PERSISTENT ORGANIC POLLUTANTS IN HARBOUR PORPOISE CALVES FROM 1990 UNTIL 2008: YOUNG WILDLIFE AT RISK?

Weijs L[1,2], van Elk N[3], Das K[4], Blust R[1], Covaci A[1,2]

[1]Laboratory of Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
[2]Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[3]Dolfinarium Harderwijk, Strandboulevard 1, 3841 AB Harderwijk, The Netherlands
[4]Laboratory for Oceanology-MARE Center, University of Liège, 4000 Liège, Belgium

Introduction

Polychlorinated biphenyls (PCBs), DDT and metabolites and polybrominated diphenyl ethers (PBDEs) are anthropogenically produced contaminants extensively used worldwide for decades[1-3]. Although these pollutants have been banned already, they are toxic to wildlife and can still be found in aquatic food chains thereby accumulating to high levels in top predators, such as marine mammals [4-7].

Harbour porpoises are small cetaceans living in the Northern hemisphere. Although harbour porpoises have, as top predators, high levels of contaminants in their tissues[7], results of Weijs et al.[8] for PCB 153 suggest that calves are possibly the most vulnerable age class among all other age classes. Neonates and calves have already a 'start' concentration from birth because transplacental and post-natal (through lipid-rich milk) transfer of pollutants. In addition, their metabolism is probably not fully developed yet, which makes it difficult for them to eliminate these compounds. Tiedeken and Ramsdell[9] have shown that fetal exposure to *p,p'*-DDE in zebra fish 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.

The aim of the present study was to investigate the presence of several contaminant classes in various tissues of harbour porpoises from the North Sea from 1990 until 2008 with special attention for neonates and calves.

Materials & Methods

Samples, chemicals and target compounds. Blubber samples were collected from 28 harbour porpoises (*Phocoena phocoena*; 3 neonates, 15 calves, 6 juveniles and 3 male adults). In addition, 6 kidney samples and 18 liver samples of these animals were analysed as well to assess the distribution of pollutants between tissues. All porpoises stranded alive between 1990 and 2008 on the North Sea coasts, but died during rehabilitation at the SOS Dolfijn rehabilitation center, Dolfinarium Harderwijk, The Netherlands. 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, 209), 7 PBDEs (IUPAC numbers: BDE 28, 47, 49, 99, 100, 153, 154) and 6 DDXs (*o,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT) were investigated. Standards were from Wellington Laboratories (PBDEs) and from Dr. Ehrenstorfer Laboratories (PCBs).

Sample preparation and analysis. The method used for the sample extraction and clean-up has been previously described[10] and is briefly presented below. Approximately 2 g of liver, 0.2 g of blubber and 3 g of kidney was spiked with internal standards BDE 77/BDE 128 (25 ng) and CB 143 (100 ng) and extracted by hot Soxhlet with 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 and analytes eluted with 20 ml hexane and 15 ml dichloromethane. The cleaned extract was evaporated to dryness and reconstituted in 150 µl iso-octane. PBDEs were measured by GC-ECNI/MS on a 30 m x 0.25 mm x 0.25 µm DB-5 column by monitoring ions *m/z* = 79 and 81 during the entire run. PCBs and DDXs were measured by GC-EI/MS on a 25 m x 0.22 mm x 0.25 µm HT-8 column by monitoring 2 ions for each homologue group. The later system (GC-EI/MS) was also used for confirmation of organobromines.

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. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio S/N 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 US National Institute of Standards and Technology.

Statistical analysis. Statistical analyses are conducted using the SPSS 18.0 statistical package (PASW Statistics 18). The level of statistical significance is defined at $p < 0.05$. Because of different age classes (neonate, calf, juvenile, adult), different genders (male, female) and different years (1990 until 2008), sample sizes of age-gender groups are sometimes very small. Due to these low sample sizes, non-parametric statistical tests are performed only for calves. To assess temporal trends between the calves, the entire group was divided into calf 1, with calves from 1990 until 1998, and calf 2, 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. In addition, gender differences for juveniles were not tested due to the small sample sizes (4 females and 2 males of which one from 1993 and one from 2006). Kruskal-Wallis was used to test the differences in lipid percentages, pollutant concentrations, patterns and percentages between the groups.

