BROMINATED AND MIXED HALOGENATED DIOXINS AND FURANS (PBDD/FS) IN FOODS – A SIGNIFICANT CONTRIBUTION TO OVERALL DIOXIN-LIKE TOXICITY FOR SOME FOODS.

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Introduction

Relatively little is known about the environmental and toxicological significance of polybromodibenzo-p-dioxins (PBDDs), polybromodibenzofurans (PBDFs) and mixed bromochloro dibenzo-p-dioxins and dibenzofurans (PBrClDD/Fs). Chlorinated analogues have been studied intensively whereas brominated and mixed bromochloro analogues have been studied to a much smaller extent. Theoretically, there are a total of 5020 brominated, chlorinated or mixed bromochloro dibenzo-p-dioxin or furan congeners (Table 1) and an additional 9180 biphenyls with 1-10 halogen substituents. All of these compounds resist chemical transformations, have a low biological degradation rate, are lipophilic, stable and persistent (D'Silva et al, 2004).

Table 1: Number of PCDD/F, PBDD/F and PBrClDD/F congeners.

	Substitution :								
Compound	Mono	Di	Tri	Tetra	Penta	Hexa	Hepta	Octa	Total
PBDD	2	10	14	22	14	10	2	1	75
PBDF	4	16	28	38	28	16	4	1	135
PCDD	2	10	14	22	14	10	2	1	75
PCDF	4	16	28	38	28	16	4	1	135
PBrClDD	0	14	84	254	420	452	252	74	1550
PBrClDF	0	28	168	496	840	880	504	134	3050
					Grand Total PXDD/F congeners				5020

Formation

There are four main categories of formation; *thermal*, *chemical*, *photochemical* and *biological*. Thermal formation may be subdivided into '*de novo*' synthesis and '*formation from precursors*'. Evidence suggests that the addition of bromine to a furnace during combustion actually increases the amount of PCDD/Fs produced, whilst maintaining high levels of formation of PBDD/Fs and PBrClDD/Fs. After the incineration of waste containing BFRs it has been shown that mixed PBrClDD/Fs predominate, in particular dibromo-dichloro substituted dibenzofuran congeners. Chemical precursors such as brominated and chlorinated phenols or PBDEs can form halogenated dioxins and/or furans. These reactions often take place at temperatures between 250 and 500°C, usually on catalytically active surfaces, but sometimes spontaneously. PBDEs can contain PBDD/Fs as chemical impurities and can form PBDD/Fs during thermal processing of materials (such as extrusion, moulding and recycling) and by degradation within the temperature range 350–400°C. It has been shown that PBDD/Fs can also be formed during UV-irradiation of decabromodiphenyl ether (Olsman et al, 2002).

Recently, there have been reports that some lower brominated PBDD congeners (tri- tetra-) can be found in the marine environment and bio-accumulate in some marine species, such as shellfish (Malmvern et al. 2005; Haglund et al. 2007). These studies targeted the PBDD rather than PBDF molecules and the occurrence of specific di- and tri- PBDDs was recorded, with lower levels of tetrabrominated congeners. The authors suggested that this specific distribution originates from marine biogenic processes. The formation of individual PBDD congeners is thought to proceed through condensation reactions of naturally occurring bromophenols, mediated by the action of bromoperoxidases in the presence of bromide (Haglund et al. 2007). This formation pathway is likely to be governed by kinetic processes which would explain the relative abundances of the individual congeners.

Toxicity of PBDD/Fs and PBrClDD/Fs
Most investigations on dioxin emissions, environmental contamination and toxicology have focused on PCDD/Fs only, and no comprehensive knowledge on PBDD/Fs or PBrClDD/F exists. On an interim basis the World Health Organisation suggests that the current toxicity equivalency factors for PCDD/Fs should also be applied to PBDD/Fs (WHO, 1998).

