Inter-species differences for polychlorinated biphenyls and polybrominated diphenyl ethers in marine top predators from the Southern North Sea: Part 2. Biomagnification in harbour seals and harbour porpoises

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

\section*{1. Introduction}

Due to their chemical stability and other properties, such as low water solubility and vapour pressure, polychlorinated biphenyls (PCBs) biomagnify in aquatic food webs, leading to increased concentrations throughout all trophic levels (Borgå et al., 2004; Burreau et al., 2006; Persson et al., 2007). Marine mammals occupy the top of these aquatic food chains and accumulate considerable amounts of PCBs in their tissues compared with their prey (Ruus et al., 1999; Fraser et al., 2002; Johnson-Restrepo et al., 2005; Wolkers et al., 2007), causing adverse health effects (De Swart et al., 2002; Johnson-Restrepo et al., 2005; Reijnders et al., 1996; Mos et al., 2007).

Harbour seals (Phoca vitulina) and harbour porpoises (Phocoena phocoena) are two representative top predators for the North Sea ecosystem. They do not migrate on a larger scale and spend their entire life in the North Sea, which makes them suitable for pollution monitoring. Due to its limited depth, high traffic and function as an endpoint of pollutants through runoff via land and rivers, the North Sea is a highly contaminated area. This is reflected in the amounts of chemicals, such as PCBs and polybrominated diphenyl ethers (PBDEs), which were measured in species representative for each trophic level of the North Sea food chain (Boon et al., 2002; Voorspoels et al., 2003, 2004). Although the behaviour of PCBs in marine food webs is relatively well studied, the biomagnification power of PBDEs in aquatic food chains is less documented (Law et al., 2006; Ramu et al., 2006). Moreover, information about biomagnifications involving marine mammals is still scarce (Wolkers et al., 2004; Johnson-Restrepo et al., 2005). While harbour seals and porpoises may reduce high PCB and

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PBDE concentrations by metabolism, there are species-specific differences in the ability for metabolic breakdown of PCBs and PBDEs, leading to different accumulation patterns (Wejs et al., submitted for publication). Since the uptake of contaminants in marine mammals depends on the diet, any comparison of metabolic capacities between harbour seals and porpoises would be incomplete without taking into account the concentrations and patterns of contaminants in their prey. Both species share an extensive part of their diets because they feed on benthic as well as pelagic fish species from the same area and have similar requirements regarding energy content and prey size (Hall et al., 1998; Santos and Pierce, 2003; Santos et al., 2004). The aim of the second part of the present study was to investigate metabolic capacities of PCBs and PBDEs in harbour seals and porpoises from the Southern North Sea by assessing the biomagnification between these predators and their prey. Calculating biomagnification factors (BMFs) generally implies awareness that several biological, such as age, gender, biotransformation, trophic position according to stable isotopes ($^\delta^{13}$N) and chemical factors, such as octanol–water partition coefficients ($K_{ow}$), are important in bioaccumulation and biomagnification processes (Börja et al., 2004). The influence of these factors on BMFs has therefore been investigated.

2. Materials and methods

2.1. Sample preparation and analysis

Complete details for the sample collection, sample preparation and analysis by gas chromatography coupled with mass spectrometry (GC–MS system) are given in the first part of this study (Wejs et al., submitted for publication) and are briefly presented below. Blubber samples were collected from 35 harbour porpoises and 28 harbour seals stranded or bycaught in the Southern North Sea between 1999 and 2004 and necropsied by T. Jauniaux (University of Leuven, Belgium). 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). The fish samples (sole – Solea solea and whiting – Merlangius merlangus) used for the calculation of BMFs were previously analysed and discussed by Voorspoels et al. (2003, 2004).

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 involves the Soxhlet extraction of blubber dried with anhydrous Na$_2$SO$_4$ and clean up of the extract by acidified silica. The following 21 PCB congeners (IUPAC numbers) were targeted: 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 153, 156, 170, 183, 187, 194 and 199. Moreover, the following 10 PBDE congeners (IUPAC numbers) were extracted by acidified silica. The following 21 PCB congeners (IUPAC numbers) were targeted: 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 153, 156, 170, 183, 187, 194 and 199. Moreover, the following 10 PBDE congeners (IUPAC numbers) were targeted for analysis: 28, 47, 66, 85, 99, 100, 153, 154, 183 and 209. The analysis was done by GC–MS operated in electron impact (for PCBs) and electron-capture negative ionization mode (for PBDEs).

