POLYBROMINATED DIPHENYL ETHERS IN DAB (*Limanda limanda* L.) FROM THE NORTH AND BALTIC SEA

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Introduction

The OSPAR-Commission, who is implementing the Convention on the Protection of the Marine Environment of the North-Atlantic Ocean, has developed a Hazardous Substances Strategy (www.ospar.org) with the objective of preventing pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances. The ultimate aim is to achieve pollution levels in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances by the year 2020. OSPAR has identified 48 chemicals for priority action and has listed 325 further substances of possible concern. For most of these substances it is not known whether they reach the marine environment and in which concentrations they might occur in water, sediment and biota. Therefore, some of these chemicals including short- and medium-chain chlorinated paraffins, chlordanes, endosulfanes, chlorpyrifos, trifluralin, and polybrominated diphenyl ethers (PBDE) were monitored in marine samples from the North Sea and the Western Baltic Sea to assess the relevance of these substances to the marine environment. Preliminary results on PBDE levels in fish liver samples collected in 2002 indicated considerable within-species as well as geographical variation[1]. Here we report the results of a two year study of a total of 14 BDE congeners determined in individual as well as pooled liver samples of dab (*Limanda limanda* L.) collected at eight locations using fully optimised and validated GC-ECNI-MS and GC-MS methods. The objective was to evaluate within-species variability of PBDE content of samples collected at a particular site, to detect site-/source-related differences in PBDE levels and to study BDE congener patterns in marine fish species.

Materials and Methods

Fish samples were collected during the regular annual research cruises of the Institute of Fishery Ecology for monitoring hazardous substances and bio-effects in fish at eight locations in the North Sea and Western Baltic Sea during late August / beginning of September in 2003 and 2004 (Figure 1). The study area covered various sampling sites subjected to different pressures. N01 (German Bight) is influenced by discharges from the River Elbe, a former titanium dioxide dumping site, and heavy shipping traffic. N04 (Dogger Bank) revealed high incidence of fish diseases possibly due to discharges originating from industrial areas, rivers and dumping sites at the English East coast. N06 (Firth of Forth) is impacted by the industrial region of Edinburgh. N22 (South of Dogger Bank) is influenced by discharges from e.g. the River Humber and from industrial sites at the English East coast. N03 (Southern Bight) located offshore the Dutch cost represents a former incineration site and might be impacted by inflow of water from the Channel, the River Thames and the River Scheldt. P01 (Danfield) and P02 (Ekofisk) represent offshore oil and gas production facilities. B01, the only sampling site in the Baltic Sea is located in the Kiel Bight and influenced mainly by heavy shipping traffic. Fishes were caught using a standardised beam trawl of specified mesh size and aperture and were transferred to a steel tank in the ship. From there they were flushed out with sea water onto a moving belt where they were sorted according to species and length range. The selected fishes were transferred to buckets filled with sea water and taken to the laboratory clean-room. Female dab (*Limanda limanda* L.) of 200 to 250 mm length without obvious external fish diseases were selected, weighed and length recorded. Then, fishes were killed and dissected. Livers were taken from the fishes, wrapped in pre-cleaned alumina foil, immediately deep-frozen at -35°C, and stored at this temperature until analysis. Moreover, otoliths were removed for age determination.

