RESULTS OF THREE YEARS OF MONITORING PLANS IN FRANCE: LEVELS OF PBDEs, PBBs, HBCDDs, TBBPA AND OTHER EMERGING/NOVEL BROMINATED FLAME RETARDANTS IN FOOD

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Introduction

Following the concern raised by EFSA[1,2] regarding the increasing use of novel and emerging flame retardants in replacement of PBDEs and HBCDDs, we have developed a multiclass and multiresidue method dedicated to PBDEs, PBBs, HBCDDs, TBBPA and other emerging/novel BFRs (nBFRs)[3]. This analytical strategy was applied to more than 600 feed and food samples originating from the French monitoring plans performed from 2014 to2016.

Materials and methods

Standards

^{13}C-BDE-28/47/99/100/153/154/209, ^{13}C-BB-153, ^{13}C-α/β/γ-HBCDD, ^{13}C-TBBPA, 2,3,5,6-Tetrabromo-p-xylène (pTBX), Tetrabromo-o-chlorotoluene (TBCT), 1,2,3,4,5-Pentabromobenzene (PBBz), 1,2,3,4,5-Pentabromo[$^{13}C_6$]benzene (^{13}C-PBBz), Pentabromotoluene (PBT), Pentabromoethylbenzene (PBEB), Hexabromobenzene (HBBz), Hexabromo[$^{13}C_6$]benzene (^{13}C-HBBz), Octabromotrimethylphenylindane (OBIND), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB), ethylhexyl -d_{17}-2,3,4,5-tetrabromo[$^{13}C_6$]benzoate (^{13}C-EHTBB), 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE), 1,2-Bis(2,4,6-tribromo [$^{13}C_6$]phenoxy)ethane (^{13}C-BTBPE), Tris(2,3-dibromopropyl)isocyanurate (T23BPIC), $^{13}C_6$-Bromophenols mixture (^{13}C-BrPS) and bromophenol mixture (BrPS) were purchased from Wellington Laboratories. 3,3',5,5'-Tetrabromobisphenol A dimethylether (TBBPA-bME) and Tetrabromobisphenol A bis(dibromopropylether) (TBBPA-bDiBPrE) were from Chiron. All calibration standards and spiking solutions were prepared by serial dilution in toluene.

Samples

Analysed samples arose from the 2014, 2015 and 2016 BFR French monitoring plans (n=610), and included 15 fish oil, 15 fish meal, 114 fish, 154 crustaceous and mollusc, 72 milk, 57 egg, 28 sheep liver and 152 meat samples. A mix of samples (poultry meat, fish, liver …) was fortified with the target nBFRs and used as quality control. Quality control and procedural blank samples were introduced in each batch of analysis to monitor the reproducibility and the sensitivity of the method.

Sample extraction and purification

Cleaned laboratory glassware was rinsed with dichloromethane prior to use and the analyses were carried out in an over-pressurized room to minimise environmental contamination. The method has already been described elsewhere[3]. Briefly, the ^{13}C-labeled internal standards were added to the samples before the extraction step (^{13}C-BDE-28/47/99/100/153/154/209, ^{13}C-BB-153, ^{13}C-α/β/γ-HBCDD, ^{13}C-TBBPA, ^{13}C-HBBz, ^{13}C-PBBz, ^{13}C-DBDPE, ^{13}C-BTBPE, ^{13}C-EHTBB, ^{13}C-BEHTEBP and ^{13}C-BrPS). Lipids were extracted from lyophilised samples by Pressurised Liquid Extraction (Büchi) using a toluene/acetone mixture (70:30, v/v). Purification steps were achieved on successive columns manually packed with neutral and acidic silica gel where BFRs were divided in two eluted fractions. The first one, containing PBDEs, PBBs, pTBX, TBCT, PBBz, PBT, PBEB, HBBz and OBIND, was further purified on Florisil® and carbon. The second, one containing HBCDDs, TBBPA and nBFRs, was further purified by partitioning between hexane and NaOH 1 N for HBCDDs and T23BPIC, and hexane and HCl 1 N for TBBPA and BRPs. ^{13}C-BDE-77, ^{13}C-BDE-138, d_{17}-HBCDD and TCBPA were added in each final fraction for recovery determination.

