DETERMINATION OF PBDEs IN HUMAN MILK FROM THE UNITED STATES
- COMPARISON OF RESULTS FROM THREE LABORATORIES -

Olaf Päpke[1], Lothar Bathe[2], Åke Bergman[3], Peter Fürst [2], Daiva Meironyté Guvenius[4], Thomas Herrmann[1], Koidu Norén[4]

ERGO Forschungsgesellschaft mbH, Geierstraße 1, D-22305 Hamburg, Germany[1] Chemisches Landes- und Staatliches Veterinäruntersuchungsamt, Sperlichstraße 19, D-48151 Münster, Germany[2]. Dept. of Environmental Chemistry, Stockholm University, S-106 91 Stockholm, Sweden[3]. Dept of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden[4]

Introduction

The extensive use of products containing flame retardants like polybrominated diphenyl ethers (PBDEs) has resulted in the release of these compounds into the environment[1]. Due to their lipophilic and persistent character PBDEs accumulate in the human body.
For many countries decreasing levels of organochlorine compounds have been reported recently in human tissue[2] while levels for polybrominated diphenyl ethers increased continuously since 1972 [3]. Due to this surprising result efforts have been undertaken to analyse this class of compounds in human samples in various countries[3,4,5,6,7].

Material and Methods

Sample collection.

In connection with a project for the determination of a single chlorinated contaminant in human milk, samples were collected in two cities (Austin and Denver) in the United States in late 2000. The milk was collected by pumping, frozen and sent in frozen status to the laboratory in Hamburg. After the chlorine contaminant project was finished, the remaining milk samples were pooled.. The total amount of pooled milk was about 300 ml, prepared from 5 to 50 ml of the single samples. This pool was analyzed for PBDEs together with 75 human milk samples originating from Germany. Due to the unexpected PBDE concentration found in the US pooled milk sample, the material was analyzed additionally in two laboratories with extensive experience in determination of halogenated contaminants in human samples. 20 ml of the pooled sample was shipped in frozen status to the laboratories in Münster and Stockholm.

Laboratory procedure, performed at the ERGO Laboratory in Hamburg

Internal and external standards PBDE No.[8] 17, No.28, No. 47, No. 49, No. 66, No. 85, No. 99, No. 100, No. 119, No. 140, No.153, No.154 and No. 183 were obtained from Promochem, Germany. Solvents were delivered by Merck (n-hexane), Baker (diethylether) and Mallinckrodt (ethanol, toluene). Silica gel and sodium sulfate were obtained from Merck.
Before extraction the internal standards PBDE 49, 119, 140 were added to the sample. 10 ml of human milk were extracted with of n-hexane/diethyl ether (10/1, v/v, three extraction steps, 10 ml each) after adding 8 ml of water and 2 ml of ethanol. The extract was dried with sodium sulfate and cleaned up by acid treated and activated silica gel (4 and 6 g, respectively), eluted with 30 ml of n-hexane/toluene, (9/1, v/v). The extract was reduced in volume by means of a stream of nitrogen. The final volume was 50 µl.

The measurement was performed by means of HRGC/LRMS (HP 6890 coupled to HP 5973, NCI mode) and HRGC/HRMS (HP 5890 coupled to VG Autospec) using HP 5 (30 m) and DB 5 (15 m) columns, respectively, for gas chromatographic separation. Two mass traces were measured per compound. By using LRMS, m/e = 79 and m/e = 81 were recorded. Using HRMS, the two most abundant masses were used for measurement (Molecular Weight for Tri –and Tetra-BDE, MW – 2 Br for Penta to Deca – BDE). The identification of PBDEs was based on retention time and correct isotope ratio. The quantification was performed by means of internal and external standards. Reduction of solvents and control of blank data is an important step in quality control when analyzing PBDEs in trace levels. Solvents and reagents were tested before the laboratory procedure. All glassware was rinsed by solvents prior to use. Silica gel and sodium sulfate were pre-washed. Rotary evaporators were not used to reduce the risk of contamination. No plastic equipments were used. For quality control a laboratory blank was run with each batch of ten samples. Quantification was only done if sample data was at least twice the blank data.

