Investigation into the occurrence of existing and novel/emerging BFRs in food

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Glossary of Main Terms

Σ PBDD/F TEQ	Sum of WHO-TEQ for individual PBDD/PBDF congeners
BDE	Brominated Diphenylether
BFR	Brominated Flame Retardant
BTBPE	Bis(246-tribromophenoxy)ethane
DBDPE	Decabromodiphenylethane
GC-HRMS	Gas chromatography - high resolution mass spectrometry
HBB	Hexabromobenzene
HBCD	Hexabromocyclododecane
HPLC-MS/MS	LC-MS in multiple reaction monitoring mode
IUPAC	International Union of Pure and Applied Chemistry
LC-MS	High Pressure Liquid Chromatography - mass spectrometry
Organic Bromine	Bromine-containing organic compounds extracted using only organic solvents
PCB/PBB	Polychlorinated biphenyl/ Polybrominated biphenyl
PBDE	Polybrominated Diphenylether
PBDD/F	Polybrominated dibenzo-*p*-dioxin/ furan
PCDD/F	Polychlorinated dibenzo-*p*-dioxin/ furan
PTV	Programmed temperature vaporisation
RM	Reference Material
SCF	EU Scientific Committee on Food
TBBPA	Tetrabromo Bisphenol A
TEQ	Toxic equivalence
Total Bromine	Sum of organic/inorganic bromine extracted by digestion
WHO	World Health Organisation
%U	Percentage Uncertainty

Executive Summary

The aim of this study was to investigate the occurrence of existing and emerging (and novel) brominated flame retardant (BFR) chemicals in foods that are commonly consumed, as well as in a range of animal feedingstuff.

In the first instance, a mass balance approach was used in an attempt to identify candidate foods and animal feeds for further investigation of BFR content and particularly to identify specific samples for the presence of emerging BFRs. Just over 400 food and feed samples were measured for total bromine content. However, the amount of bromine detected in the samples was found to be orders of magnitude greater than the sum of the commonly occurring BFRs (polybrominated diphenyl ethers-PBDEs and hexabromocyclododecane-HBCD). A more targeted mass balance approach measuring organic bromine was applied to a subset of around 50 foods, but the organic bromine levels determined, were still too high. A sub-set of samples was therefore selected on the basis of previous experience on the occurrence of BFRs in foods, and using the likely environmental contamination pathways as a guide. This exercise yielded generally marine based foods – fish and shellfish, but also processed meat and offal. These foods were studied using targeted GC-MS scanning techniques to identify the presence of emerging BFR compounds.

The outputs from this study include data on total bromine content for the full set of food samples, PBDE and HBCD concentrations for >200 samples with the highest bromine contents, and the results of the GC-MS scans to identify emerging BFRs. The data on PBDE and HBCD concentrations is particularly timely following the recommendation (2014/118/EU) from the European Commission that requests member states to collect data on these contaminants prior to evaluation and risk assessment.

PBDEs occurred in practically all of the measured food and feed samples, in the range of 0.02 ng/g to 8.91 ng/g (0.11 ng/g to 9.63 ng/g for animal feeds) for the sum of the 17 measured congeners, with highest concentration ranges, and mean values being observed in fish, processed foods and fish feeds. As in previous studies α-HBCD remained the most frequently detected HBCD diastereomer. HBCD occurrence for both, food and animal feed ranged from <0.01 ng/g to 10.1 ng/g (α-HBCD in fish) and 0.66 ng/g (α-HBCD in fish feed).

This data will allow BFR exposure to the general population to be calculated and will contribute to the EU wide risk assessment on these contaminants. It also provides current occurrence data for commonly consumed foods and animal feeds.

1. Background to Study

Brominated flame retardants (BFRs) are used (either chemically bonded, or more commonly, as additives) in industrial and domestic polymeric materials, in order to delay the onset of fire. Historically, the most commonly used BFR was polybrominated diphenyl ether (PBDE) mixtures, but as concern about potential and reported toxicological effects have been studied and reported over the last 15 years or so, the three main commercial PBDE mixtures, Penta-BDE, Octa-BDE and Deca-BDE have been progressively banned from use in some parts of the world (notably the EU). Similarly, restrictions on use or bans are considered on other high volume BFRs such as hexa-bromocyclododecane (HBCD) due to its widespread environmental presence and potential toxicity, and restrictions on usage will apply in the EU from later in 2015. Despite bans or restricted use, PBDEs and HBCD continue to be found routinely in foods, and an investigation on current levels would be useful to evaluate any future declining trends.

It was inevitable that the industrial and commercial demand arising from the bans would be filled by an increasing number of alternative flame retardants in order to comply with fire safety regulations. Some of these more recently introduced BFRs such as hexabromobenzene (HBB), bis (2, 4, 6-tribromophenoxy) ethane (BTBPE) and decabromdiphenylethane (DBDPE) have already been the subjects of investigation and have been detected in the environment (Shi et al 2009, Korner et al 2011, Yang et al 2012) and in some foods (Fernandes et al 2010, Tlustos et al 2010). This is unsurprising, given that as additives, they have a greater potential for release, and also, they have similar properties to PBDEs (chemical stability arising from halogenated aromaticity and low aqueous solubility). Similarly, there are a number of other more recent BFR chemicals that have been introduced, and with limited resources, it would prove difficult to fully investigate all of these compounds. A more targeted approach would be to focus on those BFRs where some information on environmental persistence, bioavailability and toxicity was possible. Additionally, occurrence in biota or indeed food would provide further indication. The European Food Safety Authority (EFSA) has recently published an opinion (EFSA 2012) which takes many of these considerations into account. From the available toxicological data, EFSA identified two BFRs - tris(2,3-dibromopropyl) phosphate (TDBPP) and dibromoneopentyl glycol (DBNPG), as being genotoxic and carcinogenic, but with poor environmental stability and high chemical reactivity, these compounds are not expected to occur in foods.

