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DETERMINATION OF POLYBROMINATED DIPHENYLETHERS IN SAMPLES OF RAW COWS' MILK, FISH AND EGG

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Introduction

Polybrominated diphenylethers (PBDEs) are used as flame retardants in plastics, textiles, electronic circuitry and other materials. In plastics, concentrations range from 5 to 30%. The global PBDE production is about 40'000 tons per year, and commercial products consist predominantly of penta-, octa- and decabromodiphenyl ether mixtures[1]. PBDEs are found ubiquitously in the environment and they bioaccumulate[2]. Relatively high levels have been found in fatty fish. PBDEs have also been detected in human tissues and fluids, e.g. breast milk[3]. The biodistribution of PBDEs can be compared to other persistent organic pollutants such as PCBs. However, the time trends are different: whereas environmental PCB levels have been decreasing over the last decade(s), PBDE levels appear to increase[1,2].

In experimental systems, PBDEs have resulted in hepatotoxicity and embryotoxicity, as well as in adverse effects on thyroid function. Similar effects on the thyroid have also been reported in humans after occupational exposure. Several commercial mixtures as well as individual congeners have been toxicologically investigated, and no effect levels (NOEL/NOAEL) are in the range of a few mg/kg/day. Comparing these levels with preliminary exposure estimates indicates a large margin of safety[1]. However, since these estimates are based on very preliminary and insufficient data, they need to be treated with caution. In particular more reliable estimates of human exposure are necessary. Food is thought to be one of the major sources of human exposure, but data on levels and distribution in the food supply are scarce. Therefore we initiated a small survey analyzing for 12 PBDE congeners in 5 samples of raw cows' milk, 6 samples of different fish from various regions of the world, as well as one sample of liquid egg yolk.

Methods and Materials

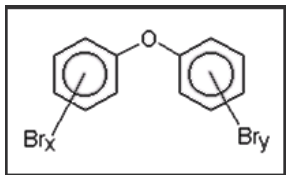
All analyses were performed following the stable isotope ratio dilution method. 13 native standards (BDE Nos. 17, 28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, 209 were obtained from Cambridge Isotope Laboratories, Andover, USA. 7 internal C13 labeled standards - BDE Nos. 28, 47, 99, 153, 154, 183, 209 - were supplied by Wellington, Canada). Solvents were supplied by Merck (n-pentane), Promochem (cyclohexane, dichloromethane) Baker (diethylether), and Mallinckrodt (ethanol, toluene). Silica gel, alumina oxide, sodium sulfate and potassium oxalate were obtained from Merck.

Cows milk: Before extraction, the mixture of 7 internal BDE standards was added to the sample (100 pg / sample for each congener). 25 ml of milk was extracted three times with 15 ml pentane, after the addition of 2 ml saturated potassium oxalate solution, 20 ml ethanol and 10 ml ether. The extract was washed with water and dried over sodium sulfate. After solvent evaporation, gravimetric lipid determination was performed.

Fish samples: A total of 10 - 200 g fish tissue (filet) was homogenized and mixed with sodium sulfate. Before column extraction, a mixture of 7 internal BDE standards was added to the sample (100 pg/sample for each congener). For column extraction a mixture of cyclohexane and

dichloromethane was applied. The extract was washed with water and dried over sodium sulfate. After solvent evaporation, gravimetric lipid determination was performed.
Egg sample: 5 g sample was mixed with 25 g of water. This was taken from a 250g sample (containing 9.3% NaCl) representative of a homogenous batch of approximately 800 to 900 Kg. Before extraction, a mixture of 7 internal BDE standards was added to the sample (100 pg/sample for each congener). Extraction was performed 3 times with acetone and pentane, 50ml each. The extract was washed with water and dried over sodium sulfate. After solvent evaporation gravimetric lipid determination was performed.
Clean up: Clean up of all lipid extracts was performed on an acid treated and activated silica gel and alumina oxide column. The final extract was concentrated by a stream of nitrogen to a final volume of 50 µl containing C13 labeled BDE 139 as a recovery standard.
The measurements were performed using high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS, HP 5890 coupled with VG Autospecat RP = 10 000). Separation was performed on a DB5 (30 m, 0,25 mm ID, 0,1 µm film) column for gas chromatographic separation. The two most abundant masses were used for measurement (M+ for Tri- and Tetra-BDE, and M-2BR+ for Penta- to Hepta-BDE). Identification of BDEs was based on retention time and isotope ratio. Quantification was performed using internal and external standards. Reduction of solvents and control of blank data is an important step in quality control when analyzing PBDEs at ultra trace levels. Solvents and reagents were tested before applying the analytical methodology. All glassware was rinsed by solvents prior to use. Silica gel and sodium sulfate were pre-washed. Rotary evaporators were not used in order to reduce the risk of contamination. No plastic equipment was used. For quality control a laboratory blank and a QC pool of human milk was run with each batch of ten samples. Quantification was only done if sample data was at least twice the blank value.

