MeO-PBDEs, HO-PBDEs and HO-PCBs in liver samples of harbor seals from the northwest Atlantic

Covaci A[1,2], Weijs L[1,2], Berger ML[3], Neels H[1], Shaw SD[3]

[1]Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[2]Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
[3]Marine Environmental Research Institute, P.O. Box 1652, Blue Hill, ME 04614, USA

Introduction

The bioaccumulative potential and toxicity of polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs) in marine mammals has been the focus of recent research. Both groups are associated with endocrine-disrupting and reproductive/neurodevelopmental effects in animals[1-3]. PCBs and PBDEs enter coastal and marine waters from multiple sources and biomagnify easily in marine food webs[4-7]. Profiles of PCB and PBDE congeners in harbor seals suggest that these animals have a higher capacity of metabolizing several congeners compared to other marine mammal species[8]. Recently, concerns have been raised about the presence and health effects of hydroxylated metabolites of PCBs and PBDEs in wildlife[9].

The objective of the present study was to investigate the levels and profiles of HO-PCBs and HO-PBDEs in liver of harbor seals *(Phoca vitulina concolor)*, apex predators in the northwest Atlantic marine ecosystem. Most of the animals investigated were pups, providing an opportunity to examine the fate of HO-metabolites resulting from placental and lactational transfer. Data regarding PBDE and HBCD contamination in the same set of samples have been presented at Dioxin 2009[10,11].

Materials & Methods

Samples. Liver samples were collected between 2001 and 2006 from 54 harbor seals (6 adult males, 20 male pups, and 28 female pups) that stranded along the northwest Atlantic coast from the eastern coast of Maine to Long Island, New York (Fig 1). Seals were weighed, and standard length and axillary girth were measured. Age was estimated based on body size. Liver samples were stored in a freezer at -20°C until analysis.

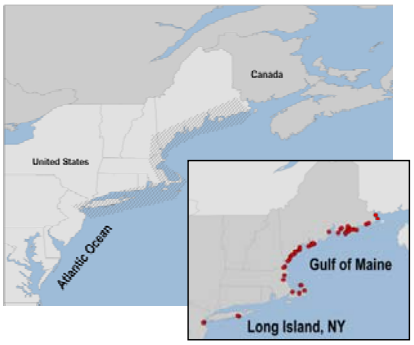

Figure 1. Map of the northwest Atlantic showing stranding locations of harbor seals

Sample preparation. Seal liver (2 - 2.5 g) was mixed with sodium sulfate and spiked with internal standards which included 4-HO-CB 159 (for the quantification of HO-PCBs and HO-PBDEs) and BDE 77 (for the quantification of MeO-PBDEs). Samples were extracted by hot Soxhlet for 2 h with a mixture of acetone/hexane (1/3, *v/v*). The extract cleaned-up on 8 g of acid silica (H_2SO_4, 44%), from which pollutants were eluted with 20 ml hexane and 15 ml DCM[12,13]. Minor adaptations were required as to separate different groups of pollutants. The cleaned extract after acid silica clean-up was evaporated to dryness, re-dissolved in 0.5 ml hexane and eluted from a pre-packed silica cartridge (500 mg, 3 mL) with 6 ml hexane (fraction 1 containing PBDEs and PCBs) and 6 ml DCM (fraction 2 containing HBCDs, MeO-PBDEs, HO-PBDEs and HO-PCBs). Both fractions were evaporated to dryness and re-dissolved in 100 µl iso-octane (Fr1) or methanol (Fr2), respectively.

Analysis. To determine the native MeO-PBDEs, fraction 2 was injected in a GC-MS operated in electron capture negative ionization (ECNI) mode equipped with a 30 m x 0.25 mm x 0.25 µm DB-5 capillary column (J&W Scientific). The ion source temperature was 170°C. The MS was used in the SIM mode with ions *m/z* 79 and 81 monitored during the entire run. Two µl of the extract were injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 ml/min). After injection of all extracts, HO-PCBs and HO-PBDEs were

derivatized by methylation using 1% trimethylsilyldiazomethane and methanol. The methylation took place at 60°C for 30 min, after which the extract was dried under nitrogen and resolubilized in iso-octane. The derivatized fraction 2 was injected in the same GC-MS system as above, with other monitored ions. The ion source temperature was 170°C. The MS was used in the SIM mode with two ions monitored for each MeO-PCB congener in specific windows, while ions *m/z* 79 and 81 were monitored for MeO-PBDEs during the entire run. Two µl of the extract were injected in cold pulsed splitless mode, splitless time 1.50 min. Helium was used at constant flow (1.0 ml/min). The values of MeO-PBDEs measured after derivatization were actually the sum of the native MeO-PBDEs and the derivatives resulting from the native HO-PBDEs. Therefore, to calculate the native HO-PBDEs, the value of native MeO-PBDEs was subtracted from the total MeO-PBDE value.