Based on high overall persistence (>500 days), the potential for bioaccumulation and identification in the environment or food, a set of BFRs were identified:

- hexabromobenzene (HBB),

- 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE),
- 5,6-dibromo-1,10,11,12,13,13-hexachloro-11-tricyclo[8.2.1.02,9]tridecene(DBHCTD),
- 1,2,3,4,7,7-hexachloro-5-(2,3,4,5-tetrabromophenyl)-bicyclo[2.2.1]hept-2-ene (HCTBPH),
- pentabromotoluene (PBT),
- pentabromobenzyl acrylate (PBB-Acr),
- pentabromoethylbenzene (PBEB)
- and 1,2,4,5-tetrabromo-3,6-dimethylbenzene (TBX)
- decabromodiphenyl ethane (DBDPE)
- octabromotrimethylphenyl indane (OBTMPI)

A further two BFRs N,N'-ethylenebis(tetrabromophthalimide) (EBTEBPI) and hexabromocyclodecane (HBCYD), with high persistence and bioaccumulation potential have not been identified in the environment or in food. It was also noted that although the modeling used by EFSA did not indicate high bioaccumulation potential, decabromodiphenyl ethane (DBDPE) and octabromotrimethylphenyl indane (OBTMPI) were likely to be bio-accumulative, because their molecular mass was not high enough to prevent bioavailability

It was also noted that chemically, other BFRs such as benzylbromides, brominated anhydrides and brominated benzylchlorides are susceptible to hydrolysis and as such, are unlikely to persist in the environment or transfer to foods (EFSA opinion 2012). Additionally as with other mass produced chemicals, the occurrence of BFRs in food is also influenced by environmental persistence, proximity to areas of high usage and disposal, and in the case of foods of animal origin, uptake and metabolism rates.

This project had 3 main aims – to measure the bromine content of foods and animal feeds, measure the concentrations of known and commonly occurring BFRs in approximately half of these sample and to further investigate a sub-set of these samples which showed the greatest discrepancies between the total bromine and the sum of the known BFRs, for the presence of emerging and novel BFRs compounds. These aims support the FSA's chemical safety programme, by investigating new industrial chemicals of concern with a view to identifying their occurrence in food. The data on PBDEs and HBCDs will also allow a refinement of existing risk assessments on the potential risk to consumers through dietary intake, and the identification of any emerging or novel BFRs will allow prioritization for further studies on food occurrence. This strategy is also in line with EFSA's recommendations (in their most recent opinion on BFRs) to investigate the occurrence of emerging and novel BFRs in food.

2. Experimental

2.1 Sample Collection and Preparation

Just over 400 samples of food (297) and animal feed (105) were collected during 2013. The food samples comprised a wide range of commonly consumed foods, the principle groups of which were eggs, processed meats, poultry and carcase meats, milk and dairy products, offals, fish and shellfish, processed foods and others including fruit, vegetable and bread. The animal feeds were mainly fish feeds, compound feeds for dairy, cattle, sheep, poultry and pigs, oilseeds, legumes, grasses, cereals and cereal by-products. Samples were generally sourced from retail outlets or commercially available in the case of the feeds. Most of the food samples were collected by Ventress Technical Limited and the feeds were supplied by ADAS UK Ltd. An overview of the samples analysed for Total Bromine (all) and PBDEs and HBCD is given in Table 2.1.

On receipt at the laboratory each sample was given a unique laboratory reference number and the sample details were logged into a database using LIMS. The samples were stored under appropriate conditions (most food samples were frozen) prior to analysis. Sample preparation for animal feeds consisted of homogenisation by milling and blending as required, to provide particle sizes of <1 mm. Some food samples were processed to isolate edible portions (in the case of shellfish and fish or meat on the bone) followed by grinding and thorough homogenisation. Where required food samples were then freeze-dried. The resulting powders were re-homogenised and aliquots used for analysis of bromine, followed by PBDE and HBCD analysis.

2.2 Measurands – Specific Analytes

Total bromine was measured in all samples; organic bromine was measured in a smaller subset of 47 samples.

The following BFRs were targeted for quantitative analysis in 207 samples:

PBDE congeners: 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, 101, 126, 169,153 and 209.

α-HBCD, β-HBCD and γ- HBCD, PeBCD and TBBPA.

A sub-set of 30 samples was screened by GC-MS (full scan) for the presence of emerging and novel BFR compounds.

2.3 Total and Organic Bromine (bromine extracted using organic solvents) – Analytical Methodology

An aliquot of 0.5g of the homogenised and freeze dried sample was used for the determination of total bromine. This was digested in tetramethyl ammonium hydroxide solution (TMAH) at 80^0 C in a heating block for four hours with regular mixing. The final digests were centrifuged to remove any suspended particulates and made up to volume with deionised water. These were then subjected to a higher dilution of 1:5 digest liquor to measurement diluent, which was an alkaline solution, containing Scandium and Antimony as internal standards. The dilutions were analysed by Inductively Couple Plasma – MS (ICP-MS) for Bromine with a set of matrix matched calibration standards using an Agilent 7700 ICP-MS with collision cell.

For the determination of organic bromine, 0.5g of the homogenised and freeze-dried sample was shaken with a 60:40 Hexane/Dichloromethane mix for 0.5 hour and allowed to stand overnight, in order to extract oils and fat from the sample material. The extraction solvent was decanted off, filtered using Glass fibre filter papers and retained. This process was repeated. The composite resultant extract was blown down using a Nitrogen concentration block and mild heating to take the extract to incipient dryness. Methanol and water mix was added to keep the oils and fats extracted in an aqueous solution. The extracts were then digested in a heating block withTMAH solution. The final digests were made up to volume with water and diluted 1:1 with an alkaline solution containing Scandium and Antimony as internal standard. The dilutions were analysed by ICP-MS for Bromine with a set of matrix matched calibration standards using an Agilent 7700 ICP-MS with collision cell.