Limited studies with PBDD/Fs have shown that all of the classic effects demonstrated for 2,3,7,8-T_4CDD and related PCDD/Fs – lethality, wasting, thymic atrophy, teratogenisis, reproductive effects, chloracne, immunotoxicity, enzyme induction, decrease in T4 and vitamin A and increased hepatic porphyrins – have been observed with PBDD/Fs. Binding affinities to the Ah receptor were varied but of a similar order to those of their chlorinated analogues. Experimentally derived potencies of PBDD/Fs and PBrClDD/Fs relative to 2,3,7,8-T_4CDD are summarised (Table 2). PBDD/Fs and PBrClDD/Fs have been shown to be equipotent or in some cases more potent than some of their chlorinated analogues; with 2,3-Dibromo-7,8-dichlorodibenzo-p-dioxin and 2,3,7,8-T_4BDD shown to elicit twice the toxic response of 2,3,7,8-T_4CDD (Table 2, with original references given in D'Silva et al, 2004).

PBDD/Fs and PBrClDD/Fs in the environment
PBDD/Fs and PBrClDD/Fs were not found, despite low LODs (<1 ppt) in a survey of salmon, osprey and human milk by Wiberg et al. (1992) and they were indicated but below the LOQ in carp in a study by Loganathan et al (1995). Watanabe et al. (2004) reported PBDD/Fs and monobromo PBrClDD/Fs in airborne dust in Osaka, Japan. PBDD/Fs and PBrClDD/Fs have also been detected in incineration fly ash in several studies, none of which have been congener specific to date. Indications were that lower halogenated congeners (X = 1 to 5) are most prevalent and of those found it appeared that mainly chloro substitution was found i.e. monobromo-polychloro and dibromo-polychloro compounds were evident with little evidence of the more fully bromo-substituted compounds. Generally the concentrations of PBDD/Fs and PBrClDD/Fs detected in the environment and in biota are very low. Many of the studies of PBDD/Fs and PBrClDD/Fs in the environment were conducted a number of years ago. Given the increasing levels of other bromine-containing molecules in the environment, such as PBDEs and other BFRs, it is not unreasonable to expect that levels PBDD/Fs and PBrClDD/Fs may also have increased.

PBDD/Fs and PBrClDD/Fs in food
The primary route of human exposure to dioxins and furans is from the diet. Although there is an increasing amount of data on PCDD/Fs that has been generated over the last couple of decades, there is very little on PBDD/Fs and PBrClDD/Fs. For this reason, combined with the impact of increased bromine use, we have investigated the presence of PBDD/Fs in foods over the last few years (Fernandes et al., 2008, 2009(a) and 2009 (b)) and recently have extended these investigations to include mixed PBrClDD/Fs and PBrClBs.

Methodology
A full description of the procedures used for the extraction and analysis has been previously reported for PBDD/Fs and PBBs (Fernandes et al., 2004; Fernandes et al., 2008). The method is based on that for chlorinated analogues and has been in place for many years, and has been verified by successful participation in many interlaboratory and proficiency testing exercises. In brief, samples were fortified with ^{13}C-labelled analogues of target compounds and exhaustively extracted using mixed organic solvents. PBDEs were chromatographically fractionated from the PBDD/Fs on activated carbon. The two fractions were further purified using adsorption chromatography on alumina. Analytical measurement was carried out using high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC-HRMS). Specific congeners of PBrClDD/Fs and PBrClBs that could be measured was limited by the availability of standards (Table 3).

Samples
PBDD/Fs in a variety of Irish food and in UK shellfish have been reported previously (Fernandes et al., 2008, 2009(a) and 2009 (b)).

Table 2: Toxicity of PBDD/Fs and PBrClDD/Fs relative to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). (from D'Silva et al. 2004)

