COOKING PROCESSES WITH FOOD: THE HEATING OF THE FLAME RETARDANT BDE 209 IN FISH GENERATED TOXIC POLYBROMINATED DIBENZOFURANS

Vetter W*, Bendig P, Blumenstein M , Hägele F

University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, D-70593 Stuttgart, Germany

Introduction

Polybrominated diphenyl ethers (PBDEs) are high volume chemicals industrially used since the 1970s mainly in fire prevention. Technical products were produced with three different average degrees of bromination, and the low and medium brominated products (e.g. DE-71 and DE-79) were recently classified as persistent organic pollutants (POPs).[1] Currently only decabromodiphenyl ether (BDE 209) is permitted for use as BFR.[1] The high production volume of BDE 209 has been linked with environmental issues. Partly due to previous analytical difficulties,[2] the detection of BDE 209 is infrequent. Part of the problem arises from the instability and partial transformation by thermal processes and UV light.[3,4] Unintentional leaching of PBDEs from the products has mainly lead to a contamination of aquatic and marine environments which in turn serve as relevant food sources. Fish is a major source for the human intake of PBDEs.[5] However, fish is scarcely consumed without being previously cooked at elevated temperatures. The fate of thermally-instable compounds such as BDE 209 under these conditions is widely unknown. Studies on the effects of the heating of fish on PBDE patterns and concentrations are scarce and incomplete. The fate of PBDEs has been correlated with PCBs.[6] Previous experiments indicated the hydrodebromination of BDE 209 when heated in fish.[7] Here we report on the formation of abundant polybrominated dibenzofurans (PBDFs) in this process.

Materials and methods

Chemicals, Standards and Samples. Supelclean ENVI-Carb, 120-400 mesh and Celite 545-AW were from Supelco (Deisenhofen, Germany). Cyclohexane and ethyl acetate (both >99.5% Sigma-Aldrich, Seelze, Germany) were combined and purified by azeotropic distillation (46/54, w/w). Origins of other chemicals and solvents were reported elsewhere.[8] Technical pentaBDE (DE-71), octaBDE (DE-79) and decaBDE (DE-83R; BDE 209 purity ~97%) were produced by Great Lakes Chemical (Indianapolis/IN, USA). Origins of BDE 47 (purity ~98%) and the internal standards 2´-MeO-BDE 68 and 6´-MeO-BDE 66 were reported by Bendig *et al*.[8] A standard mixture of 40 PBDE congeners (EO-4980) was from Cambridge Isotope Laboratories (Andover, MA, USA). BDE 199 was from Campro Scientific (Berlin, Germany). Refined sunflower oil and farmed salmon fillet (*Salmo salar*) from Chile (water content ~70%, lipid content ~5%), which was virtually free of PBDEs,[7] were purchased in a German retail store. The fish was stored frozen and thawed directly before the experiment started.

Cooking experiments. Salmon fillet (1 g) was spiked with 200 ng of decaBDE and BDE 47 (20 ng/µL in toluene). Sunflower oil (180 mg) was placed in 10 mL glass test tubes (12 mm internal diameter, 80 mm height) and the spiked fish was added. The test tube with the sample was placed in a pre-heated heating block (Labtherm, Liebisch, Bielefeld, Germany) at 200 °C for 0 (reference value), 15, 30, 45, 60, 90, 120 and 180 min, respectively. Before and immediately after the heating the test tubes were covered with aluminium foil.

Sample Extraction. The heated samples were spiked with either 200 ng 2'-MeO-BDE 68 or 6'-MeO-BDE 66 as internal standard (IS) and transferred with 80 mL cyclohexane/ethyl acetate (46/54, w/w) into 250 mL microwave vessels (borosilicate glass). Open vessel microwave assisted extraction was performed with a modified Star microwave system (CEM, Kamp-Lintfort, Germany) equipped with a water trap similarly to Batista *et al*.[9] During the extraction the temperature was increased within 10 min to 88 °C (10 min hold), then within 5 min to 95 °C (30 min hold). This program was adapted from Weichbrodt *et al.*[10]

Sample preparation. Extracts were passed through a fluted filter into pear shaped flasks (100 mL) and rinsed with five times 2 mL of cyclohexane/ethyl acetate (46/54, w/w). Then the solvent was exchanged with *iso*-octane by rotary evaporation. The sample was transferred with five times 2 mL of *n*-hexane into a 20 mL-test tube (pyrex, 16 mm internal diameter, 160 mm height) and 5 mL of conc. sulphuric acid were added for lipid decomposition. After vigorous shaking and phase separation, the organic layer was transferred into a pear shaped flask (100 mL) and the remaining acid phase was re-extracted twice with 5 mL of *n*-hexane.[7] Remaining lipids

and other polar substances were removed by adsorption chromatography with 3 g of 30% deactivated silica gel topped with 1 g sodium sulphate, eluted with 60 mL *n*-hexane.[7] Samples were then brought to 1 mL and subjected to gas chromatography coupled to electron capture negative ion mass spectrometry (GC/ECNI-MS) or electron capture detection (GC/ECD) analysis. All glassware was amber glass or covered with aluminium foil.

