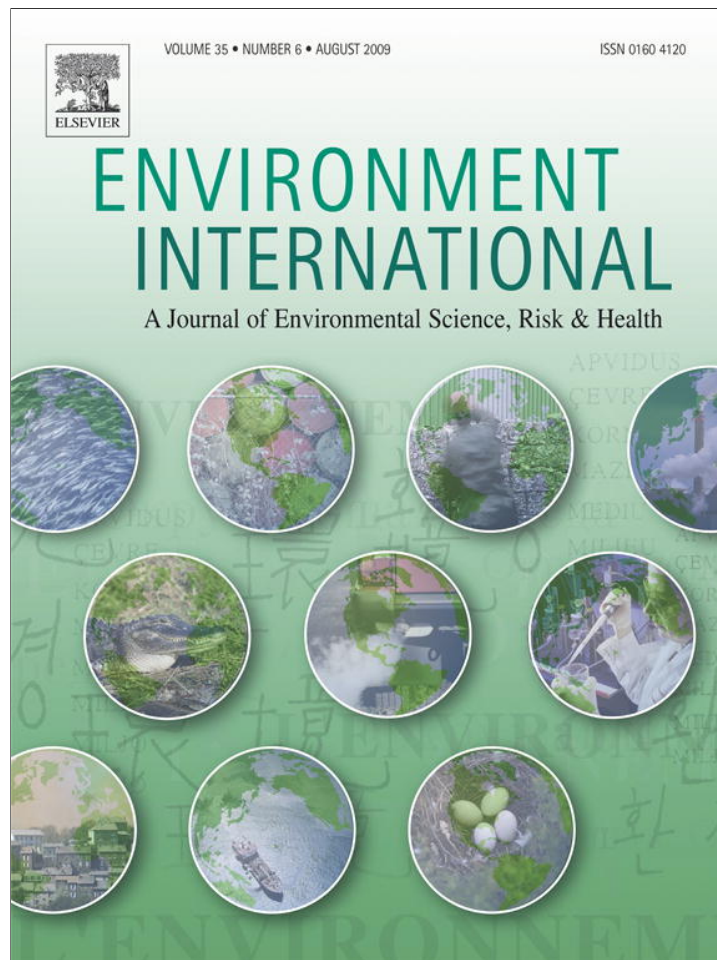


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Biomagnification of naturally-produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in harbour seals and harbour porpoises from the Southern North Sea

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ABSTRACT

Harbour seals and harbour porpoises are top predator species from the North Sea, have long life spans and hence, are known to accumulate high levels of anthropogenic contaminants. To gain knowledge about the behaviour of naturally-produced compounds in these marine mammals, the biomagnification of naturally-produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) was assessed. The biomagnification of MeO-PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) was lower in harbour seals (all biomagnification factors (BMFs) <1) compared to the same age-gender groups of the harbour porpoises (all BMFs >1). This may indicate a better metabolic breakdown of MeO-PBDEs in harbour seals, as was previously suggested for polybrominated diphenyl ethers (PBDEs). In both predators, 6-MeO-BDE 47 had the highest concentrations (range: 45–483 ng/g lw and 2–38 ng/g lw for harbour porpoises and seals, respectively) compared to 2'-MeO-BDE 68 (range: 2–28 ng/g lw and 1–6 ng/g lw for harbour porpoises and seals, respectively). In general, the highest concentrations were found in juveniles, suggesting an increased biotransformation capacity with age or the influence of dilution by growth for both species. Here we show that naturally-produced brominated organic compounds can biomagnify and accumulate in North Sea top predators, although to a lesser extent than anthropogenic lipophilic contaminants, such as polychlorinated biphenyls (PCBs) or PBDEs.

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) are one of the most important brominated flame retardants (BFRs). They represent a group of 209 congeners with differences in the number and position of the bromine atoms and are extensively used in electronic equipment, textiles, household products and transport. Although the use and production of commercial mixtures has been banned in the European Union since 2004 (for PentaBDE and OctaBDE) and 2008 (for DecaBDE), PBDEs are still widespread contaminants (Birnbaum and Staskal, 2004). There is still too limited information about the toxicity and metabolism of PBDEs in marine mammals. McKinney et al. (2006) found significant differences in the metabolism of PBDEs between rat and beluga whale (*Delphinapterus leucas*) hepatic microsomes and they assumed a different toxicity in both mammal species. Even

within the marine mammals, differences in metabolism of PBDEs were suggested between harbour seals (*Phoca vitulina*) and harbour porpoises (*Phocoena phocoena*) (Weijs et al., 2009a), indicating species-dependent metabolic capacities.

Recently, attention has shifted towards the presence in wildlife of methoxylated PBDEs (MeO-PBDEs), which are structurally related to PBDEs. MeO-PBDEs are not known to be commercially produced, nor are they reported as byproduct in industrial processes (Vetter, 2006). Yet, MeO-PBDEs have been measured in top predators, such as polar bears (*Ursus maritimus*) (Verreault et al., 2005), whale and dolphin species from the Mediterranean Sea (Pettersson et al., 2004), cetaceans from Australian waters (Vetter et al., 2002; Melcher et al., 2005), pinnipeds from the Baltic Sea (Haglund et al., 1997), harbour porpoises and harbour seals from the North Sea (Weijs et al., 2009a; Weijs et al., in press), marine mammals from Japan (Marsh et al., 2005) and beluga whales from the Canadian Arctic (Kelly et al., 2008). These PBDE-analogues are most likely of natural origin, produced by marine sponges, such as *Dysidea* sp. in Australia (Vetter et al., 2002), or red algae and cyanobacteria from the Baltic Sea (Malmvärn et al.,

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2008). Moreover, the natural origin of MeO-PBDEs has been confirmed by Teuten et al. (2005) who found a ^{14}C -enrichment of the compounds detected in blubber of a True's beaked whale (*Mesoplodon mirus*) and by the detection of MeO-PBDEs in preserved whale oil collected in 1921 (Teuten and Reddy, 2007). Despite the high concentrations of MeO-PBDEs in tissues of marine mammals, there is, to the best of our knowledge, no information available about their toxicity in marine mammals or fish. However, since MeO-PBDEs are structurally similar to compounds shown to exert toxic effects (Fu et al., 1995), adverse effects in wildlife cannot be ruled out.

