LEVELS AND PROFILES OF PCBs AND PBDEs IN HARBOUR SEAL AND HARBOUR PORPOISE FROM THE SOUTHERN NORTH SEA

Liesbeth Weijs[1,2], Adriana Gheorghe[2], Alin C. Dirtu[2], Krishna Das[3], Peter Reijnders[4], Hugo Neels[2], Ronny Blust[1], Adrian Covaci[1,2]

1 – Laboratory of Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
2 – Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
3 – MARE Center, Laboratory for Oceanology, University of Liège B6C, 4000 Liège Belgium
4 – Institute for Marine Resources and Ecosystem Studies, Dept. Ecology, PO Box 167, 1790 AD Den Burg, The Netherlands

Abstract

Harbour porpoises (*Phocoena phocoena*) and harbour seals (*Phoca vitulina*), two representative top-predator species for the North Sea ecosystem, are good indicators of coastal pollution. Concentrations of sum PCBs were 1-2 orders of magnitude higher than concentrations of sum PBDEs and covered a large range of concentrations (up to 210 µg/g lw and 5.9 µg/g lw for sum PCBs and sum PBDEs). A higher contribution of lower chlorinated congeners and non-persistent congeners, such as CB 52, CB 95, CB 101, CB 118 and CB 149 indicated that harbour porpoises are unable to metabolize these congeners. Similar to PCBs, a higher contribution of other PBDE congeners than BDE 47 was observed in harbour porpoises, suggesting that porpoises have more difficulties to metabolize these congeners. This is also supported by the higher concentrations of sum PBDEs in porpoises. In contrast, harbour seals show a higher ability to metabolize (non-persistent) PCBs.

Introduction

Persistent organohalogenated pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), enter the coastal marine ecosystems and contaminate organisms living in these waters. While PCBs have been investigated for several decades, recent concern has been raised about the high levels of PBDEs which can bioaccumulate in marine mammals and about their consequent health effects.[1,2]

Harbour porpoises (*Phocoena phocoena*) and harbour seals (*Phoca vitulina*) are good indicators of coastal pollution, because they stay in coastal waters and do not present large-scale migration. Because they have long life spans and feed high in the food chain, these species are considered to be particularly sensitive to the effects of contaminant exposure, such as reproductive, endocrine and immunological disorders.[3,4] As a result, marine mammals are exposed and consequently can accumulate high concentrations of POPs in their tissues, acting as representative sentinels for global pollution.[5]

The present study aimed at evaluating the occurrence and trends of PCBs and PBDEs in harbour porpoises and harbour seals, two representative top-predator species for the North Sea ecosystem.

Materials and methods

Samples. Blubber samples were collected from 35 harbour porpoises and 28 harbour seals stranded in the Southern North Sea between 1999 and 2004. Stranded animals were dissected and tissues were archived at the Laboratory of Oceanography, University of Liege (Belgium) at -20°C. Biological parameters, such as age, gender, weight and blubber thickness, were also recorded.

Chemicals. The following PBDE congeners (IUPAC numbering) were targeted for analysis: 28, 47, 66, 85, 99, 100, 153, 154, 183, and 209. The following PCB congeners (IUPAC numbers) were targeted: 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194 and 199. Individual standards for PBDEs (Wellington Laboratories, Guelph, ON, Canada) and PCBs (Dr. Ehrenstorfer Laboratories, Augsburg, Germany) were used for identification and quantification. All solvents used for the analysis were of pesticide-grade (Merck, Darmstadt, Germany). Sodium sulfate and silica were pre-washed with n-hexane before use.

Sample preparation. The method used for extraction and clean-up has been previously described and validated.[6,7] Between 0.3 - 0.5 g blubber was dried with approximately 8 g anhydrous Na_2SO_4, spiked with internal standards BDE 77/BDE 128 (25 ng), PCB 46/ PCB 143 (75 ng) and ^{13}C-BDE 209 (7.5 ng) and extracted for 2 h by hot Soxhlet with 100 ml hexane/acetone (3/1; *v/v*). After lipid determination (performed on an aliquot

of the extract), the extract was cleaned-up on 8 g acidified silica. After elution with 15 ml hexane and 10 ml dichloromethane, the cleaned extract was concentrated to 200 µl.