Activated carbon column chromatography. A mixture of 0.5 g Celite and 0.5 g ENVI-Carb was filled in a 1 cm internal diameter glass column.[11] One fourth (250 µL) of a purified heated sample extract (heated for 120 min with closed lid) was placed on the column and eluted with (i) 50 mL *n*-hexane (fraction 1), (ii) 50 mL *n*-hexane/toluene, 95/5 (fraction 2), and (iii) 50 mL toluene (fraction 3). Each fraction was concentrated to 250 µL and then subjected to GC/ECNI-MS analysis (5 µL injected) and the resulting chromatograms were compared with the unfractionated sample (5 µL injected).

Quality control. Procedural blanks did not show traces of PBDEs. All experiments were performed at least in duplicates or more replications. Recoveries of the spikes in unheated samples and sunflower oil (heated and unheated) were ~80% for BDE 209 and BDE 47, which compared well with the recoveries of the internal standards (~80%), and all results were recovery corrected. Repeated spiking of decaBDE (200 ng) into sample extract after heating for 120 min gave identical results for BDE 209 after subtraction of the added amount. This demonstrated that BDE 209 was neither retarded by matrix compounds nor decomposed in the injection port. Duplicate analysis varied less than 10% or the experiment was repeated.

GC/ECNI-MS. Analyses were run with an Agilent (Waldbronn, Germany) 7890 GC and 5975c MS system, equipped with a 7693A autosampler according to Bendig *et al*.[8] A DB-5HT (15 m × 0.25 mm i.d., 0.1 µm film thickness) capillary column (J&W Scientific, Folsom, USA) was used in combination with the following GC oven program: after 1 min at 50 °C, the temperature was increased by 10 °C/min to 300 °C (hold time 24 min); the total run time was 50 min. The carrier gas helium (purity 5.0) was used at a flow rate of 1.2 mL/min. The transfer line temperature was held at 300 °C, the ion source and quadrupole temperatures were maintained at 150 °C, respectively. Methane (purity 5.5) was used as reagent gas at 2 mL/min. Analyses were performed in the selected ion monitoring (SIM) mode.[7] For the analysis of the PBDFs, we recorded *m/z* 79/81 $[Br]^-$, *m/z* 639.5/641.5/643.5 (M^- of hexaBDFs), *m/z* 717.4/719.4/721.4 (M^- of heptaBDFs). Measurements in the full scan mode covered *m/z* 30-800.

Gas chromatography with electron capture detection (GC/ECD). A Hewlett-Packard 5890 series II plus system equipped with a GC PAL autosampler (CTC Analytics, Zwingen, Switzerland) was used for GC/ECD analyses. One microliter was injected in the splitless mode at 300 °C onto a 15 m × 0.25 mm i.d., 0.25 µm film thickness BGB-1 column (BGB-Analytik, Boeckten, Switzerland). The oven temperature was programmed for 2 min at 80 °C followed by 10 °C/min to 300 °C (20 min). The carrier gas flow of nitrogen (purity 5.0; Westfalen, Münster, Germany) was kept at a constant flow rate of 1.2 mL/min and the detector temperature was set to 300 °C. The makeup gas (nitrogen) was used with a flow rate of 60 mL/min.

Compounds not available as individual standards were semi-quantitatively assessed relative to the area of BDE 209 in the GC/ECD chromatogram of the unheated sample. For all results presented, confirmatory measurements were conducted by GC/ECNI-MS.

Results and discussion

When BDE 209 and BDE 47 were spiked into fish and heated (see Materials and methods), we observed no change in the concentration of BDE 47 while BDE 209 showed a continuous decrease in concentration (**Figure 1**, left side). In parallel with the transformation of BDE 209, we observed novel peaks in the chromatograms which originated from octa- and nonaBDE congeners (**Figure 2a**). HeptaBDEs were also generated and their abundance increased with time. In addition, an abundant, non-BDE transformation product was observed in the GC/ECNI-MS chromatograms. This polybrominated compound eluted between the last octaBDE and the first nonaBDE from the DB-5 column (**Figure 2a**).