Detection

PBDE congeners were separated and detected by GC-EI(+)-HRMS (7890, Agilent Technologies / JMS800D, R=10000, Jeol), using a DB-5MS column (30 m × 0.25 mm, 0.25 µm) for PBDEs, PBBs, pTBX, TBCT, PBBz, HBBz, PBT, and PBEB, and a RTX-1614 column (15 m × 0.25 mm, 0.10 µm) for BDE-209 and OBIND. HBCDD

isomers and T23BPIC were separated and detected by LC-ESI(-)-MS/MS (6410, Agilent Technologies) with a Hypersil Gold column (100 mm × 2.1 mm; 1.9 µm, Thermo), with a mobile phase composed of acetonitrile/methanol (1:1) and 20 mM ammonium acetate. TBBPA and BRPs isomers were separated and detected by LC-ESI(-)-HRMS (1260, Agilent Technologies / Thermo Exactive) with a Phenomenex Gemini column (50 × 2 mm; 3 µm), with a mobile phase composed of acetonitrile and water, both with acetic acid (0.1%). EHTBB, BTBPE, DBDPE, TBBPA-bME and TBBPA-bDiBPrE were separated and detected by GC-APCI(+)-MS/MS (APGC-Xevo TQS, Waters) with a RTX-1614 column (15 m × 0.25 mm, 0.10 µm).

Results and discussion

Occurrence levels in samples

Figure 1 illustrates the observed levels of the targeted BFRs in the different sample categories. PBDEs (except BDE-183) were detected in more than 60% of the samples. BDE-47 appeared as the most abundant PBDE in fish feed, fish and mollusc, while BDE-209 was the most abundant one in milk, egg and meat. BDE-99 and BDE-153 were also major contributors in sheep liver, as well as BB-153. α-HBCDD was highly abundant in fish oil, observed in fish and crustaceous/mollusc, and also present in egg and meat but at relatively low levels (at pg/g ww level). β and γ-HBCDD were mainly found in fish, mollusc and feed for fish. TBBPA was only detected in molluscs. Concerning the nBFRs, PBT, PBBz, HBBz and BTBPE were detected in more than half the analysed samples. BTBPE was detected in half of the mollusc samples while TBCT, DBDPE, OBIND, T23BPIC, TBBPA-bDidBrPE, TeBRPs and PeBRPs concentrations were below the limit of quantification.

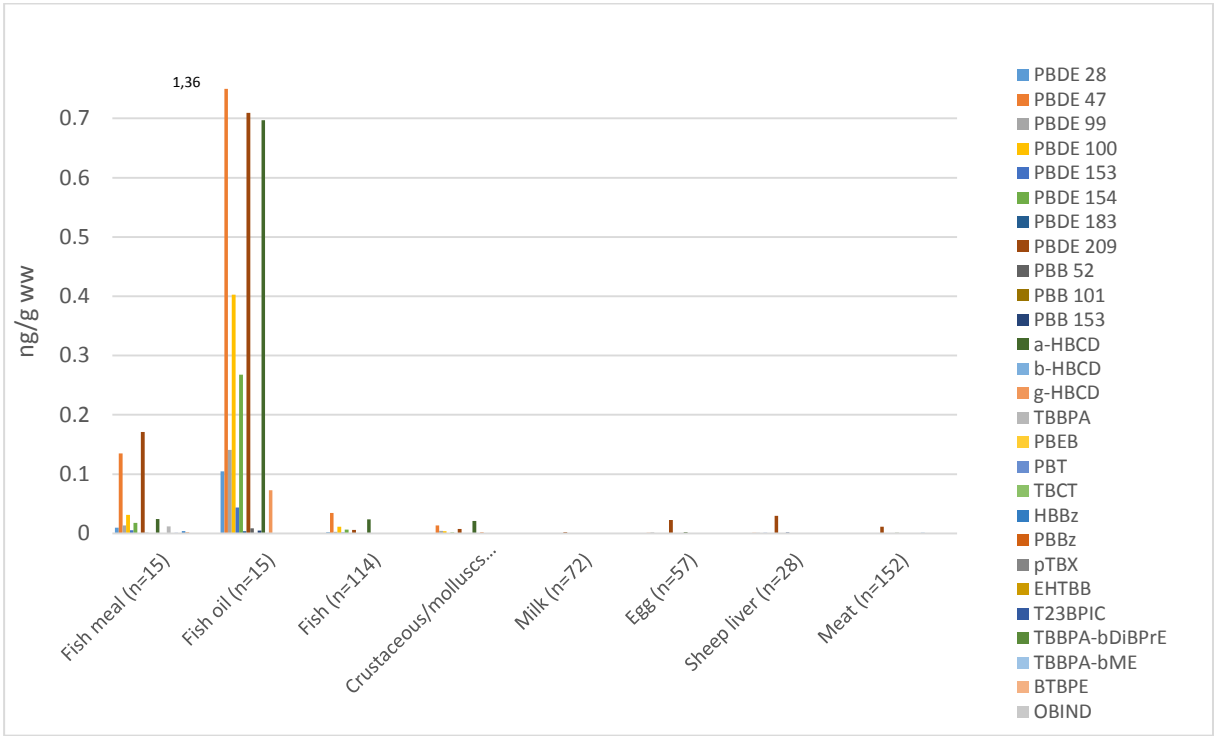

Figure 1: BFR concentrations observed in various sample categories (ng/g wet weight).

