BROMINATED CONTAMINANTS (PBDD/FS AND PBDES) IN SHELLFISH

[1]A Fernandes, [2]D N Mortimer, [2]M Gem, [3]P Dicks, [1]F Smith, [1]S White and [1]M Rose

[1] Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK.
[2] Food Standards Agency, Aviation House, 125 Kingsway, London, WC2B 6NH, UK
[3] Food Standards Agency Scotland, St Magnus House, 25 Guild Street, Aberdeen AB11 6NJ

Introduction

Bivalve shellfish are an excellent source of protein, with high levels of essential minerals, and are generally recommended by nutritionists and doctors as healthy foods. Scallops (*Pecten maximus*), Pacific and native oysters (*Crassostrea gigas and Ostrea edulis respectively*), cockles *(Cerastoderma edule)* and mussels (*Mytilus edulis*), are among the most popular bivalve species produced as food. Shellfish in general have a recognised potential for bio-accumulating contaminants and some species, such as mussels, have been commonly used as early indicators of local pollution. This is because bivalve shellfish feed by filtering nutrients from seawater and are unable to metabolise some contaminants that they ingest at the same time. The resulting bioaccumulation of persistent environmental contaminants such as brominated dioxins (PBDD/Fs) and brominated flame retardants (BFRs) is particularly relevant when species are used for food. However, there is very little information on the occurrence of these contaminants in food[1,2]. This is perhaps unsurprising given the relatively recent recognition of the global environmental distribution of these pollutants, and the difficulties in analytical accessibility[1].

Polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) are mass produced additive flame retardants (BFRs) used in a variety of plastics, textiles, surface coatings, foams and man made fibres. Their use has undoubtedly resulted in a reduction in human injury and fatality as a result of fires. However, the unrestricted application of these materials in the past has allowed diffusion of the contaminants into the environment during manufacture, use and disposal and this continues to happen. This release is evident from the occurrence of PBDEs in environmental compartments such as water, sediments and biota[2] and accompanies an increasing amount of evidence on potential detrimental human health effects[2-4]. Emerging toxicological data shows that PBDEs can cause liver and neurodevelopmental toxicity and affect thyroid hormone levels. In recent years the EU has carried out a comprehensive risk assessment under the Existing Substances Regulation (793/93/EEC) of commercial PBDE products. The outcome was a ban on the use of Penta-and OctaBDE since 2004, whilst deca-BDE remains under review until additional toxicological and occurrence data are finalised.

PBDD/Fs are formed inadvertently as by-products of incineration processes and have similar physico-chemical properties to their chlorinated analogues. They also originate from other anthropogenic sources such as the chemical manufacture of BFRs, e.g. PBDD/F are formed as by-products during the manufacture of PBDEs[2]. Studies[2,5] show that the incineration of products containing BFRs as well as thermolysis of BFR material such as PBDEs is an important source of PBDD/F emissions as are thermal processing procedures such as extrusion, moulding and recycling, and degradation. Recently, there have been reports that some tri- and tetra-brominated PBDD congeners[6,7] may be produced through biogenic formation in the marine environment. As the utilisation of BFRs continues to increase, a corresponding increase in PBDD/Fs levels can be expected. Studies on the toxicity of PBDD/Fs are limited but both, in vivo and in vitro studies demonstrate AhR agonist properties and dioxin-like effects[8,9]. Although there are a number of methods reported for the analysis of PBDEs, very few methods exist for the determination of PBDD/Fs[1,6,14].

A couple of recent studies in the UK[1,12] have investigated the occurrence of PBDD/Fs, PBDEs and PBBs (amongst other contaminants) in composite samples of shellfish and fish. The most abundant PBDE congeners detected were 28, 47, 49, 66, 99, 100, 153 and 154, and these were present in most or all of the samples analysed. PBBs were detected less frequently and more PBDF congeners, notably 2,3,8-T_3BDF and 2,3,7,8-

T_3BDF were detected than PBDDs. The data presented here complement these studies and provides further information on the occurrence of these contaminants in shellfish produced over a wider geographical area.

