



Inter-species differences for polychlorinated biphenyls and polybrominated diphenyl ethers in marine top predators from the Southern North Sea: Part 1. Accumulation patterns in harbour seals and harbour porpoises

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Harbour porpoises and harbour seals present differences in the accumulation of polychlorinated biphenyls and polybrominated diphenyl ethers.

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ABSTRACT

Harbour porpoises (*Phocoena phocoena*) and harbour seals (*Phoca vitulina*) are two representative top predator species of the North Sea ecosystem. The median values of sum of 21 polychlorinated biphenyl (PCB) congeners and sum of 10 polybrominated diphenyl ether (PBDE) congeners were 23.1 µg/g lipid weight (lw) and 0.33 µg/g lw in blubber of harbour seals ($n = 28$) and 12.4 µg/g lw and 0.76 µg/g lw in blubber of harbour porpoises ($n = 35$), respectively. For both species, the highest PCB concentrations were observed in adult males indicating bioaccumulation. On the contrary, the highest PBDE concentrations were measured in juveniles, likely due to better-developed metabolic capacities with age in adults. A higher contribution of lower chlorinated and non-persistent congeners, such as CB 52, CB 95, CB 101, and CB 149, together with higher contributions of other PBDE congeners than BDE 47, indicated that harbour porpoises are unable to metabolize these compounds. Harbour seals showed a higher ability to metabolize PCBs and PBDEs.

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1. Introduction

Since several decades, it has been shown that pollution puts a great pressure on the marine environment. Local input through rivers and runoff, together with (long-range) atmospheric transport are major factors governing the presence and distribution of anthropogenic contaminants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in the aquatic environment, including seas and oceans (Tanabe et al., 1994; AMAP, 2004; Law et al., 2003, 2006a). Due to their physical and chemical properties, these contaminants are capable of entering aquatic ecosystems and as a consequence, they can be a threat to organisms in every trophic level (Tanabe et al., 1994; Boon et al., 2002). Among

them, PCBs are the most monitored contaminants in marine mammals (Duinker et al., 1989; Hutchinson and Simmonds, 1994; Vetter et al., 1996; Severinsen et al., 2000; Kajiwara et al., 2001). PCBs have been used for a variety of applications including dielectric fluids for transformers, plasticisers, or components in glue and paint. Although their production was banned since the end of the 1970s, PCBs can still be found in wildlife. Recently, attention has been drawn towards the accumulation and effects of new persistent contaminants, such as PBDEs, in marine mammals. The PBDE commercial mixtures contain fewer congeners than the corresponding PCB mixtures. PBDEs are used as flame retardants in textiles, furniture, and plastics (de Boer et al., 2000; Birnbaum and Staskal, 2004). The use of the penta- and octa-BDE technical mixtures is currently banned in Europe (EU-directive 2002/95/EC). Several adverse effects observed in wildlife, such as endocrine dysfunction, reproductive failure, immunological impairment, developmental stress and genotoxic disorders have been linked to the presence of these contaminants (Reijnders, 1986; Gauthier

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et al., 1999; Fair and Becker, 2000; Damstra et al., 2002; Beineke et al., 2005; Das et al., 2006).

Marine mammals are top predators in aquatic food chains and are, thus, particularly vulnerable and sensitive to contaminants which are persistent in the environment and which can accumulate in high concentrations. In marine mammals, uptake of organic contaminants occurs mainly through their diet (Borgå et al., 2004), while routes such as placental transfer and lactation may affect the offspring at a critical stage of their development (Duinker and Hillebrand, 1979; Debier et al., 2003a,b; Wolkers et al., 2004).

Harbour seals (*Phoca vitulina*) and harbour porpoises (*Phocoena phocoena*) are two representative top predator species for the North Sea ecosystem. Their long life spans and population density make them suitable for monitoring pollution in the North Sea. These two species share an extensive part of their diets, such as benthic and pelagic fish species (Hall et al., 1998; Santos and Pierce, 2003). However, comparisons between the harbour seals and porpoises in the accumulation of contaminants must be made with caution. Harbour seals are more sedentary, while porpoises seem to move over larger distances and, as a consequence, concentrations of contaminants in these two species may reflect contamination on a different spatial scale (Vetter et al., 1996; Law et al., 2002; Das et al., 2004; Fontaine et al., 2007).

The movement of lipophilic contaminants in marine mammals is strongly influenced by the lipid dynamics inside the body. The investigation of the presence of PCBs and PBDEs in blubber, the subcutaneous fat layer, is therefore important to assess the overall contamination status of the animals. Blubber provides insulation for the body and acts as a metabolic energy storage site (Dunkin et al., 2005). This latter role is important in the mobilization of lipids and lipophilic contaminants, depending on the animal's condition.

In the present study, we have investigated the accumulation and biomagnification of PCB and PBDE congeners in blubber of harbour seals and harbour porpoises from the Southern North Sea. An overall objective of this study was to gain knowledge about the metabolic capacities of both harbour seals and porpoises. The first

part involves the study of PCB and PBDE concentrations and profiles and their species-dependent relationship with age and gender. In the second part (Weijs et al., in this issue), biomagnification factors for individual PCB and PBDE congeners were calculated and the influence of various factors, such as octanol–water partition coefficients and trophic position assessed through measurements of ^{15}N stable isotopes, was discussed.

