

## IDENTIFICATION OF PAST AND NOVEL BROMINATED FLAME RETARDANTS IN COMMON SOLE (*Solea solea* L.) FROM COASTAL AREAS IN FRANCE

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### Abstract

The levels of brominated flame retardants (BFRs) in juvenile common sole were investigated in selected nursery zones along the French coastline. Extensive identification of BFRs was carried out, including the novel compounds bis(2,4,6-tribromophenoxy)ethane (BTBPE) and decabromodiphenylethane (DBDPE). Among the identified polybrominated diphenyl ethers (PBDEs), BDE-47 was the predominant congener, accounting for 29% to 47% of the sum of the 7 indicative BDEs. Compared to previously reported results for different fish species, BDE-99 was low in the studied soles, which could indicate a capacity of the fish to metabolize this congener. In addition, the identification of BDE-49 and BDE-140, which are reported to be degradation products of higher-brominated congeners, indicates that juvenile soles may also metabolize specific congeners to different extents. BTBPE and DBDPE, which are to be used in the replacement of penta- / octa-BDEs and deca-BDE respectively, were detected for the first time in French coastal areas. These data are of particular interest with respect to a need for further monitoring of these compounds in the environment. Other BFRs identified in this study and no longer in use were hexabromobenzene (HBB) and 2,2',4,4',5,5'-hexabromobiphenyl (BB-153) which were generally detected at low levels which are likely to decrease in the future.

### Introduction

The level of contamination and the spatial distribution of brominated flame retardants (BFRs) in the marine flatfish sole, *Solea solea* L., was investigated in selected areas of the French coastline located in the English Channel and the Atlantic Coast. The contamination by polybrominated diphenyl ethers (PBDEs) and other BFRs was determined in muscle samples of juvenile fish (1-group). The studied areas were important nursery zones for a variety of species including the common sole and were in areas that receive inputs of contaminants from human activities to various extents. Juvenile fish were chosen as they represent the most sensitive group to contamination and because of the fact that they are location limited to their nursery zone and are thus representative of the area in which they are sampled.

This study is part of a pluridisciplinary research project aimed to characterize the behaviour of persistent organic contaminants and their effects on the common sole. To the best of our knowledge, this paper presents the first results on the contamination of marine species by novel BFRs on the French coastline.

### Materials and methods

Juvenile fish (age group 1) were collected in 2003 and 2004 during the same period of the year (autumn) at 3 sampling areas representing main nursery zones: the Seine estuary in the English Channel, the Vilaine estuary and the Pertuis Breton in the Bay of Biscay on the Atlantic Coast (figure 1). Muscles were dissected from fish of homogeneous sizes (17-22 cm length) pooled by ten individuals.

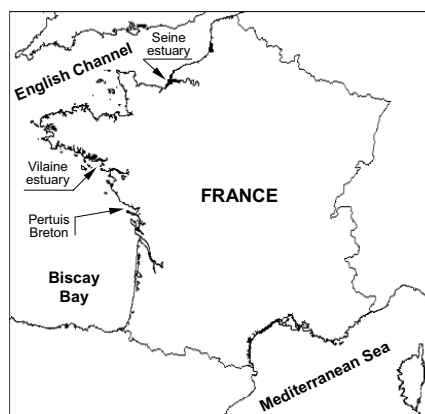


Figure 1 : Sampling locations of juvenile soles.

The chemical analyses of BFRs were conducted on 7 to 16 grams dry weight (dw) of muscle. The total amount of extractable lipids was determined gravimetrically in muscles in a separate extraction using a mix of acetone and hexane (80/20 v/v).

For the contaminant analysis, the tissue samples were homogenized, freeze-dried, and spiked with a solution of labelled recovery standards before extraction. Forty-two native compounds and twenty-three  $^{13}\text{C}$  labelled analogs in solution were obtained from Wellington Laboratories (Guelph, ON, Canada). The extraction and cleanup have been described previously<sup>2</sup>. After the initial cleanup steps on silica and alumina column and sulphuric acid treatment, the extracts were further fractionated using a high performance liquid chromatography (HPLC) system equipped with a nitrophenylpropylsilica column (Nucleosil, 5- $\mu\text{m}$  particles, 250 x 4.6 mm, Interchim, France).

