LEVELS OF SOME POLYBROMINATED DIPHENYL ETHER (PBDE) FLAME RETARDANTS ALONG THE DUTCH COAST AS DERIVED FROM THEIR ACCUMULATION IN SPMDs AND BLUE MUSSELS (*MYTILUS EDULIS*).

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Introduction

Many plastics in household equipment, car interiors and textiles are impregnated with brominated flame retardants for safety reasons. In the Netherlands, 20% of all electronic equipment contained PBDEs or PBBs in 1995 [1]. PBDEs are applied as additive flame retardants, which are not chemically bound to the product matrix[2]. Any resulting emissions would thus be highly diffuse and therefore difficult to control. The most frequently used PBDE formulation (30,000 tons in 1992) is currently decabromodiphenylether (DeBDE); other formulations are pentabromodiphenylether (PeBDE; *e.g.* Bromkal 70-5DE), and octabromodiphenylether (OcBDE).

Like related organochlorines, the PBDEs are very hydrophobic ($\log K_{ow}$ values 4-10) and resistant to degradation [3]. The BDE congeners 47, 99 and 153 (numbering according to the IUPAC nomenclature for PCBs) showed even higher bioaccumulation factors as the comparably chlorinated PCBs in blue mussels, despite their larger molecular size [4]. These congeners and BDE 100 are also widespread in biota [5-8]. The presence of these PBDE congeners in deep-sea foraging animals as sperm whales indicates that they can be regarded as globally occurring chemicals [9].

Since the production figures for technical applications of the DeBDE formulation are becoming increasingly dominating over those of the lower brominated formulations, the bioaccumulation potential of the fully brominated BDE 209 is of special significance. Although the ability of rainbow trout (*Oncorhynchus mykiss*) to accumulate BDE 209 has recently been demonstrated experimentally, the uptake efficiency was very low[10]. Also the levels in sediments of the Swedish river Viskan were not reflected in fish from the river [3].

Biomonitoring with invertebrates is commonly used for identifying geographical and temporal trends, although the concentrations of the contaminants are not only determined by the exposure levels but also by internal processes such as the physiology of the organisms including biotransformation processes. To overcome these problems, the use of semipermeable membrane devices (SPMDs) has been developed to measure contaminant concentrations in water and air[11; 12]. SPMDs sample dissolved organic contaminants at rates that are linearly proportional to their aqueous concentration. The typical rates of 4-80 L d^{-1} depend mainly on the intensity of the flow near the membrane-water interface [13-15].

Because of their possible environmental risks the inclusion of certain PBDEs in monitoring programs is appropriate. The present study was focussed on their determination in SPMDs and in mussels (*Mytilus edulis*) exposed on buoys in the Western Scheldt estuary and along the Dutch North Sea coast. The PBDE levels in clean mussels transplanted to the Western Scheldt

were also compared to those in native animals.

Methods and materials

Low-Density PolyEthylene (LDPE) lay flat tubing (2.5 cm wide, wall thickness 70 µm) that contained no additives was pre-extracted two times by soaking overnight in pentane. After air-drying the membranes were heat-sealed at one side, and 0.3 mL triolein (Sigma, 95% purity) was added. After squeezing the air out as much as possible a second heat seal was made at a distance of 30 cm from the first seal. Mounting loops were made in the (triolein-free) LDPE ends by applying a third and forth heat-seal. A number of perdeuterated PAHs (acenaphtene-D10, phenanthrene-D10, chrysene-D12) and PCBs (CB 4, 29, 155 and 204) not occurring in the environment were added to the triolein phase prior to exposure as Performance Reference Compounds (PRCs) for estimation of the *in situ* sampling rates [15-17]. After exposure, the SPMDs were stored at -20 °C. Mussels were collected from the Eastern Scheldt, a brackish basin with low contamination levels.

Exposure: Two SPMDs and 100 mussels were exposed on buoys for 42 days in Jan./Feb./Mar. and in Oct./Nov. 1999 in the Scheldt estuary and at four locations along the Dutch coast. Immediately after sampling the mussels were stored on dry ice.

Two additional batches of native mussels were collected in order to compare their concentrations with those of the transplanted mussels and to estimate the contribution of particles in the intestine to the measured PBDE concentrations. Half of the mussels was immediately frozen, whereas the other half was allowed to depurate their gut contents in flowing sand-filtered sea water for 24 hours.

Extraction: Decabromobiphenyl (BB 209) was added as an internal standard. This compound could not be detected in a series of 150 samples from Dutch environments (Pers. comm. J. de Boer). The exposed SPMDs were extracted overnight with pentane. Sub samples (~ 4 g) of wet mussel homogenate were extracted with dichloromethane/ methanol/ water [18]. The lipid content ranged from 5 to 9% on a dry weight basis. Lipids were removed from the mussel extracts by passing the extract two to four times over silica columns (2 g deactivated with 6% water, elution with 20 mL pentane). The SPMD extracts were passed once over similar silica columns.