Laboratory procedure, performed at Karolinska Institutet in Stockholm

Human milk (10 ml) and a blank sample were analysed according to the previously described method[3]. Internal standard (50 µl ^{13}C-BDE-77 10 pg/µl) and formic acid were added to the samples before extraction. Organohalogen compounds were extracted from milk with the lipophilic gel Lipidex 5000 at 35°C for 2.5 hours. The mixture was transferred to a glass column and gel was eluted with solvents of different polarity. Oraganohalogen compounds were eluted with acetonitrile and the remaining lipids with a mixture of methanol/ trichloromethane/ hexane (1/1/1, v/v/v). Both fractions were evaporated under reduced pressure and dried in a desiccator to a constant weight. The sum of these two fractions was used for calculations of the lipid content. The acetonitrile fraction was applied to an aluminum oxide column for separation of residual lipids. Organohalogen compounds were eluted with hexane and applied to silica gel. After the removal of most oraganochlorine compounds with hexane, an acidified silica gel column (silica gel, 0.1g and silica gel/ 90 % H_2SO_4, 2:1, 0.3g) was connected to the first silica column. PBDEs were eluted with dichloromethane/ hexane (1:3). Nitrogen was used to concentrate the fraction to about 30 µl. Identification and determination was performed by HRGC/HRMS (HP 5890A coupled to VG 70-250), EI, SIM. A fused silica, methyl 5% phenyl silicone, capillary column (25m) was used for gas chromatographic separation. For each PBDE congener two ions of the molecular ion or fragment ion cluster were monitored.

Laboratory procedure, performed at the CVUA Laboratory in Münster

PBDE Standards were obtained from Promochem, Germany, and Campro Scientific, The Netherlands. All organic solvents (Nanograde purity) as well as Florisil were delivered by Promochem and potassium oxalate, silica gel and sulfuric acid were obtained from Merck.

The PDBEs are extracted together with other lipophilic compounds and fat using potassium oxalate, ethanol, ethylether and pentane. After addition of the six ^{13}C-labelled internal standards PBDE No. 28, 47, 99, 153, 154 and 183, fat is removed on a silica gel column treated with sulfuric acid. Chromatography on Florisil is used to separate PBDEs and PCBs (hexane fraction) from dioxins (toluene fraction) and other more polar compounds. Analytical determination was performed by means of HRGC/HRMS (HP 5890 coupled to VG Autospec) using a 30 m DB 5 capillary column for gas chromatographic separation. Two mass traces were measured for each homologue group. The identification of PBDEs was based on retention time and correct isotope ratio for both fragments recorded. Quantification was performed by means of the ^{13}C-labelled internal standards and a five-point calibration curve.

Results and Discussion

The PBDE congeners: BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154 and BDE –183 were quantified in a pool of human milk. The results for all congeners are reported in Table 1.

Table 1: **Comparison of PBDE results in human milk measured in three different laboratories** Values in ng/g, lipid based

PBDE No	ERGO Hamburg		CVUA Münster	Karolinska Stockholm
	LRMS, NCI	HRMS, EI	HRMS, EI	HRMS, EI
17	0.03	0.07		
28	2.92	3.14	5.4	5
47	112	124	122	150
66	0.92	0.53	1.2	0,5
100	23.6	25.1	24.7	21
99	29.9	35.,4	21.7	23
85	3.18		2.6	3
154	3.40	1.60	1.4	1
153	14.2	14.1	17.2	14
138	0.34			
183	0.17	0.16	0.2	0.1
Total	190.6	204.1	196.4	217.6