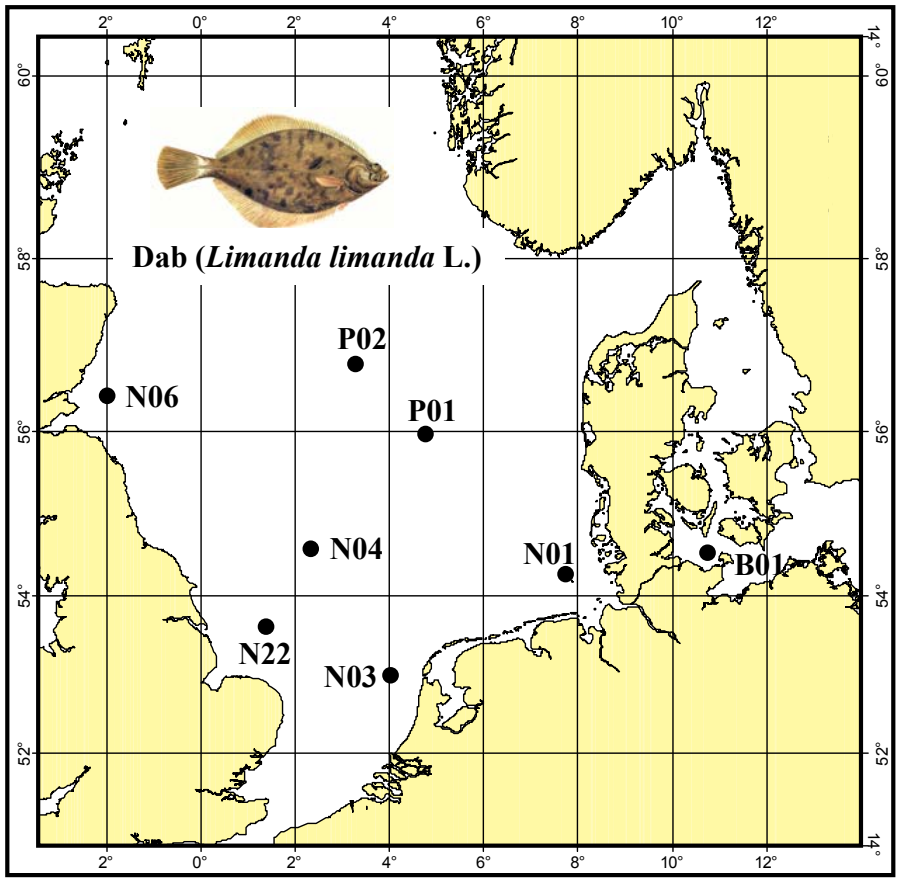

Figure 1. Sampling locations in the North and the Baltic Sea at which dab (*Limanda limanda* L.) were collected in 2003 and 2004. N01=German Bight, N03=Southern Bight, N04=Dogger Bank, N06=Outer Firth of Forth, N22= Southern Dogger Bank, P01= Danfield, oil and gas production area, P01= Ekofisk, oil and gas production area, B01= Kiel Bight

In 2003, 66 individual dab livers have been analysed, whilst in 2004, pooled samples consisting of 15 livers per site have been prepared and analysed in triplicate. Liver samples were freeze-dried and homogenized with pre-cleaned sea sand (extracted with toluene) in a mortar. An analytical procedure described previously has been applied with some modifications[2]. In brief, samples representing 0.2 to 1.0 g of lipid were extracted with toluene at 125 °C and 14 MPa using an ASE 200 accelerated solvent extractor (Dionex GmbH, Idstein, Germany) followed by gel permeation chromatography and multi-layer silica gel column chromatography clean-up. The purified extracts were evaporated to a final volume of 100 µl using an automatic evaporation device (flowtherm optocontrol, Barkey GmbH & Co. KG, Leopoldshoehe, Germany). Quantification of BDE28, BDE47, BDE66, BDE71, BDE75, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183, BDE190 and BDE209 was performed by high-resolution capillary gas chromatography-electron capture negative ionisation mass spectrometry (GC-ECNI-MS) and/or high-resolution capillary gas chromatography-electron ionisation mass spectrometry (GC-EI-MS) both in the selected ion monitoring mode under the following conditions: GC 6890+ / MSD 5973 (AGILENT, Palo Alto, CA, U.S.A.) equipped with autosampler MPS2 (CTC Analytics AG, Switzerland) and PTV injector CIS 4 plus (GERSTEL, Muelheim / Ruhr, Germany); capillary column: Rtx-CLPesticide (Restek, Bellefonte, PA, U.S.A.), 30 m x 25 mm, film thickness: 0.25 µm; pressure-pulse injection: 50 psi (0.8 min); injection volume: 2 µl; carrier gas: helium; CI ion source;

reagent gas: methane; EI: electron ionisation energy: 70 eV, ion source temperature: 230 °C. BDEs elute from the column under these conditions between 11 and 35 minutes. For congeners with three to seven bromine atoms the most prominent ions due to bromine at m/z = 79 and m/z = 81 were recorded, while for BDE209 the highly specific ions at m/z = 484.7 and m/z = 486.7 were monitored. Monofluorinated brominated diphenenyl ethers F-BDE28, F-BDE100 and F-BDE160, BDE181 and $^{13}C_{12}$-labelled BDE209 were used as internal standards. In case of GC-EI-MS detection $^{13}C_{12}$-labelled BDE28, BDE47, BDE99, BDE100, BDE153, BDE154 were used as internal standards. Quantification was done using the $(M\text{-}Br_2)^{+\cdot}$ or the molecular ion. Quality control included recovery tests, regular analyses of procedural blanks, blind replicate samples, spiked samples (standard addition) and the fish tissue certified reference material WMF-1 (Wellington Laboratories Inc., Guelph, Ontario, Canada.) as well as participation in international interlaboratory studies on the determination of PBDEs in biota (QUASIMEME). Standards were purchased from Chiron AS (Trondheim, Norway), Cambridge Isotope Laboratories Inc. (Andover, MA, U.S.A.) and Wellington Laboratories Inc. (Guelph, Ontario, Canada.), respectively.

Extractable lipids were determined gravimetrically using the toluene extracts obtained by ASE.

Pooled fish liver samples were analysed for the stable isotopes ^{15}N and ^{13}C at the Environmental Isotope Laboratory of the University of Waterloo, Canada, using a Micromass IsoChrom Continuous Flow Stable Isotope Mass Spectrometer coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108).

