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# Persistent organic pollutants and methoxylated PBDEs in harbour porpoises from the North Sea from 1990 until 2008: Young wildlife at risk?

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ABSTRACT

In the European North Sea, harbour porpoises are top predators with relatively long life spans and a limited capacity for metabolic biotransformation of contaminants compared to some other marine mammal species. As such, they are exposed to a mixture of persistent pollutants, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), DDT and metabolites (DDXs), hexachlorobenzene (HCB) and chlordanes (CHLs) that bioaccumulate in their tissues. We report here on the levels of persistent organic pollutants and of the naturally-produced methoxylated PBDEs (MeO-PBDEs) in blubber, liver and kidney of harbour porpoise neonates (n = 3), calves (n = 15), juveniles (n = 6) and adults (n = 4) of the southern North Sea. Concentrations of almost all contaminant classes decrease slightly in all age groups over the period 1990–2008. For some classes (e.g. PCBs and DDXs) however, levels seem to increase little in harbour porpoise calves. In all animals, blubber had the highest concentrations, followed by liver and kidney, whereas liver and kidney were the preferred tissues for several compounds, such as octa- and deca-PCBs. Our data suggest that harbour porpoises calves are exposed to higher or comparable concentrations of POPs and of MeO-PBDEs and somewhat different patterns of selected POPs than adults, potentially placing them, and the entire population, at a disproportionate risk for exposure-related health effects.



## 1. Introduction

In the past, persistent organic pollutants, such as polychlorinated biphenyls (PCBs), DDT and metabolites, polybrominated diphenyl ethers (PBDEs), hexachlorobenzene (HCB) and chlordanes (CHLs) were extensively used worldwide. PCBs were widely used for many applications, such as insulating and cooling in electrical equipment due to their flame retarding properties (ATSDR, 2001). Although PBDEs have a different chemical structure compared to PCBs, they also do not burn easily. Therefore, PBDEs were used often in a lot of products ranging from household products to textiles (ATSDR, 2004). PBDEs and PCBs are both a group of 209 congeners and each congener differs from the other in the number and position of the bromine or chlorine atoms, respectively.

DDXs are a mixture of isomers (*p,p′*- and *o,p′*-DDT) and metabolites (DDE and DDD). These pollutants have been proven effective in controlling pests and preventing diseases and had a main function in agriculture (ATSDR, 2002a). HCB and chlordane related compounds, such as *cis*-nonachlor and *trans*-nonachlor, and metabolites, such as oxychlordane, were also used as pesticides (ATSDR, 1995, 2002b). All contaminant classes mentioned so far are persistent, bioaccumulative and toxic. As a consequence, they are all banned globally: for PCBs, DDXs and HCB since the 1970s, for chlordane related compounds since the 1980s and for most PBDEs since 2004. Unfortunately, because of their stability in the environment, they can still be found at all levels of the terrestrial and aquatic food chains where they can be toxic to all organisms in these food webs (e.g. Hoffman et al., 1996; Michielsen et al., 1999; Ruus et al., 1999; Bondy et al., 2003; Birnbaum and Staskal, 2004; Johnson-Restrepo et al., 2005; Kelly et al., 2008; Sonne et al., 2009).

Methoxylated PBDEs (MeO-PBDEs) differ from all contaminants mentioned so far because they have a different origin. PCBs, PBDEs, CHLs and metabolites and HCB are all intentionally or unintentionally (metabolites or as byproducts) produced by humans. In contrast, MeO-PBDEs are produced by natural sources, such as algae or sponges (Vetter et al., 2002; Kelly et al., 2008; Malmvärn et al., 2008; Weijs et al., 2009a, b, c).

The marine environment acts as a sink for pollutants as it receives a lot of chemicals through run-off from the mainland or atmospheric deposition. Most compounds have the capacity to biomagnify in marine food webs and can thus be found at high levels in marine top

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predators, such as marine mammals (e.g. Blasius and Goodmanlowe, 2008; Kelly et al., 2008; Weijs et al., 2009b; Ylitalo et al., 2009; Dorneles et al., 2010). Harbour porpoises are small cetaceans that live in waters of the Northern Hemisphere. Although all harbour porpoises have, as top predators, high levels of contaminants in their tissues (Zegers et al., 2005; Pierce et al., 2008; Weijs et al., 2009a), results of Weijs et al. (2010b) for PCB 153 suggest that calves are possibly the most vulnerable age class among all other age classes because they already have a 'start' concentration from birth due to the transfer of chemicals through the placenta and via the lipid-rich milk during lactation as found previously (Debier et al., 2003; Yordy et al., 2010). In addition, their metabolism is probably not fully developed yet, which makes it difficult for them to eliminate these compounds (Sly and Flack, 2008). Several studies have investigated the impact of pre- or post-natal exposure to environmental pollutants on the overall health and development of an organism. Tiedeken and Ramsdell (2009) have shown that fetal exposure to *p,p′*-DDE in zebrafish increases the sensitivity to domoic acid-induced seizures at later stages. The same study found comparable *p,p′*-DDE levels in California sea lion fetuses and suggests a link between the DDE exposure and domoic acid toxicity in these animals. Rice (1999) found that monkeys, exposed to PCBs during lactation, experience a behavioral impairment later, whereas brominated and chlorinated dioxins may influence the fear memory of male mice after *in utero* and lactational exposure (Haijima et al., 2010).

The aim of the present study was to evaluate the bioaccumulation of PCBs, PBDEs, CHLs, DDXs (DDT and metabolites), HCB and MeO-PBDEs in harbour porpoises from the North Sea. Until now, blubber has mainly been used as the ideal matrix for the biomonitoring of lipophilic compounds such as PCBs or PBDEs. However, the distribution of chemicals inside the body of harbour porpoises depends on the biochemical properties of the compound rather than its lipophilicity alone. Therefore, we aimed at investigating the presence of these contaminants in various tissues of harbour porpoises to determine the distribution or preference of the compounds for a specific tissue. The animals investigated in the present study died from 1990 until 2008 covering a period of 18 years. Therefore, an attempt was made to look for possible time trends within these 18 years with factors such as age or gender taken into account.

## 2. Materials and methods

### 2.1. Samples, chemicals and target compounds

Blubber samples were collected from 28 harbour porpoises (*Phocoena phocoena*; 3 neonates, 15 calves, 6 juveniles and 4 adults; Supporting Information Table S1). In addition, 6 kidney samples and 18 liver samples of these animals were analysed to assess the distribution of pollutants between tissues. In the present study, calves are animals that are still drinking milk as part of their diet and that are younger than 1 year old. Juveniles are animals older than 1 year, but younger than 3 years, while adults are animals older than 3 years (Lockyer, 1995). All porpoises stranded alive on the North Sea coasts, but died during rehabilitation at the SOS Dolfijn rehabilitation center, Dolfinarium Harderwijk, The Netherlands between 1990 and 2008 (Supporting Information Table S1). In all samples, 35 PCB congeners (IUPAC numbers: CB 18, 28, 44, 47, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 146, 149, 151, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, 206, and 209), 7 PBDEs (IUPAC numbers: BDE 28, 47, 49, 99, 100, 153, and 154), 6 DDXs (*o,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT), 3 chlordanes (oxychlordane (OxC), *trans*-nonachlor (TN), and *cis*-nonachlor (CN)) and HCB were targeted. Also, 2 naturally-produced methoxylated PBDEs (2′-MeO-BDE 68 and 6-MeO-BDE 47) were investigated. PBDE standards were from Wellington Laboratories (Guelph, ON, Canada) and all other POPs were from Accustandard (New Haven, CT, USA).

### 2.2. Sample preparation

The method used for the sample extraction and clean-up has been previously described (Covaci et al., 2008) and is briefly presented in the following discussion. Approximately 2 g of liver, 0.2 g of blubber and 3 g of kidney were dried with ~8 g anhydrous $Na_2SO_4$, spiked with internal standards BDE 77/BDE 128 (25 ng) and CB 143 (100 ng) and extracted for 2 h by hot Soxhlet with 100 ml hexane/acetone (3/1; *v/v*). After lipid determination (gravimetrically, performed on an aliquot of the extract, typically 1/8), the extract was cleaned on 8 g of acidified silica. After elution of analytes with 20 ml hexane and 15 ml dichloromethane, the cleaned extract was evaporated to dryness and reconstituted in 150 µl iso-octane.

