PBDE LEVELS IN HUMAN MILK: THE SITUATION IN GERMANY AND POTENTIAL INFLUENCING FACTORS - A CONTROLLED STUDY

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Introduction

PBDEs are used as flame retardants in upholstery textiles and in polymers with end-uses in electrical and electronic equipment (computers, TV, wire cables etc.) [1]. They are believed to be persistent and to bioaccumulate. They may cause neurodevelopmental effects and endocrine disruption and may be carcinogenic. They can cross the placenta. In the EU, the use of technical penta- and octa-BDE has been banned in all applications by a directive, which will enter into force on 15 August 2004 [2]. Regulations for deca-BDE are debatable due to a lack of data.
An exponential increase of PBDE levels in breast milk from Sweden between 1972 and 1997 has been reported, which is in contrast to the continuous decline of other chlorinated POPs in breast milk [3]. Also in blood samples from Germany, an increasing trend has been observed during the period from 1985 to 1999 [4]. The knowledge about human exposure pathways, which contribute to the PBDE body burden, is very limited. Consumption of food of animal origin, inhalation or ingestion of dust and further factors possibly influencing the PBDE levels in human matrices, like age, breast-feeding or smoking are under discussion.
Only a few data on PBDE levels in breast milk from Germany have been published [5]. To fill the data gaps, a controlled study was started in 2001 to characterise the PBDE levels in human milk from Germany with special efforts to identify and quantify deca-BDE-209. Furthermore, it was intended to verify potential factors possibly influencing PBDE levels. Two main hypotheses were proposed: 1) Are PBDE levels in breast milk from mothers consuming traditional food (omnivores) higher than those found in breast milk from mothers consuming vegetarian or vegan food? and 2) Are the PBDE levels found in human milk after a three-months period of breast-feeding lower than those detected at the beginning or does breast feeding result in a lower body burden, respectively? This paper summarises preliminary results. Further analytical data and results of data evaluation will be presented at the conference.

Methods and Materials

Study design and sample collection:
143 human milk samples were collected from volunteers from Germany between November 2001 and December 2003. Samples were collected in chemically cleaned glass bottles wrapped in aluminium foil. Samples were frozen at -20 °C and stored at -80 °C. Two groups of participants were included: 1. Mothers with traditional food consumption habits (omnivores) and 2. Mothers following a vegetarian or vegan diet for the last 5 years at least. A 1st milk sample was collected during the second week after delivery (1st sampling period, N=85). In the case of continued breast-feeding we asked for a 2nd milk sample collected around the 12th week after delivery (2nd sampling period, N= 53). In addition, 5 milk samples from mothers nursing for at least 6 months or even longer were collected. All participants had to comply with a set of criteria, for instance: living in Germany since delivery, no known occupational exposure, nursing their first or second child. A few samples from mothers nursing their third child were included. The women answered a questionnaire asking for further details regarding their dietary habits, life style, profession, smoking habits, living area, age, weight, height and other data.
Statistical method and data evaluation:
All calculations were performed assuming that values below the LOQ (limit of quantification) were equal to one half of the LOQ. For testing the hypotheses, the t-test was used on log transformed data as the PBDE concentrations did not follow a normal but a lognormal distribution. The t-test for paired samples was chosen for testing the 2nd hypothesis by comparing the PBDE level of the 1st and the 2nd milk samples of mothers by whom both had been collected. Based on the reasonable assumption of decreasing trends for both hypotheses, all tests were performed as a one-tailed test with a level of significance of 0.05 by using SPSS.
Analytical method and quality control:
The method used as well as the quality assurance procedure are described elsewhere in more detail[7]. In the following a brief description is presented: Before extraction, a mixture of 7 ^{13}C-labelled internal PBDE standards (PBDE Nos. 28, 47, 99, 153, 154, 183, 209; Wellington Laboratories and Cambridge Isotope Laboratories) was added to the sample. 10 ml of human milk was extracted with n-pentane (Merck). After solvent evaporation gravimetric lipid determination was performed. The extract was cleaned up by acid treatment and passed through an activated silica gel and an alumina oxide column. ^{13}C-labelled PBDE 139 as a recovery standard was added to the extract. The measurement was performed by high-resolution gas chromatography / high-resolution mass spectrometry (HRGC/HRMS) on an HP 5890 II GC coupled with a Micromass AutoSpec mass spectrometer. Quantification was carried out by means of the isotope dilution method. Quantification was only performed if the sample level was at least twice the blank level (usually the sample level was much higher than the blank level).