As illustrated in Figure 2, PBDEs and HBCDDs were observed as the major BFR contributors (between 80 and 99% of the total amount of BFR quantified in the samples).

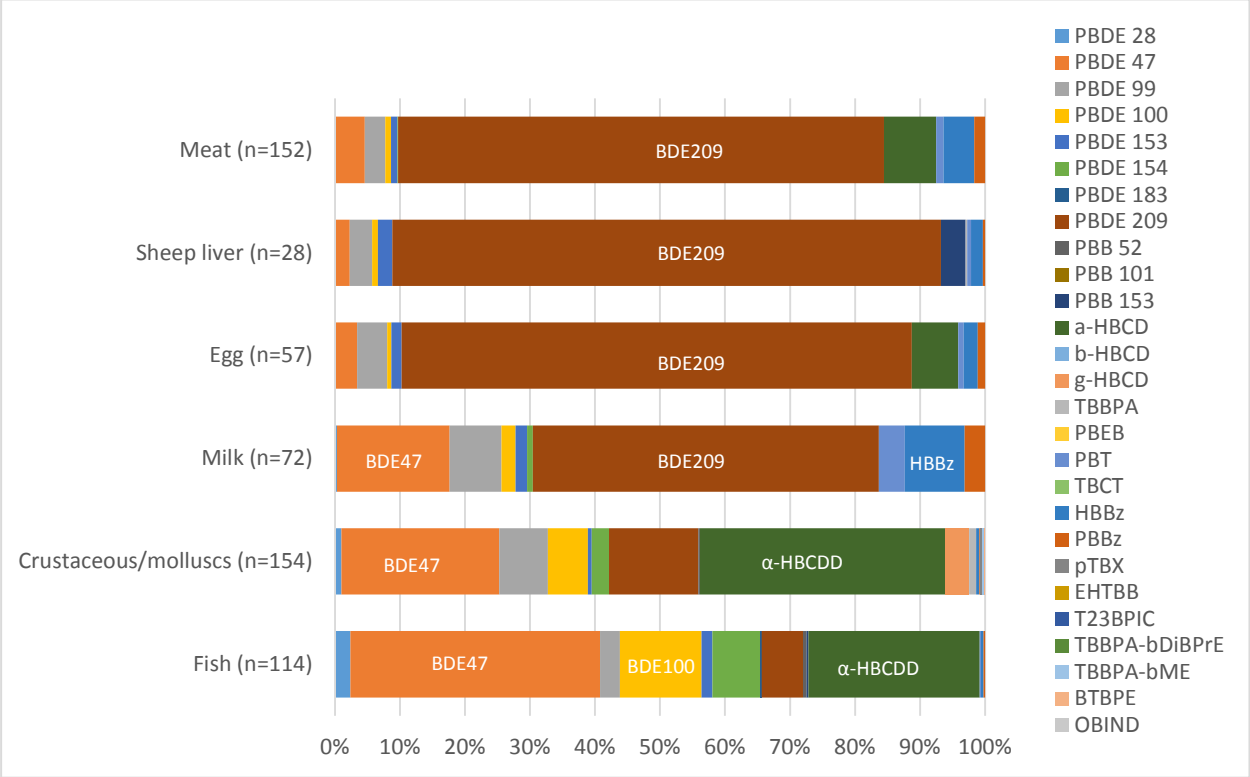

Figure 2: Median contamination profiles (relative contributions of the different targeted BFRs) observed in various sample categories (percentage of the total, ng/g ww basis).

These results show the suitability of the developed method for the simultaneous analysis of regulated compounds (PBDEs, HBCDDs, TBBPA) and a certain number of emerging/novel BFRs. Some nBFRs were observed in some foodstuffs originating from the French market, but at lower levels than those of PBDEs and HBCDDs.
The subsequent application of the developed methodology will allow monitoring further trends reflecting the evolution of the environmental contamination and/or impact of regulatory dispositions regarding these historical and more emerging/novel BFRs.

Acknowledgements
The analysis of French foods was funded by the French Ministry of Agriculture, DGAl.

References
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