DETERMINATION OF PBDES IN HUMAN MILK – ANALYSIS AND QUALITY CONTROL -

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Introduction

Polybrominated diphenylethers (PBDEs) are widely used as flame retardants in polymer materials, textiles, electronic boards and various other materials. Technical PBDE preparations are produced as mixtures of mainly penta-, octa- or decabromobiphenyl ethers[i]. PBDEs are structurally similar to other environmental pollutants, such as dioxins and PCBs. They are lipophilic and persistent compounds and widespread in the environment. For certain congeners, bioaccumulation has been observed[ii]. Recent findings of increasing levels in humans[iii,iv] showed that more detailed investigations of human milk (or other suitable matrices) will be required in order to evaluate the general human exposure to this group of environmental contaminants. Only a few data on PBDE levels in breast milk from Germany had been published[v]. To fill the data gaps, in 2001 a controlled study was started to characterize the PBDE levels in human milk from Germany with special efforts to identify and quantify deca-BDE-209[vi]. 103 samples were analyzed in this study so far including 10 hidden pool samples provided by Federal Institute for Risk Assessment to ERGO laboratory (total number of samples finally will be 157 (including 14 hidden pool samples)). This paper describes the analytical procedure applied and emphasizes on the quality control procedure.

Material and Methods

Analytical method
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/ether/ethanol (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 a HP 5890 II GC coupled with a Micromass AutoSpec mass spectrometer. Quantification was done by means of isotope dilution method. Quantification was only performed if the sample level was at least twice the blank level (usually sample level was

much higher compared to the blank level). More details concerning the analytical method will be published this year[vii].

Quality control procedure
The quality control measures applied within in the investigation (analysis of 103 human milk samples so far) included

- analysis of 17 human milk samples spiked with native PBDEs
- analysis of 16 QC-pool of human milk (one analysed with each batch of samples)
- 8 duplicate analysis of human milk samples
- Interlaboratory comparison with CVUA (Chemical and Veterinary Control Laboratory, Münster, Germany, Peter Fürst), both laboratories analyzed the same quality control pool (German pool, collected 1992, CVUA: n=10, Ergo: n=18).
- 10 blind pool samples delivered with the samples (blind pool samples could not be identified by the laboratory as being control samples)

For reasons of clearness, details of the single measures are described together with the results in the following section:

Results and Discussion

The accuracy (closeness of agreement between a test result and the true value, includes trueness and precision) of an analytical method is an important parameter to evaluate the analytical results. Therefore, extensive efforts were made to approve the validity of the analytical method used for the determination of PBDEs in human milk and the reliability of the analytical results by estimation of the trueness and the precision of the method. Hence, the quality control (QC) and quality assurance (QA) part in this type of analysis represents an important tool of the total analytical concept. In total, more then 30 % of the whole analytical effort was covered by QC/QA measures.

The trueness of the method applied

Quality assurance by the standard addition technique for human milk
The best way to estimate the accuracy of a method is to analyse certified reference materials (CRM). However, due to lack of respective materials, accuracy has to be determined by other approaches, such as the standard addition method. To demonstrate the trueness of the analytical method, a human milk QC pool with a well-known concentration was spiked with a PBDE mixture at two different levels. Table 1 gives details on the concentrations which were added.

Table 1: Concentration of PBDEs spiked by the standard addition technique

Standard addition to human milk samples (n = 17 for each spike value)		
	PBDE 47, 209 Spike value in ng/g, lipid based	Other PBDEs Spike value in ng/g, lipid based
spike concentration I	2	0,4
spike concentration II	20	4

The results of accuracy checks by the standard addition technique are given in Figure 1 (mean data). As can be seen, the recoveries for almost all compounds spiked at both concentrations are nearly equal to 100 % - confirming the trueness of the method applied. The slightly higher values found for PBDE 100 are caused by lack of a respective ^{13}C-labelled standard which was not commercially available at the time when performing this test.