2.2. Stable isotope analysis

Measurements of $^\delta^{13}$C and $^\delta^{15}$N in muscle of harbour seals and porpoises were used to investigate the influence of trophic position on biomagnification of PCBs and PBDEs. Procedure and results for $^\delta^{13}$C and $^\delta^{15}$N were presented elsewhere (Das et al., 2003, 2004,a, b, 2007). Briefly, after drying at 50 °C (48 h), muscle samples were ground into a homogeneous powder and treated with a 2:1 chloroform:methanol solution to remove lipids. CO$_2$ and N$_2$ gas were analyzed on a VG Optima (Micro–mass) IR–MS coupled to an N–C–S elemental analyzer (Carlo Erba) for automated analyses. Routine measurements are precise to 0.3‰ for both $^\delta^{13}$C and $^\delta^{15}$N. Stable isotope ratios were expressed in δ notation according to the following equation:

$$\delta X = \frac{[R_{sample}/R_{standard}]-1} \times 1000$$

where X is $^\delta^{13}$C or $^\delta^{15}$N and R is the corresponding ratio ($^\delta^{13}$C/$^\delta^{12}$C or $^\delta^{15}$N/$^\delta^{14}$N). Carbon and nitrogen ratios are expressed relative to the Vienna Pee Dee Belemnite standard and to atmospheric nitrogen, respectively. Reference materials were IAEA CH-6 (sucrose) ($^\delta^{13}$C = −10.4 ± 0.2‰) and IAEA-N1 ($^\delta^{15}$N = −0.4 ± 0.2‰), respectively.

2.3. Calculation of biomagnification factors

The BMF was defined as the ratio between the lipid-normalized contaminant concentrations in predator and prey (Ruus et al., 1999; Mackay and Fraser, 2000; Borga et al., 2004).

$$\text{BMF} = \frac{C_{\text{predator}}}{C_{\text{prey}}}$$

Biomagnification occurs when BMFs are greater than 1, indicating that predators are less capable of metabolizing these compounds compared with their prey. BMFs are usually calculated relative to only one prey species (Ruus et al., 1999; Wolkers et al., 2007), but using a diet of mixed prey items is a more realistic approach (Fraser et al., 2002; Ramu et al., 2006; Borga et al., 2007). Harbour seals and porpoises have a diet consisting of pelagic fish species, as well as benthic fish. Recent information about the diet of harbour seals and porpoises from the Southern North Sea is scarce. Studies reported previously that specific contributions of pelagic and benthic fish to the overall diet of harbour seals and porpoises may vary from season to season (more pelagic fish in winter, more benthic fish in summer for harbour seals in the Wash, UK; Hall et al., 1998) and from location to location (more pelagic fish in the Netherlands, while more than 50% benthic fish in the diet of harbour porpoises in German waters) (reviewed by Meiningier et al., 2003). All animals from the present study were bycaught or found stranded in different seasons between 1999 and 2004. Also, it is impossible to know the exact diet of an individual since they do not have to be resident in exactly one place of the Southern North Sea, an area consisting of the Southern Bight (coasts of Belgium and The Netherlands) and the German Bight (German coast). Therefore, pelagic and benthic fish species were assumed to have an equal contribution (Leonards et al., 2008). In the present study, sole and whiting, both present in the diet of harbour seals and porpoises, were taken as representative prey for benthic and pelagic prey, respectively (Hall et al., 1998; Meiningier et al. 2003; Santos and Pierce, 2003; Santos et al., 2004; Brasseur et al., 2006). The size of the fish was not taken into consideration here. Lipid-normalized concentrations of PCBs and PBDEs in sole and whiting from the Southern North Sea were previously reported (Voorspoels et al., 2003, 2004). The fish samples were collected during the same time period as the marine mammal samples, though for different purposes, and were from the same location (the Southern North Sea). Since reproduction reduces through lactation and gestation the concentrations of hydrophobic contaminants (Covaci et al., 2002; Shaw et al., 2005; Wejs et al., submitted for publication), BMFs were not calculated for the AF group of harbour seals and porpoises.

2.4. 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 for further calculations. BMFs of individual PCB and PBDE congeners were compared between the four age–gender groups (adult males–AM, adult females–AF, juvenile males–JF and juvenile females–JF) using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test, to determine differences between groups. Pearson’s r and corresponding p-values for relationships between log concentrations of individual PCBs and PBDEs were obtained using GraphPad Prism 4 (GraphPad Software, Inc.).