2. Materials and methods

2.1. Samples

Necropsy was carried out at the Department of Veterinary Pathology (Liege University) and at the IMARES Research Center at Texel (The Netherlands). Blubber samples were collected from 35 harbour porpoises and 28 harbour seals stranded or bycaught in the Southern North Sea between 1999 and 2004. The 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 (standard procedure in Jauniaux et al., 2002 and Das et al., 2004) and given in Table 1. Age classification (<3 years for juveniles and >3 years for adults) was based upon the length of the animals (for harbour porpoises; T. Jauniaux, personal communication) and the development of their gonads (for harbour seals; T. Jauniaux, personal communication).

2.2. Targeted compounds

The following PBDE congeners (IUPAC numbers) were targeted for analysis: 28, 47, 66, 85, 99, 100, 153, 154, 183, and 209. BDE 77 was used as internal standard (IS) for tetra- and penta-BDE congeners, while BDE 128 was used as IS for hexa- and hepta-BDE congeners. For BDE 209, ^{13}C -labelled BDE 209 was used as IS. The following 21 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. Internal standards used were CB 46 and CB 143. Individual standards for PBDEs (Wellington Laboratories, Guelph, ON, Canada) and PCBs (Dr. Ehrenstorfer Laboratories, Augsburg, Germany) were used for identification and quantification.

2.3. Chemicals

All solvents used for the analysis (*n*-hexane, acetone, dichloromethane, isooctane) were of pesticide-grade (Merck, Darmstadt, Germany). Sodium sulphate and silica were pre-washed with *n*-hexane before use. Extraction thimbles were pre-extracted for 1 h with the extraction mixture used for the samples and dried at 100°C for 1 h.

Table 1

Arithmetic means, standard deviations (SD) and range of biological data (length, weight and blubber thickness), concentrations of CB 153, Σ PCBs, BDE 47 and Σ PBDEs ($\mu\text{g/g}$ lipid weight) measured in blubber of harbour seals and harbour porpoises from the Southern North Sea.

| | Harbour seal | | | | Harbour porpoise | | | |
|---------------------------------------|-----------------|-----------------|-----------------|-----------------|------------------|-----------------|----------------|----------------|
| | AM | JM | AF | JF | AM | JM | AF | JF |
| <i>n</i> | 8 | 9 | 2 | 9 | 8 | 12 | 5 | 10 |
| Length (cm) | | | | | | | | |
| Mean (SD) | 139.4 (12.1) | 106.6 (9.0) | 153.0 (24.0) | 112.2 (11.9) | 145.5 (7.9) | 107.3 (7.2) | 149.4 (5.4) | 111.8 (9.4) |
| Range | 128–163 | 93–120 | 136–170 | 94–130 | 137–160 | 96–117 | 144–158 | 94–127 |
| Weight (kg) | | | | | | | | |
| Mean (SD) | 46.6 (8.9) | 23.3 (5.1) | 69.5 (41.7) | 25.0 (8.5) | 41.6 (7.1) | 19.2 (4.9) | 47.6 (10.0) | 22.8 (4.9) |
| Range | 34–58 | 17–33 | 40–99 | 10–36 | 36–58 | 11.3–26.5 | 36–60 | 15–30 |
| Blubber thickness (mm) | (<i>n</i> = 6) | (<i>n</i> = 5) | (<i>n</i> = 1) | (<i>n</i> = 7) | | | | |
| Mean (SD) | 20.5 (9.5) | 14.6 (6.6) | 50 | 10.9 (2.8) | 9.1 (5.1) | 10.8 (9.6) | 15.6 (6.9) | 14.2 (5.9) |
| Range | 11–35 | 7–20 | – | 6–15 | 1.7–18 | 4–40 | 10–26 | 3–22 |
| <i>n</i> | 8 | 8 ^a | 2 | 8 | 8 | 11 ^a | 4 ^a | 10 |
| CB 153 ($\mu\text{g/g lw}$) | | | | | | | | |
| Mean (SD) | 28.9 (23.3) | 7.2 (2.4) | 4.3 (4.3) | 10.3 (10.8) | 28.7 (12.0) | 3.9 (3.0) | 1.7 (0.6) | 3.7 (4.1) |
| Range | 0.8–65.9 | 4.7–11.8 | 1.3–7.3 | 2.2–35.2 | 11.6–46.0 | 1.2–11.5 | 1.0–2.3 | 0.2–13.4 |
| Σ PCBs ($\mu\text{g/g lw}$) | | | | | | | | |
| Mean (SD) | 72.4 (58.2) | 20.7 (6.7) | 12.5 (12.2) | 28.3 (27.6) | 82.9 (31.8) | 15.4 (10.7) | 7.3 (2.0) | 12.9 (11.9) |
| Range | 2.2–171.7 | 12.7–33.8 | 3.9–21.5 | 6.5–91.5 | 38.7–125.5 | 5.3–39.8 | 4.4–8.9 | 1.3–39.3 |
| <i>n</i> | 8 | 9 | 2 | 9 | 8 | 12 | 5 | 9 ^a |
| BDE 47 ($\mu\text{g/g lw}$) | | | | | | | | |
| Mean (SD) | 0.21 (0.11) | 0.35 (0.21) | 0.12 (0.05) | 0.42 (0.31) | 0.69 (0.46) | 1.11 (1.16) | 0.43 (0.30) | 0.45 (0.27) |
| Range | 0.07–0.40 | 0.11–0.73 | 0.08–0.15 | 0.07–0.82 | 0.11–1.43 | 0.27–3.88 | 0.15–0.79 | 0.16–0.99 |
| Σ PBDEs ($\mu\text{g/g lw}$) | | | | | | | | |
| Mean (SD) | 0.30 (0.14) | 0.44 (0.27) | 0.18 (0.09) | 0.54 (0.40) | 1.54 (0.96) | 1.73 (1.77) | 0.85 (0.60) | 0.70 (0.41) |
| Range | 0.11–0.52 | 0.13–0.87 | 0.11–0.24 | 0.09–1.15 | 0.28–3.10 | 0.50–5.93 | 0.32–1.56 | 0.22–1.48 |

J – juvenile (<3 years); A – adult (>3 years); F – female; M – male.