Results & Discussion

Due to the sometimes low sample sizes, some conclusions in this study may have only a preliminary character and deserve further investigations with larger sample sizes. All results of PCBs, DDXs and PBDEs for all tissues of all animals are shown in Table 1.

Lipid percentages. While there are no statistically significant differences between the lipid percentages in blubber of calves, juveniles and adults (median: 89.6 %; $p = 0.115$), lipid percentages of neonates are significantly lower compared to these 3 groups (median: 79.5 %; $p = 0.019$). Although the sample sizes of liver and kidney are smaller than of the blubber, there are no statistically significant difference between the lipid percentages of the kidney and liver between calves, juveniles and adults (median: 3.3 %; $p = 0.497$ for kidney, median: 5.2 %; $p = 0.637$ for liver).

PCBs. PCBs are predominant among all compounds measured in this study. Levels depend 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 of the calves of that time period are difficult to compare to these two older age classes. For animals from 2000-2008 however, concentrations of sum PCBs in blubber and liver decreased from neonates and calves to juveniles after which they increased until higher levels in adults. Trends of sum PCBs in kidneys are the opposite compared to those observed in liver or blubber. PCB 153 (16.3-28.3 %) has the highest concentrations in all samples followed by PCB 138 (11.9-16.3 %). For most samples, PCB 149 is the third congener in the PCB pattern[7,11]. In 6 samples (1 kidney, 2 blubber and 3 liver samples) however, PCB 149 is replaced by PCB 187 as third congener. Concentrations of sum PCBs are consistently higher in blubber followed by liver and kidney as found as well for PCB 153[8]. In contrast, percentages of PCB groups based on the number of chlorine atoms differ only little between the three different tissues. Ratios of concentrations between liver and blubber and between kidney and blubber reveal that higher chlorinated PCBs (octa- en deca-PCBs) prefer the liver and kidney instead of the blubber. Roughly, PCB results of the present study are higher than values of harbour porpoises from the Black Sea[11,12], but lower than harbour porpoises from Canada[13].

PCBs in the future. To assess temporal trends, even for groups with low sample sizes, the PCB 153 results of the present study, together with the individual data from Weijs et al.[7] are used in the PBPK model for bioaccumulation of PCB 153[8]. Reverse dosimetry modeling is a technique that allows to determine and predict past and future exposure scenarios by varying or sliding the input (concentration of PCB 153 in milk and fish) parameters in an existent model. Results of the simulations reveal that levels of PCB 153 in blubber of harbour porpoises of all ages from the North Sea will be below a toxicity limit of 310 ng/g lw in 2053. A limit of 310 ng/g

lw is arbitrarily chosen because it is the lowest concentration in the study of Das et al.[16] that investigated possible endocrine disruption in relationship with contaminant loads in harbour porpoises. This finding is confirmed by Law et al.[15] who also found that PCB concentrations are only decreasing slowly in blubber of harbour porpoises from the UK from 1991 until 2005. Therefore, this leads to the conclusion that PCB 153, the most persistent congener and thus representative for PCB contamination, is still an issue of concern and a continuous threat in harbour porpoises for years to come although it has been banned decades ago.

