POLYBROMINATED FLAME RETARDANTS IN HUMAN ADIPOSE TISSUE IN CZECH REPUBLIC INHABITANTS. THE PILOT STUDY.

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Introduction

The extensive use of products containing fire retardants like polybrominated diphenyl ethers (PBDEs) has resulted in the release of these components into environment. Due to their high lipophility and persistence, PBDE are expected to bioaccumulate(1,2).

The Environmental Health Monitoring System in the Czech Republic based on Resolution No. 369/ 1991 of the Government of the Czech Republic, has been run routinely since 1994. This system includes the monitoring of toxic substances or their metabolites in the human body fluids and tissues, the most important among them being the selected persistent chlorinated organic compounds like PCBs, PCDDs, PCDFs. The human adipose tissue as matrix for human exposure evaluation is not easy available. That's why we extended the analysis range and included the PBDEs in monitoring to obtain backround data of the Czech population exposure. Biological monitoring has been implemented in four towns. Two of them are characterized by high level of industrialization; the remaining two towns represent more rural areas.

The aim of the present study was developing and validation of analytical method for PBDE determination and perform pilot study of human exposure in Czech Republic.

Materials and Methods

Study group and sample collection

Sample were collected in two towns (Ždár nad Sázavou-rural area, Ústí nad Labem-industrial area) of Czech Republic in 2000 and 2001. The human adipose tissue were taken from people died at age range 23-78, the studied group involved 14 women and 10 men.

Sample preparation and analysis

The human adipose tissues samples were analysed for the content of 2,4,4'-Tri BDE(BDE 28), 2,2',4 ,4'-Tetra BDE(BDE 47), 2,2',4,4',5-Penta BDE (BDE 99), 2,2',4,4',6-Penta BDE (BDE 100),2,2',4,4',5,6'-HexaBDE (BDE 154), 2,2',4,4',5,5'-HexaBDE (BDE 153), 2,2',3,4,4', 5',6-HeptaBDE (BDE 183). Standards – native and isotope labelled PBDE - were purchased from Wellington laboratories, Canada.

The sample preparation steps are identical with them for PCDD/F determination (3). Briefly, about 10 g of fat was spiked with $^{13}C_{12}$ labelled PBDE isomers and dialyzed through semipermeable membrane. After removal of most of fat, samples were cleaned on a multilayer silica column followed by a separation on alumina column. The PBDEs were eluted in the PCDD/F and non-ortho PCB fraction. This fraction was finally cleaned up on a carbon column. After that 30µl of recovery standards ($^{13}C_{12}$ labelled 1,2,3,4-TCDD, $^{13}C_{12}$ labelled 1,2,3,7,8,9-HxCDD in nonane) were added to the sample. The samples were concentrated by evaporation to 30µl (Zymark evaporator).

The levels of individual PBDEs were determined by GC/MS – PolarisQ or GCQ, Thermoquest (Finnigan MAT, San Jose, USA) operated in MS/MS mode. MS/MS conditions were optimised as described (3). The fragment belongs to loos of 2Br from molecular ion was chosen as an parent iont. The quantification was performed at daughter ions corresponding to loos of fragment COBr. Separations were carried out on a capillary column DB-5ms (with parameters 30m or 60m x 0,25 mm x 0,25µm film thickness) from J & W Scientific, Folsom, USA with the rate of heating condition as follows: held for1 min. at 150 °C, programmed 20 °C min^{-1} to 180 °C, further 2,5 °C. min^{-1} to 300 °C and finally held for 2 min at 300 °C.

Calibration standards were made using the 6 native and 6 labelled PBDE. Samples were quantified using an isotope dilution method (internal standard method).

The method validation (including the extraction and purification steps) was performed on pork fat samples spiked with native standards. Repeatibility, recovery of spiked native compounds and average detection limits are given in Table 1.

Table 1. Validation data for PBDE congeners, performed on 5g of pork fat, spiked with native PBDE (1ng/g of fat), the whole „clean up" procedure (semipermeabile membrane, 3 column system), final volume 100µl

PBDE congener	Recovery	RSD	Average detection limit in ng/g fat
PBDE28	108 %	3,6 %	0,006
PBDE47	98 %	4,3 %	0,004
PBDE99	102 %	6,9 %	0,006
PBDE154	85 %	6,0 %	0,006
PBDE153	90 %	8,1 %	0,012
PBDE183	95 %	9,1 %	0,026

Repeatibility of the method at background level was validated by analysis of not fortified pork fat. Results are shown in table 2.

Table 2. Validation data for PBDE performed on 10g pork fat, not fortified, the whole „clean up" procedure (semipermeabile membrane, 3 column system), final volume 50µl

PBDE congener	Mean concentration in pg/g fat	RSD
PBDE28	1,6	13 %
PBDE47	294	3,7 %
PBDE100	57	5,0 %
PBDE99	366	6,3 %
PBDE154	47	13 %
PBDE153	105	7,8 %
PBDE183	86	12 %

Results and Discussion

There were PBDE congeners (BDE 28, BDE 47, BDE 100, BDE 99, BDE 154, BDE 153 and BDE 183) quantified in human adipose tissue samples. The resulting relative profiles of these isomer in sum (congener profile) are shown in Figure 1. The congener BDE 47 is occurred at the highest level, followed by BDE 153, BDE 183, BDE 100 and BDE 99. These compounds contributed approximately 32,29,18,12 and 9 % respectively, to the sum of PBDEs in the human adipose tissue samples. In several samples concentration of congener BDE 153 is higher than concentration of congener BDE 47(e.g. No.2).