^a One outlier was excluded from the data set of the respective age–gender group.

2.4. Sample preparation and clean up

The method used for the sample extraction and clean up has been previously described and validated (Covaci et al., 2002; Voorspoels et al., 2003), and is briefly presented below. Between 0.3 and 0.5 g blubber was dried with ~8 g anhydrous Na₂SO₄, spiked with internal standards BDE 77/BDE 128 (25 ng), CB 46/CB 143 (75 ng) and ¹³C-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 on 8 g of acidified silica. After elution of analytes with 15 ml hexane and 10 ml dichloromethane, the cleaned extract was concentrated to 200 µl.

2.5. Analysis

PBDEs were measured with an Agilent 6890-5973 gas chromatograph coupled with a mass spectrometer system (GC-MS). The GC was equipped with a 20 m × 0.18 mm × 0.20 µm AT-5 capillary column (Alltech, Lokeren, Belgium) and the MS was operated in electron capture negative ionisation (ECNI) mode. Methane was used as reagent gas and the ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The MS was used in the selected ion-monitoring (SIM) mode with ions *m/z* 79 and 81 (for tri- to hepta-BDEs) and *m/z* = 484.7/486.7 and 494.7/496.7 (for BDE 209 and ¹³C-BDE 209, respectively) monitored during the entire run. Dwell times were set at 40 ms. One microlitre of the cleaned extract was injected in solvent vent mode (injector temperature: 90 °C, held for 0.05 min, then with 700 °C/min to 305 °C and kept for 25 min; vent flow was set at 75 ml/min and the purge vent opened at 1.5 min). Helium was used as carrier gas at constant flow (0.8 ml/min). The temperature of the AT-5 column was kept at 90 °C for 1.50 min, then increased to 200 °C at a rate of 20 °C/min, further increased to 300 °C at a rate of 5 °C/min, kept for 15 min.

PCBs were measured with the same GC-MS system as for the PBDE determination, operated in electron ionisation (EI) mode and equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The MS was used in the SIM mode with two ions monitored for each PCB homologue group. One microlitre of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 700 °C/min), pressure pulse 25 psi, pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as carrier gas at constant flow (1 ml/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (kept for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, kept for 12 min.

2.6. Quality assurance/quality control (QA/QC)

Multi-level calibration curves were created for the quantification and good correlation (*r*² > 0.999) was achieved. The identification of each target analyte was based on their relative retention times (RRTs) to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. A deviation of the ion intensity ratios within 20% of the mean values of the calibration standards was considered acceptable. Recoveries for individual PBDE congeners were between 87 and 104% (RSD < 12%), while recoveries of PCBs ranged between 75 and 90% (RSD < 10%).

For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at three times the standard deviation of the procedural blank, which ensures >99% certainty that the reported value is originating from the sample. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. LOQs depended on the sample intake and on the analyte and ranged between 1 and 4 ng/g lipid weight (lw).

QC was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs and PBDEs in whale blubber) was used to test the method accuracy. Obtained values were not deviating more than 10% from the certified values. The QC scheme is also assessed through regular participation to interlaboratory comparison exercises organised by the Arctic Monitoring Assessment Programme and the National Institute of Standards and Technology.

2.7. Statistical analysis

Statistical analyses were conducted using the SPSS 14.0 statistical package. The level of statistical significance was defined at *p* < 0.05. Outliers in all groups, detected using Grubbs' test, were removed before further calculations. Differences in the concentrations and profiles of PCBs and PBDEs were compared between the groups (adult males, adult females, juvenile males and juvenile females) using one-way ANOVA, followed by Tukey's post hoc test. Correlation coefficients between PCBs and PBDEs were calculated using GraphPad Prism 4 (GraphPad Software, Inc.).

3. Results and discussion

3.1. PCB concentrations

Of the 21 congeners analyzed, only congener CB 31 was detected in less than 50% of the blubber samples from harbour seals and

porpoises and therefore this congener was removed from the following statistical interpretation. The remaining PCBs were measured in all samples. PCB concentrations (sum of 21 congeners) in blubber tissue ranged between 2.2–172 µg/g lw and 1.3–126 µg/g lw for harbour seals and porpoises, respectively. These minimum and maximum values represent a large range, underlying the numerous biotic factors involved in PCB lipid accumulation (e.g. age, gender and body condition). Therefore, the samples from both species were divided into four groups according to their age and gender: adult male (AM), adult female (AF), juvenile male (JM) and juvenile female (JF). The results for the \sum PCBs for harbour seals and porpoises are given in Table 1. Results for CB 153, the most persistent PCB congener in marine mammals are also shown in Table 1 to allow comparisons with other studies. Almost all conclusions drawn for the \sum PCBs were similar for CB 153 (although with other *F* and *p*-values).

For both species, the AM group contained the highest PCB concentrations probably due to bioaccumulation of these contaminants in time. Contrarily, the AF group displayed the lowest concentrations linked to the well-described transfer during gestation and lactation (Covaci et al., 2002; Wolkers et al., 2004; Shaw et al., 2005). PCB concentrations were similar between JM and JF (*p* > 0.05) suggesting that the accumulation pattern is comparable between males and females until sexual maturity.

