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LEVELS OF SELECTED PBDEs AND PCBs IN BELGIAN HUMAN MILK

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Introduction

Since few years, some concerns appeared regarding the presence of polybrominated biphenyl ethers (PBDEs) in our environment[1]. Those chemicals are used as flame retardant in virtually most of our surrounding man-made materials. Despite the great protection those chemicals offer, they also can act as precursors for polybrominated dibenzo-*p*-dioxins and furans (PBDD/Fs)[2] that are suspected to express similar toxicities than their chlorinated homologs[3]. Additionally, depending of their route of incorporation in the synthetic materials, PBDEs can relatively easily leach out during their life-time, even if no fire is involved. They can therefore become available to humans that are exposed via food consumption and air particulates breathing.

As in the case of dioxins and related compounds, PBDEs are characterized by high lipophilic properties and are willing to bio accumulate. Although increasing levels have been reported in human last years[4], very limited toxicological data are available so far[5]. It is however believed that thyroid hormone disruption, neurodevelopmental effects and, in a certain extend, cancer, can be listed as potential toxicological endpoints[6]. Developing babies and infants therefore represent one of the most sensitive layer of the population regarding exposure to those chemicals.

The present study was carried out in order to give an estimate of the current PBDE background levels in breast milk of a selected Belgian population.

Materials and methods

Samples: Breast milk samples (N=14) issued from primi and multiparae women between the ages of 26 and 38. They were collected between August 2000 and April 2001 in Belgium (most of them in the city of Liege, Wallonia) at different times of lactation. Volunteers were asked to fill out a personal questionnaire including parameters of interest. Decontaminated collection vials and breast-pump were used by volunteers for sampling and samples were stored less than one day at 4°C before freezing at –20°C until analysis. Portions of 10 ml were used for this study.

Extraction and Clean-up: Samples were pre-treated prior extraction. Milk fat globules membranes were disrupted by potassium oxalate (1mg/g milk) and acetonitrile (1:1). Water was added to the milk (1:1) in order to decrease the viscosity of the mixture. Around 30 ml of pre-treated sample was then loaded on Isolute Flash C18 cartridges (International Sorbent Technology, Hengoed, UK). These cartridges consisted of polypropylene syringe-barrels of 70 ml filled with 10 g of non-endcapped C18 bonded silica sorbent (average particle size 50 µm, 60 Å porosity). Cartridges were used on a Flashvac® extraction vacuum manifold (IST). C18 sorbent was solvated using 2 volumes of acetonitrile and 2 volumes of water at 20 ml/min prior to the addition of sample at a maximum flow-rate of 10 ml/min. SPE cartridge were washed with twice 10 ml of water and 2 ml of methanol were added (to make drying easier) prior to 1 hour of drying under reduced pressure. PBDEs and PCBs were eluted using 4 times 15 ml of hexane at a flow rate of 5

ml/min. Extracts were concentrated to 15 ml using a Turbovap II Concentration Workstation (Zymark, Hopkinton, MA, USA) prior to further clean-up. Automated multi-column clean-up was performed on the Power-Prep System (Fluid Management Systems, Waltham, MA, USA). Details concerning the system are available elsewhere[7]. The set of column consisted in multi-layer silica (acidic, neutral and basic) and basic alumina. The lipid content determinations were carried out gravimetrically on the side of the analysis using small volume of milk.

Analysis: Analyses were performed by tandem in time mass spectrometry (GC/MS/MS) using a ThermoQuest Trace GC PolarisQ ion trap mass spectrometer (Austin, TX, USA) and a Agilent (Palo Alto, CA, USA) 6890 Series gas chromatograph, the latter equipped with a Rtx 5-MS (40m x 0.18 mm x 0.18 µm) capillary column (Restek, Evry, France). Electronic impact was used as ionisation mode, with an energy of 70 eV. Quantification was performed using internal standards (isotopic dilution). Details are available elsewhere[8]. As QC samples, aliquots of a breast milk sample pool were analyzed and compared with another laboratory performing PBDE and PCB analyses.

Results and Discussion

As expected, the predominant PBDE congeners in the investigated human breast milk were BDE-47 and BDE-153, with concentrations of 1.7 ng/g and 0.4 ng/g on a lipid weight basis, respectively (Figure 1). Both BDE-47 and BDE-153 contribute for more than 75% of the measured concentrations (6 congeners).

	Concentrations (pg/g lipid weight)	S.D.
BDE-28	91.6	170.5
BDE-47	1692.2	1902.6
BDE-100	168.6	209.2
BDE-99	354.0	318.5
BDE-154	119.0	82.5
BDE-153	430.1	387.8

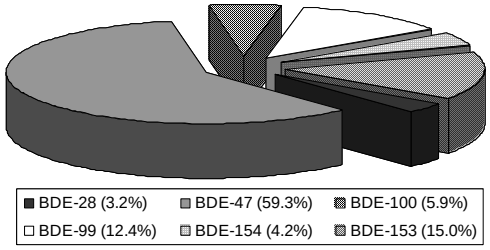

Fig. 1 : BDE concentrations and relative contributions in investigated Belgian breast milks.