Analyses of BFRs were performed by High Resolution Gas Chromatography - High Resolution Mass Spectrometry (HRGC-HRMS) with an AutoSpec Ultima (Micromass, Manchester, UK) operated in electronic impact (EI) ionisation mode at a minimum resolution of 10,000 in the selected ion monitoring (SIM) mode. The HRMS was interfaced with a Hewlett-Packard (Palo Alto, CA, USA) 6890 gas chromatograph installed with a 15 m DB1 column (J&W Scientific, CA, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness). The chromatographic conditions allowed the analysis of all compounds in 25 minutes in one run, and gave a good resolution for all compounds but BDE49 / BDE 71 and BDE 204 / BDE197 which were poorly resolved. Optimisation of separation of these compounds could be obtained using a longer 30 m column. In order to optimise the sensitivity and resolution conditions, BTBPE was analysed for its base peak (356.7948) in a single separate run. Due to overlap in some masses of  $^{13}\text{C}$ -BB153 and BDE154 when looking at the loss of 2 Br (for example 481.7388 and 481.6975), the identification and quantification of these two compounds was confirmed in another run, using different mass channels.

Quality Assurance / Quality Control procedures (blank determinations, analysis of certified material WMF-01 from Wellington laboratories) were included within every batch of eight samples. The laboratory has routinely participated in QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) intercomparison exercises. Samples were processed in the laboratory under clean low dust atmosphere and positive pressure. However, despite ultra-clean conditions, many congeners were still detected in the blank at various levels. Only results significantly different from the blank are presented in this paper.

## Results and discussion

Concentrations of PBDEs and other BFRs determined in muscle of the fish are presented in table 1 for samples collected in the Seine estuary, in the Vilaine estuary and in the Pertuis Breton. In this table, results for PBDEs are given for the sum of the seven indicative BDEs<sup>3</sup>, namely BDE-28, BDE-47, BDE-100, BDE-99, BDE-154, BDE-153, BDE-183, and for BDE-209. Other BFRs, namely HBB, BB-153, BTBPE and DBDPE are also reported. The concentrations were reported only if they were significantly different from the value determined in the procedural blank, i.e. 3 times higher than the blank.

In fish muscle, a relatively high variation in the lipid content was determined between different sites and years. For this reason, results were normalized to lipid weight (lw). The concentrations of hydrophobic organic contaminants such as PBDEs have been linked to lipid content by a positive relationship<sup>4</sup>. In these sole samples, the correlation between contaminant concentrations and lipid content was constantly positive and significant in most of the cases.

### *BDEs concentrations*

The concentration of the sum of 7 BDEs ranged from 4.05 ng g<sup>-1</sup> lw to 62.2 ng g<sup>-1</sup> lw in muscle. Although differences were detected between years, no clear evidence of a year-related tendency was observed. Sole samples from the Seine estuary present the highest concentrations for all congeners, followed by Pertuis Breton and Vilaine estuary, both from the Atlantic coast. High levels of contamination of different marine species collected in the Seine estuary by PBDEs has been reported by others<sup>5,6,7</sup>. Expressed in wet weight (ww), the levels found in soles ranged from 0.02 ng g<sup>-1</sup> ww in the Vilaine estuary (year 2004) to 1.49 ng g<sup>-1</sup> ww in the Seine estuary (year 2003) for the sum of 6 BDEs (without BDE-183). The levels found in the same species ranged from 0.1 to 0.7 ng g<sup>-1</sup> ww in the North Sea<sup>8</sup>, from 0.1 to 6.9 ng g<sup>-1</sup> ww in the Sheldt estuary, and from 0.2 to 1.1 ng g<sup>-1</sup> ww in the Loire estuary<sup>5</sup>.