Results and Discussion

Up to now, the PBDE levels of 93 human milk samples from both groups of mothers have been analysed. These analytical results and the preliminary statistical evaluation of the hypotheses are presented here. Some relevant data describing the sampling groups are summarised in Table 1. The PBDE levels analysed in breast milk samples collected between 2001-2003 are given in Table 2.
PBDE-levels in human milk from Germany
Based on all data of the first sampling period, the PBDE levels in human milk from Germany have been characterised. The concentrations of both the total PBDE and the main congeners in the

German milk samples are comparable to the current data on PBDE levels in human milk from other European countries [8-13]. However, as compared to PBDE levels in human milk from the US, the German values are lower by a factor of about 10 - 100 [14].

Table 1: Description of the sampling groups

	Omnivores	Vegetarians/ Vegans	Total
1st sampling period	N = 37	N = 25	N = 62
2nd sampling period	N = 17	N = 14	N = 31
Age of the mothers (years)	Mean: 31y, (18-40 y)	Mean: 32 y (19-41 y)	Mean: 32 y (18-41 y)
Nursing the first child	N = 29	N = 14	N = 43
Nursing the second child	N = 7	N = 9	N = 16
Nursing the third child	N = 1	N = 2	N = 3

The average of the total PBDE (sum of 9 congeners, all samples from the first sampling period) is equal to 2.2 ng/g fat (range 0.6 - 7.25 ng/g fat). In the meantime, a number of further data on PBDE levels in human milk from Germany collected during the same period have been published. While data by Weber et al. are distinctly higher (mean Σ-PBDE 7.2 ng/g fat), data by Fürst have confirmed the range of the PBDE concentrations reported in the present study (mean Σ-PBDE = 2.4 ng/g fat) [15, 6]. The mothers participating in this study belonged to the general population with no specific exposure to PBDEs. Therefore, the concentrations determined in breast milk may be considered as background levels.

Table 2: PBDE concentrations in human milk from Germany (ng/g fat) sampled between 11/2001-12/2003 (Σ-PBDE was calculated assuming values below LOQ as half the LOQ)

Nutrition habits	Omnivores and Vegetarians/ Vegans together						Omnivores	Vegetarians/ Vegans
	1st Sampling period					2nd Sampling period	1st Sampling period	1st Sampling period
Number of samples	N = 62					N = 31	N = 37	N = 25
	Mean	Median	95th Perc	Max	N<LOQ	Mean	Mean	Mean
BDE-28	0.04	0.04	0.12	0.17	9	0.04	0.05	0.04
BDE-47	0.82	0.54	3.52	4.50	1	0.78	0.99	0.58
BDE-66	0.01	0.01	0.03	0.06	14	0.01	0.02	0.01
BDE-99	0.25	0.17	0.92	1.30	2	0.25	0.30	0.16
BDE-100	0.21	0.17	0.67	1.10	0	0.19	0.23	0.18
BDE-153	0.63	0.53	1.54	1.90	0	0.50	0.66	0.57
BDE-154	0.02	0.02	0.06	0.07	0	0.02	0.03	0.02
BDE-183	0.09	0.04	0.34	0.63	12	0.06	0.10	0.07
BDE-209	0.17	0.10	0.59	1.00	37	0.11	0.17	0.17
Σ-PBDE	2.23	1.78	6.69	7.25		1.95	2.54	1.78

The share of the different congeners in the total PBDE is shown in Figure 1. BDE-47 is the predominant congener followed by BDE-153, with mean concentrations of 0.82 and 0.63 ng/g fat, respectively. Together these congeners account for about 65 % of the total PBDE. Similar congener patterns have been reported by other authors [3, 6, 9, 12].

Despite of its lower bioavailability compared with the other congeners and in contrast to results of other human milk studies of the European countries, BDE-209 has been identified and quantified in our samples [8-13]. In 40 % (25 of 62) of the samples, BDE-209 levels were above the limit of

quantification, which is about 0.1 ng/g fat. The mean concentration of BDE-209 is 0.17 ng/g fat (max: 1.0 ng/g fat). To our knowledge, it is the first time that BDE-209 concentrations in human milk samples reflecting background contamination are reported. There has been a major data gap concerning BDE-209. Up to now, BDE-209 concentrations have been determined only in those human samples that exhibited distinctly elevated PBDE levels. These include blood samples from occupationally exposed workers and some human milk samples from the US (7 of 23) [14, 17, 18].

