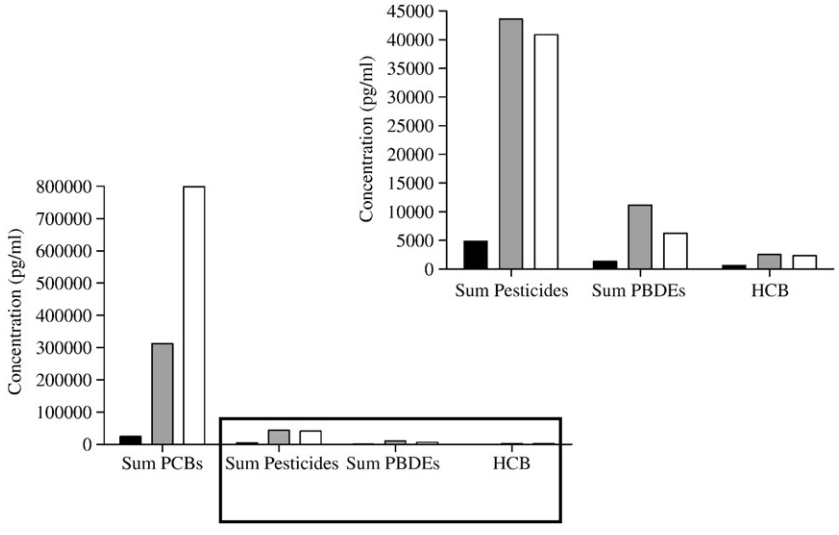

Fig. 4. Influence of body condition on concentrations of sum PCBs, sum pesticides (*p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD), sum PBDEs and HCB in serum samples of harbour porpoises (■ = median of harbour porpoises in captivity ($n = 19$), ■ = concentrations in serum of the outlier in the JF-group, □ = concentrations in serum of the wild adult male).

3.3. Influence of body condition on concentrations in serum

In addition to the 21 harbour porpoises in captivity, serum of a wild adult male harbour porpoise (stranded at the Belgian coast in 2003) was also analyzed. This animal was emaciated and necropsy revealed lung pneumonia. The outlier in the JF-group (animal P12, Table 1), which was excluded from further statistical analysis as mentioned earlier, was also emaciated. Both animals have concentrations for sum PCBs, sum PBDEs, HCB and sum DDTs that exceed by far the median values of the harbour porpoises in captivity (Fig. 4).

3.4. Naturally-produced MeO-PBDEs in harbour seals and harbour porpoises

Two naturally-produced MeO-PBDEs, 2′-MeO-BDE 68 and 6-MeO-BDE 47, were analyzed as well. Both compounds were not detected in any serum sample of the harbour seals. In harbour porpoises, no influence of age, gender and location of sampling was found and a median concentration of 221 pg/ml with 6-MeO-BDE 47 accounting for more than 84% of the total sum of MeO-PBDEs was calculated.

4. Discussion

This is the first study to report the simultaneous measurement of PCBs, PBDEs, HO-PCBs, HO-PBDEs, DDTs and naturally-produced MeO-PBDEs in blood of living harbour seals and harbour porpoises from the North Sea.

4.1. Levels

Information on the levels of PBDEs and HO-PCBs in blood of marine mammals is scarce. A comparison with results from other areas is complicated because contaminants are reported in other tissues than serum (e.g. liver, adipose tissue), because concentrations are expressed in different ways (pg/ml, pg/g wet weight (ww), pg/g lipid weight (lw)) and because not always the same congeners are analyzed. Concentrations of PCBs in serum of harbour seals in the present study (sum of 19 congeners) were approximately 3 to 6 times higher than concentrations of Scottish adult grey seal (*Halichoerus grypus*) females in late lactation when lipid mobilization is highest (sum of 26 congeners; Debier et al., 2003a). Levels of PCBs are also higher than the concentrations reported in whole blood of ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*) from Svalbard (sum of 33 congeners; Bang et al., 2001) and in whole blood of harbour seals from California (sum of 20 congeners; Young et al., 1998). In an experiment where harbour seals were fed fish from a contaminated area, the Baltic Sea, blood (fraction III containing mostly high density particles) concentrations of PCBs were associated with immune disorders and reproductive impairment (Boon et al., 1987). Since the results from the present study are more than an order of magnitude higher compared to these results, serious concerns have been raised about the health condition of the harbour seals in the North Sea at this moment. It is difficult to discuss differences in levels in serum between harbour seals and harbour porpoises, since it is unknown how (and if) living in captivity for a few months affects the contaminant concentrations. Although several PCBs and PBDEs are stable and persistent in marine mammals and the porpoises in rehabilitation from the present study received amounts of fish caught in the North Sea, it remains unclear how captivity may influence the concentrations of these pollutants. A number of 7 out of 20 (the emaciated animal excluded) porpoises were healthy at the time of sampling, while the other 13 were ill in some way (Table 1). Yet, no part of the individual variation could be assigned to the health status. Concentrations of PCBs in harbour porpoises are in general somewhat lower compared to the concentrations found in harbour seals, but still exceed the levels mentioned above in other marine mammal species.