	Seine estuary		Vilaine estuary		Pertuis Breton	
	2003	2004	2003	2004	2003	2004
% lipid	10.0	4.9	9.5	2.1	3.5	1.0
<b>BDEs</b> (ng g <sup>-1</sup> lw)						
Sum 7 BDEs	62.24	23.68	4.05	8.08	12.75	13.11
BDE-209	1.76	< blank	0.73	< blank	< blank	< blank
<b>Other BFRs</b> (ng g <sup>-1</sup> lw)						
BTBPE	2.22	< blank	0.12	0.24	0.61	< blank
DBDPE	0.18	1.15	0.19	2.30	0.25	3.90
HBB	0.21	0.64	0.03	0.50	0.19	4.30
BB-153	1.10	0.62	0.03	0.10	0.05	0.10

Table 1: Concentrations of BFRs in muscle of juvenile soles collected in the English Channel (Seine estuary) and the Biscay Bay (Vilaine estuary and Pertuis Breton). Concentrations are expressed in ng g<sup>-1</sup> lw.

BDE-209 was detected in all samples, but at levels that most often did not exceed the level found in the procedural blank. Concentrations significantly higher than in the blank (4 times) were 0.73 and 1.76 ng g<sup>-1</sup> lw at Vilaine estuary and Seine estuary respectively (blank unsubtracted). Despite its high molecular size, BDE-209 has been shown to be weakly bioavailable for biota. However, low levels are usually found because of low uptake and active metabolism occurring in fish, and its extent is species-related<sup>9,10</sup>. Furthermore, BDE-209 is more difficult to analyse and frequently exhibits high blank levels. Therefore, levels reported in the literature for this congener in biota are scarce. Voorspoels *et al.*<sup>8</sup> found this congener in fish liver from the North Sea and the Scheldt estuary at levels ranging from 3.4 to 37.2 ng g<sup>-1</sup> ww, while levels in fish from Chesapeake Bay and from Lake Winnipeg ranged from 2 to 50 ng g<sup>-1</sup> lw and 4 to 99 ng g<sup>-1</sup> lw respectively<sup>4</sup>, thus generally higher than the levels determined in our sole muscle samples.

Interestingly, other BDEs congeners, reported in the literature to be degradation products of higher-brominated congeners, were also detected in our samples. BDE-49, although poorly resolved from BDE-71 on the 15 m column, was detected in all samples. This congener has been identified as a degradation product of higher brominated congeners by reductive debromination<sup>11</sup>. BDE-140, identified by others as a metabolite of BDE209 in juvenile trout<sup>12</sup>, was also detected. Higher-masses congeners such as BDE-207 or BDE-208 were identified in our samples at low levels, although not always significantly different from those found in the procedural blank. However, both congeners may be degradation products of BDE-209 during gas chromatographic analysis<sup>4</sup>. Nevertheless, the degradation of BDE-209 during chromatography is believed to be very low, and can not solely explain the concentrations of BDE-208 determined in our samples. Further investigations are needed in order to explain their origin.

#### Congener profiles

Among the identified PBDEs, BDE-47 was the predominant congener in most samples, accounting for 29 % to 47 % of the sum of the 7 BDEs (figure 2). Compared to previously reported results for different fish species<sup>3</sup>, the levels of BDE-99 in most samples were low, accounting for 3 % to 10 % of the sum of the 7 BDEs. The ratio between BDE-99 and BDE-100 is indicative of the metabolic capacity of marine organisms<sup>8</sup>. In the soles studied here, the ratio 99:100 was between 10:90 and 30:70, which is similar to other studies for this species. The results obtained here could indicate a high degree of metabolism of BDE-99, known to be transformed to BDE-47<sup>13</sup>. However, the sample from Pertuis Breton in 2003 has a particular profile, with BDE-99 being the highest congener, and a ratio 99:100 of 70:30, which is closer to the ratio found in the technical mixture Bromkal 70-5 DE (86:14). On the other hand, BDE-154, which can degrade to BDE-99, or be the result of metabolism of BDE-183, was found at high levels in the samples (between 15 % and 36 % of the sum of the 7 BDEs). This would tend to indicate that juvenile soles might metabolize specific congeners to different extent (i.e. metabolism of BDE-99 and BDE-183 but not BDE-154). However, more specific experimental work is needed in order to better understand metabolism pathways of PBDEs in juvenile soles.