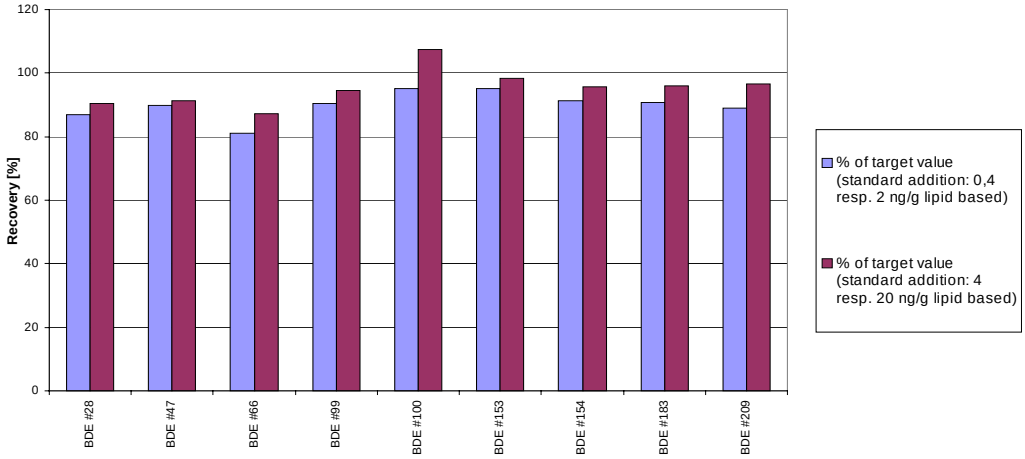

Figure 1 Mean recovery rates of the PBDE determination in spiked human milk samples

The precision of the method applied

Analysis of quality control pools (repeatability)

Each batch of samples contained a QC–pool which was analysed in parallel under the same condition. The concentration of the QC-pool is reflecting the German background level. The results of the QC-pool analyses – received during the investigation covering 103 milk samples - are presented graphically in Figure 2. For clarity reasons, only two values are given here for each pool. BDE 47 and total PBDEs are shown as blue and violet bars. The figure shows the results for the analysis in series (pool 7/15 to pool 7/20) and the relevant data for the day to day precision (pool 7/21 to pool 7/76). The day-to-day results for all individual congeners are given in Table 2. This table includes the mean values, standard deviations and relative standard deviations. It is important to mention, that the relative standard deviations for the main congeners (BDE 47, 153, 99) are below 10 %.

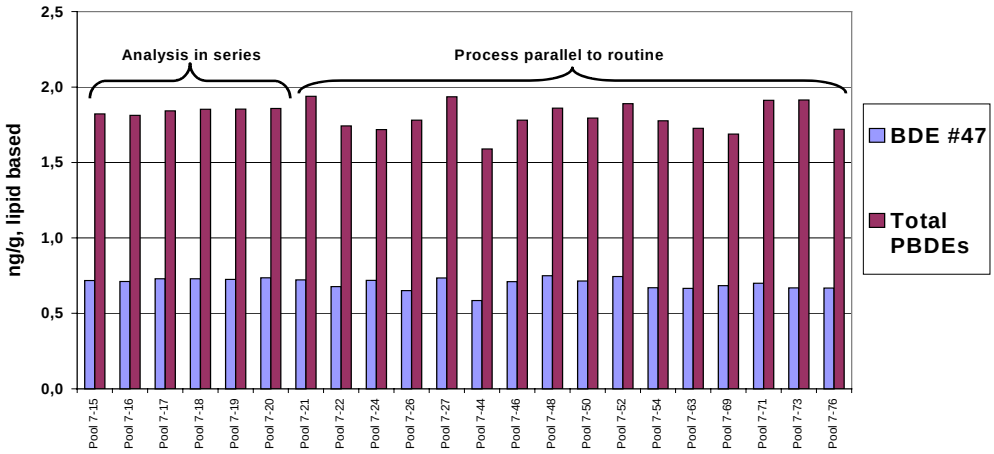

Figure 2 Results for PBDE determination in a human milk QC pool (n = 6: Analysis in series during validation; n=16: Analysis from day to day)

Table 2: Day to day precision of PBDEs determination in a human milk QC pool

	Mean [ng/g lipid based]	n	Standard deviation [ng/g lipid based]	Relative standard deviation [%]
BDE #28	0,032	13	0,0033	10
BDE #47	0,69	16	0,042	6
BDE #66	0,0082	16	0,0016	20
BDE #99	0,28	16	0,022	8
BDE #100	0,17	16	0,040	23
BDE #153	0,39	16	0,023	6
BDE #154	0,026	16	0,0015	6
BDE #183	0,075	16	0,009	12
BDE #209	0,15	13	0,033	22

Blind pool samples (repeatability)
Additionally, the repeatability was controlled by blind pool samples provided by the Federal Institute for Risk Assessment to the Ergo-laboratory. These pool samples could not be identified by the laboratory as being control samples. The whole outcome of this additional QC procedure is shown in the following

Table 3:

Table 3: Determination of PBDEs in blind QC pool (n=10, control samples analyzed from day to day, in parallel to 93 individual samples), data in pg/g, lipid based

