PCBs, PBDEs AND THEIR HYDROXYLATED METABOLITES IN SERUM OF FREE-RANGING HARBOUR SEALS (*PHOCA VITULINA*): LEVELS AND PROFILES

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Introduction

The bioaccumulative potential and toxicity of polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs) in marine mammals has been the focus of numerous papers worldwide. Although PCBs and PBDEs may undergo metabolic/enzymatic breakdown leading to methylsulfone and hydroxylated metabolites[1], recent concerns have been raised about the presence and health effects of some of these metabolites in wildlife. Harbour seals (*Phoca vitulina*) are common pinnipeds in European coastal waters. They are known to accumulate high PCB and PBDE concentrations in their tissues because of their longer life spans and top-position in aquatic food chains[2,3]. Profiles of PCB and PBDE congeners in harbour seals suggest that these animals have a higher capacity of metabolizing several congeners compared to other marine mammal species[4]. However, considering the assumed toxicity of the resulting metabolites, a higher metabolism of PCBs and PBDEs might not be an advantage after all. The objective of the present study was to investigate the levels and profiles of PCBs, PBDEs and their hydroxylated metabolites in blood of free-ranging harbour seals in order to elucidate the metabolism of PCBs and PBDEs.

Materials & Methods

Samples, chemicals and target compounds. Blood samples were collected from 31 harbour seals caught in seal-catch campaigns organised on Helgoland and Lorenzenplate (North Sea, Germany) in 2006 and 2007. Seals were physically restrained. 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 1500g during 20 min at 20°C (Multifuge 3 S-R, Kendro). In all samples, the following 22 HO-PCB congeners were investigated: 3-HO-CBs (numbers 118, 153, 138 and 180), 4-HO-CBs (numbers 120, 107, 146, 127, 130, 163, 187, 162, 202, 177, 172, 193, 198, 199 and 208), 4,4'-diHO-CB 202. Additionally two unknown HO-tetraCBs were also measured. 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).

Sample preparation. The method for serum analysis was adapted from the methods described by Covaci and Voorspoels[5] for the determination of neutral compounds in serum and by Weiss et al.[6] for the determination of phenolic compounds. A volume of serum (~ 1.5 ml) was spiked with internal standards (PCB 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 (3ml/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.

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 x 0.25 mm x 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 were injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 ml/min). For the PCB analysis, a GC-MS operated in electron impact ionisation (EI) mode was equipped with a 25 m x 0.22 mm x 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 were injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 ml/min).

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 with less than 10% 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 procedural blanks (all HO-PCBs and HO-PBDEs), LOQs were calculated for S/N=10.

Statistical analysis. Statistical analyses were conducted using SPSS 14.0 Statistical Package. Differences between possible groups were detected using one-way ANOVA followed by Tukey's post hoc test. Outliers were found by making boxplots and removed for further calculations (means and standard deviations). The level of statistical significance was defined at $p < 0.05$.

Results and discussion

At first, samples were divided according to age (between 6 and 18 months or older than 18 months) and gender (male or female), resulting in 4 groups. However, for each congener and metabolite, no statistical significant differences were found between groups (all $p > 0.05$; one-way ANOVA followed by Tukey's post hoc test). Therefore all samples were pooled.

Levels and profiles of PCBs and PBDEs. Congener BDE 28 was not detected in any investigated sample, while congeners CB 110, BDE 99 and BDE 153 were found in less than 50 % of all samples. Values of sum PCBs were more than 200 times higher compared to concentrations of sum PBDEs, with values ranging from 10573 pg/ml to 193822 pg/ml for sum PCBs and 22 pg/ml to 648 pg/ml for sum PBDEs. For PCBs, CB 153 was the most dominant or persistent congener in all samples, followed by respectively CB 138, CB 187 and CB 180 (Fig. 1A). This profile was previously also found in blood samples[7] and blubber samples[4] of harbour seals and is well conserved in this species[8-10]. 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 resulting in a different profile compared to that found in blubber[3,4].

Levels and profiles of PCB and PBDE metabolites. Although the behavior of HO-PBDEs is assumed to be similar compared to HO-PCBs[11] and biotransformation of PBDEs in beluga whales was earlier shown to occur[12], no HO-PBDEs were found in the investigated blood samples. This is a confirmation of the results of a recent study[13], 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). However, very low, yet measurable HO-PBDE concentrations were detected in beluga whale livers[14]. 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. In the present study, the highest concentration was found for the lowest chlorinated compound (4-HO-CB 107), while higher chlorinated compounds showed lower values (Fig. 2).

