PBDEs (POLYBROMINATED DIPHENYL ETHERS), HBCDs (HEXABROMOCYCLODODECANES) AND PFCs (PERFLUORINATED COMPOUNDS) IN MARINE SHELLFISH: LEVELS AND PATTERNS ALONG THE FRENCH COASTS

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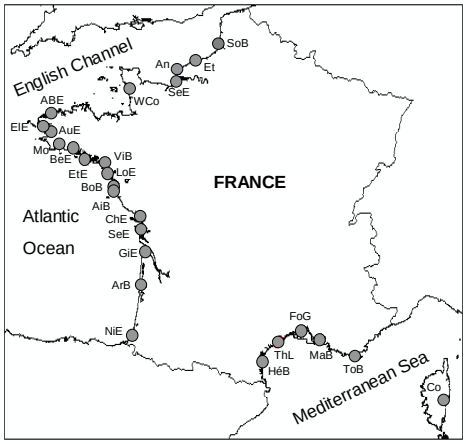
Introduction

The geographical distribution of the contamination levels of selected persistent organic contaminants was studied in marine shellfish (*Mytilus edulis*, *Mytilus galloprovincialis*, *Crassostrea gigas*) at selected sites along the French coastlines. The shellfish were obtained from specimens collected within the French Monitoring Network (Réseau national d'Observation de la Contamination CHimique -ROCCH), operated by IFREMER. In this study, samples collected in 2008 and 2010 were analysed for selected organohalogen contaminants, including the brominated flame retardants (BFRs) polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), and perfluorinated compounds (PFCs).

Materials and Methods

Sampling strategy

The analysed samples were chosen from selected sites located in the English Channel, the Atlantic and the Mediterranean coasts. The locations of the studied sites are shown in Figure 1. These sites cover main estuaries (Seine, Loire, Gironde), the Rhône delta and also smaller tributaries. To avoid possible differences of contaminant concentrations due to seasonal variations related to the physiological state of the shellfish, all samples were collected in the same way and at the same period of the year (from late November to early December). All samples were systematically depurated in filtered water for 24 hours before freezing. This allows a natural clearance of particles from the digestive tracts and mantle of the shellfish.



ATLANTIC
- ElE: Elorn Estuary
- AuE: Aulne Estuary
- Mo: Mousterlin
- BeE: Belon Estuary
- EtE: Etel Estuary
- ViB: Vilaine Bay
- LoE: Loire Estuary
- BoB: Bourgneuf Bay
- AiB: Aiguillon Bay
- ChE: Charente Estuary
- SeE: Seudre Estuary
- GiE: Gironde Estuary
- ArB: Arcachon Bay
- NiE: Nivelle Estuary

ENGLISH CHANNEL
- SoB: Somme Bay
- Va: Vaucottes
- An: Antifer
- SeE: Seine Estuary
- WCo: West Cotentin
- ABE: Aber Benoit Estuary

MEDITERRANEAN
- HéB: Hérault Bay
- ThL: Thau Lagoon
- FoG: Gulf of Fos
- MaB: Marseille Bay
- ToB: Toulon Bay
- Co:Corsica

Figure 1: Location of the study sites

Shellfish analysis

PBDEs were analysed at IFREMER's Laboratory. The analyses were conducted using approximately five grams (dry weight -dw) of the freeze-dried samples. The analytical protocols for extraction and cleanup have been described previously[1]. Samples were analysed for BDE-28, -47, -49, -66, -77, -85, -99, -100, -138, -153, -154, -183 and -209 using an Agilent 6890 series gas chromatograph coupled to an Agilent 5973 quadrupole mass spectrometer in electron capture negative ionisation mode (ECNI), using a DB-5-MS (J&W Scientific, CA)

capillary column (40 m x 0.18 mm i.d. x 0.18 µm film thickness). A DB-1 (J&W Scientific, CA) capillary column (15 m x 0.25 mm i.d. x 0.10 µm film thickness) was used to analyse BDE-209[1,2]. Quality Assurance / Quality Control procedures (blanks, analysis of replicates and certified materials) were included within every batch of six to eight samples. The laboratory also routinely participates in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) intercomparison exercises for PBDEs and obtains satisfactory results, i.e., Z scores between -2 and +2.