Congener	Mean [ng/g, lipid based]	Relative standard deviation [%]
BDE 28	0,02	32
BDE 47	**0,57**	**14**
BDE 66	0,01	111*
BDE 99	**0,21**	**12**
BDE 100	0,11	12
BDE 153	**0,28**	**7,3**
BDE 154	0,02	19
BDE 183	0,03	32**
BDE 209	n.d.	-
Sum of PBDEs analyzed	1,26	12
Lipid content [%]	3,16	4,7

n.d. = not detectable *Relevant congeners are presented in bold*
** concentration found is equal to detection limit* *** One outlier eliminated*

The relative standard deviations for the main congeners (BDE 47, 153, 99) are between 7 - 14 %. These values are only slightly higher than the relative standard deviation estimated by the QC-pool and confirm the good precision of the method.

Duplicate analyses
8 samples were analyzed in duplicate (totally separate determination of two aliquots of human milk from the same sample). In Figure 3 the results for two samples analyzed in duplicate are shown exemplarily. The precision of the method is quite satisfying. It has to be stated, that especially the relative difficult determination of the fully brominated congener, DecaBDE (BDE#209) shows surprisingly good repeatability.

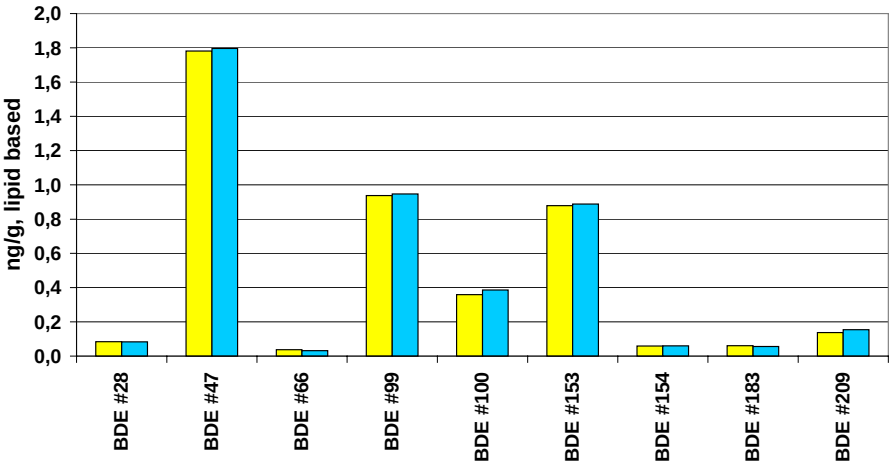

Figure 3 Example for duplicate analysis of PBDE determination in human milk

Interlaboratory comparison(reproducibility)
The outcome of the interlaboratory comparison between CVUA and ERGO is presented in Figure 4. The results show a good reproducibility of the PBDE determination between both laboratories in human milk samples reflecting German background levels.

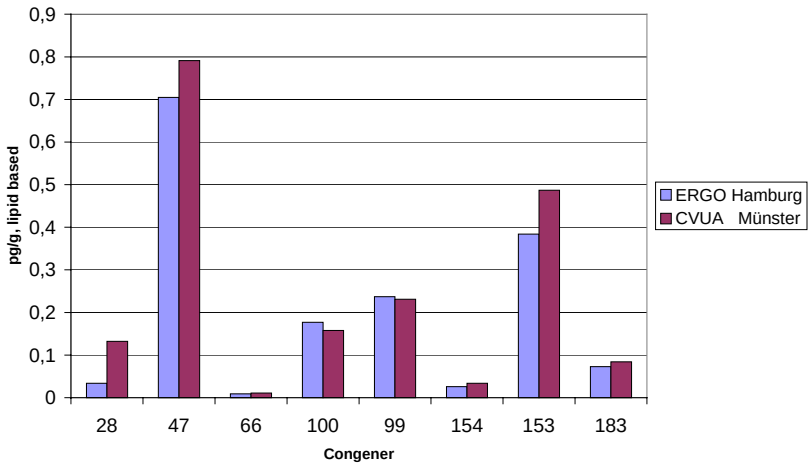

Figure 4 Comparison of PBDE analysis (mean data) in human milk measured in two different laboratories (CVUA, Münster, Germany (n=10) and Ergo, Hamburg, Germany (n=18)

Conclusion
The quality control measures applied showed that the analytical method for PBDE determination in human milk has a good repeatablity, reproducibility and trueness. The analytical method is very suitable for PBDE analysis of background contaminated human milk samples. It has successfully been applied in other studies as well[viii, ix].

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