BROMINATED DIOXINS AND BROMINATED FLAME RETARDANTS IN IRISH COW`S MILK

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Abstract
Brominated flame retardants (BFRs) and related compounds are environmental contaminants with bioaccumulating properties, which have entered the food chain. In order to allow an estimate of the dairy intake of BFRs and brominated dioxins (PBDD/Fs) by Irish consumers these chemicals have been investigated in five composite cow`s milk samples. The monitoring of eleven Tri- to HeptaBDD/F congeners confirms the existence of brominated dioxins in each milk sample and 1,2,3,4,6,7,8-HeptaBDF was the predominating congener. The upper-bound WHO-TEQ values, calculated in accordance to the TEF model established by WHO in 1998 for chlorinated dioxins, were in the range of 0.152 to 1.67 pg WHO-TEQ/g fat. Furthermore four PBDEs (BDE-47, 99, 100 and 153) have been found in all milk samples and the total amount of these PBDE congeners varied between 150 and 265 ng/kg fat. No further BFR compound (PBDEs, PBBs, HBCD and TBBP-A) could be determined in any of this sample of the series. These results indicate again the increased demand for additional studies on dietary exposure to brominated dioxins and further classes of brominated POPs.

Introduction

Brominated flame retardants (BFRs) are used to reduce the risk of fire in a great variety of materials and appliances and it is a fact that BFRs are widespread in nearly all compartments of the environment.[1] This compound class includes the recently restricted polybrominated diphenylethers (PBDEs), the banned polybrominated biphenyls (PBBs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBP-A). Polybrominated dibenzodioxins and dibenzofurans (PBDD/Fs) are unintentional by-products in chemical processes and can also be produced during various combustion processes and photolytic degradation of PBDEs and bromophenols. The toxic properties of individual PBDD/F congeners strongly depend on the bromine substitution number and position. Several brominated dioxins have shown greater toxicity than their chlorinated homologues.[2] The WHO expert panel has recommended to perform a more thorough human exposure analysis and to give high priority to the development of specific WHO-TEFs for PBDD/Fs .[3]
Dietary intake of these contaminants predominantly takes place through food of animal origin. Recently the FSA has conducted two comprehensive studies on the levels of brominated dioxins and BFRs in a variety of fish matrices as well as in the whole UK diet.[4,5] PBDE and PBDD/F levels in fatty food from Germany have also been in the focus of current investigations.[6] The PBDD/F and PBDE levels in mother`s milk and dairy products from Japan have been reported in 2005.[7] Because of the limited information available, there is great need for more data on dietary exposure to brominated dioxins and further brominated pollutants.
This study was carried out to determine the background concentrations of PBDD/Fs and BFRs (PBDEs, PBBs, HBCD and TBBP-A) in Irish cow`s milk on sale. In accordance with the above-mentioned FSA surveys, this study also includes tribrominated dioxins, as there are indications that these congeners may be more common than their chlorinated analogues. Furthermore, the investigations include analyses for polychlorinated dioxins (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs).

Material and Methods

Sample preparation
Similar to the surveys in 2000 and 2004, a series of 24 raw milk samples from so-called background stations and a further set of 13 milk samples from so-called potential impact locations were collected by the Irish EPA in 2006.[8] The milk samples were frozen in glass bottles and shipped without interrupting the cooling chain. All these 37 individual milk samples were analysed for PCDF/Ds and dl-PCBs. The five composite samples for PBDF/D and BFR analysis were pooled in the lab and consist of three individual milk samples in each case.

PBDD/F analysis
All analyses for brominated dioxins were performed by HGRC/HRMS and each analysis included the determination of the PBDD and PBDF congeners listed in Table 1.

Table 1: PBDD/PBDF congeners measured in the survey

PBDD congeners	TEF*	PBDF congeners	TEF*
2,3,7-TriBDD	-	2,3,7-TriBDF	-
2,3,7,8-TetraBDD	1	2,3,7,8-TetraBDF	0.1
1,2,3,7,8-PentaBDD	1	1,2,3,7,8-PentaBDF	0.05
1,2,3,4,7,8-/1,2,3,6,7,8-HexaBDD	0.1	2,3,4,7,8-PentaBDF	0.5
1,2,3,7,8,9-HexaBDD	0.1	1,2,3,4,7,8-/1,2,3,6,7,8-HexaBDF	0.1
		1,2,3,4,6,7,8-HeptaBDF	0.01

* TEFs quoted are the WHO-TEFs (1998) that apply to the analogue chlorinated congeners