Analysis. PBDEs were measured with an Agilent 5973 GC-MS operated in electron capture negative ionisation (ECNI) mode and equipped with a 20 m x 0.18 mm x 0.20 µm AT-5 capillary column (Alltech). Methane was used as moderating gas and the ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The mass spectrometer was used in SIM mode with ions *m/z* = 79 and 81 (tri- to hepta-BDEs) and 484.7/486.7 and 494.7/496.7 (BDE 209 and ^{13}C-BDE 209, respectively) monitored during the entire run. Dwell times were set at 40 msec. One µl extract was injected in solvent vent mode and helium was used as carrier gas at constant flow (0.8 ml/min). PCBs were measured with an Agilent 5973 GC-MS operated in electron 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, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. Two specific ions were monitored for each PCB homologue group. One µl extract was injected in cold pulsed splitless mode and helium was used as carrier gas at constant flow (1 ml/min).

QA/QC. Multi-level calibration curves (r^2 > 0.99) were created for the quantification. The analyte identification was based on their relative retention times (RRTs) to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. Recoveries of individual PBDE and PCB congeners ranged between 85 and 105 % (RSD ≤ 12 %) during the method validation. For each analyte, the mean procedural blank value was used for subtraction and the limit of quantification (LOQ) was set at 3 x SD of the procedural blank, which ensures > 99 % certainty that the reported value is originating from the sample. Therefore, LOQ was analyte-specific and depended on the sample intake. For analytes not detected in procedural blanks, LOQs were calculated for S/N = 10. LOQs ranged between 1 and 4 ng/g lipid weight (lw), except for BDE 209, for which LOQ was 10 ng/g lw. QC was performed through regular analyses of procedural blanks, random injection of standards and solvent blanks. SRM 1945 (PCBs and PBDEs in whale blubber) was used to test the method accuracy. Obtained values were deviating with < 10% from the certified values. The QC scheme is also assessed through participation to interlaboratory comparison exercises organized by AMAP and NIST.

Statistical analysis. For each species, samples were divided as a function of age (J-juvenile and A-adult) and gender (M-males and F-females), resulting in 4 groups (JM, JF, AM and AF, respectively). In each group, outliers (detected using Grubbs' test) were removed for further calculations. Concentrations and profiles of PCBs and PBDEs were compared between the groups using one-way ANOVA, followed by Scheffe's post-hoc test.

Results and discussion

Levels of PCBs and PBDEs. Congeners CB 31, BDE 66, BDE 85 and BDE 183 were detected in less than 50% of the samples, while BDE 209 was not detected in any investigated samples at concentrations higher than 10 ng/g lw. Shaw et al. have detected BDE 209 at concentrations between 2 and 8 ng/g lw in blubber of harbour seals from the NW Atlantic Ocean.[8] The remaining PCB and PBDE congeners were detected in all samples.

Table 1. Mean concentrations and standard deviations (in brackets) of CB 153, sum PCBs, BDE 47 and sum PBDEs (µg/g lw) measured in blubber of 28 harbour seals and 35 harbour porpoises from the Southern North Sea.

	Group	N	PCB 153	Sum PCBs	BDE 47	Sum PBDEs
Harbour seals	JM	9	7.2 (2.4)[a]	20.7 (6.7)[a]	0.35 (0.21)	0.44 (0.27)
	JF	9	10.3 (10.8)[a]	28.3 (27.6)[a]	0.42 (0.31)	0.54 (0.40)
	AM	8	28.9 (23.3)	72.4 (58.2)	0.21 (0.11)	0.30 (0.14)
	AF	2	4.3 (4.2)	12.5 (12.2)	0.12 (0.05)	0.18 (0.09)
Harbour porpoise	JM	12	3.9 (3.0)[a]	15.4 (10.7)[a]	1.11 (1.16)	1.73 (1.77)
	JF	10	3.7 (4.1)	12.9 (11.9)	0.45 (0.27)[a]	0.70 (0.41)[a]
	AM	8	28.8 (12.0)	82.9 (31.8)	0.69 (0.46)	1.54 (0.96)
	AF	5	1.7 (0.6)[a]	7.3 (2.0)[a]	0.43 (0.30)	0.85 (0.60)