Fig.1: Chemical structure of polybrominated diphenyl ethers PBDEs and congeners analysed:

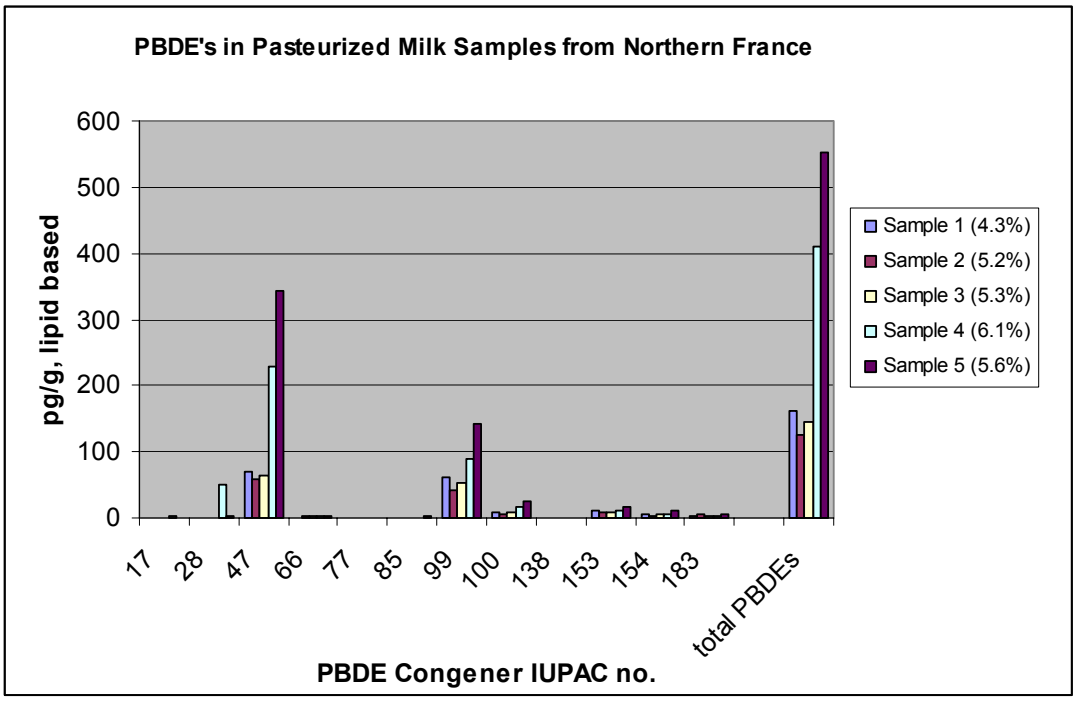


IUPAC-code	Compound	
17	2,2',4-	Tri-BDE
28	2,4,4'-	Tri-BDE
47	2,2',4,4'-	Tetra-BDE
66	2,3'4,4'-	Tetra-BDE
77	3,3',4,4'-	Tetra-BDE
85	2,2',3,4,4'-	Penta-BDE
99	2,2',4,4',5-	Penta-BDE
100	2,2',4,4',6-	Penta-BDE
138	2,2',3,4,4',5'-	Hexa-BDE
153	2,2,'4,4,',5,5,'-	Hexa-BDE
154	2,2',4,4',5,6'-	Hexa-BDE
183	2,2',3,4,4',5',6-	Hepta-BDE
209	2,2',3,3',4,4',5,5',6,6'-	Deca-BDE

Results and Discussion

The following graph summarizes the results from milk samples (A). Numbers in parenthesis in the legend are the percentage fat content of the respective sample. Total PBDEs is the sum of all quantifiable congeners.

