BIOACCUMULATION OF BROMINATED FLAME RETARDANTS IN HARBOUR SEAL

Leonards Pim [1,2], Jol Johan[1], Brandsma Sicco[1,2], Kruijt Alwin[1], Kwadijk Christiaan[1], Vethaak Dick[3], De Boer Jacob[1,2]

[1] Institute for Marine Resources and Ecosystem Studies, P.O. Box 68, 1970 AB IJmuiden, The Netherlands
[2] Current address: Institute for Environmental Studies, VU University Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
[3] Deltares, P.O. Box 177, 2600 MH Delft, The Netherlands

Introduction

The European FIRE project focused on the improvement of risk assessment of brominated flame retardants (BFRs) for human health and wildlife, and was recently completed. One of the main aims of the project was to study the food chain transfer of BFRs. The objective of current study was to investigate the transfer and accumulation of polybrominated diphenyl ethers (PBDE), hexabromocyclododecane (HBCD), and tetrabromo bisphenol A (TBBP-A) in the food web of the barbour seal.

Materials and methods

Sampling

Adult male harbour seals were selected to study the bioaccumulation of PBDEs, HBCD and TBBP-A because of the long time of exposure and to avoid the effect of BFR elimination through lactation as can occur by females. Blubber samples of 10 adult males stranded along the North Sea coast and Dutch Wadden Sea were collected. A blubber section from the outer to the inner layer was dissected and used for analysis. In addition, various fish species (e.g. plaice, flounder, sandeel, goby, whiting, herring, and sprat) from the same areas were collected. Ten to 25 individuals per fish species were homogenized as whole fish and used for analysis.

Analysis

Seal blubber samples were dried with sodium suplhate and extracted with accelerated solvent extraction (ASE) using hexane:DCM, after the addition of internal standards, BDE58, 13C-decaBDE, 13C-TBBP-A, and 13C-α,β,γ-HBCD were added. The ASE cell was filled from bottom to top with 33% H_2SO_4 deactivated silica gel (10 g), the dried sample, and sodium sulphate. The ASE extract was evaporated to 1 ml. Fish samples were extracted with Soxhlet (hexane:acetone), cleaned with GPC, sulphuric acid and silica gel. The seal and fish extracts were analysed by gas chromatography (GC) coupled to a mass spectrometer (MS) for PBDEs using ECNI mode (methane). A DB-5 GC column 15 m, 0.25-mm i.d., 0.1-µm film thickness (J&W) was used for decaBDE and a CP-Sil8 50 m, 0.25 mm, 0.25 -µm film thickness column (Chrompack) for other BDE congener analysis (BDE nos: 28, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 183, 190). Peak identification was based on the retention times and the bromine clusters of m/z 79, 81, and m/z 487 and 489 for decaBDE[1]. HBCD isomers and TBBP-A were determined with LC-MS (LCQ) using a gradient of water, acetonitril and ammoniumchloride with a Zorbax eclipse XDB-C18, 2.1 x 150 mm, 3.5 µm column (Agilent).

Results and discussion

The average concentration of the sum of PBDEs in adult male seal blubber was 232 ng/g blubber (193 to 280 ng/g). BDE47 was the dominant congener (average concentration 153 ng/g blubber), followed by BDE154 (average 34 ng/g blubber), and BDE99, BDE100 and BDE138 (13, 15, 12 ng/g blubber, respectively). Levels of BDE28 and BDE49 were much lower (0.9 and 1.9 ng/g blubber, respectively). DecaBDe was not detected in any of the seals (<3 ng/g blubber), however, frequently found in fish (<0.05 to 1.4 ng/g wet weight in whole fish). In the Wadden and North Sea decaBDE is the dominant congener in sediment, but due to a lower absorption and faster elimination/metabolisation[2,3] of decaBDE than lower brominated BDEs, decaBDE is less transferred in the food chain.

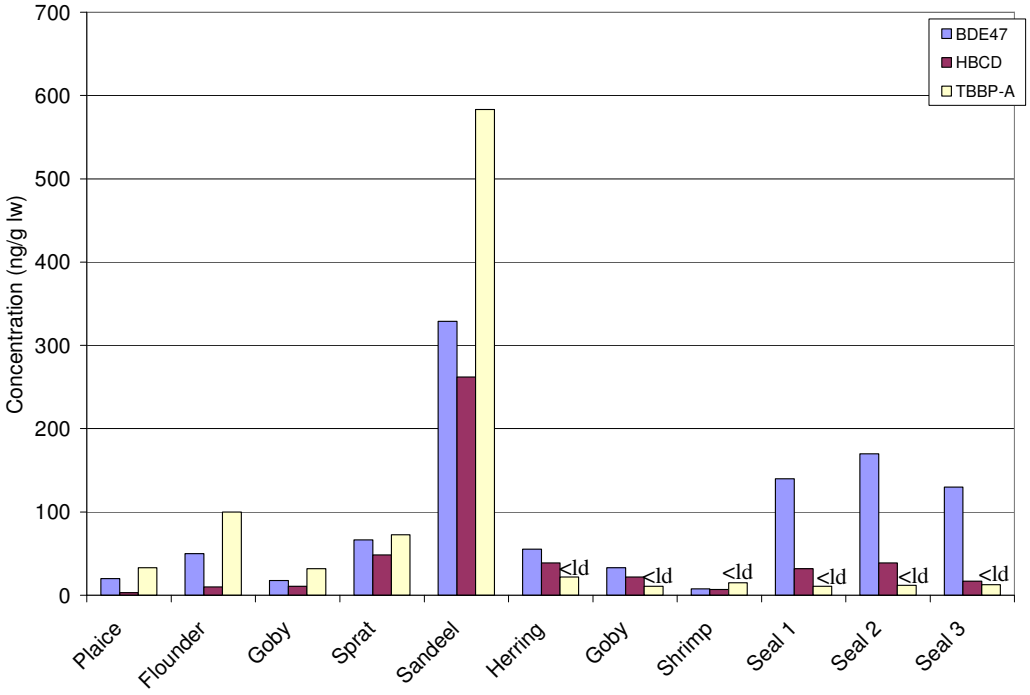

Figure 1: Levels (ng/g lipid weight) of BDE47, HBCD and TBBP-A in fish, shrimp and seal from the Wadden and North Sea. <ld: lower than the limit of detection.