Ten $^{13}C_{12}$-labelled tri to hexabromodioxins and furans were added to the freeze-dried samples, which were extracted by means of accelerated solvent extraction (ASE 300, Dionex Corp.) using a solvent mixture of n-hexane, dichloromethane and methanol. The extracted fat was used and the recoveries of the internal standards through the fat separation and all purification steps were determined by means of a further $^{13}C_{12}$-labelled standard. A PowerPrep workstation (Fluid Management Systems) was used for an automated chromatographic clean-up of the defatted extract. The PBDD/F determination was performed on HP 5890 HRGC connected to a VG AutoSpec HRMS (mass resolution typically 10,000). The instrument was operated in a selected ion monitoring mode; two ions monitored for each analyte. A 10 m DB-1 MS capillary column was used for the gas chromatographic separation. Acceptance criteria followed were analogous to those used for the PCDD/F analysis. The limits of quantification (LOQs) on fresh weight basis (fw) were in the range of 0.0007 pg/g fw for TetraBDD/Fs to 0.011 pg/g fw for HexaBDD/Fs.

BFR analysis
Seventeen PBDE congeners (BDE-17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 183 and 209), some individual PBBs (BB-52, 101, 153 and 209), the totals of Tetra to NonaBBs, hexabromocyclododecane (sum of α-, β– and γ-HBCD) and tetrabrombisphenol A (TBBP-A) have been monitored in this study. The main aspects of the analytical method used for these parameters have already been described before.[9] In brief the freeze-dried aliquots were fortified with six $^{13}C_{12}$-labelled PBDE congeners ($^{13}C_{12}$-BDE-28, 47, 99, 153, 183, 209), $^{13}C_{12}$-labelled γ-HBCD and $^{13}C_{12}$-labelled TBBP-A. The sample material was extracted with the same solvent mixture as mentioned above by means of ASE. The extracts were acid treated and further cleaned-up by liquid/solid chromatography. A GC/MS system (HP Agilent MSD 5973) was used for the BFR analysis, in which the analytes were separated on a 30 m DB-5 column. The LOQs for PBDEs were in the range of 0.1 to 1 ng/kg fw for Tri to HeptaBDEs up to 50 ng/kg fw for DecaBDE. The LOQs for PBBs were in the range of 0.2 to 3 ng/kg fw and for the sum of HBCDs the LOQ was 50 ng/kg fw.
For TBBP-A analysis an extract portion was treated with sulphuric acid followed by a derivatisation step using acetic acid anhydride. The derivatised analyte was extracted with hexane and subsequently cleaned-up by liquid/solid chromatography using silica gel. The instrumental method is almost the same as described for the other BFRs and the LOQ for the TBBP-A analysis of the milk samples was 200 ng/kg fw.

Results and Discussion
In accordance with the current EC legislation on PCDD/F and dl-PCB levels in foodstuffs (Commission Regulation 199/2006) the concentration of the brominated pollutants have also been related to the extracted fat content of the milk samples. The gravimetrically determined fat fractions varied between 3.4 % and 3.8 %.
Brominated dioxins and furans (PBDD/Fs)
The PBDD/F concentrations of the individual milk samples and the resulting mean values are shown in Table 2. The presented WHO-TEQs are calculated on the basis of the TEFs listed in Table 1.

Table 2: PBDD/F concentrations of composite milk samples and resulting mean values related to the fat content

	Sample 1 pg/g fat	Sample 2 pg/g fat	Sample 3 pg/g fat	Sample 4 pg/g fat	Sample 5 pg/g fat	Mean value* pg/g fat
2,3,7-TriBDF	< 0.08	0.68	0.16	0.13	0.15	0.24
2,3,7,8-TetraBDF	< 0.02	0.35	0.02	< 0.02	0.02	0.09
1,2,3,7,8-PentaBDF	< 0.03	1.42	0.06	< 0.03	< 0.03	0.31
2,3,4,7,8-PentaBDF	< 0.03	1.28	< 0.03	0.06	< 0.03	0.29
1,2,3,4,7,8-/1,2,3,6,7,8-HexaBDF	0.12	1.26	0.24	0.13	0.18	0.39
1,2,3,4,6,7,8-HeptaBDF	< 0.20	4.06	1.62	0.96	4.62	2.29
2,3,7-TriBDD	< 0.08	0.57	< 0.08	< 0.09	< 0.08	0.18
2,3,7,8-TetraBDD	< 0.02	0.04	< 0.02	< 0.02	0.14	0.05
1,2,3,7,8-PentaBDD	0.04	0.55	< 0.03	0.04	0.29	0.19
1,2,3,4,7,8-/1,2,3,6,7,8-HexaBDD	< 0.30	1.30	< 0.31	< 0.32	< 0.29	0.50
1,2,3,7,8,9-HexaBDD	< 0.30	0.32	< 0.31	< 0.32	< 0.29	0.31
WHO-TEQ (excl. LOQs)	0.051	1.64	0.045	0.090	0.496	0.463
WHO-TEQ (incl. LOQs)	0.152	1.67	0.172	0.179	0.571	0.549