The objectives of the present study were: 1) to investigate the occurrence and profiles of MeO-PBDEs in harbour seals, harbour porpoises and their prey constituted by various fish species from the North Sea, 2) to calculate biomagnification factors (BMFs) of MeO-PBDEs between these top predators and their prey and to compare them with BMFs of PBDEs, 3) to assess the influence of several factors, such as the trophic position of the predators, on the accumulation of MeO-PBDEs, and 5) to investigate the distribution of MeO-PBDEs between liver and muscle in various fish species and to compare it to that of PBDEs and PCBs.

2. Materials and methods

2.1. Samples

Blubber samples were collected from 35 harbour porpoises and 28 harbour seals stranded or bycaught along the Southern North Sea coast ranging from Belgium to Germany between 1999 and 2004. No detailed information on the prey consumed by the animals from the present study was available. The animals were dissected and tissues were archived at the Laboratory of Oceanography, University of Liège (Belgium) at $-20\text{ }^{\circ}\text{C}$. Biological parameters, such as age, gender, weight and blubber thickness, were also recorded and can be found in Weijs et al. (2009a). Measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in muscle of harbour seals and porpoises (Das et al., 2003, Weijs et al., 2009b) were used to investigate the influence of trophic position on the biomagnification. Liver and muscle samples were collected from flounder (*Pleuronectes flesus*), dab (*Limanda limanda*), herring (*Clupea harengus*), cod (*Gadus morhua*), pout (*Trisopterus luscus*) and whiting (*Merlangius merlangus*) caught in the North Sea in the spring of 2008. Due to its small size, the whole body of sprat (*Sprattus sprattus*) was analysed.

2.2. Materials

Standards of PBDE congeners (IUPAC no. 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183) and a mixture of MeO-PBDEs (including 6-MeO-BDE 47 and 2'-MeO-BDE 68) were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE 77 was used as internal standard (IS) for the MeO-PBDEs, the tri- to penta-BDE congeners, while BDE 128 was used as IS for the hexa- and hepta-BDE congeners. The following 21 polychlorinated biphenyl (PCB) congeners (IUPAC numbers) were analysed: 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194 and 199 using CB 143 as IS. Individual standards of PCBs (Dr. Ehrenstorfer Laboratories, Augsburg, Germany) were used for identification and quantification. All solvents used for the analysis (n-hexane, acetone, dichloromethane, iso-octane) were of pesticide-grade (Merck, Darmstadt, Germany). Sodium sulphate and silica were pre-washed with n-hexane before use. Extraction thimbles were pre-extracted for 1 h with the extraction mixture used for the samples and dried at $100\text{ }^{\circ}\text{C}$ for 1 h.

2.3. Sample preparation

The method used for extraction and clean-up was described in Weijs et al. (2009a) and Covaci et al. (2008). Briefly, approximately

0.3 g of blubber, 0.5 g fish liver or 5 g fish muscle were mixed with anhydrous sodium sulfate and spiked with internal standards. Samples were extracted for 2 h by hot Soxhlet with a mixture of acetone/hexane (1/3, v/v). The extract was evaporated and cleaned by passing through 8 g of acid silica (H_2SO_4 , 44%), using 50 mL of a mixture of hexane/dichloromethane (1/1, v/v) for elution of the analytes. The extract was evaporated and a second clean-up step on 1 g Florisil (only for liver samples) Supelclean (Supelco, Bornem, Belgium) was carried out, using 12 mL of hexane/dichloromethane (1/1, v/v) for elution. The eluate was evaporated to dryness with nitrogen and re-dissolved in 100 μL of iso-octane.

2.4. Analysis

Brominated compounds (PBDEs, MeO-PBDEs) were analysed using an Agilent 6890-5973 GC-MS operated in electron capture negative ionization (ECNI) mode equipped with a $30\text{ m}\times 0.25\text{ mm}\times 0.25\text{ }\mu\text{m}$ DB-5 capillary column. Bromine isotope ions (m/z 79 and 81) were acquired in selected ion monitoring (SIM) mode, with a dwell time of 50 ms. For confirmation of MeO-PBDEs and PBDEs and for the analysis of PCBs, the extracts were injected into a GC/MS operated in electron ionization (EI) mode and equipped with a $25\text{ m}\times 0.22\text{ mm}\times 0.25\text{ }\mu\text{m}$ HT-8 capillary column (SGE, Zulte, Belgium). The mass spectrometer was used in SIM mode with the two most intense ions (typically from the molecular cluster) acquired for each homologue group or isomer. Further analytical details, including the analysis of polybrominated hexahydroxanthenes (PBHDs) and tribromoanisole (TBA), are presented in Covaci et al. (2008).

