LEVELS AND PROFILES OF PERSISTENT ORGANIC POLLUTANTS IN SEVERAL TISSUES OF HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) FROM THE BLACK SEA

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Abstract

Harbour porpoises (*Phocoena phocoena*) are one of three cetacean species inhabiting the Black Sea. PCBs (polychlorinated biphenyls) and PBDEs (polybrominated diphenyl ethers) are two types of lipophilic compounds which are banned in Europe since the 1970s (for PCBs) and 2004 (for PBDEs). Despite this, they are still present in the environment and can cause toxic effects in wildlife, including marine mammals. Recently, attention has been drawn towards the presence of naturally-produced methoxylated PBDEs (MeO-PBDEs) in marine mammals. These compounds can biomagnify in the food chains, acting in the same way as the anthropogenically-produced PBDEs. This is the first study to report on PBDEs and naturally-produced compounds (MeO-PBDEs and PBHDs) in tissues (kidney, brain, blubber, liver, muscle) from male harbour porpoises (11 adults, 9 juveniles) from the Black Sea. Lipid-normalized concentrations decreased from muscle > blubber > liver > kidney > brain for the sum of PCBs and of PBDEs. Among the naturally-produced compounds, levels of PBHDs were higher than of MeO-PBDEs with tri-BHD and 6-MeO-BDE 47 being dominant for both groups, respectively. Concentrations of naturally-produced compounds decreased from blubber to brain, a profile which was the same as for the sum of DDXs. Concentrations of DDXs were highest, followed by PCBs, HCB, PBHDs, PBDEs and MeO-PBDEs. Levels of PCBs and PBDEs in blubber were lower than concentrations reported for blubber of harbour porpoises from the North Sea.

Introduction

Harbour porpoises (*Phocoena phocoena*) are one of three cetacean species inhabiting the Black Sea[1]. The Black Sea acts as a sink for chemicals as it receives lots of pollutants through run-off from the surrounding countries and since it is only linked to the Mediterranean Sea through the Marmara Sea[2]. Consequently, porpoises in this area are isolated from their counterparts in other European waters, migrate on a small scale and spend their entire life in the same polluted region[3].

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are two groups of lipophilic compounds which are banned in Europe since the 1970s (PCBs) and 2004/2008 (PBDEs). Despite these regulations, they are still present in the environment and can cause toxic effects in wildlife, including marine mammals. Recently, attention has been drawn towards the presence of naturally-produced compounds, such as methoxylated PBDEs (MeO-PBDEs) and polybrominated hexahydroxanthene derivatives (PBHDs) in marine mammals[4-6]. MeO-PBDEs can biomagnify in the food chains, acting in the same way as the anthropogenically-produced PBDEs[7]. To our knowledge, this is the first study to report on human-made and naturally-produced brominated compounds in various tissues of harbour porpoises from the Black Sea.

Materials & Methods

Samples, chemicals and target compounds. Blubber, liver, muscle, kidney and brain samples were collected from 20 male harbour porpoises (*Phocoena phocoena*; 9 juveniles and 11 adults) stranded or bycaught in the Black Sea in 1998. In all samples, 39 PCB congeners (IUPAC numbers: CB 18, 28, 31, 44, 47, 49, 52, 74, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 146, 149, 151, 153, 156, 158, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 196/203, 199, 203, 205, 209), 8 PBDEs (IUPAC numbers: BDE 28, 47, 49, 99, 100, 153, 154, 183), DDXs (*o,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT) and HCB were targeted. In addition, 2 naturally-produced methoxylated PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47), tri-BHD and tetra-BHD were investigated. Standards were from Wellington Laboratories (PBDEs and MeO-PBDE), from Dr. Ehrenstorfer Laboratories (PCBs) and a gift from Walter Vetter (PBHDs).

Sample preparation. The method used for the sample extraction and clean up has been previously described[8] and is briefly presented below. Approximately 2 g of liver and brain, 0.2 g of blubber and 3 g of muscle and kidney was 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 (performed on an aliquot of the extract), 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 concentrated.

Analysis. PBDEs, MeO-PBDEs and PBHDs were measured with an Agilent 6890-5973 gas chromatograph coupled with a mass spectrometer system (GC-MS). The GC was equipped with a 30 m x 0.25 mm x 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. PCBs were measured with the same GC-MS system as for the PBDE determination, operated in electron ionisation (EI) mode and equipped with a 25 m x 0.22 mm x 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.

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 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 exercise organised by the US National Institute of Standards and Technology.

Statistical analysis. Statistical analyses were conducted using the SPSS 15.0 statistical package. The level of statistical significance was defined at $p < 0.05$. For concentrations below the LOQ, a value of ½ LOQ was used. Outliers in all groups, detected making boxplots, were removed before further calculations. Non-parametric statistical tests were used since the data were found to have a non-normal distribution (Shapiro Wilk's statistical test). Differences in the concentrations and profiles of PCBs and PBDEs were compared between the two age groups (adult males and juvenile males) using Kruskal-Wallis. Spearman's correlation coefficients were calculated using GraphPad Prism 4 (GraphPad Software, Inc).