### 2.3. Analysis

PBDEs, MeO-PBDEs and CHLOR were measured with an Agilent 6890 gas chromatograph coupled with a 5973 mass spectrometer system (GC–MS). The GC was equipped with a 30 m × 0.25 mm × 0.25 µm DB-5 capillary column. The MS was operated in electron capture negative ionisation (ECNI) mode and was used in the selected ion-monitoring (SIM) mode with ions $m/z = 79$ and 81 monitored during the entire run for PBDEs and MeO-PBDEs and two specific ions for each CHLOR compound. PCBs, HCB and DDXs were measured with a GC–MS system 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 MS was used in the SIM mode with 2 ions monitored for each PCB homologue group. The latter system (GC–EI/MS) was also used for confirmation of organobromine compounds.

### 2.4. Quality assurance/quality control (QA/QC)

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 3 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 S/N 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 from the National Institute of Standards and Technology (PCBs, PBDEs and OCPs in whale blubber) was used to test the accuracy of the method. Obtained values were not deviating more than 10% from the certified values. The QC scheme was also assessed through regular participation to interlaboratory comparison exercises organised by the US National Institute of Standards and Technology.

### 2.5. Statistical analysis

Statistical analyses were conducted using the SPSS 18.0 statistical package (PASW Statistics 18). The level of statistical significance was set at $p < 0.05$. Because the animals are of different age classes (neonate, calf, juvenile, and adult), different genders (male and female) and from different years (1990 until 2008), sample sizes of paired age–gender groups are sometimes very small. Therefore, non-parametric statistical tests (Kruskal–Wallis tests) were performed only for calves. To assess temporal trends between the calves, we divided the individuals in two groups: group calf 1, with calves from 1990 until 1998, and group calf 2 group, with calves from 2000 until 2008. Because gender differences are often only apparent for juveniles and adults, there was no division made between male or female neonates and between male or female calves (Mos et al., 2006; Weijs et al., 2010b). Where possible, Kruskal–Wallis

was used to test the differences in lipid percentages and pollutant concentrations between the groups. Outliers (one adult female and one calf of 1990) were detected by making boxplots and removed from further statistical analyses.

## 3. Results

PCBs, PBDEs, naturally-produced MeO-PBDEs, DDXs, CHLs and HCB were measured in 28 blubber samples, 18 liver samples and 6 kidney samples of harbour porpoises (3 neonates, 15 calves, 6 juveniles and 4 adults) from the North Sea. All animals were stranded alive between 1990 and 2008, but died during rehabilitation.

The only adult female of this dataset was excluded from all statistical analyses. Levels of all compounds in this individual were much lower compared to the concentrations measured in all other animals of the present study and can be found for comparison reasons in Table 1. Although the low levels were probably caused by elimination of compounds through gestation/lactation during previous pregnancies, this animal was anaemic, pregnant and died at the rehabilitation center SOS Dolfijn (Harderwijk, the Netherlands) in 2007 due to acute hepatic lipidosis.

### 3.1. Lipid percentages

While there were no statistically significant differences between the lipid percentages in blubber of calves ($n=15$), juveniles ($n=6$) and adults ($n=3$) (median: 89.6%; range: 60.1–95.1%; $p=0.115$), lipid percentages of neonates ($n=3$) were significantly lower compared to these 3 groups (median: 79.5%; range: 73.9–82.5%; $p=0.019$). There were no statistically significant differences between the lipid percentages of the kidney and liver between calves ($n=2$ for kidney, $n=11$ for liver), juveniles ($n=1$ for kidney, $n=3$ for liver) and adults ($n=2$ for kidney, $n=3$ for liver) (median: 3.3%; range: 3.0–4.9%; $p=0.497$ for kidney, median: 5.2%; range: 4.5–30.5%; $p=0.637$ for liver). Samples of kidney and liver of neonates were not analysed.

### 3.2. Comparison between life history groups and temporal trends

#### 3.2.1. PCBs

PCBs and DDXs had the highest concentrations in all tissues of all animals with PCBs being predominant (Table 1). Levels of PCBs and of DDXs depended on the age class, the tissue and the period of time.

Due to a sample size of only one animal for the juvenile- and adult group for 1990–1998, results for PCBs of the calves of that time period were difficult to compare to these two older age classes. For animals from 2000 to 2008 however, concentrations of the sum of PCBs in blubber decreased from neonates (median: 16.8 μg/g lw) to juveniles (median: 9.9 μg/g lw) after which they increased to higher levels in adults (median: 24.9 μg/g lw). Levels of the sum of PCBs in liver were also higher in calves (median: 11.2 μg/g lw) compared to juveniles (median: 8.3 μg/g lw), but lower in calves compared to adults (median: 11.3 μg/g lw). Trends of the sum of PCBs in kidneys were the opposite compared to those observed in liver or blubber, because these concentrations seem to increase from calves (median: 4.0 μg/g lw) to juveniles (median: 16.5 μg/g lw), but were lower in adults (median: 6.1 μg/g lw) than in juveniles.

PCB 153 had the highest concentrations in all samples followed by PCB 138. For most samples, PCB 149 was the third congener in the PCB pattern. In 6 samples (1 kidney, 2 blubber and 3 liver samples) however, PCB 149 was replaced by PCB 187 as third congener. Percentages of these four congeners ranged from 16.3 to 28.3% for PCB 153, from 11.9 to 16.3% for PCB 138, from 7.8 to 12.3% for PCB 149 and from 4.2 to 10.7% for PCB 187. To assess temporal trends, age groups were divided into 2 time groups (1990–1998 and 2000–2008) each covering 8 years. However, only blubber samples of calves could be tested statistically because sample sizes of all other age groups, especially when they are divided into 2 time groups, were too small. Statistically, there were only differences in PCB percentages between calves from 1990 to 1999 and calves from 2000 to 2008 for PCB 151, PCB 187, PCB 177, PCB 174, PCB 180, PCB 199 and PCB 194 (all $p<0.05$) with a decrease in PCB 174, PCB 180 and PCB 194 percentages and an increase in PCB 151, PCB 187, PCB 177 and PCB 199 percentages over time. However, when all PCBs were grouped according to the number of chlorine atoms (Fig. 1), these differences for calves were no longer apparent so that there was no division in calves from 1990 to 1999 and calves from 2000 to 2008 needed. Percentages of PCB groups differed only slightly between the three different tissues (Fig. 1). In blubber or liver, percentages of lower chlorinated penta-PCBs decreased with age, whereas percentages of higher chlorinated hepta- and octa-PCBs increased slightly with age. These trends were not clear in the kidney, but this might be due to the limited sample size for the kidney. Overall, the proportion of hexa-PCBs to total PCBs remained preserved in all tissues and all age classes. Octa- and deca-PCBs were better represented in kidney and liver than in blubber.

**Table 1**
Median concentrations and range of the sum of PCBs and of the sum of DDXs (expressed in μg/g lipid weight) in blubber, liver and kidney of harbour porpoises from the North Sea between 1990–1998 and 2000–2008.

| Age class | Tissue | N[a] | ∑ PCBs[b] | | ∑ DDXs[c] | |
|---|---|---|---|---|---|---|
| | | | 1990–1998 | 2000–2008 | 1990–1999 | 2000–2008 |
| Neonate | Blubber | 1/2 | 13.7 | 16.8 (4.7–29.0) | 1.9 | 1.8 (0.5–3.0) |
| Calf | Blubber | 3[d]/11 | 10.0 (8.2–11.6) | 12.8 (4.0–25.2) | 2.2 (1.9–4.7) | 2.4 (0.8–3.6) |
| | Kidney | 0/2 | – | 4.0 (2.8–5.2) | – | 0.5 (0.4–0.5) |
| | Liver | 3/8 | 9.1 (3.0–10.5) | 11.2 (3.8–23.4) | 0.7 (0.5–2.8) | 0.9 (0.7–2.9) |
| Juvenile | Blubber | 1/5 | 19.1 | 9.9 (1.1–68.2) | 4.5 | 1.7 (0.4–6.4) |
| | Kidney | 0/1 | – | 16.5 | – | 1.3 |
| | Liver | 0/3 | – | 8.3 (6.8–36.7) | – | 0.8 (0.7–3.2) |
| Adult | Blubber | 1/2 | 81.5 | 24.9 (15.3–34.5) | 22.9 | 3.4 (2.3–4.4) |
| | Kidney | 0/2 | – | 6.1 (4.1–8.2) | – | 0.7 (0.5–0.9) |
| | Liver | 1/2 | 66.1 | 11.3 (10.0–12.5) | 9.9 | 1.3 (1.2–1.4) |
| Outlier* | Blubber | 0/1 | – | 1.1 | – | >0.1 |
| | Kidney | 0/1 | – | 0.5 | – | <0.1 |
| | Liver | 0/1 | – | 0.7 | – | <0.1 |

[a] Number before '/' is sample size of time period 1990–1998, number after '/' is sample size of time period 2000–2008.
[b] The sum of 35 PCB congeners: IUPAC numbers: CB 18, 28, 44, 47, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 146, 149, 151, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, 206, and 209.
[c] The sum of 6 DDXs: *o,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT.
[d] Considered as an outlier, calf from 1990. The sum of PCBs is 79.9 μg/g lw and the sum of DDXs is 34.6 μg/g lw.
* Adult female considered as an outlier and excluded from further statistical analyses. Animal was anaemic and pregnant and died due to acute hepatic lipidosis.

