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PBDEs IN HUMAN MILK FROM THE DUTCH 1998 MONITORING PROGRAMME

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Introduction

Since the early 1970s, Dutch breast milk is monitored for the presence of organochlorine pesticides[1]. In the 1980s, the monitoring programme has been extended with PCBs and PCDD/Fs. The programme has a five year frequency, the last round being held in 1998[2]. This year starting in June, the 2003 round is in progress.

A few years ago, a paper was published on the presence of Poly Brominated Diphenyl Ethers (PBDEs), a specific group of Brominated Flame Retardants or BFRs, in breast milk originating from Sweden[3]. This aroused interest for the situation in The Netherlands regarding the presence of these compounds in Dutch human milk. Thus, the samples collected in the 1998 round were recently analysed for the presence of a selection of the PBDEs. This paper reports on the results of this work.

Methods and Materials

The study population consisted of a representative milk sample of 339 mothers, who had given birth to their first baby in September/October 1998. The mothers were approached in co-operation with 26 maternity centres located all over The Netherlands. The respondents were asked to collect a 100 ml breast milk sample between days 6 and 10 after delivery. Of these 339 samples, 103 were analysed quantitatively for the presence of 11 PBDE congeners (nos. 17, 28, 47, 66, 85, 99, 100, 138, 153, 154 and 183).

In order to obtain the fat and the fat-soluble compounds, the milk samples were liquid-liquid extracted according to a modified AOAC method[4]. To an aliquot of the fat extracts, a solution of hexane containing six $^{13}C_{12}$-labeled internal quantitation standards (Cambridge Isotope Laboratories, Woburn, MA, USA) of the PBDEs (nos. 28, 47, 99, 153, 154 and 183) was added at levels between 2.9 and 6.9 pg/g of (extracted) fat. Clean up of the fat extracts was by injecting 1 ml of a fat solution (45 mg fat/ml hexane) onto a normal phase HPLC system equipped with a silica column and iso-hexane as mobile phase [5]. The fraction containing the PBDEs was collected, evaporated to dryness and redissolved in 0.5 ml of hexane.

GC/MS analyses were performed on a quadrupole mass spectrometer coupled to a gas chromatograph. GC separations were carried out on a non-polar column (30 m DB-5MS ; J&W Scientific, Folsom, USA; 0.25 mm ID, 0.10 µm film thickness). Samples for PBDE analysis were analysed by injecting 100 µl of the final hexane extract with an OPTIC PTV injector (ATAS, Veldhoven, The Netherlands), operated in the rapid large volume sampling mode. Ionisation of samples was performed in the electron impact mode (EI) with 70 eV electrons. Detection was performed by selected ion recording (SIR).

Quantitation of the compounds was by isotope dilution and one point calibration with the mean value of results of the analysis (n=10) of a standard solution. Selection of m/z-values for each

congener was from the molecular ion [M] cluster or from the [M – 2Br] cluster, depending on which cluster was the most intense and/or less distorted.
The efficiency of the applied extraction method was estimated by comparing the recovery of the $^{13}C_{12}$–labeled congeners from milk and from fat extracts, respectively. The ratio of the recoveries from fat extract vs the recoveries from milk (n=10) was 94-105% (RSD 10-18%), with the exception of PBDE 183 (146%, RSD=54%). The limits of determination for the 11 congeners ranged from 0.02 – 0.1 ng/g fat.
In every batch of analysis a quality control (QC) sample is analysed. The reproducibility of the method was evaluated by calculating mean values with RSDs for all PBDEs from the results of all the QC samples analysed, during the validation of the analytical method as well as during the analysis of the milk samples (n=14). RSDs ranged from 3.2 – 16.4 %, except for PBDE 183 (RSD=64.5%). The accuracy of the method was evaluated by participating in the BSEF/Quasimeme Interlaboratory Study on Brominated Flame Retardants 2002. Within the group of participating laboratories, our results were satisfactory.

Results and Discussion

TABLE 1
Characteristics of the dataset in minimum-maximum values, medians etc

	BDE17	BDE28	BDE47	BDE66	BDE85	BDE99
Number of results > LOQ	10	103	103	36	13	103
minimum value (ng/g fat)	<0.03	0,05	0,45	<0.06	<0.08	0,17
maximum value (ng/g fat)	0,13	0,43	6,50	0,32	0,17	2,70
median (ng/g fat)	**<0.03**	**0,11**	**1,19**	**<0.06**	**<0.08**	**0,37**
mean value (ng/g fat)		0,13	1,53			0,53
standard deviation (ng/g fat)		0,07	1,11			0,41
relative standard deviation		0,51	0,73			0,78

	BDE100	BDE138	BDE153	BDE154	BDE183
Number of results > LOQ	103	0	103	47	100
minimum value (ng/g fat)	0,09	<0.1	0,33	<0.08	<0.09
maximum value (ng/g fat)	1,72	<0.1	3,88	0,26	1,90
median (ng/g fat)	**0,31**	**<0.1**	**0,95**	**<0.08**	**0,41**
mean value (ng/g fat)	0,37		1,03		0,45
standard deviation (ng/g fat)	0,25		0,53		0,28
relative standard deviation	0,69		0,52		0,62

In table 1 the whole dataset is characterised. The PBDE congeners 28, 47, 99, 100, 153 and 183 are found at a concentration higher than the Limit of Quantitation (LOQ) in all or nearly all of the 103 milk samples that were analysed. PBDE 47 is the congener that occurs in the relatively highest concentration. The ratio maximum value : median for the PBDE congeners is larger than is the case for the dioxin and PCB congeners in the same samples[2].

