PBDEs, PBDD/Fs, AND PXDD/Fs IN FOOD OF ANIMAL ORIGIN FROM BADEN-WUERTTEMBERG (GERMANY) AND CORRELATIONS TO INDICATOR PCBs

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in a wide range of materials including electronic products or textiles[1]. Thermal stress (waste combustion or accidental fires) may result in polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) or mixed brominated-chlorinated dibenzo-p-dioxins and dibenzofurans (PXDD/Fs)[2]. PBDEs and their by-products have similar physical and chemical properties than polychlorinated biphenyles (PCBs) and dibenzo-p-dioxins and dibenzofurans (PCDD/Fs). They are lipophilic, persistent and bioaccumulate. PBDD/Fs and PXDD/Fs show similar toxicity as their chlorinated homolgues[3].

The human uptake of these contaminants is predominantly through food of animal origin[4]. PBDE concentrations in different food matrices have been published. Highest PBDE levels have been found in various fresh water and marine fish samples[5]. Concentrations in other food samples of animal origin were significantly below 1 ng/g fresh weight (fw)[6,7,8,9].

The aim of the study was to get an overview of the contamination of regular food samples from the German federal state of Baden-Wuerttemberg with polybrominated and mixed brominated-chlorinated contaminants. The focus was set on food of animal origin and additionally kale samples from a monitoring program. All samples were also analyzed for polychlorinated biphenyls (PCBs) to investigate possible correlations between PBDEs and indicator PCBs.

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 (CIL). 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. 6 indicator PCBs (# 28, 52, 101, 138, 153, 180) were obtained from CIL.

Sample preparation

39 food samples from the German federal state of Baden-Wuerttemberg were selected for analysis. 28 samples of animal origin (cow's milk, butter, chicken egg, meat, farmed freshwater fish and freshwater fish from the rivers Neckar and Rhine) and 12 kale samples of a monitoring program were analyzed for PBDEs, PBDD/Fs, PXDD/Fs and indicator PCBs. The eatable part of the food samples were used for analysis.

The freeze-dried fatty food samples were extracted with organic solvents (cyclohexane/toluene 1+1 for egg, meat and fish, ethanol/toluene 7+3 and tert-butylmethylether for cow's milk) in a Twisselmann hot extraction device. The extracted fat was used and the $^{13}C_{12}$-labeled PCBs, PBDEs, PBDD/Fs and PXDD/Fs directly added. Gelpermeation 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, PCBs and dibenzo-p-dioxins/dibenzofurans were

separated on Florisil deactivated with 3 % of water. PBDEs and PCBs 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 PB/XDD/Fs. Further clean-up for indicator PCBs is described elsewhere[10]. The analysis of kale samples has been described[11]. All internal standards were added prior to freeze-drying of the kale samples. Amber glassware or coverage by aluminium foil was used for all extraction and clean-up steps.

Instrumental methods
High-resolution gas chromatography (HRGC) coupled to high-resolution mass spectrometry (HRMS) was employed. In brief separation for PBDEs, PBDD/Fs and PXDD/Fs 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), for PCBs a 60-m-column was used (HT-8-PCB, 0,25 mm i.d., SGE, Victoria, AUS). The MS resolution was 10'000. The $M^{+\cdot}$ masses of DiBDE/TriBDE/PBDD/Fs/PXDD/Fs/PCBs and $[M-2Br]^{+}$ of TetraBDE, PentaBDE, HexaBDE and HeptaBDE were used.

Quality control
Blanks, a quality control sample (for PCB and PBDE) and spiked samples (for PBDD/Fs and PXDD/Fs) were checked. It was also participated in the ring tests "Food 2004, 2005 and 2006" (PCBs and PBDEs).
Interferences between PCBs and PBDEs as well as PXDD/Fs and PCBs were observed. For the latter the interfering PCBs have very similar ratios compared to PXDD/Fs.

Results and Discussion

PBDE
The total PBDE concentrations and the sum concentrations of six indicator PCBs in pg/g fw for six different food matrices are shown in table 1.

Table 1: Total PBDE concentrations in 39 food samples from Baden-Wuerttemberg and sum concentrations of six indicator PCBs in pg/g fresh weight (fw).

Sample type	Sample no.	Sum PBDE	Indicator PCB
		pg/g fw	pg/g fw
Cows' milk	C1	5,4	91
	C2	3,9	97
	C3	6,3	95
	C4	4,2	151
	mean	*5,0*	*110*
Butter	B1	220	2500
	B2	260	3000
	B3	130	3000
	B4	220	2600
	mean	*210*	*2800*
Hen's egg	E1	1800	1700
	E2	27	210
	E3	13	190
	E4	23	280
	mean	*470*	*600*
Meat	M1	11	240
	M2	100	410
	M3	25	1600
	M4	130	560
	mean	*66*	*700*
Freshwater fish			
Farmed fish	F1	1100	6200
	F2	210	2300
	F3	200	2500
	F4	120	1400
	mean	*410*	*3100*
Fish from Neckar and Rhine	F5	39000	380000
	F6	48000	460000
	F7	26000	290000
	F8	500	7700
	F9	85	2500
	F10	56000	520000
	F11	18000	280000
	mean	*27000*	*280000*

Sample type	Sample no.	Sum PBDE	Indicator PCB
		pg/g fw	pg/g fw
Kale	K1	170	2200
	K2	120	990
	K3	82	1800
	K4	6200	41000
	K5	*130,0*	*2700*
	K6	51	870
	K7	51	970
	K8	61	2500
	K9	61	1100
	K10	*73*	*1400*
	K11	110	1400
	K12	85	1300
	mean	600	4900