2.4 PBDEs and PBBs - Analytical Methodology

The method used for the preparation, extraction and analysis of samples has been reported previously (Fernandes et al 2004, 2008) and is part of the combined analysis for dioxins, PCBs and PBDEs/PBBs. In brief, samples were fortified with ^{13}C-labelled analogues of target compounds and exhaustively extracted using mixed organic solvents. As dioxin analysis was not required for this determination, no activated carbon fractionation was required and the extracts were purified using adsorption chromatography on alumina. Analytical measurement was carried out using high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) at 10,000 res.

The analytical process is UKAS accredited to ISO 17025 standards, with the inclusion of an in-house reference material and method blanks which were evaluated prior to quantitation and reporting. Further quality assurance measures included the successful participation in international inter-comparison exercises on PBDEs such as Dioxins in Food-2013, Dioxins in Food-2014 and

the EURL PT exercise on PBDEs and HBCD, held in 2014. Quality control evaluation for the accompanying data also follows the criteria described for chlorinated dioxins and PCBs (Commission Regulation (EU) No 252/2012) as applicable.

2.5 HBCD - Analytical Methodology

As reported previously (Harmer et al 2007) the analyses were carried out in duplicate and additionally with an overspike. Sample aliquots including a procedural blank and a reference material were fortified with ^{13}Carbon labelled analogues of each of the 4 analytes (TBBPA, αHBCD, βHBCD, and γHBCD), allowed to stabilise, blended with hexane:dichloromethane, 60:40 (v/v) and the matrix was hydrolysed using acid modified silica. The extract recovered from this process was filtered, washed, concentrated and solvent exchanged to a methanol:water solvent system prior to analysis by HPLC-MS/MS in the multiple reaction monitoring (MRM) mode.

The parameters used for evaluating data quality, were similar to those used for PBDE analyses. Method limits of detection, evaluated through method blank determinations were typically <0.01 µg/kg whole weight and analytical recoveries were generally within the range of 50 - 120%. There are no certified reference materials available for HBCD or TBBPA analysis in food matrices. However, aliquots of all the samples analysed were fortified with native analytes and the concentrations of recovered analytes measured, were in good agreement with fortification levels. Additionally a fortified in-house reference material was also analysed regularly with the samples and returned values that were in good agreement with fortification levels. Fera also successfully participated in the EURL PT exercise which included HBCD, in 2014.

The presence of pentabromocyclododecene (PBCD) which can occur as a metabolite or degradation product of HBCD was investigated using the above methodology, but the absence of a full set of standards (there is only a single native congener and no ^{13}C surrogates) for this analyte make this measurement qualitative/semi-quantitative.

2.6 Emerging and Novel BFRs – Screening Methodology.

Aliquots of the freeze-dried samples (3 g) were extracted by Ultraturrax blending with dichloromethane:hexane (4:6, 200 mL) and 80 g of sulphuric acid modified silica. The mixture was filtered through silanised glass wool and further washed with 50 mL of the extraction solvent. Finally, extracts were blown down to incipient dryness under nitrogen and reconstituted with ethyl acetate (100 µL).

All extracts (30) were analysed by GC-MS using an Agilent 6890 gas chromatograph (Palo Alto, California, USA) coupled with an Agilent 5973 inert mass selective detector. The MS was operated in electron impact and full scan mode (m/z 40 to 800). Sample extracts were also analysed by gas chromatography - time of flight mass spectrometry (GC-TOF-MS) using automatic tube exchange (ATEX) injection and negative chemical ionisation (NCI - in full scan mode - m/z 50 to 1000, using methane as reagent gas), and a set of samples were investigated in full scan Electron Ionisation (EI) mode as well.

Splitless injection of extracts was carried out onto a ZB-5MS column (15 m x 0.25 mm i.d., 0.25 µm film thickness; Phenomenex, USA) using the following temperature gradient: 100^{o}C (held for 3 minutes) and ramped at 10^{o}C/minute to 325^{o}C (held for 5 minutes). Then injector was kept at 280^{o}C and helium was used as carrier gas at a constant flow of 1 mL/min.

Following GC-MS analysis the generated data were processed using the 'Unknowns' software by Agilent which employs algorithms to automatically identify all the detectable substances or molecular features in GC-MS data even when analysing very complex mixtures. The software generates a list of molecular features and all the related ions of a molecular feature (isotopes, charge states) are grouped together and areas of noise are removed. Once the data has been deconvoluted, filtered and sorted, the mass spectra extracted for each peak can be compared to spectra from a National Institute of Standards and Technology (NIST) library containing over 100,000 standardised electron impact mass spectra with associated matching scores, and tentative identification can be proposed.

Following this, the GC-MS data was also screened for masses corresponding to known emerging and novel BFR compounds.

Table 2.1: Overview of Samples

Animal Feed Type	Fish Feeds	Composite Feeds	Oilseeds/Legumes	Cereals & Byproducts	Grasses
Number of Samples **(Total bromine analysis)**	27	38	21	16	3
Subset Analysed for PBDE & HBCD (& GC-MS scan)	13 (2)	18	10	8	2
Food Type	Eggs	Fish	Processed Meat	Dairy Products	Milk
Number of Samples **(Total bromine analysis)**	44	40	37	27	8
Subset Analysed for PBDE & HBCD (& GC-MS scan)	21 (2)	36 (10)	17 (2)	13	3
Food Type	Shellfish	Meat (inc. poultry & game)	Offal	Processed Foods	Other Foods
Number of Samples **(Total bromine analysis)**	44	29	42	16	10
Subset Analysed for PBDE & HBCD (& GC-MS scan)	20 (5)	14 (2)	20 (5)	7	5 (2)