Analyses of HBCDs and PFCs were conducted at LABERCA's Laboratory. PFCs analyses were done by liquid solid extraction followed by purification using dispersive solid phase extraction with Envicarb stationary phase and hydrated silica column[3]. The purified extracts were separated using liquid chromatography (LC) equipped with a C18 reverse phase column (50 mm x 2.0 mm, 3 µm) fitted with a guard column (10 mm x 2.0 mm, 3 µm) and analysed by high resolution mass spectrometry (15 000 FWHM on m/z 400) operated in the negative ion mode (Thermo Electron model LTQ-Orbitrap hybrid MS system). HBCDs were extracted using pressurized solvent extraction and purified on silica column (500 mm x 25 mm) filled with anhydrous sodium sulfate (5 g), neutral silica gel (5 g), silica acidified with 22% concentrated sulphuric acid (20 g), silica acidified with 44% concentrated sulfuric acid (25 g) and sodium sulphate (5 g). HBCD stereoisomers were analyzed using reverse-phase LC (Hypersil Gold column, 100 mm × 2.1 mm, 1.9 µm), and determined by LC-MS-MS (Agilent 6410) fitted with an electrospray ion source, operating in the negative ion mode.

Results and Discussion

PBDE levels and patterns

Among the analysed congeners, BDE-28, -47, -49, -99, and -100 were detected in all samples. BDE-153 was detected in all samples but one, BDE-154 and BDE-183 in 74% and 17% of the samples, respectively. The concentrations reported below are expressed as the sum of these eight congeners, those below the limit of detection (LOD) being counted as zero. Median PBDE concentrations ranged from 0.2 ng g^{-1} dw to 4.4 ng g^{-1} dw (0.06-1.2 ng g^{-1} ww -wet weight). The maximum concentration was recorded at the Seine estuary site (SeE, Figure 1). This estuary is known to be the most contaminated in France, due to urban, domestic and industrial activities. Samples from the Atlantic coast exhibited the lowest concentrations, with a median value of 0.6 ng g^{-1} dw, while samples from the English Channel and the Mediterranean coast showed similar median concentrations (1.3 ng g^{-1} dw and 1.2 ng g^{-1} dw, respectively, Table 1).

PBDE contamination levels in shellfish from the French coasts were in the range of those recorded recently in other European countries. For example, concentrations of 0.1-0.2 ng g^{-1} ww, 0.06-0.25 ng g^{-1} ww, and 0.2-3 ng g^{-1} ww have been reported in mussels from the Netherlands[4], Norway[5] and the UK[6], respectively. These levels are lower than those recorded in the United States or in some Asian countries[7,8], where PBDEs were, or still are, used in higher quantities.

PBDE patterns showed the predominance of BDE-47, followed by BDE-99, BDE-49 and BDE-100. These four congeners represent about 90% of the sum of the eight congeners. BDE-47 is often reported as the predominant congener in biota[9]. BDE-49, present at levels higher than those of BDE-100, has previously been detected in marine species such as shellfish and other marine species[1,10]. In fish species, its presence has been related to the degradation of higher brominated BDEs, such as BDE-99[11,12]. Its origin in shellfish is not fully explained, as the metabolic capacity of shellfish is known to be low, and degradation of BDEs into BDE-49 has not been demonstrated. BDE-99 / BDE-100 ratio, used as an indicator of the metabolising capacity of organisms[13], was above 1 in our samples (i.e., median value of 1.6), indicating a low capacity of shellfish to metabolise BDE-99.

BDE-209 was also detected at levels above the procedural blanks in 87% of the samples. Only values three times above the value of the median blank were taken into consideration, and concentrations were corrected from the median blank value. BDE-209 identification in shellfish has sometimes been attributed to the presence of particles in the digestive tract or the mantle[14]. As our samples were depurated, we can reasonably assume that BDE-209 concentrations are representative of true bioaccumulation. With the above precautions taken into account, BDE-209 was detected at levels (above LOD) between 0.04 ng g^{-1} dw and 0.5 ng g^{-1} dw (0.01-0.12 ng g^{-1} ww) with a median value of 0.09 ng g^{-1} dw (0.02 ng g^{-1} ww), representing 9% of the sum of the 9 detected congeners. BDE-209 has previously been reported in mussels from Northern Europe at similar levels to those detected in our samples, i.e., at < 0.01-0.53 ng g^{-1} ww (UK)[15], 0.12-0.46 ng g^{-1} ww (Norway)[4] and < 0.1-

0.8 ng g^{-1} ww (the Netherlands)[16]. In shellfish from the Spanish market, BDE-209 was found at < 0.06-395 pg g^{-1} ww, representing the second most abundant congener[17].