Results & Discussion

Lipids. Lipid percentages for each tissue were comparable with percentages found previously in harbour porpoises[2,9-13] and ranged in general from 1.97 - 4.93% for kidney, 7.80 - 18.5% for brain, 2.64 - 5.54% for liver, 0.22 - 7.33% for muscle and 87.6 - 97.3% for blubber in the present study. Concentrations and levels discussed here are all lipid-normalized.

PCBs. Concentrations for the sum of PCBs decreased from muscle > blubber > liver > kidney > brain (Table 1). In all tissues and in both age-groups, CB 153 was the predominant congener (17-21% of total PCBs), followed by CB 138 and CB 149. This is a well-preserved profile in harbour porpoises[14,15]. For adults, CB 99 was the fourth congener, replaced by CB 118 in juveniles. The next congeners were CB 95 or CB 180 depending on the age-group and on the tissue. CB 31 could only be detected in some samples of brain. No differences were found for the sum of PCBs between the adults and the juveniles in kidney ($p = 0.327$), brain ($p = 0.050$) and liver ($p = 0.143$) while significant differences were found in blubber ($p = 0.003$) and muscle ($p = 0.016$).

PBDEs. Profiles were comparable with previous results by Boon et al. (2002)[16] and Weijs et al. (2009)[15]. BDE 183 could not be detected in any sample, while BDE 47 was the most dominant congener in all samples representing between 50 and 72% of total PBDEs. Profiles were consistent among adults, with BDE 47 > BDE 100 > BDE 99 > BDE 154 in all tissues. In contrast, there was some variation in juveniles with BDE 100 and 99

switching places depending on the type of tissue. In accordance with sum PCBs, levels of sum PBDEs decreased from muscle to blubber, liver, kidney and brain (Table 1). No influence of age could be found as there were no significant differences in sum PBDEs (or any other PBDE congener) between adults and juveniles in all tissues (kidney: $p = 0.178$; brain: $p = 0.540$; liver: $p = 0.594$; muscle: $p = 0.700$ and blubber: $p = 0.382$).

Table 1. Medians (range) expressed in ng/g lw (lipid weight) for sum of PCBs and for sum of PBDEs in several tissues of harbour porpoises from the Black Sea.

		N	Adults	N	Juveniles
Sum PCBs	Kidney	10	4044 (2723 – 8739)	9	3384 (1851 – 7429)
	Brain	10	1614 (897 – 2282)	9	1009 (758 – 1575)
	Liver	10	8450 (4161 – 20131)	8	6508 (5311 – 13532)
	Muscle	9	17420 (9908 – 24956)	8	11707 (8479 – 15022)
	Blubber	11	13215 (8810 – 24875)	9	6956 (4896 – 13665)
Sum PBDEs	Kidney	10	15.9 (10.6 – 27.1)	9	18.4 (11.0 – 63.2)
	Brain	10	3.1 (2.4 – 5.5)	9	3.9 (2.1 – 6.2)
	Liver	10	44.7 (20.0 – 59.9)	8	44.0 (30.8 – 66.3)
	Muscle	9	80.8 (54.1 – 115)	8	80.4 (48.0 – 102)
	Blubber	11	65.6 (43.2 – 85.1)	9	57.0 (47.5 – 72.8)

Naturally-produced organobrominess. MeO-PBDEs and PBHDs have been suggested to be produced by marine organisms, such as algae and sponges[4,17], and have been reported in whale[18] and fish oil[19], fish and marine mammals[4-7]. Tri-BHD, tetra-BHD, 2'-MeO-BDE 68 and 6-MeO-BDE 47 were targeted in the present study. Among MeO-PBDEs, 6-MeO-BDE 47 was the most dominant compound, as previously also assumed for marine mammals in the northern hemisphere in general[7]. Tri-BHD had the highest concentrations for PBHDs and for all naturally-produced organobromines in total. No differences were found for the sum of MeO-PBDEs between adults and juveniles in all tissues (all $p > 0.05$) except for brain ($p = 0.041$). Considering the sum of PBHDs, levels were not different between adults and juveniles in all tissues except for blubber ($p = 0.004$). Adults had higher concentrations of sum of PBHDs than juveniles, reflecting bioaccumulation with age and possibly a limited capacity for biotransformation. In addition, the highest concentrations of sum of PBHDs, but also of sum of MeO-PBDEs, can be found in blubber, while the lowest concentrations were in brain. Concentrations of the sum of naturally-produced organobromines (Table 2) were approximately 15 times higher than concentrations of sum PBDEs (Table 1) and about 5 times lower than concentrations of sum PCBs (Table 1).

