PBDE, PBDD/F and mixed chlorinated-brominated PXDD/F in pooled human milk samples from different countries

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Introduction

Polybrominated diphenylethers (PBDEs) are flame retardants used in a wide range of materials including electronic products or textiles. Mainly three different mixtures of PBDEs, designated PentaBDE, OctaBDE and DecaBDE, are used as technical products[1]. Thermal stress (waste combustion or accidental fires) of PBDEs may result in brominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) or mixed brominated-chlorinated dibenzo-p-dioxins and dibenzofurans (PXDD/Fs)[2]. These products and there precursors are lipophilic, persistent and bioaccumulate.

PBDD/F and PXDD/F congeners show similar toxicity as their chlorinated homologues. The increasing use of brominated flame retardants has raised concern regarding environmental releases of PBDD/Fs[3,4]. At present, there are only few studies about levels of PBDD/Fs and PXDD/Fs in human milk samples[5,6]. PBDE concentrations from different countries have been published[5,7,8] which indicate that levels in the United States are 10 to 100 times higher than those from Europe. The CVUA analyzed human milk samples from 24 countries for PCDD/Fs and PCBs as reference laboratory of the 3rd round of the WHO-coordinated exposure studies[9]. As a follow-up, PBDEs, PBDD/Fs and PXDD/Fs were quantified in selected samples. The aim was to compare levels of PBDE in human milk from countries worldwide, to investigate a possible correlation between PBDD/F, PXDD/F and PBDE levels and to determine their relative contribution to total TEQs.

Materials and Methods

Standards and Chemicals

18 unlabeled (#15, 17, 28, 47, 66, 71, 75, 77, 85, 99, 100, 119, 126, 138, 153, 154, 183, 190) and nine $^{13}C_{12}$-labeled (#15, 28, 47, 77, 99, 100, 126, 153, 183) PBDE congeners were obtained from Cambridge Isotopes Laboratories. In addition the following congeners were purchased: Unlabeled: 2,3,7,8-TBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8-HxBDD, 1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF, (2-B-3,7,8-TriCDD, 3-B-2,7,8-TriCDF, 1-B-2,3,7,8-TCDD, 1-B-2,3,7,8-TCDF); $^{13}C_{12}$-labeled: 2,3,7,8-TBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8-HxBDD, 1,2,3,6,7,8-/1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF and 1-B-2,3,7,8-TCDD. OBDF, 2-B-1,3,7,8-TCDD and $^{13}C_{12}$-labeled 2,3-DiB-7,8-DiCDD were obtained from Wellington Laboratories.

Sample preparation

Extracted human milk fat from the 3rd round of the WHO-coordinated exposure study was used and the $^{13}C_{12}$-labeled PBDEs, PBDD/Fs and PXDD/Fs directly added. Gel permeation chromatography (GPC) on Bio-Beads S-X3 (Bio-Rad Laboratories, Hercules, USA) was used for lipid elimination followed by a further clean-up using a multi-layer silica-gel column with neutral silica, acidified and basic silica and n-heptane as eluent. PBDEs, PBDD/Fs and PXDD/Fs were separated on Florisil deactivated with 3 % of water. PBDEs were eluted with n-heptane, and PBDD/Fs plus PXDD/Fs with toluene. An automated clean-up-system with a mixed column of activated carbon (Carbopack B) and Celite 545 was employed for further clean up of the latter. Amber glassware or coverage by aluminum foil was used.

Instrumental methods

High-resolution gas chromatography (HRGC) coupled to high-resolution mass spectrometry (HRMS) was employed (Trace GC/MAT 95 XP (Thermo, Bremen, Germany)). Separation was carried out on a fused silica capillary column (DB-5 MS, 15 m, 0.25 mm i.d., 0.25 µm film thickness, J&W scientific, Folsom, USA). The following temperature program was used for PBDE (1 µl injected, splitless-mode, injector temperature of 300 °C): 100 °C, isothermal for 2 min, 8 °C/min to 220 °C, 5 °C/min to 320 °C, isothermal for 4 min. Injection of PBDD/Fs and PXDD/Fs: 5 µl, PTV injector, solvent split mode, injector programming: 100 °C, isothermal 0.2 min, split flow 70 ml/min, 700 °C/min to 320 °C, isothermal 10 min, splitless time 2 min. The same temperature program as for PBDE was used for PBDD/Fs. Separation of PXDD/Fs: 100 °C, isothermal 2 min, 5 °C/min to 320 °C. The MS resolution was 10'000. The $M^{+\cdot}$ masses of DiBDE/TriBDE/PBDD/Fs/PXDD/Fs and $[M\text{-}2Br]^{+}$ of TetraBDE, PentaBDE, HexaBDE and HeptaBDE were used. WHO-TEF (1997) were used for the corresponding PBDD/F and PXDD/F congeners assuming an equal toxicity.