Quality Assurance and Quality Control was performed through the analysis of procedural blanks, spiked samples and a replicate sample. No peaks corresponding to the investigated MeO-PBDEs, HO-PBDEs and HO-PCBs were observed in the procedural blanks and therefore method quantification limits (LOQs) were based on the instrumental LOQs and the analyzed amount of sample. LOQs range between 2 and 5 pg/g for various congeners.

Statistics. Differences in concentrations between groups (female pups, male pups and male adults) or regions (south and north) were tested by using ANOVA followed by a Tukey post hoc test (SPSS 18.0). The level of statistical significance was defined at $p = 0.05$. Non-detects were replaced by a value of f*LOQ with f being the detection frequency. The precursor PCBs and PBDEs of the same animals that are used for the linear relationships with HO-PCBs and HO-PBDEs respectively, are taken from Shaw et al.[10].

Results & Discussion

In addition to the 54 liver samples, 5 blubber samples (4 pups, 1 adult) were analyzed as well. In all samples, 6-MeO-BDE 47 was predominant among MeO-PBDEs. Results of MeO-PBDEs in blubber ranged from 1500 to 4400 pg/g wet weight (ww). HO-PCBs were not found in these samples, while HO-PBDEs were not targeted.

HO-PCBs. The sum of HO-PCBs ranged from 90 to 22450 pg/g ww (Table 1). HO-PCBs have been analyzed earlier in marine mammals, such as in blood of several cetacean species[14], in blubber of bottlenose dolphins[15] and in serum of harbor seals from the North Sea[16]. HO-PCBs showed a linear correlation with the precursor PCBs in liver of harbor seals from the northwest Atlantic (Fig 2A), similar to findings of Weijs et al.[16] in serum from North Sea harbor seals. Congener 4-diHO-CB 202 was not detected in any sample, while 4-HO-CB 107 was predominant in almost all samples, regardless of age or gender. For the sum of HO-PCBs, there were statistical significant differences between female pups and male pups ($p=0.015$), whereas there were no statistical significant differences between pups (both genders) and adult males.

HO-PBDEs. In liver samples of harbor seals from the northwest Atlantic, three compounds were not found in any sample (5-HO-BDE 100, an unknown HO-BDE and 4-HO-BDE 101) and two compounds (4-HO-BDE 103 and 5-HO-BDE 99) were only sporadically detected in 1 and 2 out of 25 samples, respectively. None of the other compounds (2-HO-BDE 68, 6-HO-BDE 47, 5-HO-BDE 47 or 4-HO-BDE 49) was predominant in all samples. Overall, concentrations of HO-PBDEs ranged from 70 to 1850 pg/g ww and similar to HO-PCBs and PCBs, HO-PBDEs were also correlated to PBDEs (Fig 2B). McKinney et al.[17] have shown that metabolic biotransformation of PBDEs occurs in beluga whales and they also found measurable, but low concentrations of HO-PBDEs in liver of beluga whales[18].However, in serum of harbor seals and harbor porpoises of the North Sea, HO-PBDEs were not detected[16].

MeO-PBDEs. Bioaccumulation of MeO-PBDEs in these animals was not influenced by age or gender as there were no statistically significant differences between female pups (n=28), male pups (n=20) and male adults (n=6) ($p=0.280$). Therefore, all animals were considered as one group with concentrations of sum MeO-PBDEs ranging from 18 to 1455 pg/g ww. This range is lower than concentrations of sum MeO-PBDEs in serum or blubber of

harbor seals from the North Sea[16,19] and in blubber and other tissues of ringed seals and beluga whales[20], mostly likely because the animals analyzed here are pups.

In the present study, 9 MeO-PBDEs were targeted of which two (4-MeO-BDE 101 and an unknown MeO-BDE) were not detected in any sample and three (4-MeO-BDE 49, 4-MeO-BDE 103 and 5-MeO-BDE 99) were detected in less than 50% of all samples. MeO-PBDEs are different from all other compounds investigated in the present study, because they may be produced by sponges or algae and may thus have a natural origin[21].