2.5. Quality assurance and quality control

Quality assurance and quality control were performed through the analysis of procedural blanks, replicate samples and a standard reference material (SRM 1945, PCBs and PBDEs in whale blubber). For the replicates and SRM 1945, the relative standard deviations (RSD) were $<10\%$. Multi-level calibration curves were created for the quantification and good correlation ($r^2 > 0.999$) was achieved. Recoveries of analytes were between 72 and 98% (RSD $<10\%$) as measured by spiking experiments ($n=5$) at a concentration of 20 ng/g lipid weight (lw) for each individual compound. Additionally, the method performance was assessed through successful participation to inter-laboratory studies organized by NIST (National Institute of Standards and Technology) (PCBs and PBDEs in marine mammals). Procedural blanks of PBDEs were consistent (RSD $<20\%$) and therefore the mean value of each analyte in the procedural blanks was used for subtraction. MeO-PBDEs were not present in the procedural blanks. After blank subtraction, the limit of quantification (LOQ) was set at $3*\text{SD}$ of the value obtained in the procedural blanks. Method LOQs ranged from 0.2 to 0.3 ng/g lw for individual PBDE and MeO-PBDE congeners and between 1 and 4 ng/g lw for individual PCB congeners.

2.6. Statistical analysis

Statistical analysis was conducted using SAS 9.2 for Windows. The level of statistical significance was defined at $p < 0.05$. Outliers in all groups, detected through boxplots, were removed for further calculations. Data had a non-normal distribution (Shapiro–Wilks normality test) even after log-transformation. Therefore, differences in the concentrations and biomagnification factors of MeO-PBDEs were compared between the age–gender groups AM (adult males), AF (adult females), JM (juvenile males) and JF (juvenile females) (juveniles <3 years and adults >3 years) using the non-parametrical Kruskal–Wallis test. Statistical analysis for levels of PCBs, PBDEs and MeO-PBDEs in fish was not performed due to the low sample sizes.

2.5. Calculation of biomagnification factors (BMF)

BMF was defined as the ratio between the lipid-normalized contaminant concentrations in predator and prey (Borgå et al., 2004; Weijs et al., 2009b). Biomagnification occurs when $BMF > 1$, indicating that predators are less capable of metabolizing these compounds compared to their prey or that they are able to absorb these compounds to a greater extent (Gobas et al., 1999). In contrast, $BMF < 1$ can indicate a better developed metabolism in predators compared to prey or a lesser absorption. Diets of harbour seals and porpoises vary according to the season, year and location (e.g. Hall et al., 1998; Meininger et al., 2003; Santos and Pierce, 2003; Santos et al., 2004), but consist in general of approximately 50% pelagic and 50% benthic fish (Brasseur et al., 2006; Leonards et al., 2008). In the present study, flounder and dab were taken as representative species for benthic prey, each contributing 25% to the total diet. Sprat, cod, pout and whiting were taken as representative fish for pelagic prey, each contributing 12.5% to the total diet. Since harbour seals and porpoises usually swallow their prey as a whole, they prefer smaller fish (length < 30 cm) (Santos et al., 2004). Therefore, all fishes analysed in this study were smaller than 30 cm. Liver concentrations (Table 1) were used for the calculation of BMFs, except for sprat (whole body concentrations). Herring was not included in the calculations due to the low sample size ($n = 1$). Due to the small sample size and lack of knowledge about their reproductive history, BMFs were not calculated for the AF group in both species.

2.6. Calculation of liver–muscle distribution

The preferential accumulation of compounds in liver can be expressed by the ratio of the lipid-normalized concentration in liver divided by the sum of lipid-normalized concentrations in liver and muscle (Voorspoels et al., 2003; Svendsen et al. (2007)). Values equal to 0.5 indicate no preferential accumulation, whereas higher or lower values are an indication of accumulation in liver or muscle, respectively.

3. Results

3.1. Levels of MeO-PBDEs in harbour seals and harbour porpoises

For harbour porpoises, no statistical significant differences were found between the four groups (AM, AF, JM and JF) for 2'-MeO-BDE 68 ($\chi_3 = 6.448$; $p = 0.092$), nor for 6-MeO-BDE 47 ($\chi_3 = 7.546$; $p = 0.056$) (Table 2). Male porpoises displayed higher concentrations of 6-MeO-BDE 47 compared to females ($\chi_1 = 6.178$; $p = 0.013$). No significant influences of age were observed. For harbour seals, statistics were only

Table 1

Concentrations (mean ± standard deviation, SD) expressed in ng/g lw of the sum of PCBs, sum of PBDEs and sum of MeO-PBDEs in liver, muscle and whole body of several fish species from the Southern North Sea in 2008.

Tissue	Species	n	Lipids (%)	ΣMeO-PBDEs ^a	ΣPBDEs ^b	ΣPCBs ^c
Liver	Flounder	4	2.77 ± 2.64	<LOQ	34.3 ± 36.6	1360 ± 705
	Dab	3	11.6 ± 11.6	^d	21.5 ± 5.1	707 ± 127
	Cod	3	14.5 ± 6.1	18.1 ± 20.7	42.5 ± 11.3	3690 ± 1590
	Herring	1	1.50	5.5	93	1060
	Pout	2	5.60 ± 2.13	10.2 ± 12.9	69.8 ± 62.8	4420 ± 3620
	Whiting	7	22.3 ± 7.25	157 ± 123	168 ± 40.1	8780 ± 2420
	Flounder	4	0.47 ± 0.19	<LOQ	28.9 ± 19.4	2000 ± 288
Muscle	Dab	3	1.70 ± 1.12	<LOQ	19.7 ± 10.7	757 ± 74
	Cod	6	0.53 ± 0.08	7.5 ± 3.9	12.9 ± 5.3	1290 ± 735
	Herring	2	0.94 ± 0.42	7.1 ± 0.4	79.6 ± 91.8	1460 ± 910
	Pout	2	0.46 ± 0.15	16.7 ± 2.4	49.5 ± 42.8	5060 ± 4450
	Whiting	7	0.47 ± 0.09	34.2 ± 12.4	34.2 ± 12.9	3000 ± 1160
	Flounder	4	0.47 ± 0.19	<LOQ	28.9 ± 19.4	2000 ± 288
	Sprat	3	2.50 ± 0.53	37.1 ± 48.4	45.1 ± 15.3	2980 ± 597

^a 2'-MeO-BDE 68 and 6-MeO-BDE 47.