In GC/ECNI-MS response was neither observed for the characteristic ring fragment ions of octaBDEs ($[C_6HBr_4O]^-$ at *m/z* 409 nor to the characteristic *m/z* 161 $[HBr_2]^-$. By contrast, screening of *m/z* 719.4 ($[M]^-$ of heptabromodibenzofurans (heptaBDFs) and $[M\text{-}HBr]^-$ of octaBDEs) was successful (**Figure 2b**). The higher response to $[M]^-$ (*m/z* 717.4) compared to $[Br]^-$ (**Figure 2b**) is a known feature of different PBDFs but uncommon for PBDEs. Final proof was obtained by carbon column chromatography because planar PBDFs can be absorbed with charcoal.

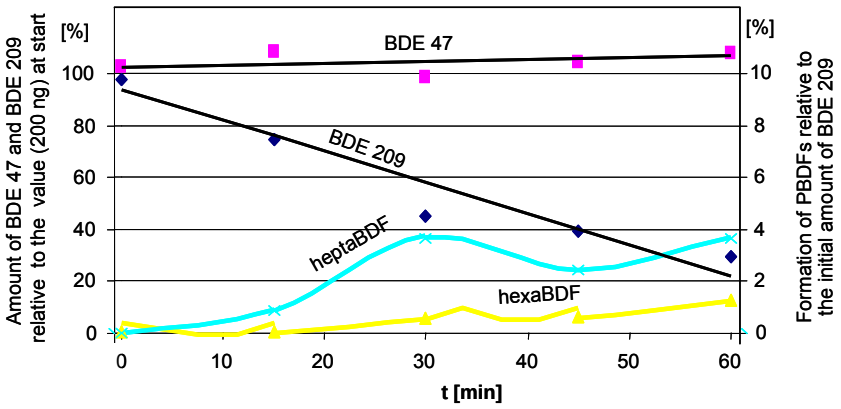

Figure 1: Fate of BDE 47 and BDE 209 (in %) during the heating of fish with spiked amounts of 200 ng (left) and formation [%] of polybrominated dibenzofurans (PBDFs) (right).

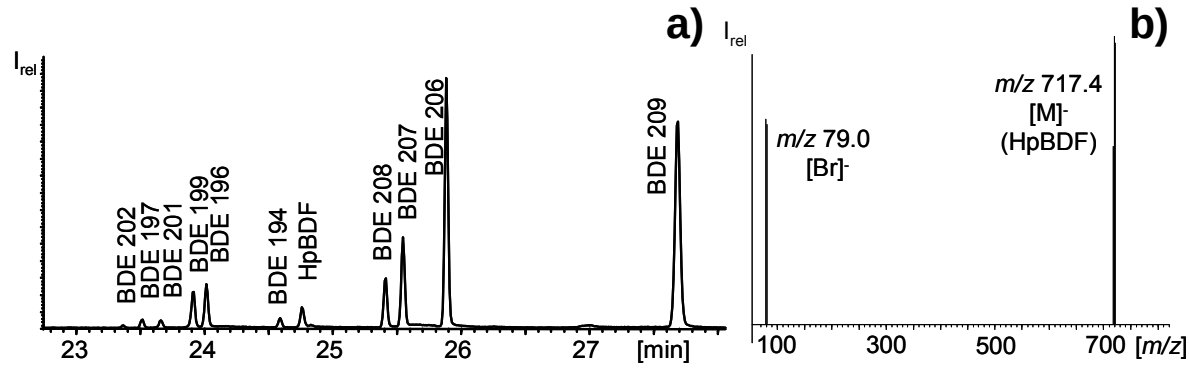

Figure 2: (a) GC/ECNI-MS-SIM chromatogram of the results of the heating of BDE-209 (200 ng spike) in fish (30 min, 180 °C) and (b) SIM mass spectrum of (*m/z* 79, 81, 717.4, 719.4, 721.4)