DDXs. The dominant DDX is *p,p'*-DDE with concentrations ranging from 58.5 to 88.4 % of the total sum of DDXs[11]. Both *o,p'*-DDT and *p,p'*-DDT are not present in samples of kidneys and are only sporadically found in some samples of liver, but are consistently analysed in all samples of blubber. Congener *o,p'*-DDE was only detected in 3 blubber samples and 1 liver sample. In 2000-2008, there is thus a decrease in sum DDXs from calves to juveniles, followed by an increase from juveniles to adults for liver and blubber. Similar to sum PCBs, the sum of DDXs is highest in the kidney of the only juvenile. In contrast to PCBs, the levels of DDXs of this study are lower compared to the DDX concentrations of harbour porpoises from the Black Sea[11,12] indicating that DDXs are probably still used by some countries surrounding the Black Sea but not in the North Sea.

Table 1. Median concentrations (minimum – maximum) of sum PCBs, sum PBDEs and sum DDXs in µg/g lipid weight in several tissues of harbour porpoises from the North Sea in 1990-2008.

Age class	Tissue	N[a]	Sum of PCBs		Sum of PBDEs		Sum of DDXs	
			1990 – 1998	2000 – 2008	1990 – 1998	2000 – 2008	1990 – 1999	2000 – 2008
Neonate	Blubber	1 / 2	13.7	16.8 (4.7-29.0)	0.1	0.5 (< 0.1-0.8)	1.9	1.8 (0.5-3.0)
Calf	Blubber	3#/11	10.0 (8.2-11.6)	12.8 (4.0-25.2)	2.6 (0.2-4.1)	0.6 (0.3-1.5)	2.2 (1.9-4.7)	2.4 (0.8-3.6)
	Kidney	- / 2	-	4.0 (2.8-5.2)		0.1 (< 0.1-0.2)	-	0.5 (0.4-0.5)
	Liver	3 / 8	9.1 (3.0-10.5)	11.2 (3.8-23.4)	0.5 (< 0.1-1.2)	0.3 (0.1-1.4)	0.7 (0.5-2.8)	0.9 (0.7-2.9)
Juvenile	Blubber	1 / 5	19.1	9.9 (1.1-68.2)	4.8	0.5 (0.3-1.5)	4.5	1.7 (0.4-6.4)
	Kidney	- / 1	-	16.5	-	0.6	-	1.3
	Liver	- / 3	-	8.3 (6.8-36.7)	-	0.3 (0.3-1.3)	-	0.8 (0.7-3.2)
Adult	Blubber	1 / 2	81.5	24.9 (15.3 – 34.5)	1.9	1.2 (0.6-1.8)	22.9	3.4 (2.3 – 4.4)
	Kidney	- / 2	-	6.1 (4.1-8.2)	-	0.2 (0.1-0.3)	-	0.7 (0.5-0.9)
	Liver	1 / 2	66.1	11.3 (10.0-12.5)	1.6	0.4 (0.3-0.5)	9.9	1.3 (1.2-1.4)

- Considered as an outlier, calf from 1990. Sum of PCBs is 79.9 µg/g lw, sum of DDXs is 34.6 µg/g lw.
[a] - Before '/' is sample size of time period 1990-1998, after '/' is sample size of time period 2000-2008.

PBDEs. As for PCBs, sample sizes of juveniles and adults from 1990-1998 are too small to compare to the results to the corresponding calves. For the sum of PBDEs in animals of 2000-2008, PBDEs in blubber and liver are highest in calves and adults, while juveniles have the highest levels of sum PBDEs in their kidneys. Levels of PBDEs are lower in calves from 2000-2008 compared to calves from 1990-1998. PBDE patterns are typically dominated by BDE 47, covering more than 30% of the sum of PBDEs, in all tissues and in all animals[7,11,14]. However, the percentage of BDE 47 decreases with age in all tissues. In all samples, BDE 47 is followed by BDE 100 although the differences between percentages of BDE 47 and BDE 100 are small in the kidneys. To investigate possible time trends, differences for percentages of each PBDE congener were tested between calves of 1990-1998 and calves of 2000-2008. Although the percentages are not statistically different for all PBDE congeners, there are

some statistical significant differences for BDE 47, BDE 100 and BDE 154 ($p = 0.006$; $p = 0.006$ and $p = 0.026$, respectively) in blubber and for BDE 154 ($p = 0.034$) in liver.