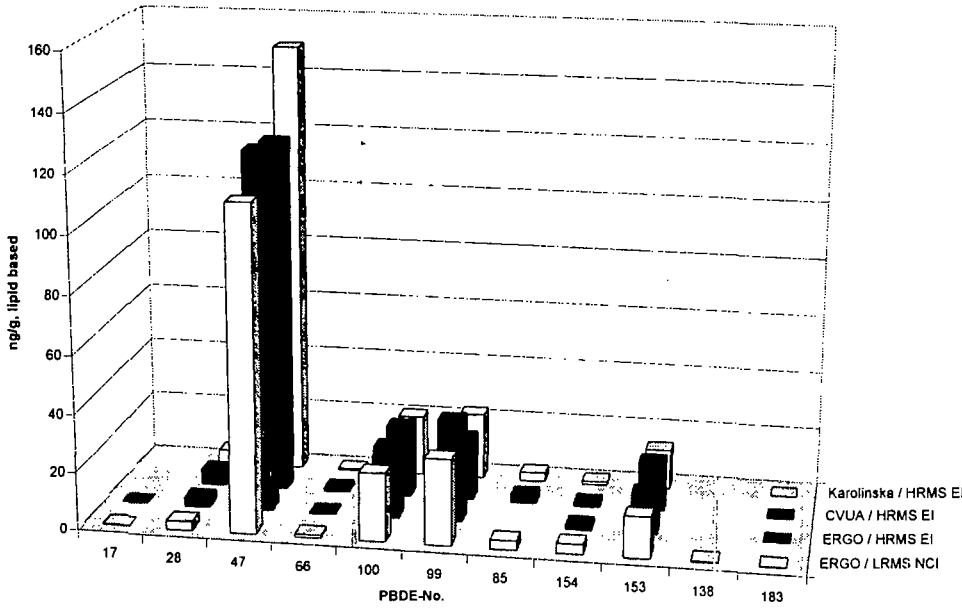
When comparing the results measured by ERGO using LRMS/NCI and HRMS/EI, it can be seen that the concentration for most congeners is quite similar using both techniques. An exception was found for BDE-154. This particular congener shows higher values when using LRMS/NCI. The high value results possibly from a co-eluting bromine containing component. This difference was found to be much more striking in the human milk samples from Germany than in the US milk pool sample. It is important to mention that the results provided by ERGO follow a method developed in 1999. At that time only a few ^{13}C-labeled standards were available. Therefore the native internal standard method was used instead of the isotope dilution method. Due to the fact that within the analyses of two projects performed in 1999/2000 and 2000/2001 the analytical methods should not be changed, ERGO continued to used the native internal standard method. Comparing the HRMS results from the 3 laboratories the differences in concentrations for most congeners are quite low and the total concentrations of PBDEs: 204, 196 and 217 ng/g lipid are similar. Due to the relative difficult determination of components with high boiling points like PBDEs the results of this comparison are quite satisfying.

The congener BDE-47 occurred at the highest level, followed by BDE-99 BDE-100 and BDE-153. These compounds contributed approximately 61-69%, 11 -17 %, 10 – 13 % and 5 - 9 %, respectively, to the total of PBDEs in the pooled US milk sample.

It is striking that a concentration of PBDEs as high as approx. 200 ng/g lipid weight is indicated for the US human breast milk. This concentration is about 40 times higher than levels reported by other authors for mean total PBDEs values (maximum values in brackets) for human samples collected recently in countries like Sweden, Germany, California and Canada have been reported at 4.0, 4.9 (12.6), 25.1 (37), 5.8 (28.5) ng/g lipid based, respectively [3,5,6,7]. It is notable that the PBDE levels in the US mothers milk pool is at least close to the levels of PCBs in Swedish mothers milk[2].At this point it can not be expressed that the values found in the present US breast milk sample is representative with respect to origin and collection time. On the other hand, we do not

have any indication for an external influence resulting at the unexpected high values. This result call for some in-dept studies on the situation in mothers milk from US women

Figure 1: **PBDEs in human milk, comparison of data from different Laboratories with different methods,** values given in ng/g, lipid based



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