3. Results and discussion

Concentrations and profiles of PCBs and PBDEs are discussed in detail in the first part of this study (Wejs et al., submitted for publication) and therefore are only briefly presented in Table 1. These results were used for further calculations.

3.1. Biomagnification factors

3.1.1. Harbour seals

As there were no significant differences in BMFs, calculated for each individual PCB and PBDE congener, between JF and JM (all p > 0.05), both groups were pooled and further referred to as ‘juvenile seals’ or ‘juveniles’.

For juvenile seals, as well as for the AM group, BMFs were generally lower for lower chlorinated PCBs (tri- to penta-CBs) and higher for higher chlorinated PCBs (hexa- to octa-CBs) as a consequence of dietary exposure (Voorspoels et al., 2004). The fact that there is no statistical difference in the BMFs of lower chlorinated compounds (such as CB 28, 52, 74 and 95) between juvenile and AM seals (all p > 0.05) shows that this metabolic capacity does not decline with age or higher body burdens (Table 2). In contrast, higher BMFs for most hexa- to octa-CB congeners indicate lower metabolic activities in harbour seals causing therefore bioaccumulation. Indeed, BMF values of these higher chlorinated PCB congeners were statistically higher in the AM group (all p < 0.05; Table 2), as a result from longer life-spans and limited biotransformation capacities for several PCB congeners.

The BMFs obtained in the present study were difficult to compare across the studies due to the unclear definition of juvenile individuals and to the use of $^\gamma$PCBs for the calculation of BMFs. Ruus et al. (1999) found BMF values of 8.7 and 28.0 for cod...
(Gadus morhua) and sandeel (Ammodytes marinus) to harbour seal, respectively, and 10.0 and 32.2 for cod and sandeel to grey seal (Halichoerus grypus), respectively. However, BMF values were calculated only for $\gamma$PCBs and for juvenile and adult animals of both genders taken together, thus making any comparison difficult. Fraser et al. (2002) calculated BMFs of individual PCB congeners for JM and JF harp seals (Phoca groenlandica) with a diet consisting of 50% polar cod (Boreogadus saida) and 50% crustaceans (Thermisto libellula). Except for some PCB congeners (e.g. CB 105 and CB 118), the BMF values found by Fraser et al. (2002) compare favourably with the present study (Table 2), suggesting that bioaccumulative capacities are comparable between pinnipeds.

BMFs of PBDEs in seals and in other marine mammals are not well established. Boon et al. (2002) reported that the biggest biomagnification step in the North Sea food web occurs from gadoid fish (cod and whiting) to marine mammals (seals and porpoises). Wolkers et al. (2004) found metabolic indices (MI) (which express BMFs relative to CB 153) greater than 1 for BDE 47 and BDE 99 and MI below 1 for BDE 100 (Phoca hispida), suggesting some metabolism for BDE 100. In the present study (Table 3), BMFs of lower brominated congeners were higher (although not significant) for juveniles than for AM harbour seals. This might be due to an increased metabolism with the animal’s body burden or a better developed metabolic capacity with age. Contrarily, BMFs of higher brominated congeners, such as BDE 153 and BDE 154, were lower for juveniles compared with the AM group.

### 3.1.2. Harbour porpoises

There were significant differences between the JM (n = 11) and JF (n = 10) porpoises for BMF values of CB 74 (p = 0.010), CB 101 (p = 0.026), CB 105 (p = 0.033) and CB 156 (p = 0.043). For these congeners, BMFs for the JM group alone were compared with the values for the AM group. For all other PCBs, JM + JF porpoises were compared with the AM group (Table 2).

Except for CB 110, BMFs of PCBs in the AM group were greater than 1 (Table 2). For this congener, the lower BMF value in the AM group may be a reflection of the fact that the prey species were able to accumulate it directly from the sea water (bioconcentration), while the AM porpoises have better developed metabolizing capacities compared with juveniles (Weijs et al., submitted for publication). The AM group had significantly higher BMFs for the most PCB (including persistent and non-persistent) congeners.
compared with juveniles (Table 2). Juveniles (JM) showed higher (although not statistically significant) BMFs for congeners 28, 101, 110, 105 and 156 than the AM group, reflecting increased metabolism for these congeners in adults (Table 2). The BMFs obtained in the present study were difficult to be compared across the studies and between different cetacean species, due to the (sometimes ambiguous) definition of juvenile individuals and the frequent use of ΣPCBs (and individual PCB congeners) for the calculation of BMFs. Ramu et al. (2006) reported BMFs for ΣPCBs of 2.4 and 2.2 for two adult male finless porpoises (Neophocaena phocaenoides) stranded along the Hong Kong coast (2000–2001) using their stomach contents. Johnson-Restrepo et al. (2005) found that the mean BMFs for ΣPCBs ranged from 148 to 863 for bottlenose dolphins (Tursiops truncatus) using a wide variety of prey, such as silver perch (Bairdiella chrysoura) spotted seatrout (Cynoscion nebulosus), striped mullet ( Mugil cephalus), and Atlantic stingray (Dasyatis sabina). Wolkers et al. (2007) calculated MI-values (BMFs relative to CB 153) in killer whales, which revealed biomagnification of persistent PCBs from groups I and IIIB, similar to BMFs calculated in our study.