Harbour porpoises from the AM group tend to have higher, although not statistically significant, concentrations of \sum PCBs than harbour seals (*F*_{1,14} = 0.200; *p* = 0.662), while the concentrations in the other groups (JM, JF and AF) were lower compared with the corresponding group of the harbour seals (*F*_{1,17} = 1.520; *p* = 0.234 for JM, *F*_{1,16} = 2.539; *p* = 0.131 for JF and *F*_{1,4} = 0.907; *p* = 0.395 for AF, respectively). An explanation can be found in differences in age, body size or in the blubber thickness. Differences in the concentrations of PCBs between the outer and inner blubber layers, with the outer layers having significantly higher concentrations, have been reported previously in grey seals (*Halichoerus grypus*) (Debier et al., 2003a), in harp seals (*Phoca groenlandica*) (Lydersen et al., 2002), in ringed seals (*Phoca hispida*) (Severinsen et al., 2000) and in bottlenose dolphins (*Tursiops truncatus*) (Montie et al., 2008). The mean blubber thickness in the AM group was 9.1 mm for harbour porpoises and 20.5 mm for harbour seals. Therefore, the probability of having samples from the outer blubber layer is greater for porpoises than for seals, with an overestimation of the reported PCB concentrations as a consequence (Fig. 1A). The difference in the body size, which influences the food intake and therefore the contaminant uptake, could be another possible explanation for variation between species (reviewed by Borgå et al., 2004). However, in the present study, no influence of body size could be detected, because there were no significant differences in body size between the same age groups of both species (all *p* > 0.1).

Due to the number and position of chlorine atoms, PCB congeners do not follow the same metabolic pathways which result in the formation of different metabolites (Letcher et al., 2000) and in differences in accumulation patterns and persistence of PCBs. This has resulted in the classification of PCBs in several groups as introduced by Bruhn et al. (1995) and Boon et al., 1997 and recently further developed by Wolkers et al. (2007) (Table 2). The most persistent congeners from the metabolic groups I and IIIB reached the highest proportions in harbour seals and porpoises, with percentages between 90–95% and 67–81%, respectively (Fig. 2). Less persistent congeners (metabolic groups IIB, IIC and IIIA) had higher contributions in the blubber of harbour porpoises (especially CB 149), but were less important in harbour seals.

Concentrations of CB 153 were higher in the present study than in similar species from other seas and oceans, indicating that the Southern part of the North Sea is still highly contaminated with PCBs, in agreement with previous published studies. Reijnders

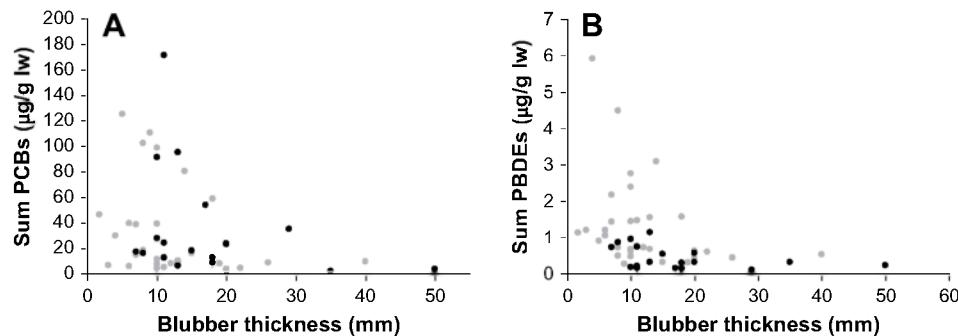


Fig. 1. Relationship between the blubber thickness (mm) and (A) the Σ PCB concentrations ($\mu\text{g/g lw}$) and (B) the Σ PBDE concentrations ($\mu\text{g/g lw}$) in blubber of harbour porpoises (●) and harbour seals (●).

(1986) measured high PCB levels causing reproductive failures in harbour seals from the Wadden Sea. Vetter et al. (1996) found the highest PCB levels in harbour seals from the Dutch Wadden Sea and concluded that this area is the major source of input of PCBs into the North Sea and North Atlantic. The same study also found decreasing PCB levels along the continental line from the North Sea to Germany, Denmark and Norway (Table 3). Similar trends have been observed in harbour porpoise with decreasing PCB and PBDE concentrations from German Baltic and North Sea to Iceland (Das et al., 2006). Covaci et al. (2002) also found that concentrations of PCBs in harbour porpoises from the Southern North Sea were higher than in porpoises from the English or Scottish coast of the North Sea.

3.2. General PCB profiles

CB 153 was the dominant PCB congener in all individuals of both marine mammal species. Profiles for harbour seals (CB 153 > CB 138 > CB 187 > CB 180 > CB 99) and harbour porpoises (CB 153 > CB 138 > CB 149 > CB 187 > CB 180) were similar for all age groups, except for AF porpoises (Fig. 3A and B). These results confirm the PCB profiles reported in the literature and reflect the differences in the accumulation of certain PCB congeners (e.g. CB 101 and CB 149) between pinnipeds (seals) and cetaceans (porpoises) (Hutchinson and Simmonds, 1994; Vetter et al., 1996; Boon et al., 1997).