^b 10 PBDEs (liver and whole body) and 6 PBDEs (muscle) were analysed.

^c 40 PCB congeners were targeted.

^d MeO-PBDEs were found in only 1 sample (1.5 ng/g lw for 6-MeO-BDE 47).

Table 2

Concentrations (median, min–max) of 2'-MeO-BDE 68 and 6-MeO-BDE 47 (expressed in ng/g lipid weight, lw) in blubber of harbour porpoises and harbour seals from the southern North Sea.

	Harbour porpoises				Harbour seals			
	AM	JM	JF	AF	AM	JM	JF	AF
2'-MeO-BDE 68								
n	8	12	10	4 ^a	1	5	4	2
Median	5.7	12	7.4	3.2	0.5	1.6	1.4	1.3
Range	2.2–10	2.3–28	3.3–17	2.1–4.0	n.a.	0.7–4.8	0.9–5.6	0.8–1.8
6-MeO-BDE 47								
n	8	12	9 ^a	5	5 ^a	9	8	2
Median	108	131	67	88	2.7	5.4	7.1	7.7
Range	74–142	88–483	45–154	49–135	1.9–5.3	3.4–23	4.1–38	4.4–11

Statistical tests (Kruskal–Wallis) were not performed for 2'-MeO-BDE 68 in harbour seals due to the low sample size in 2 out of 4 groups.

^a 1 outlier, n.a. – not applicable.

performed for 6-MeO-BDE 47 due to the small sample size of the AM and AF group. 2'-MeO-BDE 68 could only be detected in 12 out of 28 samples analysed. The significant differences between the four groups for 6-MeO-BDE 47 ($\chi_3 = 9.069$; $p = 0.028$) (Table 2) are caused by the influence of age ($\chi_1 = 6.214$; $p = 0.013$) with juveniles accumulating higher concentrations than adults. In contrast, the effect of gender was not significant ($\chi_1 = 3.556$; $p = 0.059$).

For each age–gender group, concentrations of 6-MeO-BDE 47 and 2'-MeO-BDE 68 in harbour porpoises were significantly higher compared to harbour seals (all $p < 0.05$).

3.2. Levels and ratios of MeO-PBDEs and PBDEs in fish

Total concentrations of PBDEs and MeO-PBDEs in fish were higher ($p < 0.05$) in liver than in muscle, except for herring (Table 1). The detection frequency of PBDE congeners in liver was also higher compared to muscle. BDE 183 not detected in any liver sample, while BDE 183, BDE 49 and BDE 85 were not found in their muscles. There was a clear difference in the levels of MeO-PBDEs between benthic (dab and flounder) and pelagic (herring, cod, pout, sprat and whiting) fish species. 6-MeO-BDE 47 could be detected in only one liver sample of a benthic fish (dab), while MeO-PBDEs were measured in all samples of pelagic fish. For the latter species, ratios 2'-MeO-BDE 68/6-MeO-BDE 47 were between 0.15 and 0.60. For PBDEs, a similar trend could be observed as lower concentrations (sum PBDEs) were found in the liver of benthic fish compared to the pelagic species. However, the same trend could not be found in muscle (Table 1). The highest concentrations of sum MeO-PBDEs and sum PBDEs in liver were found in

Table 3

Liver/muscle ratio (calculated as liver/(liver + muscle) concentrations expressed in lipid weight) obtained for individual PBDE and MeO-PBDE and PCB congeners in species for which both liver and muscle concentrations were calculated.

PBDEs	Flounder	Dab	Pout	Cod	Whiting
N	4	3	2	3	7
BDE 28	n.a.	n.a.	n.a.	n.a.	0.79
BDE 47	0.37	0.49	0.51	0.78	0.81
BDE 99	n.a.	n.a.	n.a.	n.a.	0.75
BDE 100	0.66	0.65	0.67	0.67	0.79
BDE 153	0.56	n.a.	n.a.	n.a.	n.d.
BDE 154	0.49	0.54	0.52	0.67	0.70
MeO-PBDEs					
	Flounder	Dab	Pout	Cod	Whiting
2'-MeO-BDE 68	n.a.	n.a.	n.a.	0.72	0.78
6-MeO-BDE 47	n.a.	n.a.	0.49	0.78	0.79
PCBs					
	Flounder	Dab	Pout	Cod	Whiting
CB 99	0.38	0.47	0.50	0.74	0.75
CB 101	0.36	0.45	0.47	0.73	0.74
CB 105	0.41	0.48	0.47	0.71	0.72
CB 110	0.37	0.43	0.45	0.71	0.73
CB 118	0.39	0.49	0.50	0.74	0.75
CB 138	0.40	0.47	0.49	0.75	0.76
CB 149	0.25	0.38	0.39	0.67	0.72
CB 153	0.41	0.52	0.52	0.77	0.78
CB 170	0.46	0.61	0.49	0.71	0.75
CB 180	0.41	0.54	0.50	0.75	0.77
CB 183	0.41	0.58	0.47	0.73	0.76
CB 187	0.42	0.54	0.50	0.74	0.76

n.a. – not applicable (concentrations < LOQ).

whiting, while levels of sum MeO-PBDEs and sum PBDEs in muscle were highest in herring and pout, respectively.