Table 1. Mean - median (range) for concentrations (ng/g ww) of HO-PCBs, HO-PBDEs and MeO-PBDEs, together with concentrations of sum PCBs and sum PBDEs as taken from Shaw et al.[10].

Concentration (ng/g ww)	Pup Liver (F)	Pup Liver (M)	Adult liver (M)	Blubber
Sum HO-PCBs	1.96 – 1.37 (0.09-6.02) (n=28)	4.96 – 3.18 (0.16-22.45) (n=20)	2.30 – 1.72 (0.44-5.29) (n=6)	< 0.02
Sum HO-PBDEs	0.32 – 0.30 (0.07-0.66) (n=10)	0.70 – 0.44 (0.11-1.85) (n=12)	0.16 – 0.14 (0.10-0.23) (n=3)	not analyzed
Sum MeO-PBDEs	0.29 – 0.20 (0.02-1.46) (n=28)	0.30 – 0.22 (0.02-1.08) (n=20)	0.09 – 0.05 (0.02-0.28) (n=6)	2.89 – 3.29 (1.50-4.40) (n=5)
Sum PCBs	2030 – 1520 (36-6900) (n=28)	6160 – 4410 (86-27960) (n=20)	2910 – 1520 (320-7240) (n=6)	16000 – 14900 (2600-27200) (n=5)
Sum PBDEs	145 – 132 (30-370) (n=10)	290 - 230 (50-910) (n=12)	70 – 40 (30-130) (n=3)	790 – 510 (110-2170) (n=5)

To assess spatial trends, animals from southern areas (southern Maine and Massachusetts Bay) were compared to animals from northern areas (mid and eastern Maine), but there were no differences in the sum MeO-PBDEs ($p = 0.258$). In the present study, 6-MeO-BDE 47 had the highest concentrations in 44 out of 54 samples, while 2'-MeO-BDE 68 was dominant in the remaining 10 samples. Of these 10 samples, 4 were from animals living in more northern areas (out of 25), while 6 were from animals living in more southern areas (out of 29).

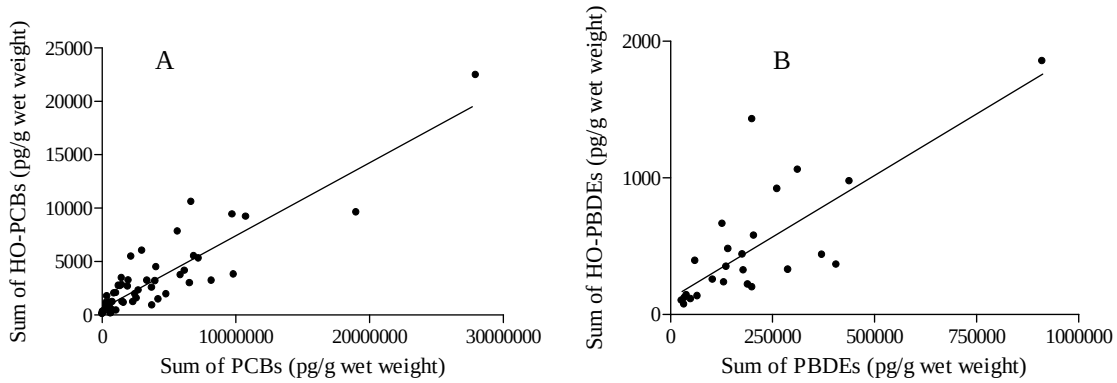

Fig 2. Linear relationships between hydroxylated PCBs and their precursor PCBs (A; $r^2=0.79$) and between hydroxylated PBDEs and PBDEs (B; $r^2=0.58$). Results of PCBs and PBDEs were taken from Shaw et al.[10].

Effects. Effects of these hydroxylated metabolites are mostly related to disturbances of hormonal and endocrine systems as they can bind to and interact with several hormone receptors and transport proteins[1,22]. As a result, toxic effects can have a great impact on the health condition of organisms in general. Hydroxylated metabolites are not particularly associated with lipids as can be seen for the parent compounds, but have a high affinity for plasma proteins. The presence of HO-metabolites in liver of pups suggests that they may be capable of metabolizing PCBs and PBDEs, but it is most probably that they have received these metabolites during transplacental/lactational transfer. Moreover, HO and MeO-PBDEs have also a natural origin from algae and sponges and therefore marine mammals may be exposed to potentially hormone active compounds also in 'clean' environments. Our results show

the increasing complexity of bioaccumulated chlorinated and brominated contaminants and/or their metabolites as an additional stress factor.

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