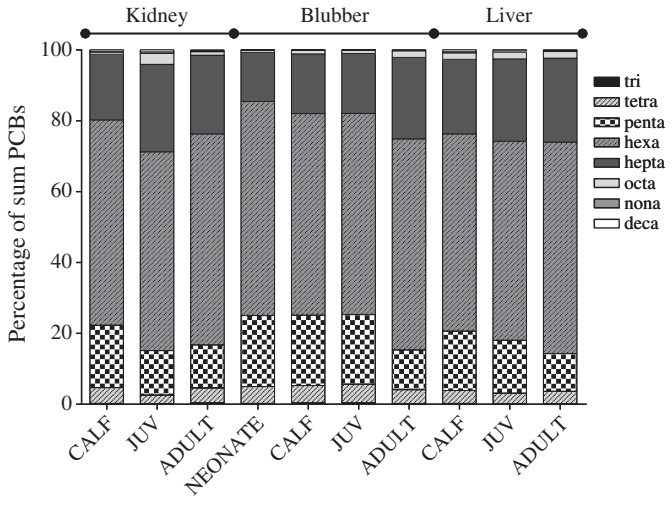


**Fig. 1.** Proportions of the different PCB groups in kidney, blubber and liver of neonates, calves, juveniles and adult harbour porpoises from the North Sea (1990–2008). PCB groups are structural homologs listed by degree of chlorination.

### 3.2.2. DDXs

Within the time period 2000–2008, there was a decrease in the sum of DDXs from calves to juveniles, followed by an increase from juveniles to adults for liver and blubber (Table 1). Similar to the sum of PCBs, the sum of DDXs was highest in the kidney of the only juvenile harbour porpoise analysed compared to the kidneys of calves and adults. When comparing blubber of neonates and calves for both time periods, concentrations of the sum of PCBs were lower in blubber of the calves compared to neonates, while levels of the sum of DDXs were lower in blubber of the neonates than those in blubber of calves.

For DDXs, the dominant DDX was *p,p′*-DDE with concentrations ranging from 58.5 to 88.4% of the total sum of DDXs. Both *o,p′*-DDT and *p,p′*-DDT were not present in samples of kidneys and were only sporadically found in some samples of liver, but were consistently detected in all samples of blubber. Congener *o,p′*-DDE was only detected in 3 blubber samples and 1 liver sample.

### 3.2.3. PBDEs and MeO-PBDEs

Sample sizes of juveniles and adults of the time period 1990–1998 were too small to compare the results to the calves of the same time period. For the sum of PBDEs in animals of 2000–2008, concentrations of PBDEs in blubber and liver were highest in calves and adults while juveniles had the highest levels of the sum of PBDEs in their kidneys (Table 2). For the sum of MeO-PBDEs, the highest levels for all tissues (liver, kidney and blubber) were found in juveniles, whereas the lowest concentrations were present in adults.

PBDE patterns were dominated by BDE 47, covering more than 30% of the sum of PBDEs, in all tissues and in all animals (Fig. 2). In all samples, BDE 47 was followed by BDE 100, although the differences between percentages of BDE 47 and BDE 100 were small in the kidney. The next congeners were BDE 99 and BDE 154, but the order depended on the tissue and the age class. Overall, the contribution of BDE 47 to the total sum of PBDEs was highest in blubber, followed by liver and kidney. In all tissues however, the percentage of BDE 47 decreased with age. Together with declining BDE 47 percentages with age, there was a slight decline in percentages of lower brominated PBDEs, e.g. BDE 28. To compensate for these decreasing BDE 47 and BDE 28 contributions, the percentages of higher brominated compounds such as BDE 100, BDE 153 and BDE 154 increased with age in each tissue. To investigate possible time trends, differences in the percentages of each PBDE congener were tested between calves of 1990–1998 and calves of 2000–2008. Although the percentages were not statistically different for all PBDE congeners, there were some statistical significant differences for BDE 47, BDE 100 and BDE 154 ($p = 0.006$; $p = 0.006$ and $p = 0.026$) in blubber and for BDE 154 ($p = 0.034$) in liver. For consistency, the calves were divided into calf 1 (calves of 1990–1998) and calf 2 (calves of 2000–2008) for all PBDE congeners (Fig. 2).

Ratios of the naturally-produced 6-MeO-BDE 47 reached up to 90% of total MeO-PBDEs, while 2′-MeO-BDE 68 only had a minor contribution (Fig. 3). Time trends were not apparent, since differences between the calf 1 and calf 2 groups were not statistically significant for blubber or liver. In each tissue type, the contribution of 6-MeO-BDE 47 was highest in juveniles and lowest in adults.

### 3.2.4. HCB and CHLs

Concentrations of these pollutants depended on the tissue, the time group and the age class (Table 2). Due to the low sample sizes in the time period 1990–1998, comparisons were only made for the animals from 2000 to 2008. For this time group, the lowest and highest concentrations of HCB and of the sum of the CHLs (sum of *trans*-nonachlor, *cis*-nonachlor and oxychlordane), respectively, were found in all tissues of adult harbour porpoises. *Trans*-nonachlor had

**Table 2**
Median concentrations and range of the sum of PBDEs, HCB, the sum of CHLOR and the sum of MeO-PBDE (expressed in ng/g lipid weight) in blubber, liver and kidney of harbour porpoises from the North Sea between 1990–1998 and 2000–2008.

| Age class | Tissue | N[a] | ∑ PBDEs[b] | | HCB | | ∑ CHLs[c] | | ∑ MeO-PBDEs[d] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1990–1998 | 2000–2008 | 1990–1998 | 2000–2008 | 1990–1998 | 2000–2008 | 1990–1998 | 2000–2008 |
| Neonate | Blubber | 1/2 | 132 | 462 (82–841) | 143 | 104 (25–184) | 150 | 186 (24–349) | 20 | 97 (13–180) |
| Calf | Blubber | 4/11 | 2586 (1479–4062) | 552 (229–1457) | 117[e] (88–135) | 100 (54–195) | 250[e] (216–269) | 268 (72–382) | 149[e] (136–236) | 138 (32–293) |
| | Kidney | –/2 | – | 119 (86–152) | – | 75 (55–94) | – | 50 (42–59) | – | 26 (18–33) |
| | Liver | 3/8 | 485 (89–1157) | 337 (135–1419) | 153 (80–195) | 184 (67–313) | 77 (59–159) | 83 (58–245) | 43 (15–173) | 70 (29–210) |
| Juveniles | Blubber | 1/5 | 4771 | 494 (280–1501) | 191 | 141 (53–214) | 680 | 194 (68–551) | 118 | 224 (67–405) |
| | Kidney | –/1 | – | 575 | – | 129 | – | 129 | – | 111 |
| | Liver | –/3 | – | 318 (290–1305) | – | 141 (114–238) | – | 90 (56–289) | – | 120 (77–265) |
| Adult | Blubber | 1/2 | 1900 | 1194 (563–1825) | 350 | 90 (88–91) | 3611 | 688 (371–1004) | 156 | 80 (67–94) |
| | Kidney | –/2 | – | 221 (114–328) | – | 56 (54–58) | – | 151 (78–225) | – | 19 (14–24) |
| | Liver | 1/2 | 1552 | 426 (329–522) | 515 | 97 (96–97) | 1808 | 270 (199–341) | 100 | 43 (39–47) |
| Outlier[*] | Blubber | –/1 | – | 42 | – | 5 | – | 9 | – | 11 |
| | Kidney | –/1 | – | 21 | – | 5 | – | 3 | – | 5 |
| | Liver | –/1 | – | 37 | – | 8 | – | 7 | – | 9 |