HBCDs

HBCDs are present in the technical mixture primarily as three stereoisomers, i.e., alpha-, beta- and gamma-HBCD, the latter being predominant. Among those three isomers, alpha-HBCD was predominant in the shellfish samples, accounting for 88% (median value) of the sum of three isomers. The predominance of alpha-HBCD in biota may be related to various uptake and selective metabolism depending on the isomers[18].

Alpha-HBCD concentrations (median values) were between 0.2 ng g^{-1} dw in the samples from the English Channel and the Atlantic coast, and 0.5 ng g^{-1} dw in samples from the Mediterranean coast (Table 1). Unlike that observed for PBDEs, samples from both the English Channel and the Atlantic coast exhibited lower median concentrations than those from the Mediterranean coast. However, the maximum concentration (1.7 ng g^{-1} dw) was recorded at the Nivelle estuary site (NIE, Figure 1), located on the Atlantic coast.

Data about HBCD contamination of the French marine coastal environment are very scarce. Our data reveal concentrations in shellfish in the 0.01-0.3 ng g^{-1} ww range (median of 0.06 ng g^{-1} ww), or 0.3–22.1 ng g^{-1} lw range (median of 3.3 ng g^{-1} lw). In the marine environment, most available data about HBCD contamination concern fish, which exhibit higher levels due to HBCD biomagnification along trophic networks[18]. HBCDs (sum of the three isomers) were found at 10-106 ng g^{-1} lw range[18] and <0.2-0.9 ng g^{-1} ww range[19] in mussels from Norway. In mussels from the Netherlands, total HBCDs ranged from < 0.1 to 0.9 ng g^{-1} ww, while mussels from the UK exhibited levels between 0.2 and 12 ng g^{-1} ww[4,6].

PFCs

Among the analysed PFCs, only PFOS (perfluorooctane sulfonate) was detected in all samples. The concentrations were in the 0.02-4.5 ng g^{-1} dw range (0.004-0.9 ng g^{-1} ww), with a median value of 0.4 ng g^{-1} dw (0.1 ng g^{-1} ww). The median concentrations were higher in samples collected in the English Channel, although the highest concentration (Table 1) was found in one sample collected in the Loire estuary on the Atlantic coast (LoE, Figure 1). The second most prevalent compound (50% of samples) was PFDA (perfluorodecanoic acid), detected at levels between 0.13-0.37 ng g^{-1} dw. Few studies report the presence of PFCs in shellfish from the marine environment. In Europe, high concentrations of PFOS have been reported (mean values from 63 to 80 ng g^{-1} ww) in mussels from north central Portuguese estuaries[20]. In the Mediterranean Sea, both PFOA (perfluorooctanoate) and PFOS were found to be below 1.2-2 ng g^{-1} in mussels, while PFOA was detected at 12-16 ng g^{-1} in clams[21]. Little accumulation was observed in transplanted mussels in estuarine areas from Northern Spain, with PFOS or PFOA at a concentration range between < LOD and 0.06 ng g^{-1} ww[22]. Five perfluorinated chemicals, including PFOS and PFOA, were below the LODs in caged oysters from the Ebro delta in Spain[23]. In Denmark, PFCs were below the detection limits (set between 0.2 and 1.4 ng g^{-1} ww, depending on the compounds) in blue mussels[24].

	ng g^{-1} dw	English Channel	Atlantic	Mediterranean
PBDEs	**Median**	1.3	0.6	1.2
	Max	4.4	2.0	1.9
	Min	0.7	0.2	0.6
HBCD	**Median**	0.2	0.2	0.5
	Max	1.1	1.7	1.0
	Min	0.1	0.03	0.3
PFOS	**Median**	0.6	0.4	0.2
	Max	2.4	4.5	0.8
	Min	0.04	0.1	0.02

Table 1: PBDE (sum of 8 congeners), HBCD (alpha-isomer) and PFOS concentrations (median, maximum, minimum in ng g^{-1} dw) in shellfish collected in 2008 or 2010 along the French coastlines

Data about the contamination of the French marine coastal environment by BFRs, and especially HBCDs, and PFCs are scarce. Further studies are needed to determine the temporal trends of these compounds on the French coastlines for previous decades. In order to achieve this, samples obtained from the French monitoring network

bank of archived samples will be further investigated.

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