a - 1 outlier removed

As a general trend, concentrations of sum PCBs were 1-2 orders of magnitude higher than concentrations of sum PBDEs and covered a large range of concentrations (up to 210 µg/g lw and 5.9 µg/g lw for sum PCBs and sum PBDEs). An overview of the PCB and PBDEs concentrations in each group is given in Table 1.

For both species, adult males (AM) contained the highest concentrations of PCBs. Except for AM, PCB concentrations tend to be higher in seals than in porpoises. For each species, PCB concentrations in JM were much lower than in AM, while AF contained lower concentrations than JF. This is probably due to gestation and lactation, which have been shown to be the most important pathways for elimination of POPs in female marine mammals.[6,9] For PBDEs, harbour porpoises contained higher concentrations for each group than seals.

Profiles of PCBs and PBDEs. Ratios between the concentrations of individual PCB congeners and concentration of PCB 153 for each animal within the 4 groups were used to construct PCB profiles (Figure 1). A higher contribution of lower chlorinated congeners and non-persistent congeners, such as CB 52, CB 95, CB 101, CB 118 and CB 149 indicate that harbour porpoises are unable to metabolize these congeners. However, for the group AM, these congeners had a lower contribution suggesting an increased metabolic capacity with the PCB body burden increase, probably due to the activation of the P450 enzyme family.[10] Persistent PCB congeners (CB 138, CB 170, CB 180 and CB 187) seemed to have a similar contribution in both species. The percent distribution of PCB congeners in seals also tended to be more consistent (lower SDs) between samples than in porpoises.

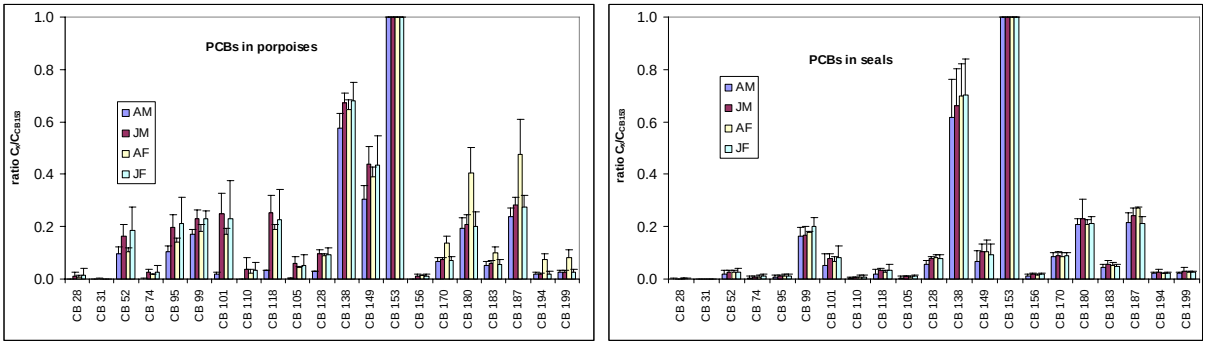

Figure 1. Mean ratios between concentration of each PCB congener and CB 153 in porpoises and seals. Error bars represent SD.