Ratios between concentrations in liver and muscle were not calculated for sprat (whole fish was analysed) and herring (only 1 liver sample). For the other species, ratios obtained for MeO-PBDEs (except for flounder and dab), PBDEs and PCBs are summarized in Table 3.

Due to low detection frequency, ratios of MeO-PBDEs were only calculated for whiting, cod and pout. Results found for whiting and cod were comparable and indicated similar accumulation of MeO-PBDE congeners in the liver (0.72 ± 0.03 and 0.78 ± 0.02 for cod, and 0.78 ± 0.05 and 0.79 ± 0.02 for whiting, for 2'-MeO-BDE 68 and 6-MeO-BDE 47 respectively; expressed as mean \pm standard deviation (SD)). In pout, the average ratio for 6-MeO-BDE 47 was 0.49 ± 0.01 . Due to low detectability of other PBDE congeners, ratios could be calculated only for BDE 47, BDE 100 and BDE 154 in all fish species. A preferential accumulation in liver has been seen for these congeners in whiting (range: 0.70–0.81), cod (range: 0.67–0.78) and pout (range: 0.51–0.67). Although BDE 47 had the highest concentrations in all tissues of all fish species, it had the lowest ratios in flounder, dab and pout compared to other compounds. For cod and whiting, the opposite trend was seen, with the highest ratios for BDE 47. The ratios for MeO-PBDEs and PCBs compared favourably with PCB ratios for PCBs (Table 3).

3.3. Biomagnification of MeO-PBDEs and PBDEs

BMFs calculated for harbour porpoises were higher for 6-MeO-BDE 47 than for 2'-MeO-BDE 68, although this was not so obvious for harbour seals (Table 4). Median BMF values were all <1 in harbour seals, while being >1 in harbour porpoises. Statistically significant differences were found for 6-MeO-BDE 47 between the three groups of harbour porpoises ($\chi^2 = 6.275$; $p = 0.043$) and harbour seals ($\chi^2 = 8.839$; $p = 0.012$) (Table 4). For harbour porpoises, these differences were more located between the JM and JF groups in harbour porpoises ($\chi_1 = 5.172$; $p = 0.023$). For harbour seals, age is more important since there were no statistical significant differences between JM and JF, while both age-gender groups differed significantly from the AM group ($p = 0.014$ and $p = 0.008$ for comparison with JM and JF respectively). No significant differences in BMFs of 2'-MeO-BDE 68 were found between the three age-gender groups of harbour porpoises ($\chi^2 = 2.820$; $p = 0.244$). Interspecies comparisons were only possible for 6-MeO-BDE 47, since 2'-MeO-BDE 68 was only sporadically measured in harbour seals. For all three groups, harbour porpoises were found to have statistically significant higher BMFs compared to the corresponding group of harbour seals ($p = 0.003$, $p = 0.001$ and $p = 0.001$ for comparisons between AM, JM and JF groups of both species, respectively). These differences seem to correspond with the differences in trophic position of both species (Fig. 1A and B for 2'-MeO-BDE 68 and 6-MeO-BDE 47, respectively).

BMFs of BDE 47, BDE 100 and BDE 154 were calculated to facilitate the interpretation of the biomagnification of naturally-produced MeO-PBDEs. These calculations were made using the concentrations of PBDEs in the same harbour seals and porpoises, as described in Weijs et al. (2009a) and the concentrations of their prey from the present study (Table 1). Results show that there are no statistical significant differences between BMFs calculated in the present study and BMFs calculated in Weijs et al. (2009b) for the age-gender groups and each individual PBDE congener (Fig. 2). Similar to the conclusions stated by Weijs et al. (2009b), BMF values of PBDEs in the AM group were higher than in the JM group for both species and that harbour porpoises had higher BMFs than harbour seals for the same age-gender group.

There were clear differences between BMFs of MeO-PBDEs and of PBDEs (Fig. 2). For AM harbour porpoise, BMFs of MeO-PBDEs were 2 to 65 times lower than BMFs of PBDEs (Fig. 2A), while they were only 2 to 7 times lower than BMFs for juvenile harbour porpoises (Fig. 2B). BMFs of MeO-PBDEs were 10 to 60 times lower for AM harbour seal than BMFs of PBDEs in the same age-gender group (Fig. 2C) and for juveniles, 6 to 40 times lower compared to BMFs of PBDEs (Fig. 2D).

Table 4
Biomagnification factors (median, min–max) of 2'-MeO-BDE 68 and 6-MeO-BDE 47 (ng/g lipid weight) between harbour porpoises and harbour seals and their prey from the southern North Sea.

	Harbour porpoise			Harbour seal		
	AM	JM	JF	AM	JM	JF
2'-MeO-BDE 68						
n	8	12	10	1	5	4
Median	1.1	2.5	1.5	0.1	0.3	0.3
Range	0.4–2.0	0.5–5.0	0.7–3.3		0.1–1.0	0.2–1.1
6-MeO-BDE 47						
n	8	12	9 ^a	5 ^a	9	8
Median	5.2	6.3	3.3	0.1	0.3	0.3
Range	3.6–6.9	4.2–23.3	2.2–7.4	0.1–0.3	0.2–1.1	0.2–1.9

Statistical tests were not performed for 2'-MeO-BDE 68 due to the low sample sizes.

^a 1 outlier.