3. Results and Discussion

As the volume of data generated for this project is very large, it has been presented in Annexe A. Summaries of the data for total bromine, organic bromine, PBDE and HBCD occurrence are given in Tables 3.1 to 3.4. In total, 402 samples were collected and these were all measured for total bromine and a smaller sub-set of 47 samples were measured for organic bromine (Tables 3.1 and 3.2, and Annexe A). 207 samples were measured for PBDE and HBCD (& TBBPA) occurrence (Annexe A) and descriptive statistics for these analytes are summarised by food group in Tables 3.3 and 3.4. Concentration units reflect current convention as reported in recent literature for these contaminants. Concentration data for PBDEs and HBCD in the summary tables is provided on an upper bound whole weight basis. The Annexe provides data for PBDEs and HBCD on both, a whole as well as fat weight basis. The reporting limits (quoted as "<") for most analytes are estimated as a dynamic parameter incorporating current method blanks and are therefore the limits of determination that prevail during measurement. Data on the reference materials that were analysed for PBDEs concurrently with the samples are also presented in Annexe A, along with other QA data for HBCD. In general the results of analysis of reference materials for PBDEs and HBCD were within established acceptable limits.

The data on total bromine content (Table 3.1) on food reveals a concentration range of <0.01 µg/g for processed foods (specifically rapeseed oil) to 418 µg/g for shellfish (winkles). In general, the mean values for the animal feed groups reflect the occurrence range more consistently than they do for food. Higher concentrations occur in foods and feeds of marine origin – fish, shellfish and fish feed. This is unsurprising as bromide occurs in relatively high concentration in seawater (around 65 ppm) and this is likely to influence the levels in seafood. The bromine contents recorded for individual foods are not directly comparable to an earlier study (Owen et al, 2000) on the bromine content of total diet samples, but there is reasonable correlation between the data for the various food groups, except for milk which appears to show a relatively high total bromine content in the present study (37 µg/g on average). The highest mean value (67.5 µg/g) for the animal feed occurred in the grasses, although this value may not be representative due to the limited number of samples (n=3) and the strong influence of the concentration of a single high value (which was 3-4 times the level of the other two samples). The range of occurrence in animal feed was 0.62 µg/g (oilseed rape) to133 µg/g (Lucerne pellets).

Organic bromine content data is presented in Table 3.2, for a selection of foods and animal feeds. It is practically difficult to determine organic bromine and there is little or no data on organic bromine content in foods, in the open literature. This parameter was determined in order to see if there might be a closer relationship between this value and the sum of the concentrations of the

most commonly used BFRs (PBDE and HBCD). The determination was carried out by ICP-MS on food and feed samples initially extracted with organic solvents and then digested as per the procedure for total bromine determination. There was very good correlation for shellfish and fish feed (R^2 = 0.9999 and 0.95 respectively) between total and organic bromine, but poor correlation for all other food and feed groups (although the R^2 value for the 'Other Foods' group was 0.999, the data set, particularly for the organic bromine, consisted of a number of values below the LOD so the data is likely to be fortuitously correlated).

PBDEs occurred in all feeds and foods (apart from a sample of skimmed goat's milk), most notably in fish and shellfish where most congeners were detected (Annexe A and summarised in Table 3.3). Similarly, among the animal feed samples, the widest range of PBDE congeners were observed in samples of fish feed. As observed in other studies (Tlustos et al 2008, Fernandes et al. 2009, 2014, Schecter et al 2010) the most abundant and frequently occurring congeners were BDE 47, BDE 99, BDE 100 BDE 209, and to a lesser extent BDE 49, BDE 153 and BDE 154. The occurrence in animal feed is similar, although BDE 209 is the most abundant congener with near-universal occurrence. This is probably due to the fact that feed samples show the effects of direct environmental impact (deposition on feeds of terrestrial origin and bioaccumulation in fish feeds), whereas many foods are subject to additional removal mechanisms such as metabolism and selective uptake (in foods of animal and marine origin). The relatively high frequency of BDE 209 occurrence may be attributable to the fact that it was the last of the PBDE commercial mixtures to be subjected to a ban on production and use. The concentrations (whole weight basis) ranged from 0.02 ng/g to 8.91 ng/g for the sum of the 17 measured congeners, with highest concentration ranges and mean values being observed in fish and processed foods (Table 3.3). The corresponding range for animal feeds is 0.11 ng/g to 9.63 ng/g for the sum of the 17 measured congeners. Fish feeds, composite feeds and grasses showed higher concentrations as compared to oilseed and cereal based feeds. PBDE concentration data on animal feed is scarce so comparison is difficult, but the data reported here for food is consistent with that reported in other studies (Food Standards Agency 2006A, Food Standards Agency 2006B, Tlustos et al 2008, Fernandes et al. 2009, 2014, Schecter et al 2010). Apart from fish, shellfish and fish feeds, PBBs which were also measured along with the PBDEs, were rarely detected in any of the other foods or feeds which is consistent with their low utilisation in the UK.

Although a widely used BFR, the frequency of detection for HBCD was lower in the food and animal feed samples that were measured for PBDEs (Annexe A). Fish, fish feed, shellfish, and processed meats showed the most frequent detections and also the highest concentrations. As in previous studies, alpha-HBCD remained the most frequently detected HBCD diastereomer (Food

Standards Agency 2006A, Food Standards Agency 2006B, Fernandes et al 2008). Although rarely detected in foods (a few positive detections in shellfish and fish were observed), TBBPA was detected in some samples of composite feed. This could be due to the same reasons of direct environmental impact mentioned earlier for PBDEs, or it may be incorporated during the production process. HBCD occurrence for both, food and animal feed ranged from <0.01 ng/g to 10.1 ng/g (alpha-HBCD in fish) and 0.66 ng/g (alpha-HBCD in fish feed). As mentioned earlier, TBBPA was generally not detected at an LOD of 0.01 ng/g; where it did occur e.g. composite feeds, the occurrence ranged from 0.01 ng/g to 0.16 ng/g.