Ratios between the concentrations of individual PCB congeners and concentration of CB 153 for each animal within the four age-gender groups were used to construct relative PCB profiles in order to be able to make comparisons between the two species:

$$R_{153}(\text{CB}_x) = \frac{[\text{CB}_x]}{[\text{CB}_{153}]}$$

Table 2

Classification of the PCB congeners analyzed in the present study according to Bruhn et al. (1995), Boon et al. (1997) and Wolkers et al. (2007).

| Metabolic group | Description | Cytochrome P450 induction | PCB congeners in the present study |
|-----------------|--|---------------------------------------|------------------------------------|
| I | No vicinal <i>o,m</i> or <i>m,p</i> H-atoms | 2B ^a | 153, 180, 183, 187, 194, 199 |
| IIA | Vicinal <i>m,p</i> H-atoms and ≤ 1 <i>o-Cl</i> | 2B/3A and 1A (maximum 1 <i>o-Cl</i>) | None |
| IIB | Vicinal <i>m,p</i> H-atoms and 2 <i>o-Cl</i> | | 52, 101, 110 |
| IIC | Vicinal <i>m,p</i> H-atoms and 3 <i>o-Cl</i> | | 95, 149 |
| IIIA | No vicinal <i>m,p</i> H-atoms and ≤ 1 <i>o-Cl</i> | 1A and 2B ^a | 28, 31, 74, 105, 118, 156 |
| IIIB | No vicinal <i>m,p</i> H-atoms and ≥ 2 <i>o-Cl</i> | 2B/1A ^a | 99, 128, 138, 170 |

^a Boon et al. (1997), *o* – *ortho*, *m* – *meta*, *p* – *para*.

For harbour seals, the JM, JF and AF groups showed similar and higher ratios for all PCBs than the AM group (Fig. 3A), suggesting a better-developed metabolic capacity with age or an increased metabolism with higher blubber concentrations for adult males.

For harbour porpoises, a higher contribution of higher chlorinated congeners, such as CB 170, CB 180, CB 183, CB 187, CB 194 and CB 199, was observed for the AF group (Fig. 3B). This might be the result of a selective transport of lower chlorinated PCB congeners to their offspring and, as a consequence, an enrichment of the higher chlorinated PCB congeners in the blubber of AF individuals. Similar to our observations, Debier et al. (2003b) reported higher contributions of higher chlorinated PCBs in blubber of adult female grey seals and higher proportions of lower chlorinated PCBs in milk. The same study also assumed a higher contribution of lower chlorinated PCBs in blubber of pups. Indeed, juvenile porpoises from our study had higher ratios for lower chlorinated congeners (CB 28, CB 52, CB 74, CB 95, CB 99, CB 101, CB 118, CB 110 and CB 105), probably due to their limited capacity for metabolic breakdown and as a result of selective transfer of PCB lower chlorinated congeners during lactation. For all congeners, the AM group showed the lowest ratios in harbour porpoises suggesting a better-developed capacity for PCB metabolism for this group.

Compared to harbour seals, harbour porpoises had a higher proportion of lower chlorinated (less persistent) congeners, such as CB 52, CB 95, CB 101, CB 118 and CB 149. In both species, persistent PCB congeners (CB 138, CB 170, CB 180 and CB 187) had a similar contribution. All together, this means that harbour seals are able to metabolize lower chlorinated PCB congeners in a more efficient way than harbour porpoises. This finding, namely a distinction

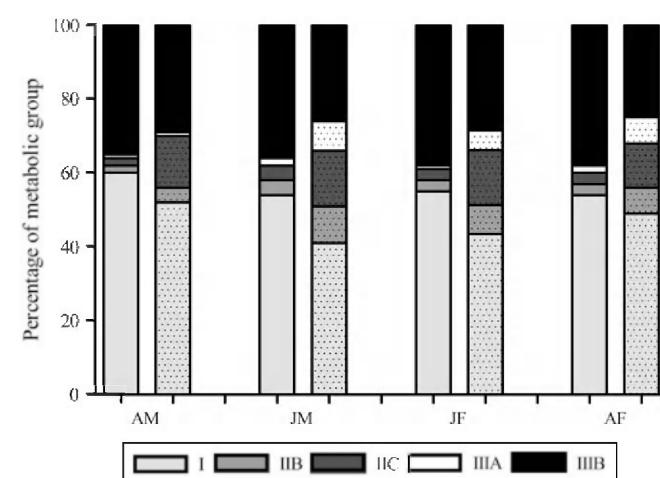


Fig. 2. Percentages of the metabolic groups (see Table 2) in the four age-gender groups of harbour seals (columns without dots) and porpoises (columns with dots).

Table 3Mean concentrations and standard deviations (between brackets) in $\mu\text{g/g}$ lipid weight of CB 153 in blubber tissue of harbour seals and porpoises.

| Species | Location | Year | n | CB 153 ($\mu\text{g/g}$ lipid weight) | | | | Reference |
|------------------|--------------------|-----------|------------|--|-----------|-----------|-------------|---|
| | | | | AM | JM | AF | JF | |
| Harbour seal | Canada | 1996–2000 | 8 | 10.6 (5.1) | | | | Hobbs et al., 2002 Wolkers et al., 2004 Present study |
| | Norway | | 6–4 | 0.61 | | 0.12 | | |
| | Southern North Sea | 1999–2004 | 8–8–2–8 | 28.9 (23.3) | 7.2 (2.4) | 4.3 (4.3) | 10.3 (10.8) | |
| Harbour porpoise | Baltic Sea | 1985–1993 | 4–13 | 20 (13) | 6.6 (3.6) | | | Berggrena et al., 1999 |
| | Kattegat-Skagerrak | 1988–1990 | 7–10 | 5.7 (2.3) | 4.8 (2.5) | | | |
| | | 1978–1981 | 5 | 19 (12) | | | | Berggrena et al., 1999 |
| | Norway | 1988–1990 | 8 | 5.6 (4.6) | | | | Berggrena et al., 1999 |
| | United Kingdom | 1999–2004 | 16–18–8–15 | 3.7 (3.2) | 4.4 (7.4) | 2.2 (1.5) | 2.9 (1.6) | Law et al., 2006b |
| | Southern North Sea | 1999–2004 | 8–11–4–10 | 28.7 (12.0) | 3.9 (3.0) | 1.7 (0.6) | 3.7 (4.1) | Present study |