4.2. Patterns

Profiles of PCBs and DDTs in serum of harbour seals were similar to the patterns found in whole blood samples (Young et al., 1998) and in blubber or liver (Kajiwara et al., 2001; Shaw et al., 2005; Weijs et al., 2009a), suggesting that the contaminant's profiles are well conserved in this species, regardless of the tissue (Hutchinson and Simmonds, 1994; Vetter et al., 1996; Boon et al., 1997). For PBDEs, patterns in blood differed from patterns in blubber (Shaw et al., 2007; Weijs et al., 2009a) probably due to a selective retention of some congeners in other tissues than blood. HCB was not detected in any serum sample but seems to be a minor contaminant in blubber of several other pinnipeds from other areas as well (Ruus et al., 1999; Kajiwara et al., 2001; Hobbs et al., 2002; Shaw et al., 2005). Formation of PCB metabolites may occur via direct insertion and/or NIH shift (Letcher et al., 2000). Since HO-PCBs were not detected in liver of fish species caught in the North Sea in 2008 (Covaci, unpublished data), concentrations found in harbour seal serum are most probably the result of intrinsic metabolic breakdown of PCBs into HO-PCBs in the marine mammals. ΣHO-PCBs/ΣPCBs ratios smaller than 1 were also found for bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon (Florida, USA) and Charleston (Montie et al., 2008), for ringed seals from Québec, Canada (Sandau et al., 2000) and for bowhead whales (*Balaena mysticetus*) from Alaska (Hoekstra et al., 2003). In contrary, ratios greater than 1 were detected in blood of polar bears (*Ursus maritimus*) from Canada (Sandau et al., 2000) and Greenland (Sandala et al., 2004; Gebbink et al., 2008) as a result of a high capacity to form HO-PCBs (Table 3). The pattern found in the present study was dominated by 4-HO-CB 107 followed by 4-HO-CB 162 and 4-HO-CB 146. Although these results agree well with results from Løken et al. (2008), they are different from patterns reported in liver, brain, blood and adipose tissue of polar bears (Sandala et al., 2004; Gebbink et al., 2008), in plasma of bowhead whales (Hoekstra et al., 2003) and in bottlenose dolphins (Montie et al., 2008).

PCBs can be divided into several metabolic groups according to their structure and affinity for Phase 1 cytochrome P450 enzyme subgroups. Different patterns are therefore caused by the presence and activity of these enzyme subgroups and are considered to be species specific. Meijer et al. (2008) measured HO-PCBs in maternal and cord serum of humans and concluded that HO-PCBs can be transferred to the offspring. Debier et al. (2003a) found that young animals have a lower ability to detoxify contaminants compared to adults because their metabolism is primarily focused on their growth and overall development. As a consequence, regardless of their metabolic capacities, young animals are probably exposed to HO-PCBs and may experience the possible effects of these compounds as well.

The PCB, PBDE and pesticide patterns found in serum of harbour porpoises agree well with profiles found in liver and blubber (Covaci et al., 2002; Weijs et al., 2009a) and seem also to be highly species

Table 3
Means and standard deviations (SD) of the concentrations (ng/g wet weight) of HO-PCBs in tissues of four marine species.