[a] Number before '/' is sample size of time period 1990–1998, number after '/' is sample size of time period 2000–2008.
[b] The sum of 7 PBDEs: IUPAC numbers: BDE 28, 47, 49, 99, 100, 153, 154.
[c] The sum of 3 CHLs (*cis*-nonachlor, *trans*-nonachlor, oxychlordane).
[d] The sum of 2 congeners: 2′-MeO-BDE 68 and 6-MeO-BDE 47.
[e] Sample size of 3 animals. One animal considered as an outlier, calf from 1990. HCB is 2288 ng/g lw, the sum of CHLOR is 5223 ng/g lw, and the sum of MeO-PBDEs is 599 ng/g lw.
[*] Adult female considered as an outlier and excluded from further statistical analyses. Animal was anaemic and pregnant and died due to acute hepatic lipidosis.

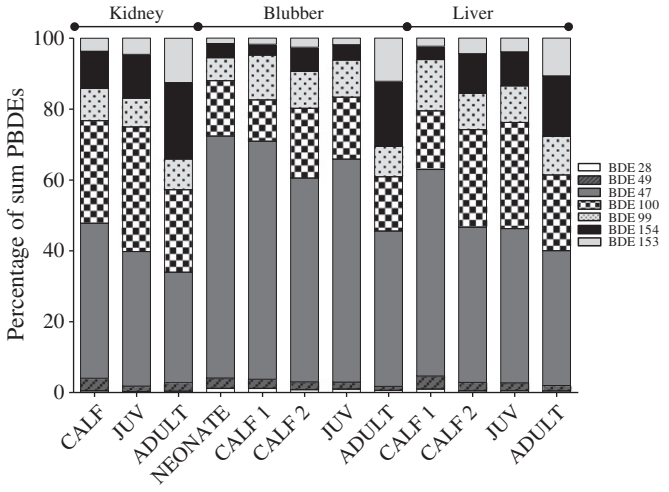


**Fig. 2.** Proportions of the different PBDE congeners in kidney, blubber and liver of neonates, calves, juveniles and adult harbour porpoises from the North Sea (1990–2008). The calves are divided into calf 1 (1990–1998) and calf 2 (2000–2008) because there are statistical significant differences for select PBDE congeners between these two groups.

the highest levels among all CHLs with percentages of more than 50% of total CHLs.

### 3.3. Comparison between tissues

The ratios of pollutant concentrations in liver and blubber were calculated for 17 animals (11 calves, 3 juveniles, and 3 adults) and kidney/blubber ratios were calculated for 5 animals (2 calves, 1 juvenile, and 2 adults) for each individual compound (Supporting Information, Table S2). Ratios>1 were indicative for a preferential accumulation of the compound in liver or kidney, and ratios<1 indicated preferential accumulation of the compound in blubber. Roughly, higher chlorinated PCBs, higher brominated PBDEs and HCB were preferentially stored in liver, whereas all other compounds were found mainly in blubber. However, the preference of these compounds for the liver disappeared when the animals grew older as liver/blubber ratios become smaller than 1. The only exception was HCB which was present in the liver rather than in blubber regardless of the age. In contrast, only higher chlorinated PCBs were present in the kidneys more than in blubber. CHLs and MeO-PBDEs were preferentially stored in blubber in all animals investigated in the present study.

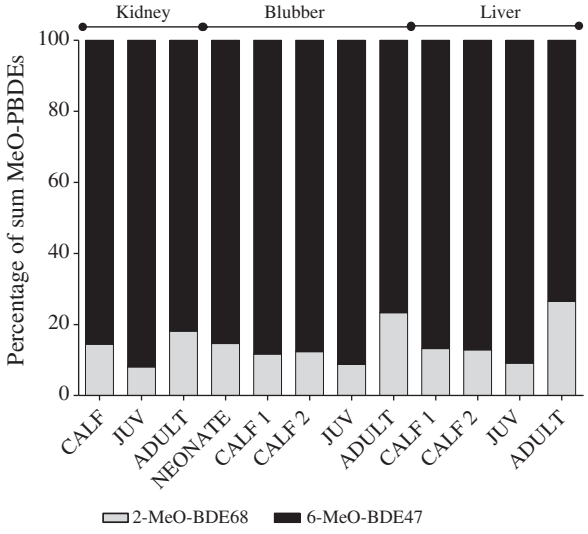


**Fig. 3.** Proportions of the two MeO-PBDEs, 6-MeO-BDE 47 (■) and 2′-MeO-BDE 68 (▒) in kidney, blubber and liver of neonates, calves, juveniles and adult harbour porpoises from the North Sea (1990–2008). The calves are divided into calf 1 (1990–1998) and calf 2 (2000–2008).

### 3.4. Metabolic biotransformation of dominant compounds

To investigate their capacity for metabolic biotransformation of some compounds, ratios between the most dominant compounds (*p,p′*-DDE and BDE 47) and PCB 153 were calculated (Fig. 4). PCB 153 was chosen due to its low, if any, metabolic breakdown in marine mammals. Results show that *p,p′*-DDE/PCB 153 ratios were only higher than 1 in blubber of five calves (two animals of 1990–1998 and three animals of 2000–2008) and in blubber of one juvenile harbour porpoise. Ratios were lower than 1 in all other animals. Ratios of *p,p′*-DDE/PCB 153 did not differ statistically between calves of 1990–1998 and calves of 2000–2008 ($p=0.151$ for blubber; $p=0.059$ for liver). Ratios of BDE 47/PCB 153 were only higher than 1 in one calf and in one juvenile animal. These ratios were statistically different between the two groups of calves in blubber ($p=0.039$), but not in liver ($p=0.099$).

## 4. Discussion

This study investigated the distribution of several POP classes and MeO-PBDEs in blubber, kidney and liver of harbour porpoises from the North Sea and focused particularly on the youngest members of the population, namely the neonates and calves. All animals were found alive, but died during rehabilitation in SOS Dolfijn, Dolfinarium Harderwijk, The Netherlands between 1990 and 2008. Except for the outlier (* in Table 1 and Table 2), all adults were males. For juveniles and neonates, a division according to gender was not taken into account because it would have limited the sample sizes even further. Calves were not divided in groups according to their gender either, because gender differences only become more apparent at older stages.

### 4.1. Levels and time trends

Because all animals died in different years, from 1990 until 2008, and belong to different age classes (neonate, calf, juvenile, and adult), levels and patterns of POPs were difficult to compare to the literature, especially when other studies included different compounds and congeners in the sums of the chemicals. Moreover, all animals in the present study (Table S1) died at the rehabilitation center, so possible interference of exhaustion, starvation or diseases on the POP results cannot be ruled out, although the influence of diseases on POP levels in marine mammals is not fully understood yet. Jepson et al. (2005) and Hall et al. (2006) found higher levels of PCBs in blubber of harbour porpoises that died due to infectious diseases compared to harbour porpoises that died because of physical trauma. On the other hand, Weijs et al. (2009d) analysed serum samples of harbour porpoises in rehabilitation, of which some animals were sick and others were healthy, but found that only starvation had an influence on the contaminant levels.