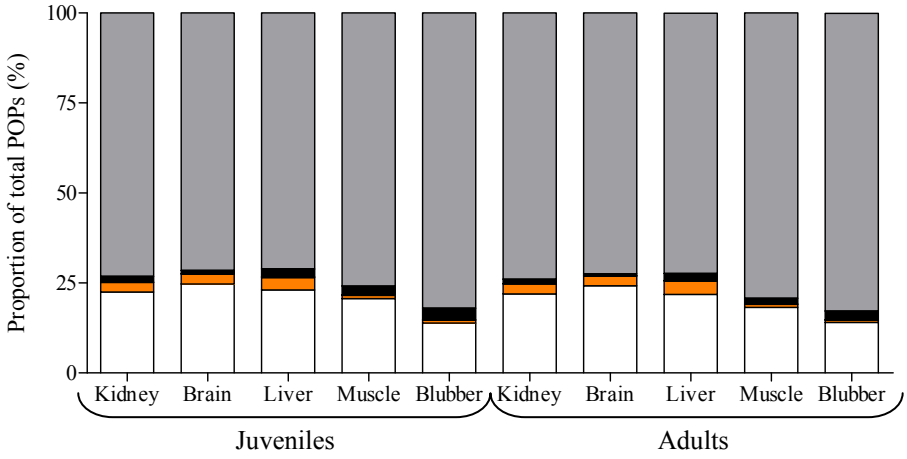

Figure 1. Overview of the proportions of sum PCBs (□), HCB (■), sum PBDEs (■), sum DDXs (■) and sum of naturally-produced organobromines (■) in tissues of harbour porpoises from the Black Sea.

Other POPs. HCB (hexachlorobenzene) and sum of DDXs (*o,p'*-DDT, -DDE, -DDD and *p,p'*-DDT, -DDE, -DDD) were included in the analyses as well. Results show that, for both age-groups, HCB levels were highest in liver, while sum DDXs were highest in blubber. Brain had the lowest concentrations of HCB and DDXs. Among DDXs, *p,p'*-DDE and *p,p'*-DDD were the most dominant compounds, contributing to more than 90 % to the sum of DDXs.

Table 2. Medians (minimum-maximum) expressed in ng/g lw for sum of naturally-produced organobromines (MeO-PBDEs and PBHDs) in several tissues of harbour porpoises from the Black Sea.

		N	Adult	N	Juvenile
Sum MeO-PBDEs	Kidney	10	10.6 (7.6 – 19.9)	9	11.7 (9.9 – 44.9)
	Brain	10	2.3 (0.6 – 5.6)	9	3.1 (2.2 – 7.0)
	Liver	10	18.2 (11.6 – 35.7)	8	21.7 (16.9 – 61.6)
	Muscle	9	39.0 (20.8 – 72.8)	8	45.7 (21.4 – 122.4)
	Blubber	11	46.6 (30.7 – 73.1)	9	52.9 (44.9 – 79.9)
Sum PBHDs	Kidney	10	211.7 (139.1 – 445.4)	9	229.5 (95.3 – 618.4)
	Brain	10	36.2 (16.9 – 53.7)	9	32.5 (20.8 – 55.9)
	Liver	10	742.9 (318.0 – 1443.6)	8	599.3 (503.8 – 1323.9)
	Muscle	9	1502.3 (1002.6 – 2497.2)	8	1341.1 (830.8 – 1962.9)
	Blubber	11	2203.7 (1534.6 – 3537.6)	9	1509.1 (944.82 – 2206.0)

Additional findings. Levels of PCBs and PBDEs in blubber and liver were lower than concentrations reported for blubber and liver, respectively, of harbour porpoises from the North Sea[12,15] and were also lower than levels of PCBs measured in blubber of adult males from the Black Sea in 1993[11]. In contrast, concentrations of sum DDXs exceed by far the concentrations found in liver of harbour porpoises from the North Sea[12]. For both age-groups, sum of DDXs > sum of PCBs > HCB > sum of naturally-produced organobromines > sum of PBDEs for kidney, brain and liver. For muscle and blubber, the contribution of sum of naturally-produced organobromines is higher compared to HCB (Figure 1). A higher presence of DDXs compared to PCBs was reported previously[2] for harbour porpoises from the Black Sea, being different from the North Sea.

To check for possible relationships between the concentrations of sum of PCBs, sum of PBDEs, sum of DDXs, sum of naturally-produced organobromines and HCB and the feeding ecology (assessed through stable isotope analyses, $\delta^{13}C$ and $\delta^{15}N$), correlations were established. Measurements of $\delta^{13}C$ and $\delta^{15}N$ in muscle of 17 out of 20 harbour porpoises were used to investigate the influence of trophic position on the levels and profiles of the POPs in the present study. Procedure and results for $\delta^{13}C$ and $\delta^{15}N$ for these animals were presented elsewhere[20,21]. However, none of these correlations were significant (Spearman; all $p > 0.05$).

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