An attempt was made to estimate the concentrations of pentabromocyclododecene (PBCD), which the literature suggests, is a breakdown product or metabolite of HBCD. The estimated concentrations have been reported, but the data remains qualitative/semi-quantitative and only a few positive identifications were made in fish and shellfish, in a similar range as TBBPA.

In order to establish that the extraction procedure used for the investigation of emerging and novel BFRs was effective, four different food matrices, meat, milk oily fish and white fish were over-spiked with a set of emerging/novel BFR standards (PBT, TBX, PBEB, DBHCTD, HCTBPH and PBB-Acr) and extracted using the procedure described in Section 2.6. Given that many of these compounds responded at low ng/g levels (a short, 15 m GC column was used in order to facilitate this levels of response), over-spiking for PBT, PBEB, TBX, HCTBPH, DBHCTB and PBB-Acr was done at concentrations ranging between 31 and 83 ng/g, and for OBTMPI (which showed much poorer response) at 0.5 or 1.3 µg/g. Although a standard was obtained for EBTEBPI, the compound failed to display any response either by GC-MS or LC MS and was excluded from this procedure. The extraction method was considered to be effective as most of the BFRs (PBT, TBX, PBEB, DBHCTD, HCTBPH and OBTMPI) were unambiguously detected in the final extracts of these matrices.

The full scan data obtained for the 30 selected samples was processed using the Agilent Unknowns software and yielded responses for brominated compounds for each sample. The spectra for these were then searched against the NIST library (after deconvolution, background subtraction and correlated *m/z* clustering procedures, and manual inspection of the data to confirm that the signals were real peaks) and the top ten matches for each ion cluster were considered. There were a number of library matches that suggested the presence of bromine containing compounds, however upon inspection of the mass spectra this could not be confirmed due to the high level of chemical background. Although spectral characteristics corresponding to the most intense characteristic ion for the available emerging/novel BFR compounds were used as guides to inspect the chromatograms for all of the extracted samples, none of these could be positively identified in the extracts.

Additional qualitative full-scan analysis using GC-TOF-MS in both NCI and EI (a smaller selection of samples) mode was also carried out on these samples. No positive identifications corresponding to the prioritised BFR compounds, or indeed other BFR compounds, were made in EI mode, confirming the earlier full scan data. The NCI mode was used to target the characteristic ions for bromine (m/z 79/81). A number of signals corresponding to the presence of bromine were observed, but these did not correspond to any of the available novel/emerging BFR compounds. Further data analysis targeted the samples where data was available for both ionisation modes. Where bromine peaks in NCI mode corresponded to a peak in EI mode, a detailed library search was carried out for possible identification, but no positive assignments were possible. A software package (MassHunter) was used to generate molecular formulas (See Annexe A:scan) for some peaks detected in NCI (assuming that the highest masses detected were molecular ions). This yielded a number of tentative molecular formulas (generally averaging a 5-6 carbon skeleton), but for many of these, the proposed accompanying presence of nitrogen and sulphur in addition to chlorine and bromine, did not match any of the available novel/emerging BFR compounds.

Discussion

The original requirement for this work envisaged a mass balance approach to investigating the occurrence of emerging and novel organic brominated contaminants. This was the approach that was primarily pursued in the execution of this work until the real differences between the total bromine content of the samples and the sum of the concentrations of the most commonly used BFRs became evident. Occurrence for the latter is generally in the sub to low part per billion level, whereas total bromine in food and animal feed was found to occur at the low parts per million to hundreds of parts per million, levels. In many cases these difference can span 4-6 orders of magnitude. Differences of this extent make it difficult to prioritise samples for further investigation based purely on the mass balance approach using total bromine content.

In an attempt to refine this approach, an attempt was made to determine the organic bromine content of a selection of the samples. As described earlier, this was done by extracting the samples with organic solvent using a moderately polar dichloromethane:hexane extraction solvent. This solvent has been successfully used before for the determination of a range of BFRs (PBDEs, HBCD, TBBPA, HBB, BTBPE, DBDPE, etc.) and allows for efficient extraction whilst maintaining a relatively low proportion of unwanted co-extractives. The real issue however with measuring organic bromine is a practical one relating to instrumentation. It is known that many BFR compounds (as a general rule, those with higher bromine atoms) tend to adsorb and degrade during analysis (Fernandes et al, 2009B). Hence the analysis of compounds like BDE-209 and

DBDPE requires very clean contact surfaces (in the injector, GC column transfer lines and MS sources) as well as good control and understanding of temperatures at which degradation starts to occur. In the ICP-MS system (the most practical way to measure bromine) used to measure bromine, there are internal transfer lines that are beyond usual operator control and may allow adsorption to occur. The lines are held at ambient temperatures so degradation is unlikely to occur. Notwithstanding these difficulties, good correlation was observed between total bromine and organic bromine for samples of shellfish and fish feed. However, the concentrations that were obtained for organic bromine were still too high (e.g. average organic bromine values of > 800 ng/g and >400 ng/g for eggs and fish feed respectively, compared to 0.2 ng/g and 2.8 ng/g for the sum of PBDEs, HBCD, TBBPA and PeBCD) to allow a mass balance calculation. It would be difficult to know what contributes to the high concentrations of total organic bromine without chemical analysis of food/feed samples, but it is known for example that some inorganic bromides e.g. bromides of alkaline earth elements, are known to be soluble in organic solvents, and may be co-extracted along with organo-bromine compounds. As mentioned earlier, these could occur in foods, such as marine based foods (which show the highest organic bromine contents).