PXDD/F Congener	TEF (Trout mortality)	Relative Potency (DR-CALUX)	Relative Potency (EROD)	Comparative Potency (EROD)	Relative Potency (AHH)	Relative Potency (EROD)	Relative Hydroxylapatite Receptor binding
2,3,7,8-Tetrachlorodibenzo-p-dioxin	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2-monobromodibenzo-p-dioxin					< 0.01		< 0.01
2,7 / 2,8-dibromodibenzo-p-dioxin					< 0.01	< 0.01	0.07
2,3,7-tribromodibenzo-p-dioxin	0.02				0.02	0.02	0.86
2,3,7,8-tetrabromodibenzo-p-dioxin	1.14 -2.54	0.54	0.65	1.00	0.14	0.34	0.67
2,4,6,8-tetrabromodibenzo-p-dioxin					0.01	< 0.01	0.01
1,3,7,8-tetrabromodibenzo-p-dioxin	0.01				< 0.01	< 0.01	0.50
1,2,3,7,8-pentabromodibenzo-p-dioxin	0.08 - 0.14	0.49	0.30		0.12	0.12	0.15
1,2,4,7,8-pentabromodibenzo-p-dioxin					0.02	< 0.01	0.06
1,2,3,4,7,8-hexabromodibenzo-p-dioxin	0.01	< 0.01					
2-bromo-3,7,8-trichlorodibenzo-p-dioxin	0.65	0.39	0.94		0.09	0.10	0.09
1,2-dibromo-7,8-dichlorodibenzo-p-dioxin							
2,3-dibromo-7,8-dichlorodibenzo-p-dioxin		0.72	0.69		1.83	1.46	0.68
2,8-dibromo-3,7-dichlorodibenzo-p-dioxin	0.68				0.11	0.15	2.24
1-bromo-2,3,7,8-tetrachlorodibenzo-p-dioxin		0.24	0.60				
2,7-dibromodibenzofuran							
2,3,7,8-tetrabromodibenzofuran	0.25	0.49	0.62				
1,2,3,7,8-pentabromodibenzofuran	0.04	0.41					
2,3,4,7,8-pentabromodibenzofuran	0.07	0.09	0.05				
1,2,3,4,7,8-hexabromodibenzofuran	< 0.01	0.02					
1,2,3,4,6,7,8-heptabromodibenzofuran		0.002					

Results and Discussion

In the absence of TEFs for PBDD/Fs, PBBs, PBrClDD/Fs and PBrClBs, values for chlorinated analogues have been used in line with the recommendation from WHO discussed above. There is evidence that some of these values are only approximations, and so this must be seen as indicative. Results are reported here on a fat weight basis.

PBDD/Fs were found in the majority of samples analysed as part of a survey of Irish food, apart from penta- and hexa-BDDs which were not found in any of the samples. The frequency of occurrence was lower than that observed for PBDEs in the same samples. As observed in other studies (Food Standards Agency 2006c; Fernandes et al. 2008, 2009(b)), PBDFs were more prevalent than PBDDs, as might be anticipated given the environmental occurrence and chemistry of formation. The most frequently found congeners were 2,3,7,8-TBDF, 23478-PBDF, 2,3,8-TBDF and 1,2,3,4,6,7,8-HpBDF of which the first two contributed most to the TEQ. This contrasts with PCDD/Fs in similar food matrices, where most 2,3,7,8-Cl-substituted PCDDs and PCDFs are usually present. As generally found for PBDEs, the highest levels and greatest frequency of occurrence was seen in samples of liver (range 0.18–3.49 ng kg^{-1} TEQ) which is consistent with the functionality of this organ in the processing of lipids. The lowest levels were observed for milk (range 0.09–0.30 ng kg^{-1} TEQ) and poultry fat (range 0.15–0.27 ng kg^{-1} TEQ), although eggs showed a higher frequency of occurrence. The TEQ values for PCDD/Fs were comparable to values recorded for similar foods in the last survey carried out for Irish food in 2004 (Food Safety Authority of Ireland 2004) with reported values of 0.08 – 0.62 ng kg^{-1} PCDD/F TEQ for fats, and 0.32–4.04 ng kg^{-1} PCDD/F TEQ for liver.

Table 3. PXDD/F and PXB analytes selected for measurement (including labelled internal and sensitivity standards)