Since there were no significant differences in the BMFs of individual PCB congeners (all p > 0.05) between the JM and AM groups, both groups were pooled and further used as “juveniles”. Increasing BMFs with higher number of bromine atoms were seen in the AM harbour porpoises (Table 3) indicating lower ability to metabolise higher brominated PBDEs. The lack of this trend for juveniles and the significant differences between AM and juveniles for the highest brominated compounds, might be an indication for comparable metabolic capacities for lower and higher brominated PBDEs in juvenile harbour porpoises (Table 3). Information in literature about BMFs of PBDEs in marine mammals is scarce. Wolkers et al. (2004) reported MI close to 1 for BDE 47, BDE 99 and BDE 100 in beluga whales (Delphinapterus leucas), which differs from the harbour porpoises in the present study. Johnson-Restrepo et al. (2005) found BMFs of sum PBDEs ranging from 29 to 150 for bottlenose dolphins and a variety of prey (see above).

### 3.3.1. *Inter-species comparison*

Harbour porpoises seem to have difficulties with metabolizing lower chlorinated PCBs, such as CB 52 and 95, but show slightly lower BMFs for higher chlorinated PCBs, e.g. CB 194 and 199. The difference between harbour seals (pinnipeds) and porpoises (cetaceans) (p = 0.014 for comparison between juveniles and p < 0.001 between AM) for CB 149 has been described before (Hutchinson and Simmonds, 1994; Vetter et al., 1996). For most hexa- to octa- CBs, juvenile harbour seals have higher BMFs compared with juvenile harbour porpoises (all p < 0.05). Taking into consideration that these differences are diminished for AM seals and porpoises (all p > 0.1), this probably means that the capacity to metabolize PCBs is higher for AM harbour seal than for AM porpoises. Significant differences were obtained for CB 156 between juvenile seals and porpoises (p < 0.001) and AM of both species (p = 0.003).

There were large inter-species differences between harbour seals and porpoises for the biomagnification of PBDEs. In general, biomagnification occurs mainly in harbour porpoises. The AM porpoises had higher BMFs (all p < 0.05, except for BDE 28, p = 0.058) than AM seals. BMFs in juvenile porpoises and seals were similar for BDE 47 and 153 (p = 0.059 and 0.717, respectively) and different for other PBDEs (all p < 0.05).

### 3.2. Influence of Kow on the biomagnification factors

Physical factors, such as the octanol–water partition coefficient (Kow), might predict to a certain extent the biomagnification of lipophilic contaminants, such as PCBs and PBDEs (Fisk et al., 1998; Borgá et al., 2004).

Relationships between BMFs and log Kow of individual PCB or PBDE congeners were investigated for the juveniles and AM in harbour seals and porpoises, but no clear trends could be established (Fig. 1).

For PCBs in harbour seals, significant regressions (p < 0.001) were found for the AM group (R² = 0.600) and the juvenile group (R² = 0.563) (Fig. 1A). Interestingly, the regression coefficients improved substantially (R² = 0.815 and 0.774 for adult males and juveniles, respectively) when several PCB congeners (CB 99, CB 118, CB 105, CB 149 and CB 156) were manually removed. CB 99 has a higher potential for biomagnification than the other penta-CB congeners, probably as a result of the position of the chlorine atoms which makes this congener as persistent as CB 138 (Boon et al., 1997). Congeners CB 118 and CB 105 (penta-CBs) and CB 149 and CB 156 (hexa-CBs) biomagnify to a lesser extent due to their molecular structure, which suggests faster metabolism (Boon et al., 1997). In contrast to PCBs, there is no clear relationship between BMFs of PBDEs and log Kow in harbour seals (R² = 0.218; p = 0.351 for juveniles and R² = 0.406; p = 0.173 for adult males) (Fig. 1B).