Thus the mass balance approach attempted here posed practical difficulties to the selection of candidate samples for further investigation. The sum of the measured BFRs (PBDEs, HBCD, TBBPA, and PeBCD) showed very low concentrations relative to both total and organic bromine. The original approach to take a selection of the samples with the highest discrepancies was clearly not feasible despite attempts at refinement. Consequently, samples were selected based on the potential of the different sample types to show the presence of the new BFRs, using extensive previous experience on the occurrence of BFRs in foods, as well as the likely environmental contamination pathways, as a guide e.g. samples where the environmental impact is likely to be important – fish and shellfish; animal tissues that are targeted for storage – meat and organs such as liver; and processed foods where contamination may arise during preparation. This exercise yielded generally marine based foods – fish and shellfish which are known to show occurrence of a wide range of environmental contaminants, but also terrestrially based foods such as meat and offal.

The data obtained from the GC-MS scans (see example in Figure 1 and Figure 2) of the selected samples did not yield any positive identifications, either for the emerging/novel BFR compounds obtained as standards or for the wider group that was targeted. Although responses for brominated signals were obtained, it was not possible to unambiguously assign these to any BFR compounds. Given the experience of earlier investigations on emerging BFR compounds such as HBB, BTBPE and DBDPE which showed that occurrence for these compounds was generally at sub-ppb (ng/g)

levels (Fernandes et al 2010, Tlustos et al 2010), it is possible that scanning techniques are not sensitive enough to pick these up at low ppb levels. Although acid modified silica was used to degrade the matrix and minimise the amount of lipid that was co-extracted (it was established that the available emerging/novel BFR compounds were resistant to this treatment), the volume of other nutrients and similar material that survives this procedure is still relatively large and presents high chemical backgrounds to the MS identification processes.

As with other environmental contaminants, the occurrence of BFRs in food is determined not only by the volumes of the commercial products that have been used, but also environmental persistence, proximity to areas of high usage and disposal, and in the case of foods of animal origin, uptake and metabolism rates. Thus for example, HBB, BTBPE and DBDPE, which have been investigated before, show occurrence (in foods) at low concentrations (sub-ppb levels), when detected (Fernandes et al 2010, Tlustos et al 2010, Zhou et al 2010), but are found at higher concentrations in environmental media such as soils, sediments and in biota (Shi et al 2009, Yang et al 2012, Korner et al 2011), often in contaminated locations.

The presence of signals from bromine containing compounds in some of the sample extracts could indicate the presence of BFRs. While it was not possible to positively identify any of the known BFR compounds it is likely that targeted analyses may be more successful in investigating the occurrence of these compounds. As reference standards for a number of these BFRs are now available, it should be feasible to develop more sensitive methodology (e.g. using HRMS) that would allow detection at lower levels. Although beyond the scope of this screening study, a complementary approach that could be used would be the use of more selective extraction techniques that would exclude a greater proportion of co-extracted material.

This data obtained for the measured BFR compounds will allow exposure to the general population to be calculated and will contribute to the EU wide risk assessment on these contaminants. It also provides current occurrence data for commonly consumed foods and provides new data on animal feeds that have not been investigated for BFR occurrence before.

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Table 3.1: Summary of Total Bromine Content in Foods and Animal Feeds

N	Description	Range µg/g	Mean µg/g
	Food		
44	Eggs	4.6 - 24.1	11.8
37	Processed meat products	0.03 - 38.2	8.0
16	Other processed foods	<0.01 - 28.3	4.4
8	Milk	16.44 - 73.7	37.2
27	Dairy and related products	0.49 - 37.1	11.1
44	Shellfish	4.44 - 418.2	77.1
29	Meat inc. poultry & game	2.2 - 18.7	7.1
42	Offal	3.35 - 50.2	15.4
40	Marine Fish	9.48 - 82.8	26.9
10	Other foods (fruit/veg. etc.)	0.12 - 46	11.0
	Animal Feed		
38	Composite Feed	2.12 - 11.2	5.6
27	Fish Feed	8.15 - 85.3	35.8
21	Oilseeds and Legumes	0.62 - 10.8	5.0
16	Cereals and Byproducts	1.8 - 19.9	5.7
3	Grasses	30.94 - 132.5	67.5

Table 3.2: Summary of Organic Bromine Content in Foods and Animal Feeds

N	Description	Range µg/g	Mean µg/g
	Food		
5	Fish	0.045 - 0.98	0.43
2	Shellfish	0.763 - 6.66	3.71
4	Eggs	0.64 - 1.02	0.86
4	Milk/dairy	<0.025 - 0.03	0.03
10	Meat/offal/ products	<0.025 - 0.24	0.08
5	Other foods	<0.025 - 0.11	0.04
	Animal Feed		
5	Fish feeds	0.08 - 0.67	0.44
12	Veg based feeds	<0.025 - 0.12	0.04