#### 4.1.1. PCBs and PBDEs

Compared to the levels of the sum of PCBs in blubber of harbour porpoises from the Bay of Fundy, Canada, concentrations of the sum of PCBs in blubber of harbour porpoises from the present study, with respect to their age classes, were relatively low (Gaskin et al., 1983; Table 3). However, compared to the levels of PCBs in blubber of harbour porpoises from the Black Sea (Tanabe et al., 1997; Weijs et al., 2010), concentrations found in the present study were high (Table 3). Within the North Sea, results of PCBs in adult males from 1999 to 2004 (82,900 ng/g lw; Weijs et al., 2009a and Table 3) did not differ much from the results of adult males from 1990 to 1998 (81,500 ng/g lw)

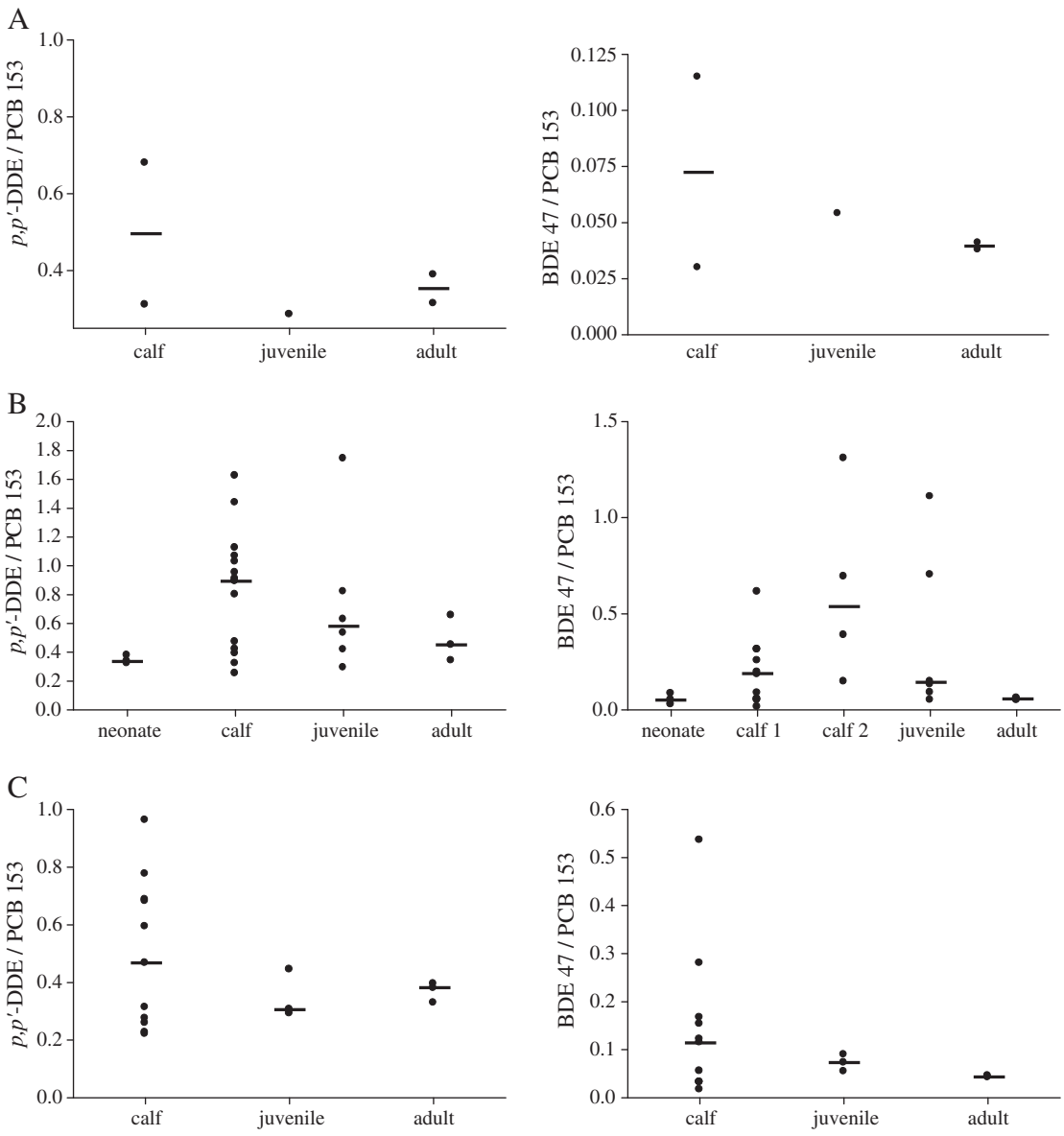


**Fig. 4.** Ratios of *p,p′*-DDE/PCB 153 and BDE 47/PCB 153 in (A) kidney, (B) blubber and (C) liver of harbour porpoises from the North Sea from 1990 until 2008. BDE 47/PCB 153 ratios in blubber of calves from 1990 to 1998 differed significantly from ratios of calves from 2000 to 2008 ($p = 0.039$). Therefore the 'calf-group' was divided into calf 1 (1990–1998) and calf 2 (2000–2008). ● = individual data, ▬ = median value.

from the present study. Whereas Law et al. (2010), who investigated PCBs in blubber of harbour porpoises from the UK from 1991 until 2005, found that concentrations were only decreasing slowly over that time period, the results of the present study are not that clear. Here, there is a decline in levels of PCBs and PBDEs for animals from 1990 until 2008, except for the calves. Basically, only levels of the sum of PBDEs were lower in calves from 2000 to 2008 than in calves from 1990 to 1998. It has been observed that concentrations of all other contaminant types in calves decreased slightly or were even increasing over the time period from 1990 until 2008 (Table 1 and Table 2). Since all contaminant types, except for the PBDEs and MeO-PBDEs were banned before 1990, the reported decline in juveniles and adults can be considered as expected. Although these molecules are stable and persistent in the environment, their levels will decrease eventually as they are phasing out very slowly. However, this would also be true for calves in theory and this is in contrast with the slight increases over time that were found for several contaminant types in this particular age class. A possible explanation for this might be that calves are essentially feeding at a trophic position higher than their mothers, so they are actually 'consuming' the tissues of their mothers. This has been reported for a range of cetaceans and pinnipeds (e.g. for bottlenose dolphins in Knoff et al., 2008 and for fur seals in Hobson et al., 1997). Higher concentrations of several chemicals in marine mammal-eating killer whales and polar bears, which are two predators feeding at a higher trophic level as well, compared to other marine mammals have been found previously (Ross et al., 2000; Kelly et al., 2007). Due to this higher trophic level, the phasing out step might go even slower. The production and use of PBDEs have been restricted in 2004/2008 for most congeners which are more at the end of the sampling design of the present study. As such, higher concentrations in 2008 compared to 1990 would seem normal. Nevertheless, concentrations of PBDEs are decreasing in calves, juveniles and adults. This can be an artifact from the low sample sizes for juveniles and adults in this study, but that would not explain the decrease in calves. Compared to PCBs, PBDEs accumulate less in marine mammals from the North Sea and on top of that, levels of PBDEs are found to decrease with age, not only in females, but also in males (e.g. Shaw et al., 2008; Weijs et al., 2009a) indicating that the

**Table 3**
Summary of temporal and spatial trends of POPs and MeO-PBDEs (expressed in ng/g lw) previously reported for harbour porpoise blubber, including results of the present study.

| Year | ∑ PCBs | ∑ DDXs | ∑ PBDEs | ∑ CHLs | ∑ MeO-PBDEs | HCB | Location, reference |
|---|---|---|---|---|---|---|---|
| 1971 | 94,943[a] | | | | | | Bay of Fundy, Canada, Gaskin et al. (1983)[b] |
| 1972 | 96,552[a] | | | | | | |
| 1973 | 74,598[a] | | | | | | |
| 1975 | 83,908[a] | | | | | | |
| 1976 | 87,471[a] | | | | | | |
| 1977 | 103,103[a] | | | | | | |
| 1989–1991 | 12,329[c] | 6071[c] | | 4024[c] | | | Bay of Fundy, Gulf of Maine, Westgate et al. (1997)[d] |
| | 14,753[e,f] | 8012[e,f] | | 5741[e,f] | | | |
| | 15,776[e] | 7376[e] | | 5624[e] | | | |
| | 28,400[a] | 12,188[a] | | 9906[a] | | | |
| 1990–1998 | 13,700[g] | 1900[g] | 132[g] | 150[g] | 20[g] | 143[g] | North Sea, present study[h] |
| | 10,000[c] | 2200[c] | 2586[c] | 250[c] | 149[c] | 117[c] | |
| | 19,100[e] | 4500[e] | 4771[e] | 680[e] | 118[e] | 191[e] | |
| | 81,500[a] | 22,900[a] | 1900[a] | 3611[a] | 156[a] | 350[a] | |
| 1993 | 13,122[e] | 54,981[e] | | 689[e] | | 368[e] | Black Sea,[b] Tanabe et al. (1997) |
| | 23,524[a] | 101,878[a] | | 1212[a] | | 498[a] | |
| 1993 | 5087[e] | 3372[e] | | 840[e] | | 419[e] | Japan,[b] Tanabe et al. (1997) |
| | 12,360[a] | 9326[a] | | 1612[a] | | 433[a] | |
| 1998 | 6956[e] | 40,891[e] | 57[e] | | 53[e] | 394[e] | Black Sea,[h] Weijs et al. (2010a) |
| | 13,215[a] | 77,329[a] | 66[a] | | 47[a] | 575[a] | |
| 1999–2004 | 12,900[e,f] | 2496[e,f] | 700[e,f] | | | 249[e,f] | North Sea,[h] Weijs et al. (2009a) |
| | 15,400[e] | 3517[e] | 1730[e] | | | 375[e] | |
| | 82,900[a] | 8387[a] | 1450[a] | | | 312[a] | |
| 2000–2008 | 16,800[g] | 1800[g] | 462[g] | 186[g] | 97[g] | 104[g] | North Sea, present study[h] |
| | 12,800[c] | 2400[c] | 552[c] | 268[c] | 138[c] | 100[c] | |
| | 9900[e] | 1700[e] | 494[e] | 194[e] | 224[e] | 141[e] | |
| | 24,900[a] | 3400[a] | 1194[a] | 688[a] | 80[a] | 90[a] | |