Species	*n*	Mean ± SD	Tissue	HO-PCB/PCB	Reference
Bowhead whales	10	1.52 ± 0.31[a]	Plasma	0.547	Hoekstra et al. (2003)
Bottlenose dolphins	10	3.9 ± 3.2	Plasma	0.016	Houde et al. (2006)
	42	18 ± 21	Plasma	0.082	
	32	209 ± 211	Plasma	0.679	
	5	94 ± 103	Plasma	0.470	
	12	33 ± 21	Plasma	0.236	
	35	64 ± 53	Plasma	0.435	
	21	168 ± 110	Plasma	0.644	
Polar bears	20	60 ± 8[a]	Adipose	0.011	Gebbink et al. (2008)
		1020 ± 132[a]	Blood	25.5	
		18 ± 3[a]	Brain	0.122	
		355 ± 36[a]	Liver	0.114	
	19	182.3 ± 72.1	Blood	8.30	Sandala et al. (2004)
Harbour seals	5	0.49[b]	Liver	0.009	Løken et al. (2008)
		2.37[b]	Plasma	0.344	
	47	4.35[b]	Serum	0.086	Present study[c]

[a] Standard error or SE.
[b] Median values.
[c] Expressed in ng/ml.

specific. In contrast to harbour seals, HO-PCBs were only occasionally measured in serum of harbour porpoises. Houde et al. (2006) detected HO-PCBs in plasma of bottlenose dolphins, while Hoekstra et al. (2003) found HO-PCBs in plasma of bowhead whales. The difficulties of harbour porpoises to form HO-PCBs, can therefore not be extrapolated to other cetaceans.

When comparing harbour seals and harbour porpoises, it is very clear that the porpoises have difficulties in forming HO-PCBs and that they accumulate a higher number of PCB and PBDE congeners compared to the seals. Troisi et al. (2001) analyzed PCB and DDE methyl sulfones in lung and uterus of a cetacean (striped dolphins — *Stenella coeruleoalba*) and a pinniped species (harbour seal) (all morbillivirus epizootic victims) and found higher concentrations of methyl sulfones in both tissues of harbour seals compared to striped dolphins. Further, it seems that harbour porpoises have only one possible way for metabolic breakdown of PCBs, namely formation of $MeSO_2$-PCBs (Chu et al., 2003). In contrast, harbour seals can form HO-PCBs and $MeSO_2$-PCBs and both metabolites to a greater extent than harbour porpoises. Both classes of PCB metabolites including the precursor PCBs are potential endocrine-disruptors and are associated with endocrine-related effects, such as cytotoxicity, competitive binding with several receptors and disruption of hormone homeostasis (Letcher et al., 2000; Sandala et al., 2004). So far, no classification regarding toxicity of PCBs, HO-PCBs and $MeSO_2$-PCBs is available. Therefore, it remains debatable whether metabolic transformation capacities can improve the overall health condition of an organism. Further toxicity tests with PCBs, HO-PCBs and $MeSO_2$-PCBs are needed to assess and to compare the condition of harbour seals and harbour porpoises at this moment.

Although biotransformation of PBDEs in beluga whales (*Delphinapterus leucas*) was earlier shown to occur (McKinney et al., 2006a), no HO-PBDEs were found in the investigated blood samples of harbour seals and harbour porpoises. This is a confirmation of the results of a recent study (Meijer et al., 2008), performed in blood of pregnant women and their infants in The Netherlands, which was also unable to detect a HO-PBDE (6-HO-BDE 47). Moreover, no HO-PBDEs were found in ringed seal blubber and beluga whale blood and liver (Kelly et al., 2008). In contrast, detectable but not quantifiable HO-PBDE concentrations were reported in beluga whale livers (McKinney et al., 2006b) and very low yet measurable concentrations of 0.01 to 0.1 ng/g lipid equivalent were found in blubber and milk of beluga whales (Kelly et al., 2008). However, since concentrations of PBDEs in harbour seals are lower than levels in harbour porpoises, a greater capacity for debromination of PBDEs in harbour seals is assumed as previously shown for BDE 209 in grey seals (Thomas et al., 2005).