Sampling and Analysis

Individual sub-samples of mussels, Pacific oysters and scallops were collected from coastal locations around Scotland between January and March 2006. Further samples of Pacific and native oysters, mussels and cockles from England, Wales and Northern Ireland were collected during the latter half of 2006 to January 2007. The individual samples were shucked and the flesh obtained from this process was representatively pooled and homogenised to yield 60 analytical samples – 17 mussels, 19 oysters, 10 scallops and 4 cockles. As scallops are sometimes retailed as the adductor muscle alone, these samples were divided into separate adductor and gonad portions prior to pooling. The composite samples were freeze-dried and ground, and the resulting powders were thoroughly mixed before taking sub-samples for analysis. The following analytes were measured:
Polybrominated diphenylethers (PBDEs) - IUPAC numbers 17, **28, 47**, 49, 66, 71, 77, 85, **99**, 100, 119, 126, 138, **153, 154**, **183** and **209**. PBB congeners: IUPAC numbers 15, 49, **52**, **77**, 80, 101, **126**, 169, **153** and 209. Ten, 2,3,7,8-Bromo substituted PBDD/Fs – tetra to hepta substituted congeners as well as 2,3,7-TBDD and 2,3,8-TBDF. (Those for which ^{13}Carbon labeled standards were used as internal or sensitivity standards are shown in bold type and these also included 6 PBDD/F congeners). Robustly validated methodology, accredited to the ISO17025 standard, for the analysis of these contaminants has been detailed elsewhere[1,10]. Analytical measurement sets included blanks and reference materials, and data quality was ensured by continuous successful participation[1,10,13] in international inter-calibration exercises (e.g. Dioxins in Food, Quasimeme).

Results and Discussion

The large volume of data produced in this work precludes publication here; hence a summarised version of the results is presented in Table 1. Data for the PBDEs has been presented as the sum of the 17 congeners measured, but also includes detail on the most significant congeners in terms of occurrence and interest – BDE-47, BDE-99, BDE-100 and BDE-209. Data for the 10, 2378-Br substituted PBDD/Fs[1,10] have been condensed into TEQs using chlorinated dioxin WHO-TEFs$_{1998}$. The use of analogous chlorinated dioxin WHO-TEFs to estimate toxicity arising from PBDD/Fs and non-ortho PBBs is an interim measure[11] until reliable TEF values that cover all the congeners that show dioxin-like toxicity become available in the literature. However, it provides data to allow comparisons with other studies that have used this approach[1,12-14]. The other data included in Table 1 are levels of the tri-brominated PBDD/F compounds as these are not included in the TEF scheme. Data for PBBs is not presented here as values were very low (0.001µg/kg on average for ortho-substituted PBBs with BB-52 as the most dominant congener, and 0.01ng/kg on average for the non-ortho substituted PBBs). All data is presented as upper bound whole weight concentrations. In general, all of the samples showed the occurrence of all of the contaminant groups under investigation, with the exception of some PBDD and PBB congener groups (penta and hexa-brominated compounds) which were not detected.

PBDD/Fs were observed in all samples with lower brominated congeners generally predominating. In terms of WHO-TEQ, relatively higher values on average were observed in scallop gonad tissue (0.083ng/kg) than in oysters (0.031ng/kg and 0.038, for pacific and native oysters respectively), mussels (0.055ng/kg) and cockles (0.018ng/kg). However in terms of the occurrence of individual PBDD/Fs, mussels generally showed a more complete range of detectable congeners, particularly the PBDFs (Figure 1) and almost all samples showed the presence of tri-bromo dioxins and furans. In particular, 2,3,7-tribromo dioxin was the predominant congener in oysters, and native oysters (*Ostrea edulis*) showed relatively elevated levels (up to 14.5ng/kg whole weight). There have been reports of similar, high, tri-BDD levels in mussels from the Baltic sea by researchers in Sweden[6,7], who report that apart from anthropogenic activity, biogenic processes involving the dimerisation or biotransformation of precursor molecules such as brominated phenols and hydroxylated PBDEs may give rise to the formation of tri-BDD (and to a lesser extent tetra-BDD) congeners. It should particularly be noted that the concentrations of tri-bromo substituted PBDD/F congeners reported here have not been included in the summed TEQs, as there are no recognised analogous WHO-TEF values for tri-chloro substituted PCDD/Fs. In general,

the occurrence of PBDFs predominates that of PBDDs, reflecting the environmental distribution of these contaminants. Similarly, the relative concentrations of the flame retardants – PBBs (low levels) and PBDEs (higher levels) is consistent with the greater and more recent usage of PBDEs in the UK. The low levels of PBBs observed are likely to arise from long-range marine and aerial transport. A fuller description of congener distribution and correlation with likely sources for PBDEs and PBDD/Fs is given elsewhere[13].