[a] Adult.
[b] Mean values.
[c] Calf.
[d] Mean values, calculated with lipid percentage of 85% for blubber.
[e] Juvenile.
[f] Juvenile female.
[g] Neonate.
[h] Median values.

capacity for metabolic breakdown of PBDEs might increase with age. As a consequence, the transfer of PBDEs from mother to offspring will reduce with each pregnancy even more than for PCBs.

#### 4.1.2. CHLs and HCB

Concentrations of CHLs found in the present study were, for the two time periods in the North Sea, slightly higher for the youngest animals (neonates and calves), but lower in 2000–2008 than in 1990–1998 for juveniles and adults (Table 2). CHL levels were also lower in 2000–2008 for juveniles and adults compared to concentrations in juveniles and adults from the Black Sea or Japan (Tanabe et al., 1997; Table 3). The highest concentrations were found in 1989–1991 in harbour porpoises from the Bay of Fundy/Gulf of Maine (Westgate et al., 1997; Table 3). HCB levels experienced a decreasing trend in the North Sea from 1990 until 2008, but were in both time periods lower compared to HCB in harbour porpoises from the Black Sea (Tanabe et al., 1997; Weijs et al., 2010a; Table 3).

#### 4.1.3. MeO-PBDEs

This paper is the first to report on MeO-PBDEs in blubber, liver and kidney of harbour porpoise calves. Results are therefore difficult to compare. Weijs et al. (2009c) measured MeO-PBDEs in juvenile and adult harbour porpoises, but not in calves or neonates while Weijs et al. (2009d) analysed MeO-PBDEs in serum of harbour porpoises. In addition, MeO-PBDEs have a natural origin and were not banned from production or use like PCBs or PBDEs. Temporal trends for MeO-PBDEs depend therefore only on the presence of algae. To our knowledge, information about the presence of MeO-PBDEs producing algae in the North Sea from 1990 until 2008 does not exist. So, although no conclusions can be drawn about the presence of MeO-PBDEs in harbour porpoises over time, the data suggest that these compounds are present in liver, blubber and kidney of harbour porpoises of all age classes from neonates to adults. For neonates, this would mean that MeO-PBDEs can be transferred from the mother to the offspring through the placenta similar as for anthropogenically produced PBDEs. The levels in calves also suggest that MeO-PBDEs are available for bioaccumulation in calves through their milk diet. For the juveniles and adults however, concentrations depend largely on the presence of these sources in their habitat.

### 4.2. Patterns

Patterns or percentages are, for some POP classes such as PCBs, DDXs, PBDEs and CHLs, difficult to compare to the literature, because they depend on the number of compounds (e.g. for CHLs) or congeners (e.g. for PCBs and PBDEs) that were analysed. Overall, the most dominant compounds were PCB 153 for PCBs, *p,p′*-DDE for DDXs, 6-MeO-BDE 47 for MeO-PBDEs and BDE 47 for PBDEs. PCB 153, BDE 47 and *p,p′*-DDE were commonly found as the most persistent congeners among PCBs, PBDEs and DDXs respectively, not only in harbour porpoises from the North Sea, but also in harbour porpoises from other areas (Weijs et al., 2010a) or even other marine mammal species around the world (e.g. Johnson-Restrepo et al., 2005; Shaw et al., 2008; Weijs et al., 2009a; Dorneles et al., 2010). Among MeO-PBDEs, 6-MeO-BDE 47 was the most dominant congener. In contrast to PBDEs, PCBs and DDXs, MeO-PBDEs are not anthropogenic chemical mixtures with fixed contributions of each congener. MeO-PBDEs have a natural origin and as a consequence, they depend on the geographic position of the natural sources, such as sponges or algae (Vetter et al., 2002; Malmvärn et al., 2008). In the Northern Hemisphere, 6-MeO-BDE 47 is often found in marine mammals as the main MeO-PBDE congener (Kelly et al., 2008; Weijs et al., 2009c), whereas 2′-MeO-BDE 68 is dominant in the Southern Hemisphere. Because the harbour porpoises only inhabit waters of the Northern Hemisphere, the 6-

MeO-BDE 47 is always found at higher concentrations in harbour porpoises than the other congener, 2′-MeO-BDE 68. Overall, and especially compared to MeO-PBDE concentrations in cetaceans from Brazil (Dorneles et al., 2010), levels reported in the present study were low.

In humans, *trans*-nonachlor and oxychlordane are the predominant compounds among all CHLs measured (Bondy et al., 2003). However, in the present study, *trans*-nonachlor had consistently higher concentrations than *cis*-nonachlor or oxychlordane in blubber, liver and kidney of all animals, while there was little difference between *cis*-nonachlor and oxychlordane.

### 4.3. Metabolism

Possible metabolic biotransformation was investigated for the most dominant compounds by calculating the ratios of BDE 47/PCB 153 and *p,p′*-DDE/PCB 153. PCB 153 is considered to be highly persistent, not only in harbour porpoises, but also in other marine mammals. Weijs et al. (2010) suggested that there is little metabolic biotransformation of PCB 153 in harbour porpoises, if any, and probably only at higher age. With the limited sample sizes for each age–gender group in mind, medians of BDE 47/PCB 153 and *p,p′*-DDE/PCB 153 ratios were, highest in calves, regardless of the tissue, while the lowest ratios were found in the adults (Fig. 4). The only exception was the *p,p′*-DDE/PCB 153 ratio in the liver, where ratios of juveniles were lower compared to ratios of adults. Again, this could mean that the calves have no or a lesser developed ability for metabolic biotransformation of BDE 47 or *p,p′*-DDE compared to juveniles and adults. It is not certain whether this ability for metabolic biotransformation develops with age or is induced by higher concentrations. However, similar or even higher concentrations of chemicals in some calves compared to adults (Tables 1 and 2) together with the higher BDE 47/PCB 153 and *p,p′*-DDE/PCB 153 ratios in calves in general (Fig. 4), lead to the preliminary conclusion that higher concentrations, at least for BDE 417 and *p,p′*-DDE, alone are not capable of inducing metabolic biotransformation in harbour porpoises although this should be investigated with a larger dataset.

Next to an absence of or a lesser developed capacity for metabolic breakdown of some chemicals in calves, the higher ratios in calves could also indicate a more recent exposure. It has been established that POPs are selectively offloaded into milk (e.g. Debier et al., 2003; Yordy et al., 2010). Higher levels of BDE 47 and of *p,p′*-DDE compared to PCB 153 in milk would therefore also lead to higher BDE 47/PCB 153 and *p,p′*-DDE/PCB 153 ratios. In the Black Sea, harbour porpoises from all age–gender groups have higher concentrations of DDXs compared to PCBs and much lower levels of PBDEs than PCBs (Weijs et al., 2010a). These patterns were also been found in milk samples obtained from adult females from the Black Sea (Weijs et al., 2010b). In harbour porpoises from the North Sea however, levels of DDXs (or *p,p′*-DDE) and of PBDEs (or BDE 47) are typically lower compared to PCBs (or PCB 153). There are no milk samples available for harbour porpoises from the North Sea. Nevertheless, assuming the same pattern in milk as in the animals as found for milk and harbour porpoises from the Black Sea, a lesser developed metabolism for calves seems a more plausible explanation for the observed higher BDE 47/PCB 153 and *p,p′*-DDE/PCB 153 ratios in calves.