between lower and higher chlorinated compounds for harbour seals, agrees with findings of Boon et al. (1997) and Hobbs et al. (2002).

3.3. PBDE concentrations

Of the 10 congeners analyzed, congeners BDE 85 and BDE 183 were detected in less than 50% of the blubber samples from both harbour seals and porpoises. BDE 66 was measured in all samples from harbour porpoises, but in less than 50% of harbour seals. BDE 209 could not be detected in any investigated sample at concentrations higher than 10 ng/g lw (LOQ). This agrees with previous reports which could not detect BDE 209 in marine mammals (Boon et al., 2002) or which have infrequently measured BDE 209 at concentrations between 1 and 8 ng/g lw in seals (Thomas et al., 2005; Shaw et al., 2007). Since very low or not detectable concentrations of BDE 209 were found in fish species which are prey for the two studied marine mammal species (Voorspoels et al., 2003) and the half-life of BDE 209 in blood of grey seals was estimated between 8.5 and 13 days (Thomas et al., 2005), it is plausible to assume that BDE 209 does not bioaccumulate in aquatic biota. However, this congener is of particular concern, because it de brominates (in fish) to lower brominated PBDE congeners (such as BDE 154 and BDE 155), which are more water soluble and probably more persistent in biota (Stapleton et al., 2004). The remaining congeners were measured in all samples from both species. Results for \sum PBDEs and BDE 47, the most persistent PBDE congener in marine mammals, are given in Table 1. Statistical comparisons for \sum PBDEs and BDE 47 were similar, although with different F and p-values.

The highest concentrations of \sum PBDEs for all age–gender groups were observed in harbour porpoises (range 0.22–5.93 $\mu\text{g/g}$ lw) compared with harbour seals (range 0.09–1.15 $\mu\text{g/g}$ lw) (Table 1).

For harbour porpoises, males were more contaminated than females ($F_{1,32}=4.942$; $p=0.033$) with the JM group having the highest ($1.73 \pm 1.77 \mu\text{g/g}$ lw) and the group JF the lowest ($0.70 \pm 0.41 \mu\text{g/g}$ lw) mean concentrations. For harbour seals, juveniles tended to have higher \sum PBDE concentrations than adults suggesting that the capacity for metabolic breakdown increases with age or with higher body burdens. Yet these findings were not statistically significant. This finding contrasts with the higher concentrations measured in adult (age > 5 years) ringed seals compared to subadult specimens (age < 5 years) from East Greenland (Vorkamp et al., 2004), but agrees with results from harbour seals from UK waters (Law et al., 2006b; MAFF, 1994) (Table 4).

Similar to PCBs, PBDE concentrations in the AF group were lower than the other age–gender groups, supporting the hypothesis that adult female animals reduce their contaminant loads through gestation and lactation (Covaci et al., 2002; Law et al., 2002).

Similar to PCBs (see above), a decrease in the PBDE concentrations was observed with the increase in the blubber thickness of harbour porpoises, but not of harbour seals (Fig. 1B). This, together with the fact that the blubber thickness was lower for harbour porpoises, suggests that the probability of having samples from the outer blubber layer was greater for porpoises than for seals. As a consequence, a higher frequency of high PBDE concentrations was observed for low blubber thickness values (Fig. 1B).

The PBDE concentrations measured in the present study are similar or slightly higher than in other studies, though information about PBDEs in marine mammals (reviewed by Law et al., 2003, 2006a; Das et al., 2006) is scarce compared to PCBs (Table 4). Kajiwara et al. (2006) investigated PBDEs in several small male cetaceans from Asian waters and reported concentrations between 0.006 $\mu\text{g/g}$ lw in blubber of spinner dolphins (*Stenella longirostris*) from India (between 1990 and 1992) and 6 $\mu\text{g/g}$ lw in Indo-Pacific

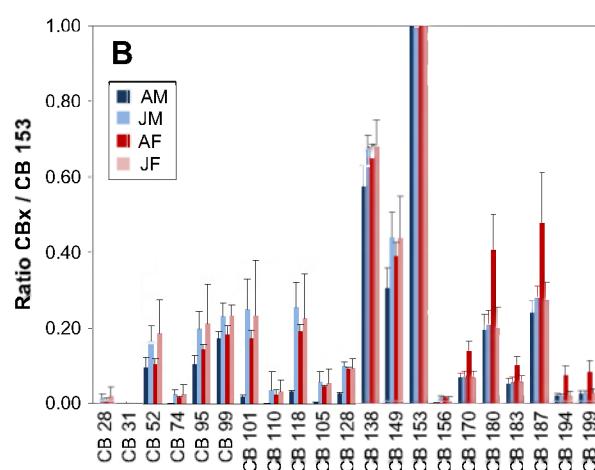
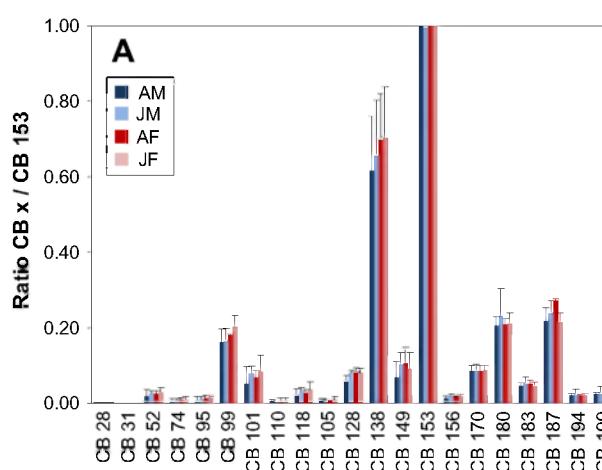