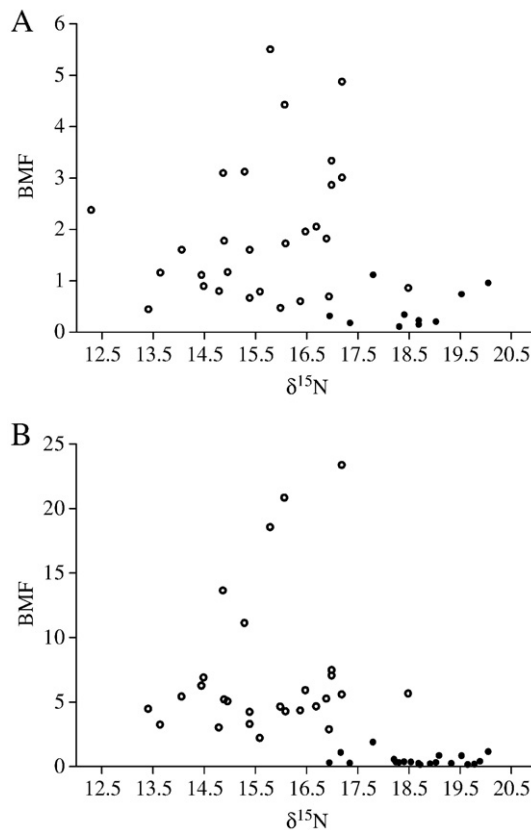


Fig. 1. 2D scatterplots with isotopic signatures ($\delta^{15}\text{N}$) and BMFs of (A) 2'-MeO-BDE 68 and (B) 6-MeO-BDE 47 for harbour seals (●) and harbour porpoises (○). Stable isotope data are from Das et al. (2003) and Weijs et al. (2009b). No significant correlations ($r^2 < 0.1$, $p > 0.1$) between BMF and $\delta^{15}\text{N}$ were observed.

3.4. Occurrence of other brominated compounds in harbour seals and harbour porpoises

In addition to the two MeO-PBDE congeners, other compounds were also analysed. TBA was not detected in any marine mammal sample investigated, while 2,7-dibromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene (triBHD) and 2,5,7-tribromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene (tetraBHD) were found in 26 and 4 samples of harbour porpoises, respectively. Concentrations of triBHD in the harbour porpoises ranged from 11–126 ng/g lw, while concentrations of tetraBHD were from 23–79 ng/g lw. TriBHD and tetraBHD were below LOQ in all harbour seal samples.

4. Discussion

Compared to other data reported in the literature for marine mammals, levels of MeO-PBDEs in harbour seals and harbour porpoises in the present study are relatively low. Concentrations of 6-MeO-BDE 47 and of 2'-MeO-BDE 68 in blubber of harbour porpoises from this study were lower than values found in beluga whales from Canada (Kelly et al., 2008) and other cetacean species from Australia (Melcher et al., 2005) and Japan (Marsh et al., 2005). Concentrations of MeO-PBDEs in harbour seals are comparable or lower compared to findings in grey seals (*Halichoerus grypus*) and ringed seals (*Phoca hispida*) from the Baltic Sea (Haglund et al., 1997), monk seals from Africa and Weddell seals from Antarctica (Vetter et al., 2002) and ringed seals from Canada (Kelly et al., 2008). The predominant MeO-PBDE congener in harbour seals and porpoises from the present study was 6-MeO-BDE 47, in accordance with findings in grey seals and ringed seals from the Baltic Sea (Haglund et al., 1997), ringed seals and beluga whales from Canada (Kelly et al., 2008), minke whales and Baird's beaked whales from Japan (Marsh et al., 2005) and monk seals from Africa and Weddell seals from Antarctica (Melcher et al., 2005). However, in most marine mammals from Australian (Melcher et al.,