### 4.4. Implications for monitoring and toxicity

Although blubber is the most convenient matrix to analyse for lipophilic compounds, such as PCBs or PBDEs, not all congeners have the same properties or molecule sizes. Both factors determine to a large extent where the specific congeners or compounds will accumulate inside the body. The ratios of concentrations of pollutants from liver to blubber and from kidney to blubber, calculated in this study (Supporting information, Table S2), showed that HCB, higher brominated PBDEs and higher chlorinated PCBs accumulate selectively in the liver or the kidneys rather than in blubber. Not because these compounds are less lipophilic, but probably because their molecule sizes are too large so that they cannot pass through the membranes (Kannan et al., 1998; Ikonomou et al., 2002). For monitoring purposes, these preferences should be taken into account as the levels of some compounds in blubber might be an underestimation of the concentrations to which the animal is actually exposed to.

Concentrations of PCBs in blubber from this study fall within the range of 10.0 and 81.5 μg/g lw (Table 1). This range is comparable with or higher than levels in blubber of harbour seal pups which are associated with depressed vitamin A levels (Mos et al., 2007). These concentrations are also much higher than concentrations from harbour porpoises from the German and Danish North Sea and from Iceland and Norway, which are linked to interfollicular fibrosis in harbour porpoise thyroids, splenic depletion and thymic atrophy (Beineke et al., 2005; Das et al., 2006). There is no doubt that the concentrations measured here are a threat to all animals of this study. However, the high concentrations found in the calves are of particular concern. It has been suggested already that exposure to a mixture of POPs, such as PCBs and PBDEs, might have an impact on the central nervous system (Montie et al., 2009). The first months of the animals' lives are critical and important for their further development. Tiedeken and Ramsdell (2009) have found that fetal exposure of zebrafish to *p,p′*-DDE can increase the sensitivity towards domoic acid in fish. That study also suggested a possible link between fetal *p,p′*-DDE exposure of California sea lions and a higher domoic toxicity in these animals. Relationships between pre- or post-natal exposure to pollutants and developmental impairment or health problems have been found in monkeys and mice as well (Rice, 1999; Haijima et al., 2010). Calves continue to assimilate organic pollutants through milk, but are possibly not capable of dealing with these burdens as their ability for metabolic breakdown is not yet fully operational according to the BDE 47/PCB 153 and *p,p′*-DDE/PCB 153 ratios. If exposure to pollutants at very young ages negatively influences the animals' development with respect to sensitivity for diseases at older stages, than neonates and calves are the most vulnerable age classes of the entire population.

## 5. Conclusions

Because of the temporal variation of POPs in marine mammals and due to the different age classes of the harbour porpoises, sample sizes in the present study are often too small to perform statistical analyses. Some conclusions in this study may therefore have only a preliminary character and deserve further investigations with larger sample sizes. Overall, biomonitoring studies should keep in mind that not all lipophilic compounds prefer blubber as main storage place. Some compounds accumulate readily in kidneys or liver so that these tissues give a more accurate view of the actual concentrations the animals are exposed to than blubber. In general, concentrations of all pollutants decreased in all groups from 1990 until 2008 except in calves where only levels of PBDEs decreased. Higher concentrations of pollutants alone are possibly not enough to induce metabolic breakdown because these higher levels are also present in calves and these animals do not seem to have a well developed mechanism for metabolic biotransformation of contaminants. This leaves the younger members of the population, the calves, as probably the most vulnerable animals.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2010.09.035.

## References


Agency for Toxic Substances and Disease Registry (ATSDR). Chlordane. US Department of Health and Human Services; 1995. Availably at: http://www.atsdr.cdc.gov/toxfaqs/index.asp.

Agency for Toxic Substances and Disease Registry (ATSDR). Polychlorinated biphenyls. US Department of Health and Human Services; 2001. Available at: http://www.atsdr.cdc.gov/toxfaqs/index.asp.

Agency for Toxic Substances and Disease Registry (ATSDR). DDT, DDE, and DDD. US Department of Health and Human Services; 2002. Available at: http://www.atsdr.cdc.gov/toxfaqs/index.asp.

Agency for Toxic Substances and Disease Registry (ATSDR). Hexachlorobenzene. US Department of Health and Human Services; 2002. Available at: http://www.atsdr.cdc.gov/toxfaqs/index.asp.

Agency for Toxic Substances and Disease Registry (ATSDR). Polybrominated diphenyl ethers. US Department of Health and Human Services; 2004. Available at: http://www.atsdr.cdc.gov/toxfaqs/index.asp.

Beineke A, Siebert U, McLachlan M, Bruhn R, Thron K, Failing K, Müller G, Baumgärtner W. Investigations of the potential influence of environmental contaminants on the thymus and spleen of harbor porpoises (*Phocoena phocoena*). Environ Sci Technol 2005;39:3933–8.

Birnbaum LS, Staskal DF. Brominated flame retardants: cause for concern? Environ Health Perspect 2004;112:9-17.

Blasius ME, Goodmanlowe GD. Contaminants still high in top-level carnivores in the Southern California Bight: levels of DDT and PCBs in resident and transient pinnipeds. Mar Pollut Bull 2008;56:1973–82.

Bondy G, Armstrong C, Coady L, Doucet J, Robertson P, Feeley M, Barker M. Toxicity of the chlordane metabolite oxychlordane in female rats: clinical and histopathological changes. Food Chem Toxicol 2003;41:291–301.

Covaci A, Losada S, Roosens L, Vetter W, Santos FJ, Neels H, Storelli A, Storelli MM. Anthropogenic and naturally-occurring organobrominated compounds in two deep-sea fish species from the Mediterranean Sea. Environ Sci Technol 2008;42:8654–60.

Das K, Vossen A, Tolley K, Vikingsson G, Thron K, Müller G, Baumgärtner W, Siebert U. Interfollicular fibrosis in the thyroid of the harbour porpoise: an endocrine disruption? Arch Environ Contam Toxicol 2006;51:720–9.

Debier C, Pomeroy PP, Dupont C, Joiris C, Comblin V, Le Boulengé E, Larondelle Y, Thomé JP. Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*. Mar Ecol Prog Ser 2003;247:237–48.

Dorneles PR, Lailson-Brito J, Dirtu AC, Weijs L, Azevedo AF, Torres JPM, Malm O, Neels H, Blust R, Das K, Covaci A. Anthropogenic and naturally-produced organobrominated compounds in marine mammals from Brazil. Environ Int 2010;36:60–7.

Gaskin DE, Frank R, Holdrinet M. Polychlorinated biphenyls in harbor porpoises *Phocoena phocoena* (L.) from the Bay of Fundy, Canada and adjacent waters, with some information on chlordane and hexachlorobenzene levels. Arch Environ Contam Toxicol 1983;12:211–9.

Haijima A, Endo T, Zhang Y, Miyazaki W, Kakeyama M, Tohyama C. *In utero* and lactational exposure to low doses of chlorinated and brominated dioxins induced deficits in the fear memory of male mice. Neurotoxicology 2010;31:385–90.

Hall AJ, Hugunin K, Deaville R, Law RJ, Allchin CR, Jepson PD. The risk of infection from polychlorinated biphenyl exposure in the harbor porpoise (*Phocoena phocoena*): a case–control approach. Environ Health Perspect 2006;114:704–11.

Hobson KA, Sease JL, Merrick RL, Piatt JF. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Mar Mammal Sci 1997;13:114–32.

Hoffman DJ, Melancon MJ, Klein PN, Rice CP, Eisemann JD, Hines RK, Spann JW, Pendleton GW. Developmental toxicity of PCB 126 (3, 3′, 4, 4′, 5-pentachlorobiphenyl) in nestling American kestrels (*Falco sparverius*). Fundam Appl Toxicol 1996;34:188–200.

Ikonomou MG, Fernandez MP, Knapp W, Sather P. PCBs in dungeness crab reflect distinct source fingerprints among harbor/industrial sites in British Columbia. Environ Sci Technol 2002;36:2545–51.

Jepson PD, Bennett PM, Deaville R, Allchin CR, Baker JR, Law RJ. Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. Environ Toxicol Chem 2005;24:238–48.