Graph A

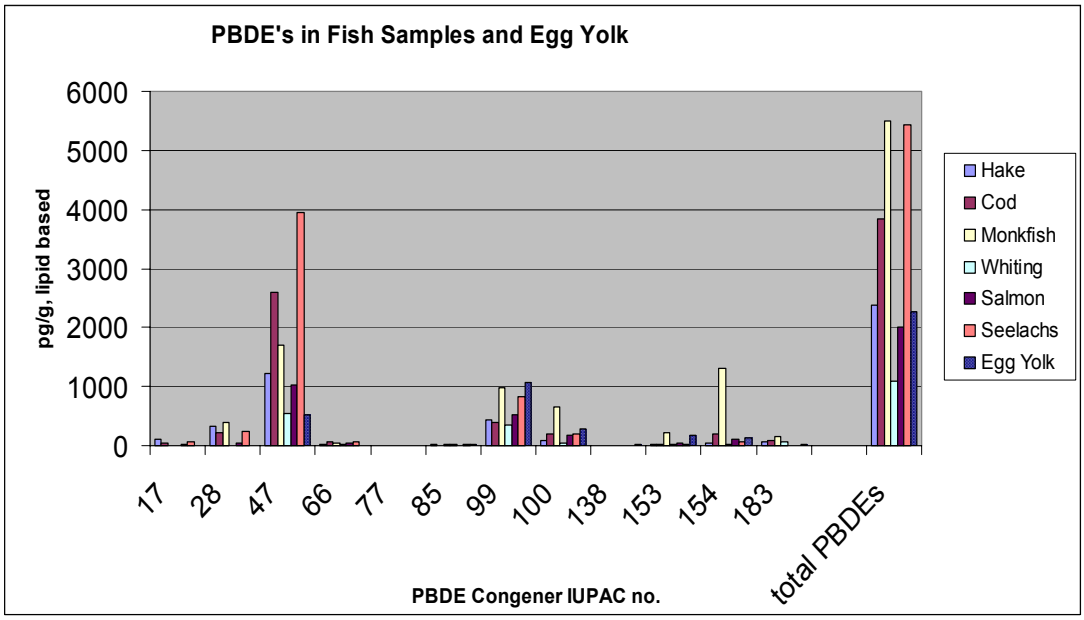


Each milk sample is representative for the load of one milk collection truck in Northern France (Districts of Pas de Calais and Nord). The area was chosen because it is highly industrialized and known for increased background levels in dioxins and PCBs, which was confirmed by our analysis (data not shown). Mean dioxin/furan + PCB levels in these samples was 2.3 WHO-TEQ (1.6–3.5). The sample with the highest dioxin and PCB levels also has the highest PBDE levels (sample 5), however it appears that dioxin/PCB and PBDE levels do not directly correlate. Samples 1-3 are from the district of Pas de Calais, whereas samples 4 and 5 with the highest PBDE levels (with PBE-47 more than 50% of total PBDEs) are from District of North, possibly indicating a specific source in this area.

The following table shows the type and origin of the fish and the egg sample, the graph below (B) summarizes the results from fish and the egg yolk sample:

Species	Origin	Fat content (%)
Hake (Colin)	North sea	0.07
Cod (Cabillaud)	North sea	0.12
Monkfish (Lotte)	China	0.06
Whiting (White Merlu)	Peru	0.10
Salmon (Saumon)	Norway	2.5
Pollock (Seelachs)	Russia	0.1

Graph B



Due to potential blank influence, 2,2',3,3',4,4',5,5',6,6'-deca BDE-209 was not quantified in the samples. The highest detected levels in all samples (except egg yolk) was from the tetra BDE-47. In milk, BDE-47 represented between 43% and 62% of the total PBDE's, while for fish, this varied between 31% and 72% and egg yolk was 23%. Simarlarly, a study on human breast milk carried out in Sweden showed that fish contained the highest amount of BDE-47 from amongst different dietary sources of PBDEs[3]. Another study also showed that BDE-47 was the predominant congener found in freshwater fish in Germany[4]. Absorption of PBDEs depends on the degree of bromination. The higher the degree of bromination the lower the degree of uptake. One of the most abundant congener found in environmental samples is 2,2',4,4'-tetraBDE-47, indicating a selective bioconcentration of this congener, since other tri- and tetra-brominated congeners are present in commercial mixtures[2,5]. This is also reflected in our samples, where this congener is found at high levels in all samples. Second highest levels detected are the 2,2',4,4',5-penta BDE-99. In the egg yolk sample this congener constitutes the highest level, contributing 47% to total PBDEs.

References

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