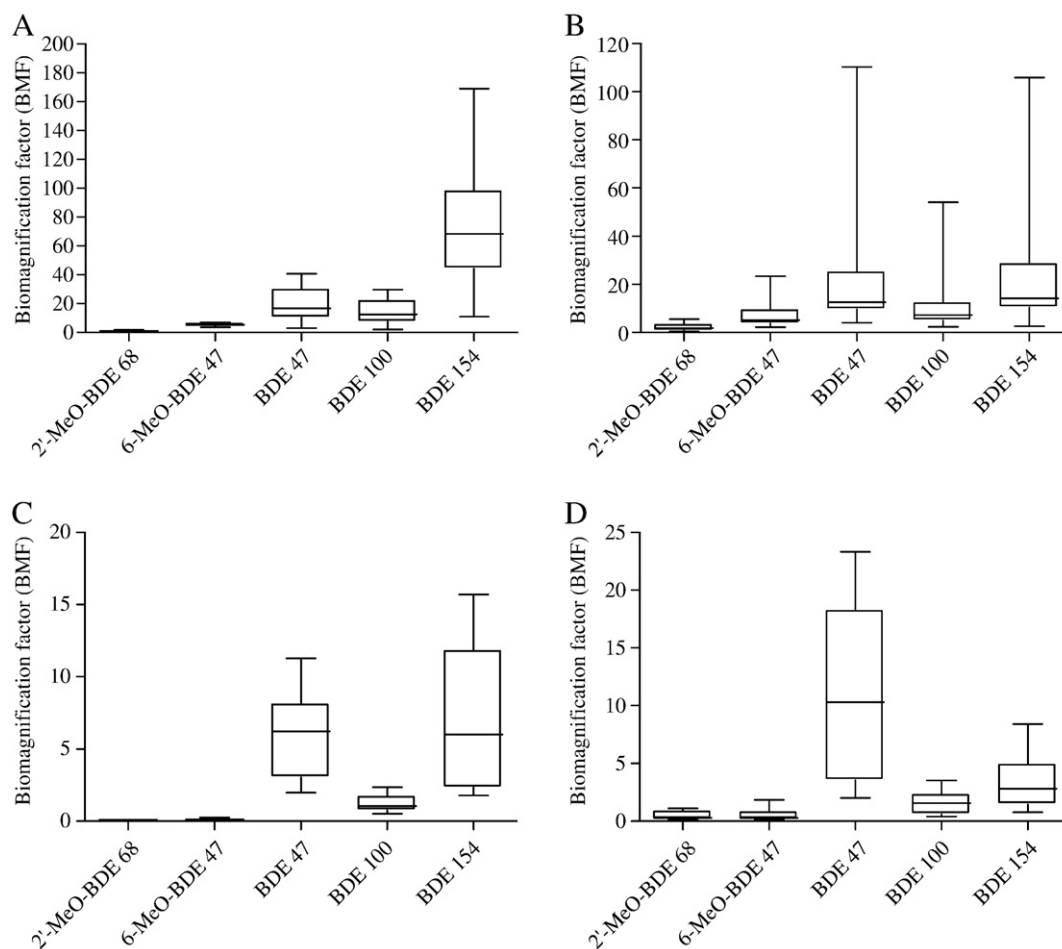


Fig. 2. Comparison between the BMFs of the naturally-produced MeO-PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) and the anthropogenic PBDEs (BDE 47, BDE 100 and BDE 154) in (A) adult harbour porpoise males, (B) juvenile harbour porpoises, (C) adult harbour seal males and (D) juvenile harbour seals.

2005) and Brazilian waters (Dorneles et al., submitted for publication), 2'-MeO-BDE 68 had the highest concentrations. This data supports the hypothesis raised by Vetter (2006) that higher contributions of 2'-MeO-BDE 68 are caused by sponges and higher proportions of 6-MeO-BDE 47 are an indication of the presence of algae or associated organisms.

In the present study, adult females had the lowest concentrations of MeO-PBDEs, followed by the adult males and juveniles (males and females) (Covaci et al., 2002; Shaw et al., 2005). The same pattern was previously found for PBDEs (Weijs et al., 2009a), indicating possible maternal transfer to offspring for the AF group. Kelly et al. (2008) analysed MeO-PBDEs in male and female beluga whales and also found higher concentrations in males compared to females. The influence of age however, was not taken into consideration. Since the groups JM and JF contained the highest concentrations of MeO-PBDEs, it is possible that the metabolism of MeO-PBDEs in marine mammals is better developed with age. In a similar way as for other lipophilic compounds, such as PCBs and PBDEs (Weijs et al., 2009a), concentrations of MeO-PBDEs in blubber decreased with the blubber thickness (Fig. 3A and B) and with the body size (growth dilution) (Fig. 3C and D), suggesting dilution of pollutants with increased size and blubber layer.

For the fish species, concentrations of 2'-MeO-BDE 68 and 6-MeO-BDE 47 in the herring muscle were very similar to concentrations found by Haglund et al. (1997) for 2 year old herring from the Baltic Sea (5.8 and 0.8 ng/lw for 6-MeO-BDE 47 and 2'-MeO-BDE 68, respectively). The MeO-PBDE concentrations in herring of the present study were higher than levels found by Asplund et al. (2004) in

muscle of herring from the North Sea (0.66–0.86 and 0.20–0.35 ng/g lw for 6-MeO-BDE 47 and 2'-MeO-BDE 68, respectively) and lower than concentrations of herring from the Baltic Sea (10.6–19.9 ng/g lw for 6-MeO-BDE 47; 2.06–5.82 ng/g lw for 2'-MeO-BDE 68). The levels of 6-MeO-BDE 47 in cod liver were similar to those found by Sinkkonen et al. (2004). Similar to reported patterns in fish (Haglund et al., 1997; Asplund et al., 2004; Sinkkonen et al., 2004; Covaci et al., 2007; Kelly et al., 2008), 6-MeO-BDE 47 was the dominant MeO-BDE congener in all analysed fish. This finding provides support for the presence of algae as source of MeO-PBDEs in the North Sea. Muscle PBDE concentrations found for herring, cod and whiting were similar to concentrations found by Boon et al. (2002) and Voorspoels et al. (2003), although lower values were found for BDE 47 in the present study. Interestingly, a statistically significant relationship was found between concentrations of 6-MeO-BDE 47 and 2'-MeO-BDE 68 with respect to BDE 47 concentrations in muscle and liver. Such relationship, also seen by Verreault et al. (2005) and Kelly et al. (2008), would indicate that these compounds accumulate in a similar manner in the analysed species.

The comparable accumulation of MeO-PBDEs, PBDEs and PCBs in fish liver or muscle was evidenced through the calculation of liver/muscle ratios (Svendsen et al., 2007). We are not aware of other studies which have investigated such ratios for MeO-PBDEs. The ratios for MeO-PBDEs found in whiting, cod and pout were in most cases > 0.5, indicating a preferential accumulation in liver (Table 3). Similarly, ratios for PBDEs in whiting and cod were also > 0.5, as previously reported (Boon et al., 2002; Voorspoels et al., 2003; Vives et al., 2004; Luo et al., 2007; Corsolini et al., 2008). Ratios of PBDEs in dab and pout