α-HBCD isomer was detected in three of the ten seals only, at an average concentration of 29 ng/g blubber. TBBP-A was not detected in any of the seals (<5 ng/g blubber). On the contrary, in fish both α-HBCD and TBBP-A were frequently detected; levels of TBBP-A were sometimes higher than BDE47 (Figure 1). This implies that seal metabolizes both HBCD and TBBP-A.

Estimating daily intake and biomagnification factors

To estimate the average daily intake (EDI) of BFRs in adult seals (82 kg), the average daily food intake (4100 g/day), the food intake per item, the fraction of each food item, and the average concentration of PBDEs or HBCD in each food item were used according to:

$$EDI = \sum_{i=1}^{n} F_i \cdot I_i \cdot C_i$$

EDI: Estimated daily intake (ng/day)
F_i: Food fraction food item *i*
I_i: Food intake per item *i*
C_i: Average PBDE or HBCD concentration in food item *i*

An estimation of the food composition of harbour seal in the Wadden and North Sea was used[4]; flatfish (45%) and whiting (23%) are the main food items of harbour seal in these areas[4]. For the sum of PBDEs the EDI was 12000 ng/day, with the main contributions from flatfish (26%), whiting (26%), and sandeel (36%). The high

contribution of PBDEs by sandeel to the EDI, which was only a minor fraction in the diet (10% only), was a result of relatively high levels of PBDEs in this species compared to other fishes (Fig. 1). This indicates the importance of using the diet as the basis of intake calculations. The EDI of HBCD was 5400 ng/day.

The concentrations of BDEs and HBCD in the diet were estimated based on the diet levels of PBDEs or HBCD in the individual species, and the diet composition as:

$$C_{diet} = \sum_{i=1}^{n} (C_i \cdot F_i)$$

C_{diet} = BDE or HBCD concentration (wet weight) in diet
C_i = BDE or HBCD concentration in species *i*
F_i = Fraction of species *i* in diet

Next, the concentration in the diet (C_{diet}) was corrected for the lipid content of the total diet to get the concentration of BDEs or HBCD in the diet on a lipid weight basis (C_{dietlw}) according to:

$$L_{diet} = \sum_{i=1}^{n} (L_i \cdot F_i)$$

L_{diet} = lipid content of total diet
L_i = Lipid content of species *i*
F_i = Fraction of species *i* in diet

$$C_{dietlw} = C_{diet} \cdot L_{diet}$$

The biomagnification factors (BMFs) of BDEs and HBCD were estimated by the concentration of BDEs or HBCD in seal blubber ($C_{sealblubber}$) and the concentration in the diet on a lipid weight basis as:

$$BMF = \frac{C_{sealblubber}}{C_{dietlw}}$$

The BMFs for adult male harbour seals for individual BDEs and α-HBCD are given in Table 1. BDE47 and BDE154 biomagnify in seals, while BMFs of BDE28 and BDE49 were lower than 1 indicating that these congeners are probably metabolized by seals. This is according to the rules for PCB metabolisation for seals[5] in which BDE28 and BDE49 have vicinal H-atoms at the *orth-meta* and *meta-para* position, respectively, and are easily metabolized by seals. The BMF for α-HBCD is 0.3 indicating that HBCD is not biomagnified in seals, but is probably metabolized. Recently, we confirmed this hypothesis by the identification of a metabolite of HBCD, monohydroxy-HBCD, in seal blubber.

Acknowledgements
We want to acknowledge S. Brasseur (IMARES) for providing the seal samples. The authors gratefully acknowledge financial support by the European Commission under the project FIRE (QLRT-2001-00596).

References
1. De Boer J, Wester PG, van der Horst A, Leonards PEG. *Environ Poll* 2003; 122:63-74.
2. Hardy ML. *Chemosphere* 2002; 46:757-777.

3. Stapelton HM, Alaee M, Letcher RJ, Baker JR. *Environ Sci Technol* 2004; 38(1):112-119.
4. Brasseur, S., I. Tulp, P. Reijnders, C. Smit, E. Dijkman, J. Cremer, M. Kotterman, E. Meesters, 2004. Alterra-report 905.
5. Boon, J.P., van der Meer, J., Allchin, C.R., Law, R.J., Klunsoyr, J., Leonards, P.E.G., Spliid, H., StorrHansen, E., Mckenzie, E.C., Wells, D.E. *Arch Environ Contam Toxicol* 1997; 33 (3): 298-311.

Table 1: Estimated biomagnification factors (BMF) based on diet content, diet levels, and seal levels.

Compound	**BMF**
BDE28	0.1
BDE47	1.8
BDE49	0.1
BDE99	1.1
BDE100	0.7
BDE154	2.4
α-HBCD	0.3