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.[8] suggests that there is little metabolic biotransformation of PCB 153 in harbour porpoises, if any, and probably only at higher age. Results show that BDE 47/PCB 153 and *p,p'*-DDE/PCB 153 ratios are highest in calves, regardless of the tissue, while the lowest ratios can be found in the adults. The only exception to this are the *p,p'*-DDE/PCB 153 ratios that are lowest in the liver of juveniles compared to adults. This would mean that the calfs 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 of pollutants. However, the sometimes very high concentrations in calves compared to juveniles or adults, leads to the conclusion that higher concentrations alone are not capable of inducing metabolic biotransformation in harbour porpoises. The first months of the lives of any organism are critical and determining for their further development. Calves receive loads of pollutants through milk, but are possibly not capable of dealing with these burdens as their metabolism is not yet fully operational. 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.

Acknowledgements. SOS Dolfijn (Harderwijk, The Netherlands) is greatly acknowledged for providing tissues of the harbour porpoises investigated in this study. LW and AC are financially supported by the Scientific Research Foundation–Flanders (FWO). KD is a research associate at FRS-FNRS (Fonds pour la Recherche Scientifique).

References:

1. Agency for Toxic Substances and Disease Registry (ATSDR). (2001); Polychlorinated biphenyls.
2. Agency for Toxic Substances and Disease Registry (ATSDR). (2002); DDT, DDE, and DDD.
3. Agency for Toxic Substances and Disease Registry (ATSDR). (2004); Polybrominated diphenyl ethers.
4. Johnson-Restrepo B, Kannan K, Addink R, Adams DH. (2005); *Environ Sci Technol.* 39 : 8243-8250.
5. Kelly BC, Ikonomou MG, Blair JD, Gobas FAPC. (2008); *Environ Sci Technol.* 42 : 7069-7077.
6. Sonne C, Gustavson K, Rigét FF, Dietz R, Birkved M, Letcher RJ, Bossi R, Vorkamp K, Born EW, Petersen G. (2009); *Chemosphere.* 77 : 1558-1568.
7. Weijs L, Dirtu AC, Das K, Gheorghe A, Reijnders PJH, Neels H, Blust R, Covaci A. (2009a); *Environ Pol.* 157: 437-444.
8. Weijs L, Yang RSH, Covaci A, Das K, Blust R. *Environ Sci Technol.* Submitted.
9. Tiedeken JA, Ramsdell JS. (2009); *Environ Health Perspect.* 117 : 68-73.
10. Covaci A, Losada S, Roosens L, Vetter W, Santos FJ, Neels H, Storelli A, Storelli MM. (2008); *Environ Sci Technol.* 42: 8654-8660.
11. Weijs L, Das K, Neels H, Blust R, Covaci A. (2010); *Mar Pol Bul.* 60: 725-731.
12. Tanabe S, Madhusree B, Öztürk AA, Tatsukawa R, Miyazaki N, Özdamar E, Aral O, Samsun O, Öztürk B. (1997); *Mar Pol Bul.* 34 : 338-347.
13. Gaskin DE, Frank R, Holdrinet M. (1983); *Arch Environ Contam Toxicol.* 12 : 211-219.
14. Weijs L, Das K, Siebert U, van Elk N, Jauniaux T, Neels H, Blust R, Covaci A. (2009b); *Environ Int.* 35: 842-850.
15. Law RJ, Bersuder P, Barry J, Deaville R, Reid RJ, Jepson PD. (2010); *Mar Pol Bul.* 60: 470-473.
16. Das K, Vossen A, Tolley K, Vikingsson G, Thron K, Müller G, Baumgärtner W, Siebert U. (2006); *Arch Environ Contam Toxicol*. 51: 720-729.