* Upper-bound values for the individual PBDD/F congeners

The PBDD/F concentrations of the individual milk samples and the resulting mean values are shown in Table 2. The WHO-TEQs are calculated on the basis of the above-mentioned TEFs for the Tetra to HeptaBDD/Fs.
On the basis of the rather low LOQs of the applied analytical method brominated dioxins as well as furans could be determined in any sample. There is no consistent congener pattern recognisable, but 1,2,3,4,6,7,8-HeptaBDF is the predominating congener. Furthermore, the data in Table 2 show significant variations in the PBDD/F concentrations of this sample series and the corresponding upper-bound WHO-TEQs are in the range of 0.152 to 1.67 pg WHO-TEQ/g fat. Taking into account the mean values for each congener which are also listed in Table 2 these data comply with the PBDD/F levels recently quoted for cow`s milk samples from the U.K.. However, in consideration of somewhat higher LOQs only TriBDF and HeptaBDF could be detected in the FSA survey.[6]

Brominated flame retardants (BFRs)

PBDEs were determined above the limits of quantification in every composite milk sample tested and the lower-bound total PBDE contents (Σ-PBDE) were in the range of 150 to 265 ng/kg fat. The congener patterns were nearly identical for all samples of this series. The relative contribution of the four determined congeners (BDE-47, 99, 100 and 153) are shown in Figure 1.
The main contributors to the total PBDE load were BDE-47 and BDE-99, contributing 85 % to the Σ-PBDE on average. In addition, considerable concentrations of BDE-100 and BDE-153 were determined in each sample.

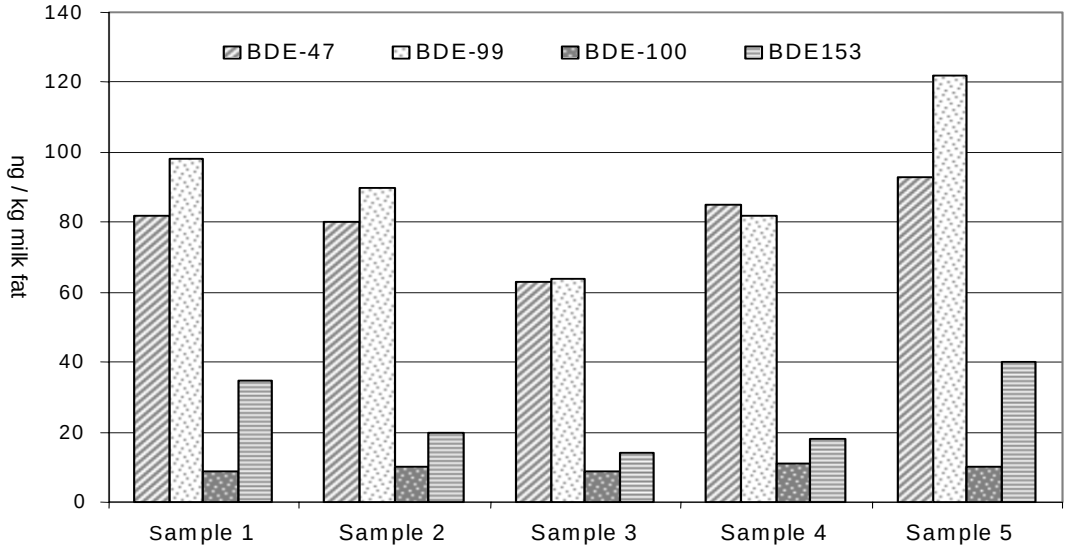

Figure 1: Concentrations of four PBDE congeners (BDE-47, 99, 100, 153) in composite milk samples related to the fat content

These data show some deviations compared with the results of our foregoing study.[9] The total PBDE contents are lower than the levels determined before and BDE-47 is not the predominant congener, but shows even slightly lower concentrations than BDE-99. Furthermore, BDE-28 and 183 could not be detected in any sample.
For BDE-47 and BDE-99 in cow`s milk almost the same pattern is documented in the above-mentioned FSA study. In contrast to this analogy the total PBDE amount found in the FSA survey is about five times higher, which can particularly be attributed to a significant level of DecaBDE.[6]
The milk samples have also been analysed for PBBs, HBCD and TBBP-A, but these brominated compounds could not be detected in any sample. These findings are in accordance with the few available literature data.

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