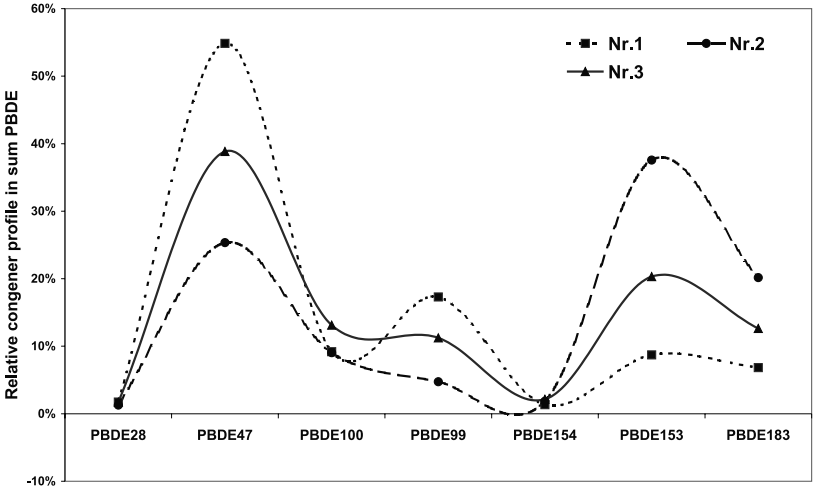

Figure 1. Individual BDE congener in sum of PBDE in human adipose tissue (examples of 3 samples). The points are smoothed for clarity

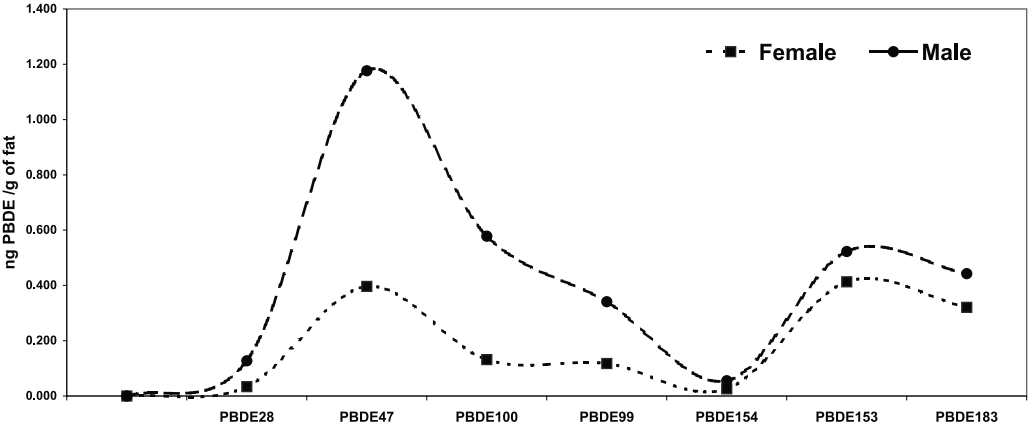

Figure 2. Average concentration of PBDE congeners in men and women

In studied group lower levels of PBDEs were observed in women compared to men (Figure 2). It correlates with the lower blood concentration observed in German female and male blood (4). The major congeners found in human adipose tissue are BDE 47,153,183,100 and 99. The concentration range in human adipose tissue in male and female are presented in Table 3.

This study shows measured concentration of selected PBDE within range 0,004-3,69 ng/ g fat. Published results (4) show dominant PBDE 47 congener in human blood. Higher brominated BDE (e.g. BDE 183) were found more contributing on sum of PBDE in some samples. However, qualitative profiles of PBDE in human adipose tissue are vary and sample number does not alows to educe exact conclusion.

Table 3. Concentration of PBDE congener in human adipose tissue

	Concentration in ng/g fat						
Sex	Male			Female			Concentration range
PBDE congener	Min	Max	Mean	Min	Max	Mean	(ng/g fat)
PBDE 28	0,028	0,53	0,13	0,004	0,145	0,034	0,004-0,53
PBDE 47	0,230	3,69	1,18	0,087	0,821	0,400	0,087-3,69
PBDE 99	0,075	1,14	0,340	0,015	0,233	0,117	0,015-1,14
PBDE 100	0,088	2,35	0,590	0,013	0,330	0,132	0,013-2,35
PBDE 154	0,012	0,110	0,055	0,002	0,054	0,026	0,002-0,11
PBDE 153	0,520	1,89	0,520	0,080	1,47	0,413	0,08-1,89
PBDE 183	0,075	1,59	0,440	0,049	1,74	0,320	0,049-1,74

This study shows measured concentration of selected PBDE within range 0,005-3,69 ng/fat. Published results (4) show dominant PBDE 47 congener in human blood. From presented results in this pilot study there are apparent higher-brominated BDE (e.g. BDE 183) as well.

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