Analysis: The levels of 15 individual PBDEs were determined by GC/MS. The GC was a Hewlett Packard 6890; the mass-selective detector a Hewlett Packard 5973. GC specifications: split-splitless injection, split valve closed for 1.5 min. $T_{injector}$ 270°C. Column: stationary phase CP Sil-8, 25 m * 0.25 mm * 0.25 µm (Chrompack, NL). Carrier gas He; linear gas velocity 74 cm s^{-1}, constant flow programmed. Oven temperature program: 90°C (1.5') / 20°C min^{-1} / 190°C (0') / 4.5°C min^{-1} / 270°C (5') / 10°C min^{-1} / 320°C (10').MSD specifications: negative chemical ionization (NCI) in the SIM mode at the m/z ratios of both bromine isotopes (79 and 81) and m/z=487 (for BDE 209 only). Ionization gas CH_4. $T_{ion\ source}$ 210°C; $T_{transferline}$ 320°C; $T_{quadrupole}$ 160°C.

Results and discussion

The effective water sampling rate of the SPMDs was estimated as 16 L d^{-1} from the elimination data of the PRCs. The distribution of the aqueous PBDE concentrations is summarized in Fig. 1. Differences between the two sampling periods were a factor of 4-8 for BDE 209, but less than a factor of 1.7 for BDEs 47, 99 and 153. The concentrations of BDE 209 ranged from 0.1 (blank level) to 1 pg L^{-1} in February, and from 0.4 to 4 pg L^{-1} in October; the highest levels were measured at the most in-shore station in the Western Scheldt (Hansweert). The BDEs 47, 99 and 153 were more evenly distributed over the entire study area. Aqueous concentrations for these congeners were respectively about 1, 0.5 and 0.1 pg L^{-1} in both sampling periods.

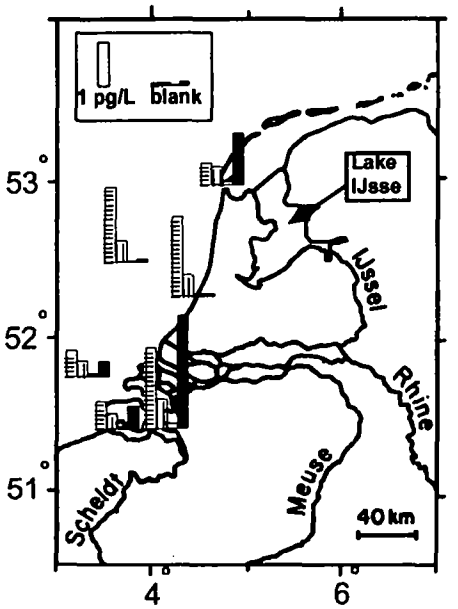

Fig 1 Aqueous concentrations of BDEs 47 (hatched horizontally), 99 (hatched vertically), 153 (white) and 209 (black) in January and October 1999.

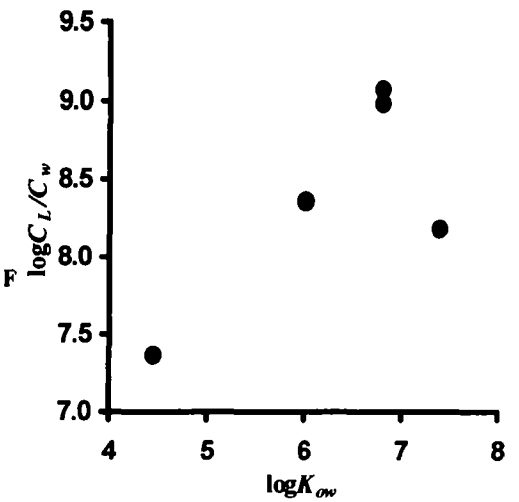

Fig. 2. BCF values (ml g^{-1} lipid) as a function of K_{ow} for BDEs 28, 47, 99, 100 and 153 for native mussels from the most in-shore station (Hansweert) in the Western Scheldt.

Without depuration, the BDE 209 levels in the mussels were completely dominated by the concentrations of the ingested particles, as evidenced by the drastic, although highly variable, decrease of this compound after 24 hr. depuration from 3350 to 50, or 1580 to 480 ng g^{-1} extractable lipid weight in two separate experiments. Thus, a useful bioconcentration factor (BCF) could not be calculated for BDE 209. In contrast, the concentrations of the other congeners were affected <5% by depuration. The BDE concentrations accumulated by native mussels at the most in-shore station in the Scheldt estuary, were higher by factors of 2-34 when compared with the levels attained by the transplanted (clean) mussels during the 42 days of exposure. This confirmed experimental data showing that the equilibration time scale for uptake of PBDEs is much larger than 42 days [4]. Therefore, the BCFs were calculated using the average aqueous concentrations from the two SPMD sampling periods and the lipid normalized concentrations in native mussels from this area. The BCF values attained a maximum of 10^9 ml g^{-1} lipid for the pentabromo BDEs 99 and 100, and started to decrease again for the hexabromo BDE 153 (Fig. 2). A 2,5-Br substitution of PBDEs on at least one ring always results in a maximum effective molecular cross-section (EMCS) of 9.6 Å [19], just above the value of 9.5 Å that was suggested to limit the uptake via biomembranes [20]. Thus, these field results show that the bioaccumulation of PBDEs cannot be fully explained by the EMCS of the different congeners, since the BDE congeners 99, 153 and 209 all have an identical EMCS of 9.6 Å. Molecular weight and water solubility/hydrophobicity are likely additional factors necessary to explain the measured BCFs in a better way.

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