Analyte	Configuration	Halogenation Level	Ion 1	Ion 2
DIOXINS				
	2-Br-7,8-Cl-DD	Tri	329.8850	331.8830
	2-Br-3,7,8-Cl-DD	Tetra	365.8431	367.8410
	2,3-Br-7,8-Cl-DD	Tetra	409.7935	411.7914
	1-Br-2,3,7,8-Cl-DD	Penta	399.8041	401.8021
	2-Br-1,3,7,8-Cl-DD	Penta	399.8041	401.8021
	2-Br-3,6,7,8,9-Cl-DD	Hexa	433.7652	435.7631
^{13}C LABELLED				
	8-Br-2,3-Cl-DF	Mixed Br/Cl	**325.9304**	**327.9283**
	2,3,7,8-TCDD	Cl dioxin	**331.9368**	**333.9339**
	3-Br-2,7,8-Cl-DF	Mixed Br/Cl	**361.8893**	**363.8864**
	2,3-Br-7,8-Cl-DD (IS)	Mixed Br/Cl	**421.8337**	**423.8308**
	1-Br-2,3,7,8-Cl-DD (IS)	Mixed Br/Cl	**411.8444**	**413.8423**
	4-Br-2,3,7,8-Cl-DF	Mixed Br/Cl	**393.8504**	**397.8474**
FURANS				
	2-Br-7,8-Cl-DF	Tri	313.8901	315.8881
	2-Br-6,7,8-Cl-DF	Tetra	349.8491	351.8461
	3-Br-2,7,8-Cl-DF	Tetra	349.8491	351.8461
	2,3-Br-7,8-Cl-DF	Tetra	393.7986	395.7956
	1-Br-2,3,7,8-Cl-DF	Penta	381.8122	385.8072
	4-Br-2,3,7,8-Cl-DF	Penta	381.8122	385.8072
	1,3-Br-2,7,8-Cl-DF	Penta	427.7596	429.7566
BIPHENYLS				
	4'-Br-3,3',4,5-Cl-B	PCB 126 Ana	369.8299	371.8279
	3,4-Br-3',4',5'-Cl-B	PCB 126 Ana	413.7793	415.7783
	3',4',5-Br-3,4-Cl-B	PCB 126 Ana	457.7297	459.7277
	4'-Br-2,3',4,5-Cl-B	PCB 118 Ana	369.8299	371.8279
	4'-Br-2,3,3',4,-ClB	PCB 105 Ana	369.8299	371.8279
	4'-Br-2,3,3',4,5-Cl-B	PCB 156 Ana	403.7909	405.7889
^{13}C LABELLED				
	4'-Br-3,3',4,5-Cl-B (IS)	**PCB 126 Ana**	**381.8701**	**383.8681**
	4'-Br-2,3',4,5-Cl-B (IS)	**PCB 118 Ana**	**381.8701**	**383.8681**
	4'-Br-2,3,3',4,5-Cl-B (IS)	**PCB 156 Ana**	**415.8312**	**417.8292**
SENSITIVITY STANDARDS				
	1,2,3,4 –TCDD (SS)	Tetra	**331.9368**	**333.9339**
	1,2,3,7,8,9-HxCDD (SS)	Hexa	**401.8559**	**403.8530**

Whilst concentrations of individual congeners varied with food type, the widespread occurrence in the relatively cleaner environment of Ireland underlines the ubiquity of these contaminants (Ireland generally shows food contamination levels below the European average for environmental contaminants such as dioxins and PCBs, except following specific contamination incidents). The foods of animal origin that were measured in the survey of Irish food were generally as expected; ie those likely to show the presence of these contaminants due to their lipophilicity.

PBDD/Fs were detected in most shellfish samples at varying concentrations depending on the species. The calculated TEQs were higher in scallop gonad than in mussels (0.083 ng/kg compared to 0.055 ng/kg whole weight, respectively), although mussels showed a more complete range of detectable congeners, particularly the PBDFs. Relatively lower TEQs were detected in the scallop adductor muscle and this may relate to the lower lipid content of the adductor. The contribution to TEQ was derived mainly from 2,3,7,8-TBDF, 2,3,4,7,8-PBDF and 2,3,7,8-TBDD.

This composition was similar to the TEQ distribution of PCDD/Fs where, for fish and shellfish, these congeners, along with 1,2,3,7,8-PCDD, are often the main contributors. Most samples in this study showed the presence of tri-bromo PBDD/Fs. In particular, 2,3,7-tribromo dioxin was the predominant congener in oysters, native oysters (Ostrea edulis) showing relatively elevated levels (up to 14.5 ng/kg whole weight).