When the results are compared with the results of a Swedish study for the presence of PBDEs in breast milk[6], also carried out in 1998, they are quite comparable. Relatively most abundant congeners in the Swedish study are 47, 99, 100 and 153. The Σ median of these 4 congeners in this study is 3.13 ng/g fat, in the Dutch study this Σ median is 2.82 ng/g fat. PBDE 47 is the compound that occurs in the relatively highest concentration in both studies.
In Canada and the United States at the end of the 1990s and in the years 2000-2001, much higher concentrations of the PBDEs were found in breast milk[7,8]. Σ concentrations of >50 ng/g fat, up to almost 300 ng/g fat, are no exception.

The correlation between the levels of PBDEs and several other organohalogen compounds is studied by Principle Component Analysis (PCA). The projection of the compounds on the first 2 PC's are shown in figure 1. The majority of PCDDs/Fs and PCBs project on the first PC. Most of the PBDEs project on the second PC. This indicates that PCDDs/Fs and PCBs have a strong mutual correlation and also that PBDEs have a strong mutual correlation, but the correlation between the two group of compounds is weak. This is illustrated by a graphical presentation of the correlation matrix of some major components as shown in figure 2. Both figures also show that the correlation of PBDE 153 with the other (lower brominated) PBDEs is relatively weak.

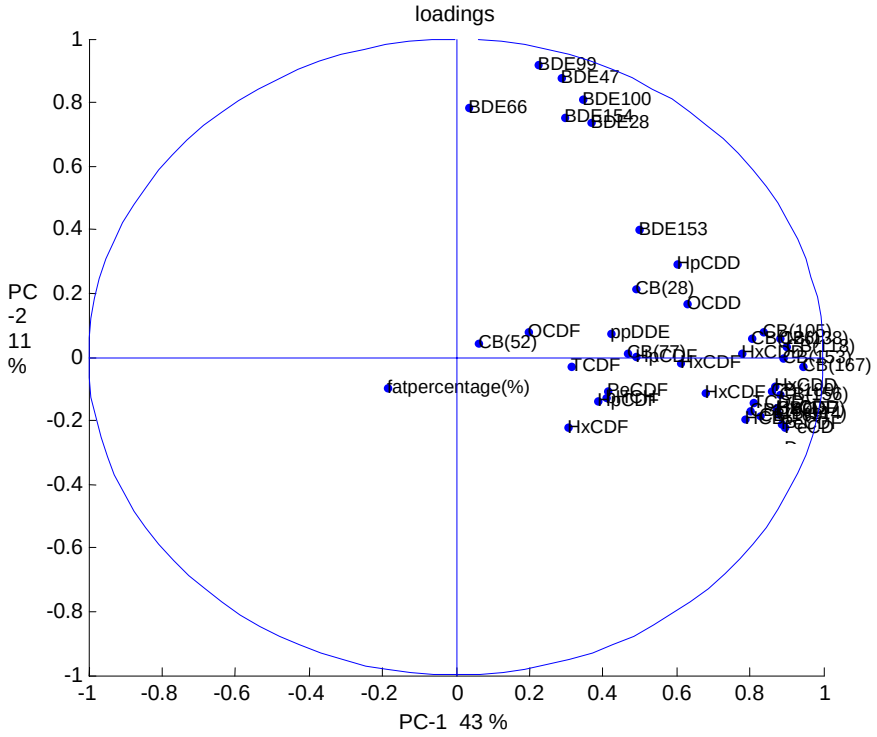

Figure 1 Projection of organohalogen compounds on the first 2 principle components

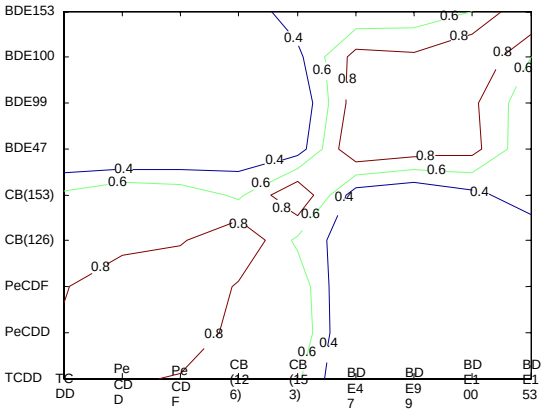

Figure 2 Grafical presentation of the correlation between several organohalogen components of interest

Future research

The same PBDE congeners as studied in this paper will also be analysed in the 2003 breast milk samples that were collected very recently. Also remaining samples from previous monitoring rounds, e.g. from 1988 and 1993, will be analysed accordingly. When all these data are available, we will be able to look at the time trend in The Netherlands for the period 1988-2003.
In the near future we are planning to look also at some other BFRs as the Hexabromocyclododecane (HBCD) isomers and Tetrabromobisphenol-A (TBBP-A) and its metabolite by developing an analytical method based on LC-MS. Decabromobiphenylether (PBDE 209), although by some authors assumed not to accumulate in the food chain due to its bulkiness, will also be investigated for its presence in breast milk in due time.

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