This method finally verified the occurrence of the heptaBDF in the heated fish samples (see Materials and Methods). While 96±6% of most PBDEs (BDE 196, BDE 197, BDE 201, BDE 203, BDE 207 and BDE 208) were mainly eluted with *n*-hexane (fraction 1) and ~50% of BDE 206 and BDE 209 mainly with *n*-hexane/toluene (fraction 2), the heptaBDF was not eluted at all. A close inspection of the GC/ECNI-MS chromatograms of the fractionation on charcoal showed a significant decrease in the abundance of BDE 180. GC/ECNI-MS-SIM analysis recording the $[M]^-$ of hexaBDFs confirmed the co-elution an hexaBDF isomer with BDE 180 (data not shown). The amounts of the PBDFs were estimated with the GC/ECD response of BDE 209. Using this method we found that that cooking of the fish spiked with 200 ng BDE 209 (30 min, 180 °C) generated ~1% of the hexaBDF and ~4% of the heptaBDF (**Figure 1**, right side). Since only one (abundant) heptaBDF was observed in the chromatograms, we predicted the most plausible structure on the basis of the following observations. PBDFs are formed from PBDEs by the elimination of HBr in *ortho*-positions of the two phenyl moieties. Thus, the precursor has to bear hydrogen in one *ortho*-position. The formation of only one heptaBDF would be fulfilled if the second hydrogen is located in *para*-position of the second ring (see structure 3 in **Figure 3**). In fact, the major nonaBDE transformation product was identified as BDE 206 (**Figure 2a**) which lacks one *ortho*-substituent **(Figure 3)**.[7] The reaction commenced by Br elimination in the second ring,[12] and lead mainly to the octaBDEs BDE 196 and BDE 199 (**Figure 2a**). BDE 199 was likely the direct precursor of the heptaBDF (**Figure 3**). One essential brick in this interpretation is that BDE 199 can only produce one heptaBDF which was actually observed while all other potential octaBDE precursors including BDE 196 (one hydrogen in *ortho*-position in one ring and the second hydrogen in *meta*-position) would suggest the formation of two heptaBDFs. The structure of the hexaBDF could not be determined.

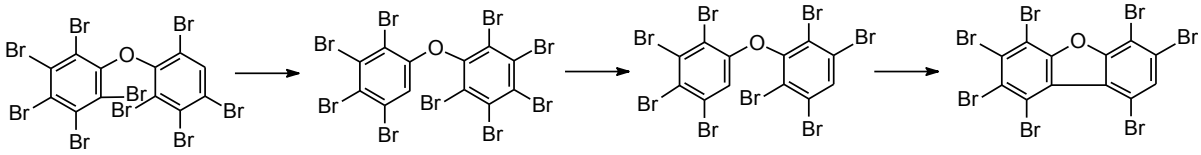

Figure 3: Potential formation and structure of the heptaBDF from BDE 209 by two times hydrodebromination followed by a dehydrobromination reaction

The toxic equivalency factor (TEF) of the potential 1,2,3,4,6,7,9-heptaBDF has not been assessed yet. Behnisch *et al*. assessed the toxicity of 1,2,3,4,7,8-hexaBDF and 1,2,3,4,6,7,8-heptaBDF *in vitro* to be ~ 1/70 or ~1/420 respectively of 2,3,7,8-tetraCDD.[13] This translates into TEFs of ~ 0.015 and 0.0024, respectively. Assuming similar TEFs for the two PBDFs detected in our samples would thus be linked with a significant increase in the toxic potential of the fish because the heptaBDF was detected without previous enrichment. Using the TEF values of Behnisch *et al*.[13] we estimated the TEQ originating from the two PBDFs as follows. The heating of 1 g fish spiked with 200 ng BDE 209 produced 4% (8 ng) heptaBDF and 1% (2 ng) hexaBDF. The resulting TEQ would be 2 ng hexaBDF x 0.015 plus 8 ng heptaBDF x 0.0024 = 49.2 pg TEQ / g fish. Consumption of a 150 g diet of fish with this BDE 209 level would amount to 7,380 pg TEQ after heating. This TEQ strongly exceeds the highest tolerable intake limit for an 80 kg person of 4 pg TEQ / kg body weight. This high value as not unexpected since the heptaBDF could be directly detected as a major compound in the GC/ECNI-MS chromatograms of the fish after heating. Assuming that the same reaction would take place with any fish and irrespective of the level of BDE 209, we checked the literature for BDE 209 levels in fish. For instance, 0.7 ng/g BDE 209 were reported in farmed salmon (on a wet weight basis).[14] Consumption of a heated 150 g-portion of such fish could generate ~4,200 pg heptaBDF and 1,050 pg hexaBDF or ~26 pg TEQ. Occasionally, even higher BDE 209 concentrations were reported in fish and seafood.

Noteworthy, the direct determination of PBDFs in the sample chromatograms was only possible when the fish was heated with BDE 209. Heating of fish with several hepta- and octaBDEs did not produce directly measurable amounts of PBDFs. This reaction was unique to BDE 209 (and potentially BDE 206 and BDE 199). In addition, the heptaBDF was only formed in the presence of fish while the heating of BDE 209 in plant oil did not produce transformation products.[8] In any case, the heating of BDE 209-contaminated fish can be a significant contribution to the human uptake of toxic compounds.

References

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