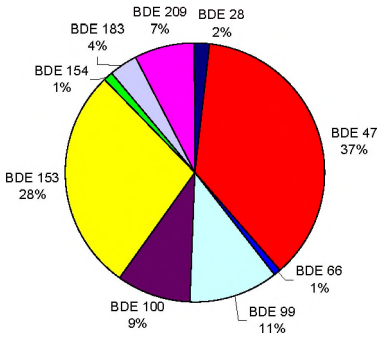

Figure 1: Shares of single BDE congeners in total PBDE in human milk from Germany

Influence of nutrition habits on PBDE levels - testing hypothesis 1

As in the case of organochlorine POPs, it has been discussed that the consumption of food of animal origin could be a relevant exposure pathway. Dietary intake data have been estimated [5, 19]. Nevertheless, no significant influence of dietary intake on PBDE levels in human milk could be observed [20]. To clarify the impact of consuming food of animal origin on human PBDE levels, human milk from omnivores and from vegetarians or vegans was sampled and analysed. This evaluation is based on the data from the 1st sampling period. When comparing the PBDE levels found in the group of omnivores with those from the group of vegetarians (see Table 1), a clear tendency becomes apparent. The mean levels of Σ-PBDE and of the main congeners in milk samples of vegetarians/ vegans are lower by about 30 % and 15-40 %, respectively.

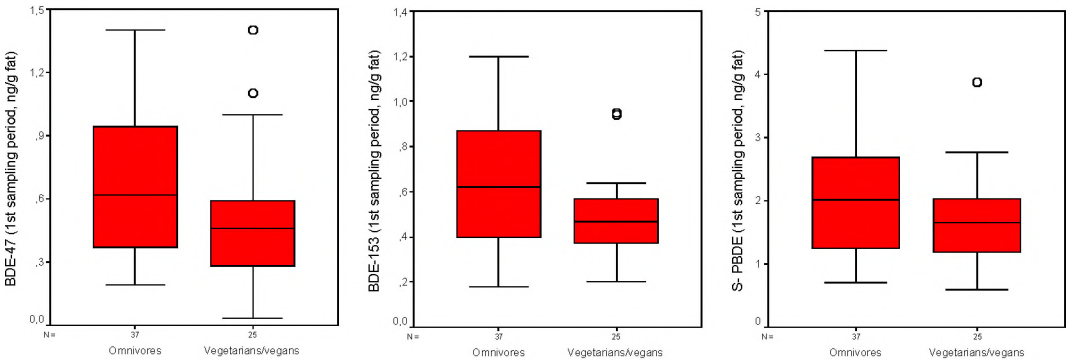

Figure 2: Comparison of concentrations of BDE-47, BDE-153 and total PBDE (S-PBDE) in human milk of omnivores vs. vegetarians/vegans (1st sampling period)

Using the one-tailed t-test, the differences between both groups are significant for Σ-PBDE and most of the congeners in human milk from the 1st sampling period (Figure 2).

Influence of breast-feeding on PBDE levels - testing hypothesis 2
While the levels of organochlorine POPs in human milk decrease with the duration of nursing, no correlation of the PBDEs could be found with the time of nursing or the number of children in human milk from the US [14]. Our analytical data indicate a slight decline of the mean PBDE levels between the 1st and the 2nd sampling period (2nd week after delivery and around 12th week after delivery) (Table 2). The mean of Σ-PBDE decreased by about 13 %, when all samples were included. Nevertheless, when comparing the Σ-PBDE levels of the 1st and the 2nd milk samples of those mothers by whom both had been collected, by using the t-test for paired samples, the declining tendency during about 10 weeks of breast-feeding is not significant. Nevertheless, longer breast-feeding periods are assumed to have a clear impact because significantly lower Σ-PBDE levels in human milk from mothers nursing their second or third child compared with mothers nursing their first child can be demonstrated by the results of the t-test (Figure 3). Further human milk samples from some mothers nursing their child for longer than 6 months will be analysed.

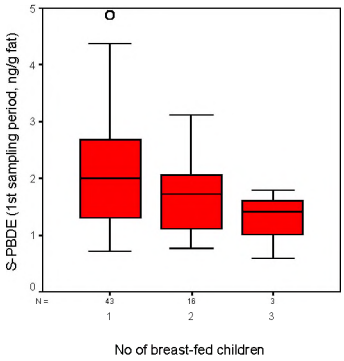

Figure 3: Dependence of total PBDE (S-PBDE) on the number of breast-fed children (1st sampling period)

Summarising these preliminary results, it is remarkable that BDE-209 has been quantified in 40 % of the human milk samples, corresponding to background level. Testing the hypotheses, significantly lower PBDE levels have been observed in the group of mothers preferring vegetarian or vegan nutrition and furthermore in the group of mothers nursing their second or third child. However, it has to be pointed out that the share of mothers nursing their second or third child is higher in the group of vegetarians/vegans (see Table 1). Therefore, further and more detailed data evaluation including more samples and analytical results is in progress.

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