4.3. Naturally-produced MeO-PBDEs

Vetter (2006) raised the hypothesis that higher contributions of 2′-MeO-BDE 68 are caused by sponges or associated organisms, whereas higher proportions of 6-MeO-BDE 47 are an indication of the presence of algae or associated organisms. The dominance of 6-MeO-BDE 47 in the present study was also found in blubber of minke whales (*Balaenoptera acutorostrata*) (Marsh et al., 2005), in male ringed seals and beluga whales (Kelly et al., 2008), in grey seals and ringed seals (Haglund et al., 1997) and in pre-industrial whale oil (Teuten and Reddy, 2007). Reversed patterns were found in blubber of striped dolphins (Marsh et al., 2005) and several marine mammal species from Oceania (Melcher et al., 2005) and Brazil (Dorneles et al., submitted for publication). A possible explanation for the presence of MeO-PBDEs only in harbour porpoises (21 porpoises in rehabilitation and the wild emaciated porpoise) in the present study might be that they feed on offshore prey coming within the southern part of the North Sea, while harbour seals feed more inshore as evidenced by their stable isotope signatures (Das et al., 2003; Weijs et al., 2009b). Higher concentrations of MeO-PBDEs in cetaceans in continental shelf and oceanic environments compared to species from estuarine areas were also recently found in Brazilian waters (Dorneles et al., submitted for publication).

4.4. Blood as biomonitoring tool

In marine mammals, or in all mammals for that matter, the blood is responsible for the transport of all kinds of molecules, such as lipids and proteins, from one organ to the other inside the body. The concentrations of these molecules in blood however may change as a result of several factors like the feeding status, metabolism and the overall health condition of the animal. These factors are also important for explaining the variation of pollutants in blood due to the (high) lipophilic nature of, for example, PCBs, PBDEs and the affinity of HO-PCBs for proteins. To date, no information about the concentrations of pollutants before and after a meal in marine mammal blood is available. However, although the feeding status of the animals at the moment of blood sampling was unknown, it can not be ruled out for explaining the individual variation among the animals. It is impossible to discuss the influence of the other two factors, namely metabolism and health condition, without taking the role of blubber into account. In general, blubber has a double function. It provides insulation for the body (Dunkin et al., 2005) and it also stores energy in the form of lipids (Koopman et al., 1996; Kastelein et al., 1997). A depletion of the blubber, caused by seasonal changes in blubber thickness or more extreme cases such as emaciation or complete fasting during lactation, may lead to lipid mobilization throughout the body (Debier et al., 2003a,b; 2006), reflected in higher levels of lipids/lipophilic pollutants in the blood. Harbour seals and porpoises do not fast during lactation or other periods of their reproductive cycle (Burns, 2002), but have a seasonally variation in blubber thickness (Lockyer, 2007). In the present study however, for the harbour seals, sampling was only done once every year, making it impossible to compare between seasons of the same year. For the harbour porpoises, being in rehabilitation may suppress this seasonality. The very high concentrations found in serum of the two porpoises (one from a rehabilitation centre, one found stranded), both suffering from emaciation and lung pneumonia, were considered to be a reflection of the depletion of the blubber. These conditions, however, are more exceptional than general (Siebert et al., 2001) and can be seen relatively easy for harbour porpoises as these animals develop a 'neck' after a few days without feeding (Kastelein et al., 1997). So far, biomonitoring of PCBs and PBDEs was mainly done in blubber of marine mammals. As concentrations in blubber were found to be correlated with blubber thickness (Debier et al., 2003a; Montie et al., 2008; Weijs et al., 2009a), these results are equally dependent on the blubber thickness as concentrations in blood. It was not possible to correlate concentrations in blood to concentrations in blubber in the present study. In contrast to blubber, blood has the advantage that sampling can more easily occur in living animals which is always more realistic.

5. Conclusions

HO-PCBs are particularly bound to proteins, so that blood is the ideal substrate for detecting HO-PCBs. The presence of HO-PCBs in serum of harbour seals suggests that these animals are capable of metabolizing PCBs, while harbour porpoises are not. In general, higher numbers of compounds (PCBs, PBDEs, HCB, 2′-MeO-BDE 68, 6-MeO-BDE 47 and DDTs) were detected in serum of harbour porpoises. Despite the fact that correlations between levels in serum and blubber, as storage compartment for the lipophilic compounds, could not be made, profiles of PCBs and PBDEs in serum were comparable with profiles in blubber. Within each species, variation between individuals remained limited, even without knowing the feeding status (time between feeding and sampling, amount of food ingested, etc.) of each individual and without taking the season of sampling into account. Concentrations in serum were assumed to be correlated with the body condition, in particular the emaciation, of the animals.

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