Fig. 3. Ratios between mean concentrations of each individual PCB congener and CB 153 ($R_{153}(\text{CB}_x)$) in harbour seals (A) and harbour porpoises (B). Error bars represent SD.

Table 4Mean concentrations and standard deviations (between brackets) in $\mu\text{g/g}$ lipid weight of BDE 47 in blubber tissue of harbour seals and porpoises.

| Species | Location | Year | n | BDE 47 ($\mu\text{g/g}$ lipid weight) | | | | Reference |
|------------------|--------------------|-----------|-------------|--|-------------|-------------|-------------|-------------------------------|
| | | | | AM | JM | AF | JF | |
| Harbour seal | California | 1989–1998 | 6–4 | 2.04 (2.41) | | 0.20 (0.15) | | She et al., 2002 |
| | North Sea | | 9 | | 1.24 | | | Boon et al., 2002 |
| | Southern North Sea | 1999–2004 | 8–9–2–9 | 0.21 (0.11) | 0.35 (0.21) | 0.12 (0.05) | 0.42 (0.31) | Present study |
| Harbour porpoise | North Sea | | 9 | | 0.86 | | | Boon et al., 2002 |
| | United Kingdom | 1992–2004 | 31–31–24–22 | 0.52 (0.53) | 1.02 (1.23) | 0.74 (1.07) | 1.23 (1.21) | MAFF, 1994; Law et al., 2006b |
| | Southern North Sea | 1999–2004 | 8–12–5–9 | 0.69 (0.46) | 1.11 (1.16) | 0.43 (0.30) | 0.45 (0.27) | Present study |

humpback dolphins (*Sousa chinensis*) from Hong Kong (between 1997 and 2001). The same study found PBDE concentrations (sum of 10 congeners) ranging from 0.024 to 0.100 $\mu\text{g/g}$ lw in male harbour porpoises ($n=3$) from Japan. Despite the small sample size, these results are an order of magnitude lower than these for harbour porpoises from the present study. Shaw et al. (2007) reported mean PBDE concentrations in blubber tissue of harbour seals stranded in the North-Western Atlantic between 1991 and 2005 and found concentrations almost 10 times higher as in present study (3.65 $\mu\text{g/g}$ lw for harbour seal pups ($n=13$), 2.94 $\mu\text{g/g}$ lw for juveniles ($n=14$), 1.39 $\mu\text{g/g}$ lw for AM ($n=7$) and 0.33 $\mu\text{g/g}$ lw for AF ($n=8$)). This is probably a reflection of the higher usage of the penta-BDE technical mixture in North America compared to Europe (Law et al., 2003).

3.4. General PBDE profiles

BDE 47 was the most abundant congener in all analyzed samples of both species similar to previous findings for the same species (Boon et al., 2002; Covaci et al., 2002; Shaw et al., 2007) and other marine mammals (sperm whales *Physeter macrocephalus*, de Boer et al., 1998; ringed seals and beluga whales *Delphinapterus leucas*, Wolkers et al., 2004; bottlenose dolphins, Johnson-Restrepo et al., 2005; Californian sea lions *Zalophus californianus*, Stapleton et al., 2006).

Profiles for JF and JM harbour porpoise were similar, namely BDE 47 > BDE 100 > BDE 99 > BDE 154 > BDE 153. For AF and AM harbour porpoises, this pattern changed into BDE 47 > BDE 99 > BDE 100 > BDE 154 > BDE 153. These profiles are comparable with results from Boon et al. (2002) for harbour porpoises, but differ from bottlenose dolphins (Johnson-Restrepo et al., 2005).

Profiles for harbour seals are different, with the AM harbour seal showing the following pattern, BDE 47 > BDE 153 > BDE 99, BDE 154 > BDE 100. The profiles for other age–gender groups are different from that of the AM group BDE 47 > BDE 99 > BDE

100 ~ BDE 153 > BDE 154. Similar results were also found by Shaw et al. (2007).

Ratios between the concentrations of individual PBDE congeners and the concentration of BDE 47, the most persistent and dominant PBDE, for each animal within the four age–gender groups were used to construct relative PBDE profiles for harbour seals and porpoises (Fig. 4A and B):

$$R_{47}(\text{BDE}_x) = \frac{[\text{BDE}_x]}{[\text{BDE}_{47}]}$$

For harbour seals (Fig. 4A), the JM and JF groups showed lower proportions of all measured PBDEs, while the AM and AF groups had slightly higher contributions of BDE 99, BDE 100, BDE 153 and BDE 154, combined with lower concentrations of this congeners, which is a reflection of the higher concentrations of BDE 47 in juveniles compared to adults.