Ratios between the concentrations of individual PBDE congeners and concentration of BDE 47 for each animal within the 4 groups were used to construct PBDE profiles (Figure 2). Similar to the PCB profiles, a higher contribution of PBDE congeners relative to BDE 47 was observed in porpoises. Apparently, porpoises have more difficulties to metabolize these congeners. This is also supported by the higher concentrations of sum PBDEs in porpoises. The distribution of PBDE congeners in seals tended to be more consistent (lower SDs) between samples than in porpoises. A less efficient metabolism of PCBs and PBDEs in cetaceans (porpoises) compared to pinnipeds (seals) could lead to greater biomagnification of these POPs and subsequently to possible adverse effects.[10] However, metabolization can also lead to increased toxicity of metabolites.[11]

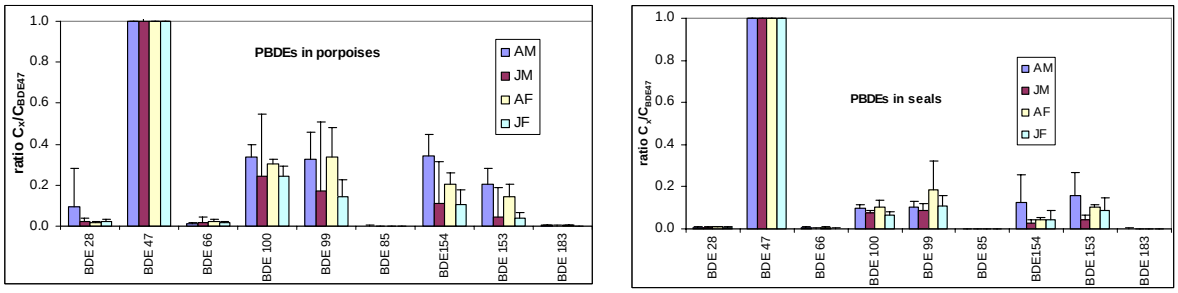

Figure 2. Mean ratios between concentration of each PBDE congener and BDE 47 in porpoises and seals. Error bars represent SD.

Bioaccumulation potential. For harbour porpoises, the mean concentrations of less bioaccumulative PCB congeners 28, 74, 101, 110, 105, 118 and 156, were lower in AM than in JM, leading to negative log ratio between the concentrations. Contrarily, for more bioaccumulative PCB congeners, such as CB 99,138, 149, 153,

170, 180, 183, 187, 194 and 199, the range of ratios between concentrations in AM and JM was 2.2 - 7.4. A significant positive correlation ($r = 0.70$; $p = 0.011$) between log ratio mean PCB concentration in AM and JM vs. log K_{ow} for (only) bioaccumulative PCB congeners was observed for porpoises (Figure 3).

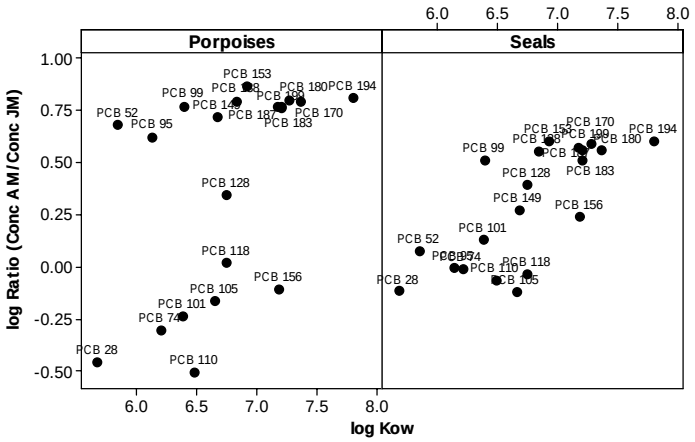

Figure 3. Relationships between log ratio mean concentration of PCB congeners in AM and JM vs. log Kow.

This relationship is similar with the correlation between the biomagnification power (BMP) and log K_{ow} revealing that the ratio between congener concentration in AM and JM can be used as an indicator of the congener-specific and gender-specific BMP for the specific part of the food chain represented by porpoises.[12] In other words, the increase in concentration with age reflects the change in position in the food chain, suggested by the large range in $\delta^{15}N$ (12.1 - 18.1) similar to almost 2 trophic levels difference along the food web observed for the porpoises.[13] In contrast, seals show a higher ability to metabolize (non-persistent) PCBs. This hypothesis is supported also by the inter-species differences in PCB profiles.

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