Johnson-Restrepo B, Kannan K, Addink R, Adams DH. Polybrominated diphenyl ethers and polychlorinated biphenyls in a marine foodweb of coastal Florida. Environ Sci Technol 2005;39:8243–50.

Kannan K, Nakata H, Stafford R, Masson GR, Tanabe S, Giesy JP. Bioaccumulation and toxic potential of extremely hydrophobic polychlorinated biphenyl congeners in biota collected at a superfund site contaminated with Aroclor 1268. Environ Sci Technol 1998;32:1214–21.

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. Food web-specific biomagnifications of persistent organic pollutants. Science 2007;317:236–9.

Kelly BC, Ikonomou MG, Blair JD, Gobas FAPC. Hydroxylated and methoxylated polybrominated diphenyl ethers in a Canadian Arctic marine food web. Environ Sci Technol 2008;42:7069–77.

Knoff A, Hohn A, Macko S. Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. Mar Mammal Sci 2008;24:128–37.

Law RJ, Bersuder P, Barry J, Deaville R, Reid RJ, Jepson PD. Chlorobiphenyls in the blubber of harbour porpoises (*Phocoena phocoena*) from the UK: levels and trends 1991–2005. Mar Pollut Bull 2010;60:470–3.

Lockyer C. Investigation of aspects of the life history of the harbour porpoise, *Phocoena phocoena*, in British waters. In: Bjorge A, Donovan GP, editors. Biology of the phocoenids, 16. Reports of the International Whaling Commission, Special Issue; 1995. p. 189–98. Cambridge.

Malmvärn A, Zebühr Y, Kautsky L, Bergman Å, Asplund L. Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-*p*-dioxins in red alga and cyanobacteria living in the Baltic Sea. Chemosphere 2008;72:910–6.

Michielsen CCPPC, van Loveren H, Vos JG. The role of the immune system in hexachlorobenzene-induced toxicity. Environ Health Perspect 1999;107:783–92.

Montie EW, Reddy CM, Gebbink WA, Touhey KE, Hahn ME, Letcher RJ. Organohalogen contaminants and metabolites in cerebrospinal fluid and cerebellum gray matter in short-beaked common dolphins and Atlantic white-sided dolphins from the western North Atlantic. Environ Pollut 2009;157:2345–58.

Mos L, Morsey B, Jeffries SJ, Yunker MB, Raverty S, De Guise S, Ross PS. Chemical and biological pollution contribute to the immunological profiles of free-ranging harbor seals. Environ Toxicol Chem 2006;25:3110–7.

Mos L, Tabuchi M, Dangerfield N, Jeffries SJ, Koop BF, Ross PS. Contaminant-associated disruption of vitamin A and its receptor (retinoic acid receptor α) in free-ranging harbour seals (*Phoca vitulina*). Aquat Toxicol 2007;81:319–28.

Pierce GJ, Santos MB, Murphy S, Learmonth JA, Zuur AF, Rogan E, Bustamante P, Caurant F, Lahaye V, Ridoux V, Zegers BN, Mets A, Addink M, Smeenk C, Jauniaux T, Law RJ, Dabin W, Lopez A, Alonso Farré JM, Gonzalez AF, Guerra A, Garcia-Hartmann M, Reid RJ, Moffat CF, Lockyer C, Boon JP. Bioaccumulation of persistent organic pollutants in female common dolphins (*Delphinus delphis*) and harbour porpoises (*Phocoena phocoena*) from western European seas: geographical trends, causal factors and effects on reproduction and mortality. Environ Pollut 2008;153:401–15.

Rice DC. Behavioral impairment produced by low-level postnatal PCB exposure in monkeys. Environ Res Section A 1999;80:113–21.

Ross PS, Ellis GM, Ikonomou MG, Barrett-Lennards LG, Addison RF. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. Mar Pollut Bull 2000;40:504–15.

Ruus A, Ugland KI, Espeland O, Skaare JU. Organochlorine contaminants in a local marine food chain from Jarfjord, Northern Norway. Mar Environ Res 1999;48:131–46.

Shaw SD, Brenner D, Berger ML, Fang F, Hong CS, Addink R, Hilker D. Bioaccumulation of polybrominated diphenyl ethers in harbor seals from the northwest Atlantic. Chemosphere 2008;73:1773–80.

Sly PD, Flack F. Susceptibility of children to environmental pollutants. Ann NY Acad Sci 2008;1140:163–83.

Sonne C, Gustavson K, Rigét FF, Dietz R, Birkved M, Letcher RJ, Bossi R, Vorkamp K, Born EW, Petersen G. Reproductive performance in East Greenland polar bears (*Ursus maritimus*) may be affected by organohalogen contaminants as shown by physiologically-based pharmacokinetic (PBPK) modelling. Chemosphere 2009;77:1558–68.

Tanabe S, Madhusree B, Öztürk AA, Tatsukawa R, Miyazaki N, Özdamar E, Aral O, Samsun O, Öztürk B. Persistent organochlorine residues in harbour porpoise (*Phocoena phocoena*) from the Black Sea. Mar Pollut Bull 1997;34:338–47.

Tiedeken JA, Ramsdell JS. DDT exposure of zebrafish embryos enhances seizure susceptibility: relationship to fetal p, p′-DDE burden and domoic acid exposure of California sea lions. Environ Health Perspect 2009;117:68–73.

Vetter W, Stoll E, Garson MJ, Fahey SJ, Gaus C, Müller JF. Sponge halogenated natural products found at parts-per-million levels in marine mammals. Environ Toxicol Chem 2002;21:2014–9.

Weijs L, Dirtu AC, Das K, Gheorghe A, Reijnders PJH, Neels H, Blust R, Covaci A. 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. Environ Pollut 2009a;157:437–44.

Weijs L, Dirtu AC, Das K, Gheorghe A, Reijnders PJH, Neels H, Blust R, Covaci A. 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. Environ Pollut 2009b;157:445–51.

Weijs L, Losada S, Das K, Roosens L, Reijnders PJH, Neels H, Blust R, Covaci A. Biomagnification of naturally-produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in harbour seals and harbour porpoises from the Southern North Sea. Environ Int 2009c;35:893–9.

Weijs L, Das K, Siebert U, van Elk N, Jauniaux T, Neels H, Blust R, Covaci A. Concentrations of chlorinated and brominated contaminants and their metabolites in serum of harbour seals and harbour porpoises. Environ Int 2009d;35:842–50.

Weijs L, Das K, Neels H, Blust R, Covaci A. Occurrence of human-made and naturally-produced organohalogenated compounds in several tissues of Black Sea harbour porpoises. Mar Pol Bul 2010a;60:725–31.

Weijs L, Yang RSH, Covaci A, Das K, Blust R. Physiologically based pharmacokinetic models for lifetime exposure to PCB 153 in male and female harbour porpoises (Phocoena phocoena): Model development and validation. Environ Sci Technol 2010b;44:7023–30.

Westgate AJ, Muir DCG, Gaskin DE, Kingsley MCS. Concentrations and accumulation patterns of organochlorine contaminants in the blubber of harbour porpoises, *Phocoena phocoena*, from the coast of Newfoundland, the Gulf of St Lawrence and the Bay of Fundy/Gulf of Maine. Environ Pollut 1997;95:105–19.

Ylitalo GM, Baird RW, Yanagida GK, Webster DL, Chivers SJ, Bolton JL, Schorr GS, McSweeney DJ. High levels of persistent organic pollutants measured in blubber of island-associated false killer whales (*Pseudorca crassidens*) around the main Hawaiian Islands. Mar Pollut Bull 2009;58:1922–52.

Yordy JE, Wells RS, Balmer BC, Schwacke LH, Rowles TK, Kucklick JR. Life history as a source of variation for persistent organic pollutant (POP) patterns in a community of common bottlenose dolphins (*Tursiops truncatus*) resident to Sarasota Bay, FL. Sci Total Environ 2010;408:2163–72.

Zegers BN, Mets A, Van Bommel R, Minkenberg C, Hamers T, Kamstra JH, Pierce GJ, Boon JP. Levels of hexabromocyclododecane in harbor porpoises and common dolphins from western European seas, with evidence for stereoisomer-specific biotransformation by cytochrome P450. Environ Sci Technol 2005;39:2095–100.