The sum of the 6 BDE congeners is equal to 2.85 ng/g lipid weight. This is inside the range of recently reported values for Belgian adipose tissues[9]. A significant difference was however present between the two studies for BDE-153 concentration that was about 6-fold higher for the adipose tissue study. The ratio of BDE-47 over BDE-153 was 3.9 for the present study versus 0.6 for the adipose tissue study. Additional samples of adipose tissues and breast milk should be collected in the country in order to assess any potential regional differences in the congener distribution. Furthermore, differences between breast milk and adipose tissue congener distribution are sometimes observed and make inter-matrix comparison delicate.

A rapid calculation yield to a PBDE (6 congeners) intake value of 7 ng/kg of body weight/day or 4.4 µg total over the 3 first months of breast feeding (600 ml of milk/day, 7 kg baby, 3.5% fat).Based on a previous market basket study[5] reporting dietary intake of 0.73 ng/kg of body weight/day (70kg adult, 5 congeners), infants would then receive about 10-fold higher intake than adults.

As illustrated in Figure 2, the concentrations of the 6 reported PBDE congeners are similar to the ones measured in various countries, excluding the U.S.A. and Canada for which levels are much higher. Unfortunately, no anterior data on PBDE levels in human breast milk are available in Belgium and no time trend can be extracted yet. Additional sample collection will allow to see if Belgium is undergoing a reduction in levels as reported in Sweden since 1999[16].

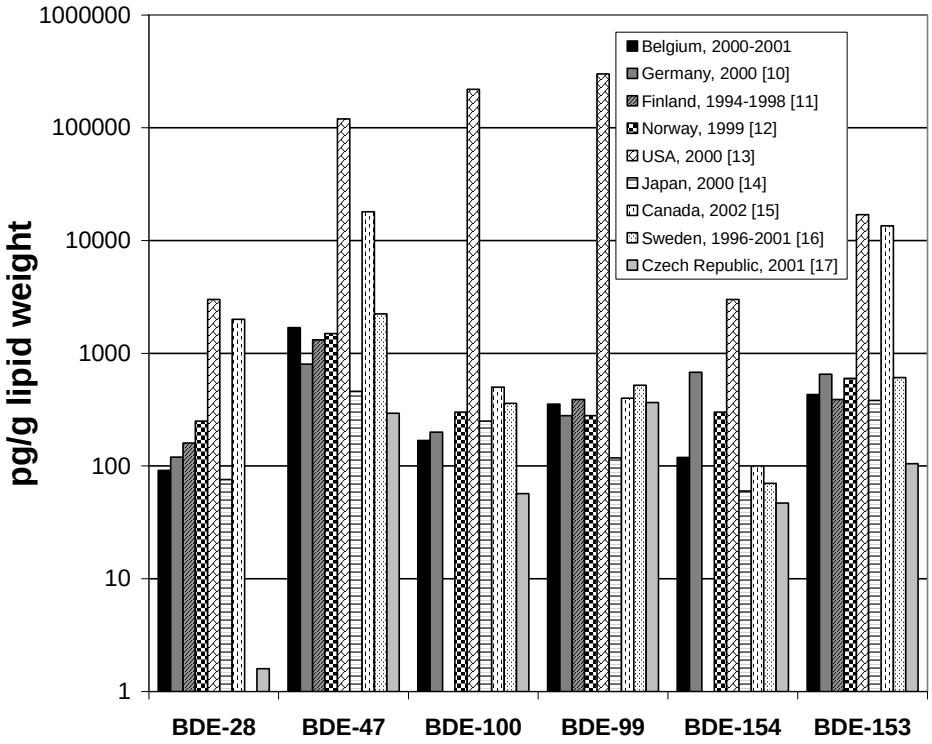

Fig. 2 : Comparison (Log scale) of the Belgian data with some reported BDE concentrations in human breast milk (except Norway (serum), Japan (adipose tissue) and Czech Republic (adipose tissue)).

Although a correlation was observed for PCDD/F and PCB levels with age and parity[18], no significant correlations were recorded for PBDEs. Such a difference is most probably connected to higher variability in exposure routes.

The concentrations of the major PCB congeners were 105±63 ng/g, 79±44 ng/g and, 90±52 ng/g for PCB-153, PCB-138 and, PCB-180 on a lipid weight basis, respectively. Although the congener distribution is very similar, those concentrations are about half of the concentrations measured in the adipose tissue study[9]. This is not surprising since the mean age of the subject was about 15 years lower and that some multiparae mothers were included in the present study. The interesting aspect is that such a difference is not observed for PBDEs, as illustrated in Table 1. PCB and PBDE distributions in human are clearly not affected by the same parameters and respond to different influences.

Table 1: Difference in behavior of major PCB and PBDE concentrations regarding age and sex.

	Breast milk 2000-2001 mean age 30.4 years women only		Adipose tissue[9] 2000 mean age 47.2 years men and women	
	Concentration (ng/g lipid weight)	Contibution (%)	Concentration (ng/g lipid weight)	Contibution (%)
PCB-153	105.11	38.4	221.1	46.6
PCB-138	78.9	28.8	105.1	22.1
PCB-180	89.73	32.8	148.3	31.3
BDE-47	1.69	68.4	1.45	34.4
BDE-99	0.35	14.2	0.28	6.6
BDE-153	0.43	17.4	2.49	59

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