Results and Discussion

With the exception of BDE71, BDE138, and BDE190, all congeners analysed for were found at detectable concentrations at least in some of the samples. ΣBDE concentrations (tri- to hexabrominated congeners) in individual liver samples ranged from 2.0 to 42.2 ng/g wet weight (mean 4.7 to 18) corresponding to 7.2 to 115 ng/g lipid weight (mean 13.2-55.8). BDE levels varied by a factor of four to eight in individual female dabs (n=9 to 13), three to five years old belonging to the same narrow length range (20 to 25 cm) and collected at the same site. This high variability in BDE concentrations between fishes of the same species, sex, and length range indicates variable exposure of individual dabs to PBDEs and has to be taken into account when discussing geographic differences in PBDE levels based on a low number of samples and when planning monitoring programmes. BDE-patterns in dab livers collected at different sites were similar, with BDE47 as dominant congener followed by BDE100 and BDE154 (Table 1). BDE99 vulnerable to metabolism in freshwater fish[3] was found at much lower concentrations than it would be expected from its percentage composition in the technical penta-mix formulation. This suggests that there are similar metabolic pathways for this congener in marine fish species. The calculated concentration ratios of BDE100/BDE99 were between 3.2 and 4.3 for most locations and in agreement with observations of Voorspoels et al.[4] who reported similar BDE100/BDE99 ratios in dab from Belgium. Samples from the German Bight (N01) and Baltic Sea (B01) showed BDE100/BDE99 ratios of 1.6 and 0.5 that cannot be explained satisfactory for the time being. Analysis of pooled samples with each pool consisting of 15 livers revealed pronounced differences in liver BDE concentrations of dab from various locations (Table 1). Although the food of dab is variable with local conditions, it feeds on any benthic invertebrate abundant locally, which is small enough to be captured (small crustaceans, molluscs, polychaetes and worms), the differences seen in BDE concentrations in this study indicated geographical differences in contamination level. The highest BDE concentrations were found offshore the British coast (N04, N22) and in the central North Sea (P01) near a Danish oil and gas production area. Lowest BDE concentration were seen in dab from the Baltic Sea and the German Bight (B01, N01) although the latter location is impacted by discharges from the River Elbe and fairly polluted with other halogenated contaminants (unpublished results). This can be explained by the obligation of the German polymer industry to restrict the use of brominated flame retardants voluntarily, which has been in effect for many years. Reported BDE concentrations in dab liver from the Belgian North Sea and the Western Scheldt Estuary[4], a heavily polluted region, ranged from 2.8 to 18.6 ng/g wet weight with the highest concentration seen in a sample from estuary of the River Scheldt. The Tee estuary has previously been shown to be a major source of BDE congeners representing technical penta-mix formulation to the North Sea[5,6], but in the light of the data reported here the oil and gas production areas have also to be considered a possible major source of tri- to hexa BDE congeners to the central North Sea. Nitrogen stable isotope ratios $\delta^{15}N$,

which have been used widely as integrative measure of trophic position[7], vary from 9.5 to 15.2 ‰ (Table 1), a fairly wide range for one species, pointing at variable food chains. The highest value was seen in dab from the German Bight (N01) and indicated the longest food chain even though BDE concentrations were amongst the lowest. Dab samples from locations with high BDE contamination showed lower $\delta^{15}N$ values. These results provide further evidence that the differences in BDE levels seen between various locations are mainly site-related and not caused by differences in the underlying food chains.

Table1. BDE concentrations in liver tissue of dab (L*imanda limanda* L.) collected at eight locations in the North and Baltic Sea between 14 August and 22 September 2004 expressed in ng/g wet weight; CV% = coefficient of variation of triplicate analysis of pooled samples; 15 livers were pooled per sampling location; LOQ = limit of quantification; $\delta^{15}N = ([^{15}N/^{14}N_{sample}/ {}^{15}N/^{14}N_{standard}]-1)*1{,}000$.

Sampling Site	B01	N01	N03	N04	N06	N22	P01	P02
BDE28	0.05	0.07	0,14	0.17	0.10	0.16	0.13	0.05
BDE47	1.78	2.41	5.13	6.99	4.68	7.18	6.37	5.21
BDE66	0.07	0.08	0.14	0.25	0.14	0.23	0.19	0.12
BDE71	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE75	0.03	0.04	0.06	0.06	0.04	0.04	0.05	0.02
BDE85	0.03	0.05	0.05	0.04	0.03	0.05	0.05	0.03
BDE99	0.43	0.28	0.32	0.33	0.33	0.41	0.44	0.39
BDE100	0.23	0.44	1,11	1.27	1.16	1.30	1.78	1.70
BDE138	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE153	0.08	0.07	0.13	0.12	0.17	0.21	0.13	0.13
BDE154	0.14	0.17	0.44	0.37	0.48	0.58	0.45	0.24
BDE183	0.05	0.04	0.04	0.04	0.08	0.05	0.06	0.04
BDE190	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE209	<LOQ	<LOQ	<LOQ	0.05	<LOQ	<LOQ	<LOQ	<LOQ
Σ tri to hexaBDE	2.83	3.60	7.52	9.61	7.12	10.15	9.60	7.9
CV %	6.4	4.2	10.7	2.4	3.3	6.2	1.7	5.9
Lipid content (%)	23.5	32.1	35,2	33.0	25.9	20.4	33.1	10.8
$\delta^{15}N$ (‰)	11.88	15.15	13.83	10.20	11.09	12.58	11.18	9.47

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