Table 3.3: Summary of PBDE Occurrence* in Foods and Animal Feeds

N	Description		Range (ng/g)	Median (ng/g)	Mean (ng/g)
	Food				
21	Eggs	Σ 17 PBDEs	0.06 - 0.45	0.12	0.15
		Σ EC 10 PBDEs	0.04 - 0.44	0.11	0.14
36	Fish	Σ 17 PBDEs	0.17 - 8.91	1.78	2.12
		Σ EC 10 PBDEs	0.17 - 8.68	1.72	2.05
17	Processed meat	Σ 17 PBDEs	0.04 - 0.57	0.16	0.19
		Σ EC 10 PBDEs	0.03 - 0.56	0.15	0.19
13	Dairy products	Σ 17 PBDEs	0.02 - 0.26	0.08	0.1
		Σ EC 10 PBDEs	0.01 - 0.25	0.07	0.09
3	Milk	Σ 17 PBDEs	<0.04 - 0.07	0.05	0.05
		Σ EC 10 PBDEs	<0.03 - 0.05	0.03	0.04
20	Shellfish	Σ 17 PBDEs	0.04 - 2.2	0.22	0.45
		Σ EC 10 PBDEs	0.04 - 2.13	0.21	0.43
14	Meat (inc. poultry & game)	Σ 17 PBDEs	0.08 - 0.59	0.16	0.2
		Σ EC 10 PBDEs	0.08 - 0.58	0.15	0.19
20	Offal	Σ 17 PBDEs	0.04 - 0.46	0.08	0.11
		Σ EC 10 PBDEs	0.03 - 0.45	0.07	0.11
7	Processed foods	Σ 17 PBDEs	0.16 - 5.84	0.26	1.09
		Σ EC 10 PBDEs	0.15 - 4.73	0.25	0.92
5	Other foods	Σ 17 PBDEs	0.05 - 0.31	0.19	0.18
		Σ EC 10 PBDEs	0.04 - 0.3	0.18	0.17
	Animal Feed				
18	Composite Feed	Σ 17 PBDEs	0.19 - 9.63	0.43	1.03
		Σ EC 10 PBDEs	0.17 - 9.62	0.42	1.01
13	Fish Feed	Σ 17 PBDEs	1.21 - 4.66	1.9	2.25
		Σ EC 10 PBDEs	1.16 - 4.56	1.83	2.18
18	Oilseeds & Cereals	Σ 17 PBDEs	0.11 - 0.82	0.24	0.33
		Σ EC 10 PBDEs	0.09 - 0.80	0.22	0.31
2	Grasses	Σ 17 PBDEs	0.56 - 1.5	-	1.03
		Σ EC 10 PBDEs	0.54 - 1.47	-	1.01

*Upper bound, whole weight concentrations.

Table 3.4: Summary of HBCD Occurrence in Foods and Animal Feeds

N	Description		Range (ng/g)	*Median (ng/g)	*Mean (ng/g)
	Food				
21	Eggs	α-HBCD	<0.01 - <0.02		
		β-HBCD	<0.01		
		γ-HBCD	<0.01 - <0.02		
		TBBPA	<0.01		
36	Fish	α-HBCD	<0.01 - 10.13	0.62	1.36
		β-HBCD	<0.01 - 0.08	0.01	0.02
		γ-HBCD	<0.01 - 0.08	0.015	0.02
		TBBPA	<0.01 - <0.03	0.01	0.01
17	Processed meat	α-HBCD	<0.01 - 0.35	0.02	0.06
		β-HBCD	<0.01		
		γ-HBCD	<0.01		
		TBBPA	<0.01		
13	Dairy products	α-HBCD	<0.01 - 0.09	0.02	0.02
		β-HBCD	<0.01 - <0.03		
		γ-HBCD	<0.01 - 0.06	0.01	0.02
		TBBPA	<0.01		
3	Milk	α-HBCD	<0.01		
		β-HBCD	<0.01		
		γ-HBCD	<0.01		
		TBBPA	<0.01		
20	Shellfish	α-HBCD	<0.01 - 3.41	0.02	0.23
		β-HBCD	<0.01 - 0.02	0.01	0.01
		γ-HBCD	<0.01 - 0.04	0.01	0.01
		TBBPA	<0.01 - <0.05	0.01	0.02
14	Meat (inc. poultry & game)	α-HBCD	0.01 - 0.07	0.01	0.02
		β-HBCD	<0.01		
		γ-HBCD	<0.01 - <0.02		
		TBBPA	<0.01 - <0.02		
20	Offal	α-HBCD	<0.01 - 0.01	0.01	0.01
		β-HBCD	<0.01		
		γ-HBCD	<0.01		
		TBBPA	<0.01 - <0.02		
7	Processed foods	α-HBCD	<0.01 - <0.07		
		β-HBCD	<0.01 - <0.02		
		γ-HBCD	<0.01 - <0.5		
		TBBPA	<0.01 - 0.15		
5	Other foods	α-HBCD	<0.01 - <0.02		
		β-HBCD	<0.01		
		γ-HBCD	<0.01		
		TBBPA	<0.01		

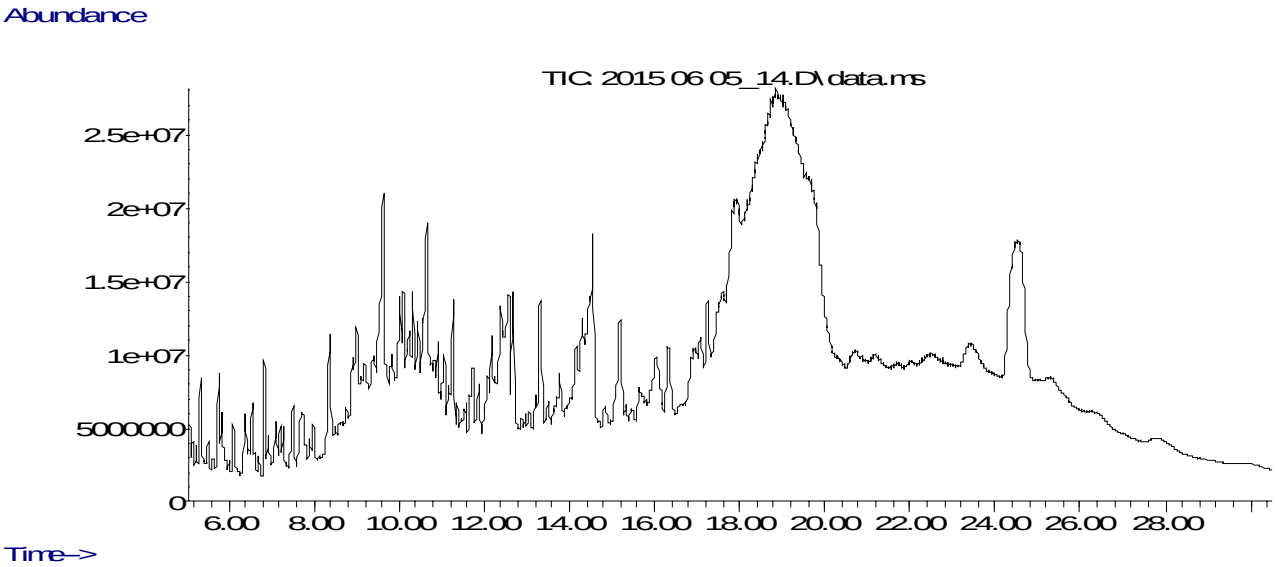
*Median and mean values are only quoted where sufficient data occurs above the LOD