In harbour porpoises, there was no significant linear relationship between BMFs of PCBs and log Kow (R² = 0.029, p = 0.48 and R² = 0.033, p = 0.44, for juveniles and adult males, respectively) (Fig. 1C). In contrast, a borderline significant linear relationship was found between BMFs of PBDEs and log Kow in the AM group (R² = 0.64; p = 0.056), not for juveniles (R² = 0.47; p = 0.135) (Fig. 1D). A similar increase in the biomagnification power and the number of bromine atoms (n = 3–6) for PBDE congeners (directly correlated to log Kow) was observed by Burreau et al. (2006) in marine food webs from the Baltic Sea and the Atlantic Ocean.

From our results, it appears that the Kow values can only partly predict BMFs for PCB and PBDE congeners. For a better understanding of the biomagnification processes for individual congeners, several other parameters, such as the molecular structure, molecular weight and the number and position of the halogen atoms, should be taken into account as well.
3.3. Influence of stable isotope on the biomagnification factors

When investigating biomagnification in a food web, information about food sources (feeding ecology) and the position in the food chain can be obtained using measurements of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes (Kelly, 2000; Das et al., 2003). While stable isotopes of $^{14}N$ provide a continuous measure of trophic level position, the $^{13}C$ stable isotopes give information on the carbon sources, with isotopic signals characteristic for location of prey. Thus, relationships between concentrations of lipophilic contaminants, which are expected to biomagnify in the food chain, and the trophic levels can be established (Fisk et al., 2001).

Fig. 2 shows the relationship between the $\delta^{13}C$ and $\delta^{15}N$ stable isotopes of juveniles and adult males in the two investigated species from the Southern North Sea. Harbour seals were found to have significantly higher $\delta^{13}C$ values than the harbour porpoises ($F_{1,48} = 91.831; p < 0.001$). Moreover, the higher $\delta^{13}C$ values for seals indicated also preferential in-shore feeding compared to the off-shore feeding of porpoises. These differences in the stable isotope signatures between harbour seals and porpoises are more likely caused by differences in their prey’s isotopic signature and not by different diets, since harbour seal and porpoise diets contain largely the same prey species (Hall et al., 1998; Meininger et al., 2003; Santos and Pierce, 2003; Santos et al., 2004; Brasseur et al., 2006). The $\delta^{15}N$ and $\delta^{13}C$ values in marine predator tissues are determined initially by the isotopic composition of the baseline phyto- and zooplankton sources which is measured in the particulate organic matter (POM). A mean $\delta^{15}N$ value of $5_{\text{ave}}$ was found for off-shore POM, increasing up to $9_{\text{ave}}$ at the North Sea coasts (Das et al., 2003). In-shore feeding of harbour seals, as revealed by the $\delta^{13}C$ values, is therefore responsible for higher $\delta^{15}N$ values, even with the same prey species as for the harbour porpoises.

The two species had also inverse positions for juveniles and adult males, probably due to a change in the diet with age. There was no difference in the $\delta^{15}N$ values ($F_{1,23} = 2.832; p = 0.016$) between juveniles ($n = 18$) and adult male ($n = 7$) harbour seals (Das et al., 2007). No difference could be detected in trophic status, assessed through $\delta^{15}N$ analysis between juvenile ($n = 21$) and adult male ($n = 7$) harbour porpoises ($F_{1,26} = 1.772; p = 0.195$).

Only few correlations between the logarithmic concentrations of individual PCB or PBDE congeners and the $\delta^{13}C$ and $\delta^{15}N$ values were found to be significant at $p < 0.05$ (Table 4). Trophic position, an important biological factor in the bioaccumulation process as mentioned earlier (Borga et al., 2004), is therefore probably not sufficient to explain the variation in the BMFs between juveniles and adult males. However, the low number of significant correlations between BMF and $\delta^{15}N$ values is not surprising since
the present study did not investigate different levels in the trophic web, but instead looked closer to only one position in the food chain.

4. Conclusions

The biomagnification of lipophilic contaminants, such as PCBs and PBDEs, in marine mammals is influenced by several factors from which age and gender seem to be the most important. Furthermore, the present study evidenced interspecies-specific abilities for metabolic breakdown of contaminants. Harbour porpoises have more difficulties for metabolizing several PCB and PBDE congeners than harbour seals, probably due to a less efficient hepatic metabolism. However, factors which may influence biomagnification, such as trophic position, measured through stable isotope analysis $\delta^{13}N$, could only partially predict biomagnification for these two species.

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