Quality control

Blanks, a quality control sample (for PBDE) and spiked samples (PBDD/Fs and PXDD/Fs) were checked. It was also participated in the ring test "Food 2004" (PCDD/Fs and PBDEs).

Results and Discussion

So far, human milk samples of 17 different countries have been analyzed (see Table 1). The PBDE congeners # 15, 28, 47, 85, 99, 100, 153, 154, 183 could be determined in almost all 17 human milk samples (for PCDD/F and PCB levels, see ref. 9).

Table 1: Total PBDE concentrations in human milk samples from 17 countries and reagent blank (ng/g fat) and percentage of the dominant congeners (%)

Country	Sum PBDE	BDE 15	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183
	[ng/g fat]	[%]							
Belgium	2.1	1.4	3.6	42.6	9.7	9.5	29.7	1.0	1.3
Brazil	0.8	3.5	2.8	42.9	16.7	13.8	15.4	2.1	1.7
Bulgaria	0.7	1.2	8.0	38.7	16.2	7.7	21.2	4.9	0.3
Croatia	2.0	1.2	2.7	49.5	15.7	10.1	15.0	2.7	0.4
Czech Republic	0.8	0.9	4.6	50.4	10.8	10.2	12.9	1.3	1.0
Finland	5.1	2.1	6.4	59.2	9.6	8.0	12.0	1.0	0.4
Germany	5.8	0.6	2.4	53.6	21.3	8.1	10.0	0.9	0.5
Hungary	0.7	1.8	3.8	42.5	14.2	9.3	24.2	1.8	0.9
Ireland	10.3	0.3	2.2	59.7	14.7	9.9	9.6	0.9	0.6
Italy	2.9	1.2	2.9	39.3	17.3	8.9	22.7	2.7	0.3
Luxemburg	3.0	1.1	4.0	50.5	9.4	9.9	22.1	0.9	1.0
Philippines	3.2	1.0	4.1	42.4	9.7	9.1	24.5	6.4	0.5
Romania	1.1	0.5	3.1	46.2	14.9	8.2	19.4	3.4	0.4
Slovakia	0.9	0.7	3.5	41.2	12.9	9.7	21.6	4.1	0.6
Spain	2.7	0.7	2.1	45.9	15.4	10.1	21.1	1.3	0.5
Ukraine	1.1	1.2	5.0	50.7	13.2	10.0	14.4	2.5	0.5
USA	373.6	0.4	2.3	62.6	15.7	10.5	4.7	0.8	0.0
Mean	24.3	1.1	3.6	48.6	14.7	9.4	16.9	2.1	0.6
Median	2.4	1.0	3.1	49.5	14.9	9.5	15.4	1.3	0.5
Minimum	0.7	0.3	2.1	38.7	9.4	7.7	4.7	0.8	0.0
Maximum	373.6	3.5	8.0	62.6	21.3	13.8	29.7	6.4	1.7
Reagent blank	0.07	0.9	3.4	13.3	51.3	7.3	10.8	3.1	9.8

The PBDE levels in human milk sample from the USA were a factor of 35 to 500 higher than those in other countries.The predominant congener in all samples was BDE 47 (39 % (Bulgaria) to 63 % (USA)). Different patterns were found for BDE 99 and 153. The fraction of BDE 153 was between 4.7 % (USA) to 29.7 % (Belgium). Samples with a higher percentage of BDE 47 had less BDE 153. BDE 99 was second abundant in these samples. Figure 1 compares the relative congener contribution for the two samples with high differences in the percentage of BDE 153.

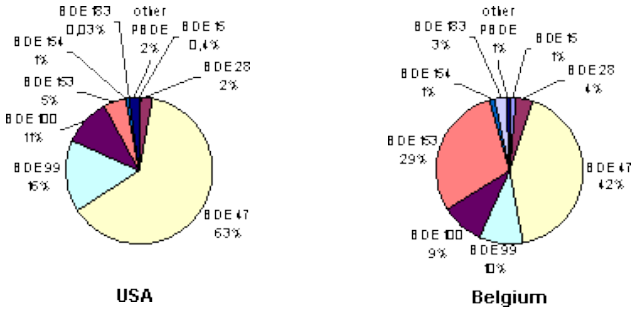

Figure 1: Relative BDE contribution to total PBDE in the human milk from USA and Belgium.