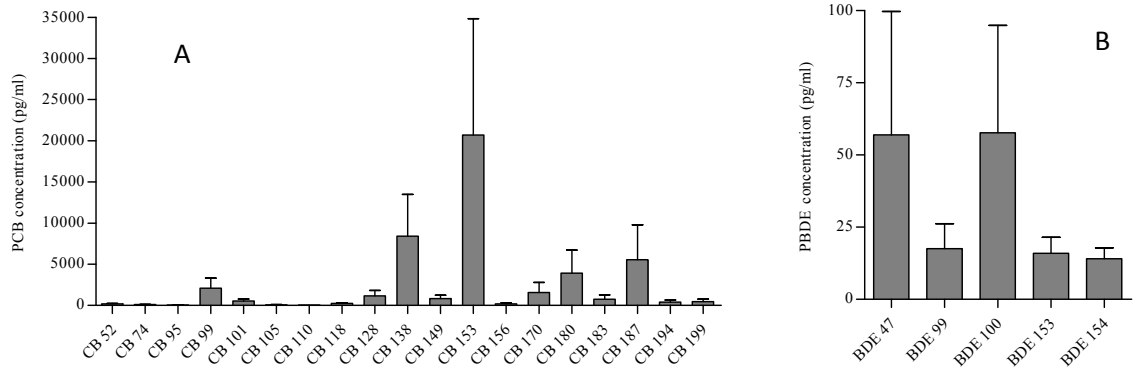

Fig. 1. Mean concentrations of precursor PCBs (A) and PBDEs (B) in serum of harbour seals. Error bars represent standard deviation.

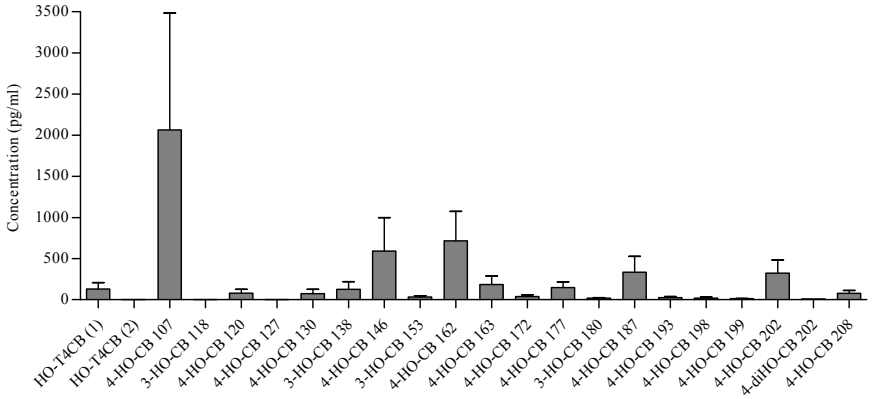

Fig. 2. Mean concentrations of HO-PCBs in serum of harbour seals. Error bars represent standard deviation.

Relationships between metabolites and precursor compounds. In general, levels of sum PCBs were 10 times higher than levels of their metabolites (ratio ΣHO-PCBs/ΣPCBs = 0.103). Ratios smaller than 1 were also found for bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon (Florida, USA) and Charleston[15], for ringed seals from Québec, Canada (*Phoca hispida*)[16] and for bowhead whales (*Balaena mysticetus*) from Alaska[17]. In contrary, ratios greater than 1 were detected in polar bears (*Ursus maritimus*) from Canada[16] and Greenland[18]. Formation of PCB metabolites may occur via direct insertion and/or NIH shift[1]. In this study, a good correlation (R^2 = 0.81) was found between the sum of HO-PCBs and their possible precursor congeners (Fig. 3), suggesting a metabolism of precursor PCBs independent of the body burden. However, unknown amounts of HO-PCBs might also be ingested directly through food or transferred from mother to offspring[13].

Comparison with harbour porpoise. In addition to the 31 harbour seals, serum of an adult male harbour porpoise (stranded at the Belgian coast in 2003) was included in the analysis as well. This animal had a concentration of sum HO-PCBs of 202 pg/ml, which was more than 25 times lower compared to the harbour seals and a concentration of sum PCBs of 800 ng/ml which was almost 5 times higher than the harbour seals. Although it is impossible to draw firm conclusions, this suggests that metabolization of PCBs in harbour porpoises occurs much slower than in harbour seals.

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HO-compounds	PCB precursors	
	direct insertion	NIH-shift
4-HO-CB107	CB 107	**CB 118, 105**
3-HO-CB118	**CB 118**	?
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**

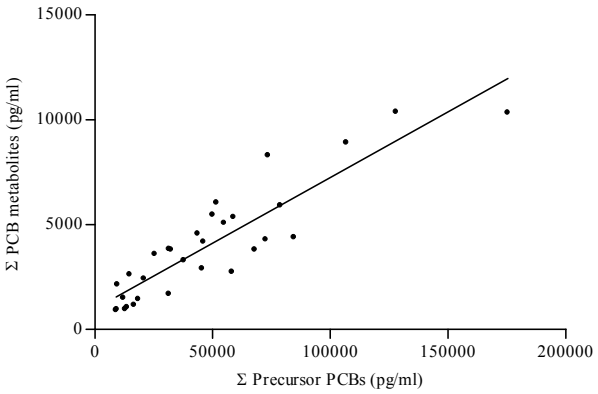

Fig. 3. Relationship between hydroxylated PCB-metabolites and their possible precursor congeners as stated in the table. Only congeners in bold were measured in present study and were therefore included in the calculations. Possible precursors were taken from Jaspers et al[19]. *(*) double insertion.*

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