PBDEs could be determined in all 39 food samples. The sum PBDE levels ranged between 0.004 and 56 ng/g fw. The highest sum PBDE concentrations were found in freshwater fish from the rivers Neckar and Rhine (average 27 ng/g fw). These levels were up to two orders of magnitude higher than those of farmed freshwater fish from Baden-Wuerttemberg. In a Swiss study sum PBDE concentrations between 1.6 and 7.4 ng/g fw were found for whitefish from Swiss lakes and between 0.74 and 1.3 ng/g fw for farmed fish[12].
The PBDE levels for the other food matrices were between 0.004 (for cow's milk) and 1.8 ng/g fw (hen's eggs). For hen's eggs and kale one sample each with one to two orders of magnitude higher level was found. The mean PBDE concentrations were in the range of those found in market basket studies from other European countries[5,6,7].

The congener pattern varied significantly between the different food matrices. The relative contribution of the four major congeners (BDEs # 47, 99, 100, 153) in food samples are shown in figure 1.

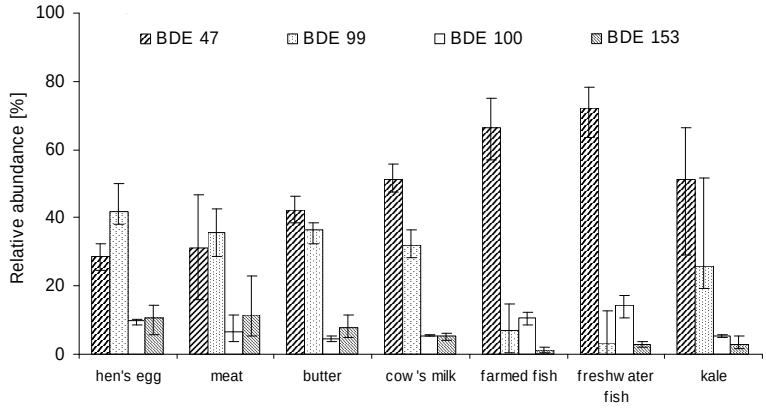

Figure 1: Relative contribution of four major PBDE congeners (# 47, 99, 100, 153) to total PBDE in % in seven different food matrices. Minima and maxima are also shown.

The predominant congener in fish samples was BDE 47 with an average relative contribution to the sum PBDE of 70 % (range 57 to 78 %). Second abundant congener was BDE 100 with a relative contribution below 16 %. In all other food samples BDE 99 was the second most abundant congener with a relative contribution between 19 to 50 % (16 to 66 % for BDE 47). In hen's eggs the relative contribution of BDE 99 was considerably higher than of BDE 47. Other congeners were mainly below 10 %.

Indicator PCBs:
Comparing the sum PBDE levels with the sum of six indicator PCBs a linear correlation over five orders of magnitude could be observed. The levels of indicator PCBs were at least a factor of 4 above the sum PBDE levels and mostly between 10 and 25 times higher except for one egg sample (E4). In this sample the sum PBDE concentration was slightly above the sum of six indicator PCBs. In figure 2 the levels of PBDEs are compared with indicator PCBs.

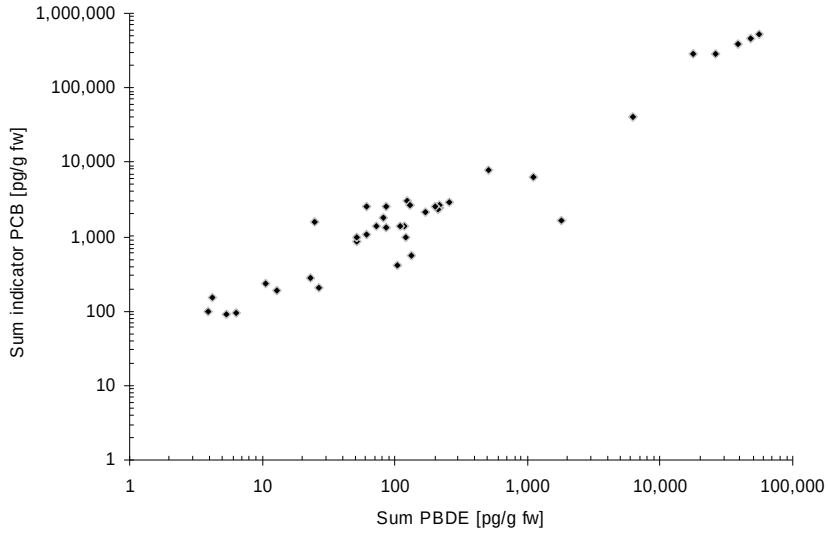

Figure 2: Comparison of sum concentration of PBDE with the sum of six indicator PCBs (#28, 52, 101, 138, 153, 180) in food samples. Concentrations in pg/g fresh weight (fw).

PBDD/F and PXDD/F:
PBDD/F and the toxicologically most interesting tetra- and pentasubstituted PXDD/F could not be detected in any sample. The detection limits of tetra- und pentasubstituted PBDD/F and PXDD/F were in the range of 0.0006 to 0.02 pg/g fw depending on the lipid content and about one order of magnitude higher than those of their chlorinated homologues. Even in the high contaminated fish samples from the rivers Neckar and Rhine PBDD/F or PXDD/F could not be found.

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