A PBDE quality control sample from "Food 2004" and reagent blanks were regularly analyzed. The average deviation from the consensus value of the control sample was 11 % in average (single congeners CV: 2.5-17 %). PBDE method blanks for the presented congeners were minimum a factor of 2 below the measured results.

PBDD/F:

The WHO-TEFs (1997) were also applied for PBDD/F congeners. An overview of the “WHO”-PBDD/F- TEQs is presented in table 2. “Upper bound” means inclusion of the limits of quantification in the TEQ calculations and “lower bound” exclusion.

Table 2: “WHO”-TEQ values in pg/g lipids of the pooled human milk samples from the 17 countries for PBDD/F, PCDD/F and their ratios. Blanks are given for comparison.

	PBDD/F-TEQ		PBDD/F- to PCDD/F-TEQ ratio	Blank (n=7)	
	lower b.	upper b.		lower b.	upper b.
Mean	0.32	1.08	0.13	0.05	0.41
Median	0.27	0.94	0.13	0.03	0.42
Min	0.04	0.45	0.06	0	0.22
Max	0.63	2.64	0.25	0.02	0.59

The average upper bound “WHO”-PBDD/F-TEQ is ca. two to three times higher than the blank. The limit of quantification (LOQ) of not detected congeners contributed to more than half to the upper bound “WHO”-PBDD/F-TEQ for most samples. “WHO”-PBDD/F-TEQ provided about 12 % to the overall sum. Dominant PBDD/Fs congeners were 2,3,7,8-TBDF (average concentration 0.7 pg/g fat, range < 0.1-2.7 pg/g) and 2,3,4,7,8-PeBDF (average 0.23 pg/g fat, range < 0.1-1.1 pg/g). 2,3,7,8-TBDF was detected in almost every sample. 2,3,7,8-TBDD (concentrations 0.06-0.28 pg/g fat) and 1,2,3,7,8-PeBDD (0.14-1.0 pg/g fat) as well as 1,2,3,7,8-PeBDF and 1,2,3,4,7,8-HxBDF were present in some samples. The recoveries of most $^{13}C_{12}$-labeled standards were between 73 and 112 % except for $^{13}C_{12}$-1,2,3,4,7,8-HxBDF (mean 48 %) and $^{13}C_{12}$-OBDD (mean 42 %).

Figure 2 compares levels of PCDD/Fs and PBDD/Fs expressed as WHO-TEQ with PBDE concentrations. No correlation between PCDD/F and PBDD/F or PBDD/F and PBDE was found. PBDD/F levels in human milk from the United States were comparable to the other countries within the same order of magnitude.

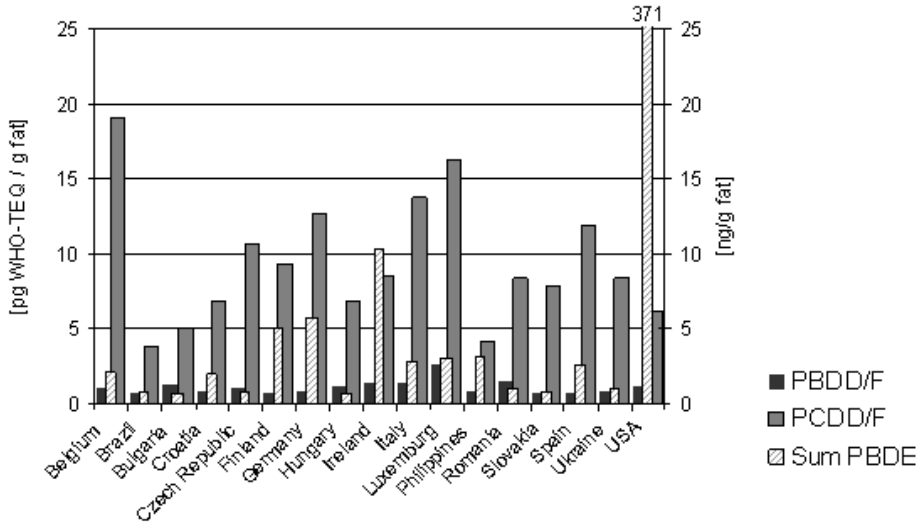

Figure 2: Comparison of "WHO"-PBDD/F- and WHO-PCDD/F-TEQs (pg/g fat WHO-TEQ, upper-bound limit) and PBDEs (ng/g fat) in the pooled human milk samples

PXDD/F:

The toxicologically most interesting tetra- and penta-substituted PXDD/Fs congeners were selected for analysis. They could not be detected in any sample. The limit of quantification of 0.05 pg/g fat for the TXDD/F and PeXDD/F congeners was comparable to those of PBDD/Fs. Recoveries of the $^{13}C_{12}$-labeled standards were between 72-105 %.

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