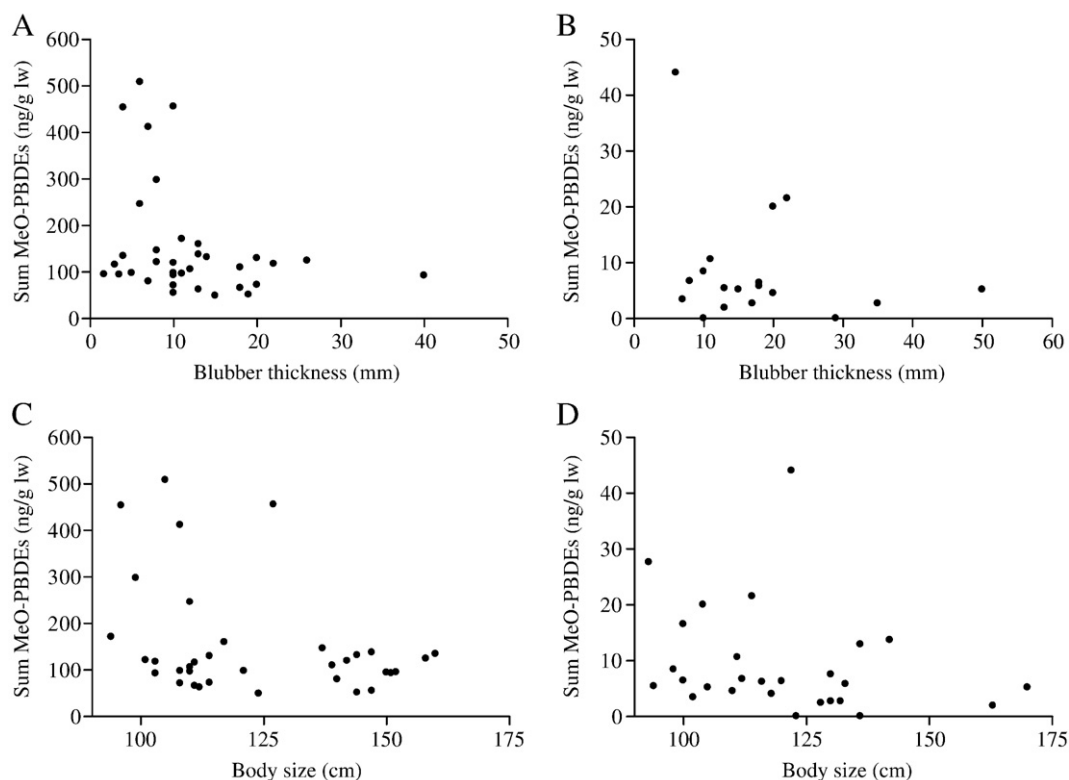


Fig. 3. Relationship between the concentrations (ng/g lipid weight) of the sum of MeO-PBDEs and the blubber thickness (mm) for harbour porpoises (A) and harbour seals (B) and between the concentrations of the sum of MeO-PBDEs and the body size (cm) for harbour porpoises (C) and harbour seals (D).

were below the values reported in dab and pout (Voorspoels et al., 2003) or in swordfish (Coriolini et al., 2008). Whiting and cod had higher ratios for MeO-PBDEs, PBDEs and PCBs compared to the other fish species studied, similar to what was found by Boon et al. (2002) and (Voorspoels et al. 2003). This different behaviour was attributed to the type of fish, as gadoid fish accumulate lipids in the liver (Boon et al., 2002; Serrano et al., 2003; Bureau et al., 2006). In the present study, strong relationships were found between the liver/(muscle + liver) ratios for PCBs, PBDEs and MeO-PBDEs with the liver lipid content (though not statistically significant for MeO-PBDEs, $p=0.251$), suggesting that higher lipid percentages are important driving factors for the accumulation of pollutants in liver.

The trophic position of harbour seals and harbour porpoises in the North Sea food chain has been extensively discussed previously (Das et al., 2003, 2004; Weijs et al., 2009b). Stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) can give important information about the diet of an organism, but also about its position in the food chain ($\delta^{15}\text{N}$) and the location of feeding ($\delta^{13}\text{C}$). Harbour seals were found to be higher in the food chain than harbour porpoises and feeding more in-shore than off-shore (Das et al., 2003). Dorneles et al. (submitted for publication) found the lowest concentrations of MeO-PBDEs in cetaceans from estuaries compared to species from the continental shelf and oceanic environments. This seems to be confirmed in the present study. BMFs for both MeO-PBDEs were <1 in harbour seals, suggesting possible breakdown of MeO-PBDEs in harbour seals rather than in harbour porpoises or a higher absorption rate in harbour porpoises. The biomagnification of anthropogenic PBDEs in both marine mammal species is higher compared to the biomagnification of naturally-produced MeO-PBDEs. Other brominated compounds (triBHD and tetraBHD) were not found in any harbour seal sample, but were also not consistently measured in the fish species of the present study. The detection of triBHD in most harbour porpoises might indicate a better developed metabolism in harbour seals. This better developed metabolism in harbour seals, as indicated here and previously in Weijs et al. (2009a,b), was also found by Tittlemier et al.

(2002), Vetter et al. (2002) and Pangallo and Reddy (2009). The latter study could detect halogenated 1'-methyl-1,2'-bipyrroles (MBPs) in all cetacean species analysed (including one harbour porpoise), but not in two pinniped species.

5. Conclusions

To the best of our knowledge, this is the first study to report levels of naturally-produced MeO-PBDEs in marine mammals from the North Sea. Within the limits of the relatively small sample sizes for the predators as well as prey species analysed, we tentatively concluded that organic compounds produced by a natural source can biomagnify and accumulate in top predators, although to a lesser extent than anthropogenic pollutants. The calculation of the BMFs is crucially depending on the composition of the prey species involved in the diet. Marine mammals are very individualistic in their feeding behaviour, therefore our results should be considered as having a preliminary character. In any case, results show that the biomagnification of MeO-PBDEs is lower in harbour seals compared to the same age-gender groups of the harbour porpoises, which might be an indication for a better metabolic breakdown of brominated compounds in harbour seals, as previously suggested for PBDEs.

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