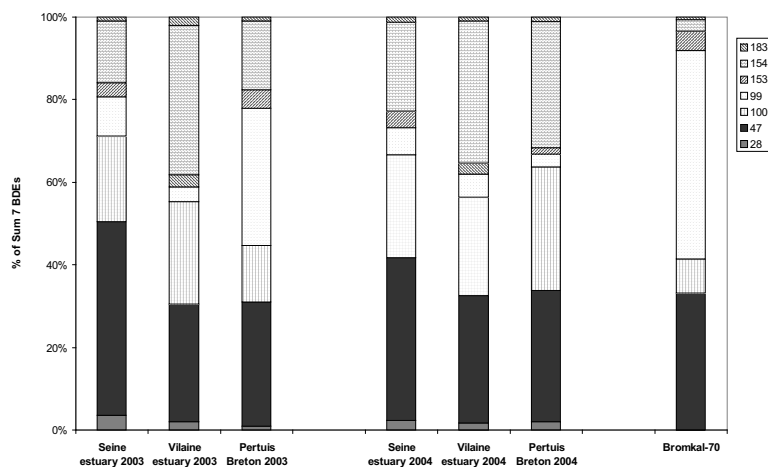


Figure 2: PBDEs profiles in muscle samples and in Bromkal 70-5 DE.

#### Other BFRs

Concentrations of other BFRs, namely BTBPE, DBDPE, HBB and BB-153 ranged from 0.12 to 2.22 ng g<sup>-1</sup> lw, 0.18 to 3.90 ng g<sup>-1</sup> lw, 0.03 to 4.30 ng g<sup>-1</sup> lw, and 0.03 to 1.10 ng g<sup>-1</sup> lw respectively. To our knowledge, these compounds have been scarcely identified in marine samples so far. BTBPE was firstly produced in the 1970s. It has been detected in indoor air samples in the United States and in sediment<sup>14</sup>. Concentrations in different fish species from Lake Winnipeg were between the method detection limit and 3.72 ng g<sup>-1</sup> lw<sup>4</sup>. The occurrence of this compound in the environment is likely to be increasing in the future as it will replace penta-BDE and octa-BDE products<sup>4, 14</sup>. DBDPE was introduced in the early 1990s and used as an alternative to BDE-209 in plastics and textiles. It has been identified in sewage sludge, sediment and indoor air<sup>15</sup> and more recently in Lake Winnipeg food web<sup>4</sup>, at levels closed to the method detection limit and up to 3.3 ng g<sup>-1</sup> lw in biota. HBB is another flame retardant which has been used in the past in plastics, textiles and woods. According to Szymanska *et al.*<sup>16</sup>, HBB was the most widely used of the polybromobenzenes. It has been identified in river and estuary sediments in Japan<sup>17</sup>. Probably because of the past usage of this compound, the levels reported here are low, except for the sample collected in 2004 in the Vilaine Bay. HBB was also identified at low levels in Bromkal 70-5 DE. BB-153, the main constituent of technical hexabromobiphenyl, was generally found at low levels compared to PBDEs, the higher levels being detected in samples from the Seine estuary. Like HBB, this compound was used in the past, but polybrominated biphenyls are no longer used in Europe. BB-153 was studied in human serum from the United States, showing a decreasing trend during 1985-2002<sup>18</sup>.

The results presented here provide reference values to survey the contamination of juvenile benthic fish by different BFRs. BTBPE and DBDPE were identified for the first time in samples from the French coasts. In addition to their present usage, these compounds will be utilized in the future as alternatives to other brominated compounds. They are thus of particular interest with respect to further monitoring in the environment. Other BFRs identified in this study, no longer in use (BB-153 and HBB), were generally detected at low levels, and are likely to decrease in the future.

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