PBDEs were positively detected in all the shellfish species, and although the relative distributions of the lower to mid brominated congeners (BDEs 47, 99, 100) measured in this study were very similar across the species, the distribution of the higher brominated congeners varied. This was particularly evident for BDE-209 with low levels observed in oysters, and higher levels in mussels and particularly in cockles. The two groupings of congeners can arise from the use of commercial mixtures such as "penta" and "deca" which is predominantly BDE-209. Analysis of the congener distribution also shows that the levels of BDE-47 correlate well (R> 0.95) with the total PBDE level for all species except cockles. As observed elsewhere, BDE-47 is the major component of "penta". On the other hand, BDE-209 only shows a strong correlation with mussels (R= 0.97). These distributions of BDE congeners within the species may be indicative of the differences in bio-accumulative potential between species, with mussels showing the potential to bio-accumulate both penta as well as deca. On average, higher concentrations of PBDEs were observed in mussels compared to the other species, with the lowest levels being observed in scallop adductor tissue. The congener distribution showing the predominance of BDEs 47, 99, 100 and, to a lesser extent, BDEs 154 and 209, is similar to that observed in other studies[1,2,12,13,15] and reflects the use of commercial mixtures. The occurrence of BDE 209 appears to be species selective with the highest values occurring almost exclusively in mussels and cockles.

The dietary intake of these contaminants as a result of shellfish consumption is currently under evaluation. An assessment of comparable data[1] for the samples collected in Scotland that form part of this study, found it unlikely that toxicity arising from these contaminants alone was of concern. However, a more complete evaluation of risk would require 2 additional sets of information - A; the contribution from other contaminants with a similar mode of action such as PCDD/Fs, PCBs, PCNs etc, and B; perhaps of greater relevance in the context of this work – the re-evaluation of the PBDD/F TEQ through the use of more appropriate TEFs, specific to PBDD/Fs, and including the contribution from the tri-brominated compounds.

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Table 1: Summary of contaminant concentrations in shellfish (whole weight basis)

		BDE-47 ng/g	BDE-99 ng/g	BDE-100 ng/g	BDE-209 ng/g	Sum of 17 PBDEs ng/g	237-T_3BDD pg/g	238-T_3BDF pg/g	PBDD/F TEQ pg/g
Pacific Oysters n=14	Mean	0.21	0.10	0.08	0.02	0.59	0.33	0.17	0.031
	Median	0.13	0.07	0.06	0.02	0.39	0.29	0.13	0.025
	Min	0.04	0.02	0.01	0.01	0.12	0.09	0.01	0.011
	Max	0.79	0.32	0.25	0.07	2.13	0.74	0.51	0.121
Native oysters n=5	Mean	0.18	0.06	0.07	0.01	0.47	6.13	0.03	0.038
	Median	0.10	0.05	0.04	0.01	0.34	5.30	0.03	0.033
	Min	0.04	0.02	0.02	<0.01	0.12	0.73	0.01	0.014
	Max	0.38	0.11	0.15	0.03	0.94	14.53	0.05	0.065
Mussels n=17	Mean	0.32	0.16	0.09	0.09	0.82	0.07	0.15	0.055
	Median	0.14	0.07	0.06	0.04	0.44	0.06	0.09	0.037
	Min	0.06	0.03	0.02	0.01	0.17	0.02	0.02	0.013
	Max	1.34	0.55	0.34	0.53	3.23	0.21	0.40	0.205
Scallops Gonad (adductor) n=10	Mean	0.12 (0.02)	0.09 (0.02)	0.05 (0.01)	0.02 (0.02)	0.42 (0.08)	0.13 (0.02)	0.25 (0.03)	0.083 (0.032)
	Median	0.07 (0.01)	0.05 (0.01)	0.03 (0.01)	0.02 (0.02)	0.27 (0.07)	0.11 (0.01)	0.25 (0.03)	0.056 (0.033)
	Min	0.03 (0.01)	0.02 (0.01)	0.01(<0.01)	0.01 (0.01)	0.12 (0.04)	0.09 (0.01)	0.10 (0.01)	0.04 (0.022)
	Max	0.39 (0.04)	0.32 (0.04)	0.11 (0.01)	0.04 (0.03)	1.25 (0.16)	0.28 (0.04)	0.44 (0.05)	0.233 (0.041)
Cockles n=4	Mean	0.04	0.03	0.02	0.10	0.23	0.02	0.01	0.018
	Median	0.03	0.03	0.02	0.07	0.23	0.01	0.01	0.019
	Min	0.01	0.01	<0.01	0.02	0.06	0.01	<0.01	0.010
	Max	0.07	0.06	0.04	0.22	0.40	0.04	0.02	0.026

Figure 1: Typical profile for lower brominated PBDD/Fs in mussels
(most co-eluting peaks fulfill acceptance criteria for positive identification as PBDD/Fs)