Table 3.4 cont'd: Summary of HBCD Occurrence in Foods and Animal Feeds

N	Description		Range (ng/g)	Median (ng/g)	Mean (ng/g)
	Animal Feed		-		
18	Composite feeds	α-HBCD	0.01 - <0.06	0.025	0.03
		β-HBCD	<0.01 - <0.03		
		γ-HBCD	<0.01 - <0.07	0.02	0.03
		TBBPA	<0.01 - 0.16	0.04	0.05
13	Fish Feeds	α-HBCD	0.22 - 0.66	0.33	0.40
		β-HBCD	<0.01 - 0.04	0.01	0.01
		γ-HBCD	<0.02 - 0.05	0.03	0.03
		TBBPA	<0.01 - <0.04	0.01	0.02
18	Oilseeds & Cereals	α-HBCD	<0.01 - 0.08	0.03	0.04
		β-HBCD	<0.01 - <0.04	0.01	0.02
		γ-HBCD	<0.01 - 0.06	0.01	0.02
		TBBPA	<0.01 - 0.18		
2	Grasses	α-HBCD	<0.06 - 0.08		
		β-HBCD	<0.03 - 0.03		
		γ-HBCD	<0.04 - 0.11		
		TBBPA	<0.06 - <0.10		

*Median and mean values are only quoted where sufficient data occurs above the LOD

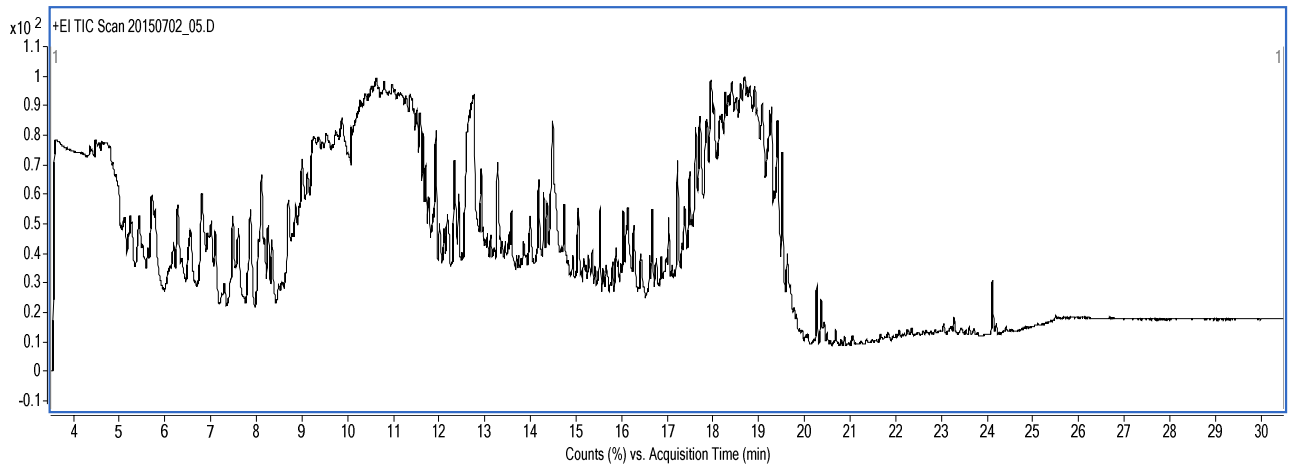
Figure 1: Full scan data for fish sample (mackerel)



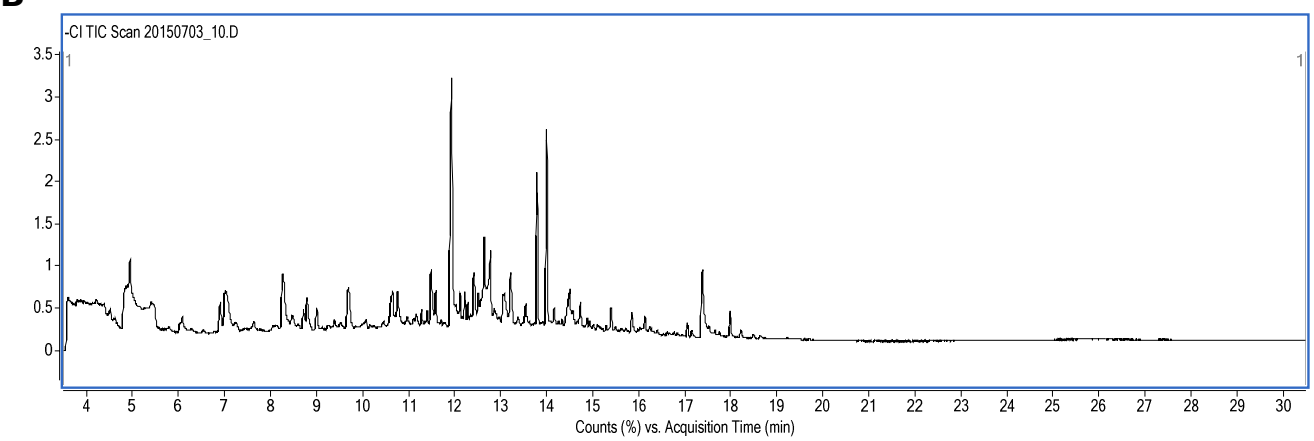
(Available novel/emerging BFRs elute over this time range)

Figure 2: GC-TOFMS full scan data for sprat sample in EI mode (A) & NCI mode (B)

A



B



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