The toxicology of the tribrominated PBDD/Fs is the subject of continuing international study, with available toxicological data indicating that these compounds elicit more potent biological responses than their chlorinated analogues. 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 TEF values for trichloro substituted PCDD/Fs. Moreover, although the levels of tri- and tetra- BDD/BDF congeners are elevated in comparison to the other 2,3,7,8-Br substituted compounds, they occur at a relatively low level in comparison to other non-laterally substituted tri- and tetra- congeners that were present in the samples. The greater abundance of these congeners (some are orders of magnitude higher in occurrence), merits further research, both in terms of toxicology (none is currently available), as well as occurrence in other marine and aquatic species, particularly those used as food. Although PBDD/Fs exposure in humans has been reported, data on the tri-brominated compounds was not available. However, apart from the levels reported in this work, tri-brominated compounds have also been detected in other foods, which makes it likely that humans are exposed to these compounds. Apart from 2,3,7-tribromoBDD, the occurrence of PBDFs generally predominates that of PBDDs, reflecting profiles observed for environmental samples, which in turn mirror the formation chemistry of these molecules through thermodynamic pathways that are a feature of combustion processes.

PBDE commercial mixtures are reported to be one of the major sources of PBDD/Fs (D'Silva et al. 2004), which occur as contaminants in the mixtures. Hayakawa et al. (2004) report a positive correlation between these two groups in atmospheric deposition. There is very little information on pathways for PBDD/Fs or the occurrence of these compounds in sediments or soils, but given the similarities in physical and chemical properties to the chlorinated dioxins, it is not unreasonable to expect similar transfer mechanisms, although the PBDD/Fs are more likely to suffer degradation due to the higher susceptibility of the C–Br bond. In these samples, apart from the set of cockles (which should be interpreted with care due to the low number of samples), there is poor correlation between concentrations of PBDD/F and PBDEs. This is perhaps unsurprising given the various processes of degradation, absorption, /metabolism and/or bio-accumulation that define the occurrence of both these sets of contaminants in shellfish.

Results from the UK shellfish survey showed congener profiles expected in relation to the hypothesis of biogenic formation (see above), and also significant levels of PBDFs. The congener profiles for the PBDFs (tri- to penta-brominated) had similarities to those observed for environmental samples, in that a wide range of PBDF congeners were observed. This congener profile suggests an origin from industrial activity and it is likely that the observed profile and levels are due to combustion processes, such as incineration. The predominance of PBDFs over PBDDs has also been observed in other studies involving combustion. Thus, it is likely that the PBDD/Fs observed in these samples reflect both types of sources, biogenically mediated (Haglund et al. 2007) as well as anthropogenic.

Results obtained from the analysis of PBrClDD/Fs and PBrClBs in common items of retail food confirm the presence of these contaminants, but a higher frequency of detection and relatively higher values of these contaminants were observed for samples of shellfish, fish and liver.

A notable observation was made from the high resolution ion chromatograms – in a manner similar to the chlorinated and brominated dioxin occurrence in some types of sample, the compounds selected for analysis do not occur in isolation. Other congeners are also observed for some samples – in particular shellfish, which are less able to metabolise some of the non-planar congeners.

Conclusion

The universal presence of PBDD/Fs, PBBs, PBrClDD/Fs and PBrClBs in foods has been observed. A general lack of data for foods restricts comparisons, and leaves the question of trends unanswered. This is particularly relevant given the recent increase in bromine and brominated compound usage over the last two decades, especially with respect to BFRs. The dioxin-like toxicity arising from the occurrence of these new compounds are lower than those reported for chlorinated dioxins, and estimates of dietary intake of these contaminants shows that it is unlikely that toxicity arising from these contaminants alone is of concern. However, the TEQ from PBDD/Fs, PBBs, PBrClDD/Fs and PBrClBs is an important contribution to the overall burden of human exposure to dioxin-like TEQ that results from PCDD/Fs, PCBs, PCNs and other similar halogenated aromatic compounds, and may be particularly significant for high consumers of some specific food groups. The exact magnitude of the contribution will remain indicative until a comprehensive set of TEFs for PBDD/Fs, PBBs, PBrClDD/Fs and PBrClBs including the contribution from the tri-substituted compounds, becomes available.

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