For harbour porpoises (Fig. 4B), the same trends as found for harbour seals were observed. Juveniles seemed to have lower contributions of all measured PBDE congeners than adults, probably because of a higher 'start concentration' of BDE 47 from lactation and gestation (assuming that similar to PCBs, the less lipophilic congeners will be mostly abundant in milk), together with a minimal metabolism at that age. In general, harbour porpoises had a higher contribution of congeners BDE 99, BDE 100, BDE 153 and BDE 154 compared to harbour seals. Although congeners BDE 28, BDE 66 and BDE 183 were infrequently detected, their concentrations were higher in porpoises than in seals, indicating that harbour porpoises have difficulties with metabolizing PBDEs.

3.5. Relationship between PCBs and PBDEs

Johnson-Restrepo et al. (2005) reported a significant correlation ($r=0.83$, $p<0.01$) between PCB and PBDE concentrations in fish and a higher correlation coefficient for the relationship between

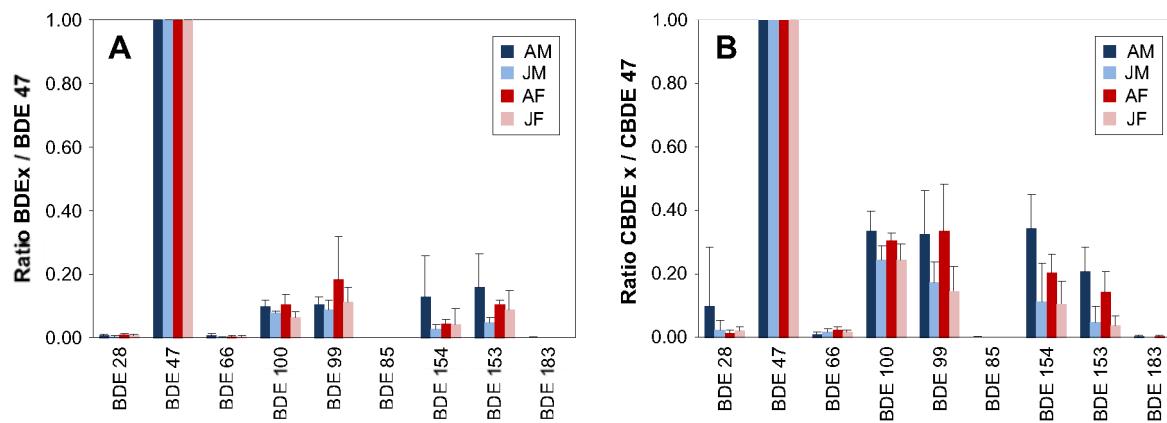


Fig. 4. Ratios between mean concentrations of each individual PBDE congener and BDE 47 ($R_{47}(\text{BDE}_x)$) in harbour seals (A) and harbour porpoises (B). Error bars represent standard deviations.

PCBs and PBDEs in dolphins and sharks from coastal Florida. Shaw et al. (2007) found a highly significant correlation ($r=0.82$, $p < 0.01$) between PCBs and PBDEs in harbour seals from the North-Western Atlantic coast. In the present study, no significant correlations between PCBs and PBDEs or between CB 153 and BDE 47 in harbour seals or harbour porpoises could be found (all $p > 0.05$), all age groups together or separate. This could be an indication for a different accumulation and biomagnification through the food chain, but it may also reflect the variation in accumulation within each age–gender group.

3.6. Adverse effects

The PCB and PBDE concentrations, found in the present study, can be a serious threat for harbour seals and porpoises. Mean concentrations for PCBs and PBDEs in harbour porpoises in the present study (Table 1) are more than 2 (for AF) to 20 (for AM) times higher for PCBs and about 10 times higher for PBDEs compared to concentrations from stranded or bycaught harbour porpoises from European coasts which are associated with interfollicular fibrosis, splenic depletion and thymic atrophy (Beineke et al., 2005; Das et al., 2006). Furthermore, PCB concentrations in almost all age–gender groups of both species are more than an order of magnitude higher than levels of PCBs negatively associated with vitamin A (a dietary hormone essential to growth, development, reproduction and immune function) concentrations in plasma and blubber of free-ranging harbour seals from British Columbia (Canada) and Washington State (USA) (Mos et al., 2007).

4. Conclusions

Harbour porpoises and harbour seals, two representative top predator species for the North Sea ecosystem, are good indicators of coastal pollution, because they have long life spans, feed high in the food chain and do not present large-scale migration. We found that factors, such as age and gender, among others, are important for the bioaccumulation of PCBs and PBDEs in marine mammals. The AM group had the highest concentrations of PCBs, but not of PBDEs, probably because of an increased metabolism with age or body burden. The AF group could eliminate considerable amounts of PCBs and PBDEs by gestation and lactation resulting in low concentrations. However, the transfer of PBDEs to the offspring needs more attention in the future. Juvenile animals had mixed trends in concentrations with the lowest concentrations for PCBs, but the highest for PBDEs. Harbour seals, members of the pinnipeds, and harbour porpoises, members of the Cetacea, are from an evolutionary point of view different and have therefore a different ability for metabolic breakdown reflected by the different PCB or PBDE profiles. Harbour porpoises have more difficulties of metabolizing lower halogenated and less persistent PCB and PBDE congeners than harbour seals probably due to less efficient cytochrome P450 enzymes. This could lead to bioaccumulation of these contaminants to a greater extent in harbour porpoises and subsequently to possible adverse effects.

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