THE GERMAN ENVIRONMENTAL SPECIMEN BANK - APPLICATION IN TREND MONITORING OF POLYBROMINATED DIPHENYL ETHERS IN HUMAN BLOOD

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Introduction

The German environmental specimen bank (ESB) was established in 1985 as a permanent institution for the systematic collection, processing, characterization and storage of environmental samples from marine, limnic and terrestrial ecosystems as well as human samples. Responsibility and funding are within the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety and the administrative coordination of the Federal Environmental Agency[1,2]. Blood and other human specimens are collected since 1981 from a group of about 100 unexposed persons in defined peripheral conditions. The subjects have to complete standard questionnaires about family and health status, occupational exposure, nutrition, smoking and drinking habits and the use of medicine. Every step in the procedure from sampling to transport, preparation, chemical analysis and long-term storage is carried out according to obligatory Standard Operating Procedures[3].

The extensive use of products containing fire retardants like polybrominated diphenyl ethers (PBDEs) has resulted in the release of these components into the environment. Due to their lipophilic and persistent character PBDEs accumulate in the human body.
For Swedish human milk decreasing levels of organochlorine compounds have been found while levels for brominated diphenyl ethers increased continuously since 1972[4]. The availability of the samples stored in ESB opened the possibility to have a close look at the human situation of the development of halogenated compounds in humans in Germany. The aim of the present study was to determine the level and the time trend of PBDEs over the past fourteen years (1985-1999) in human blood from Germany.

Material and Methods

Study group and sample collection. The study was conducted on blood samples archived by the German environmental specimen bank. Whole blood from 20 subjects (10 male and 10 female) participating in the monitoring programs 1985 (BO28 11/85), 1990 (BO57 12/90), 1995 (BO74 11/95) and 1999 (BO91 02/99), respectively, was choosen. To avoid variation of body burden with age the study was restricted to subjects born in Germany in the age range 20-30 years. Blood (about 80 ml) was collected by punctation of the cubital vein, fractionated into 20 ml-vials anticoagulated with heparin and long-term stored at -85°C. 20 ml of the blood was available to the analytical laboratory for the determination of halogenated components like PCDD/Fs, PBDEs, PCBs and others.

Laboratory procedure. BDE-28, -47, -49, -66, -85, -99, -100, -119, -140, -153 and -154 were obtained from Promochem, Germany. Solvents were delivered by Mallinckrodt (ethanol, toluene), Merck (n-hexane), and Baker (diethylether). Silica gel and sodium sulfate were obtained from Merck. Before the extraction the internal standards PBDE 49, 119, 140 were added to the sample. 5 to 10 ml of whole blood were extracted by means of n-hexane/diethylether (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 by means of sodium sulfate and cleaned up by acid treated and activated silica gel (4 gresp. 6 g), 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 10 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) resp. DB 5 (15 m) columns for gas chromatographic separation. Two mass traces were measured per compound. The identification of PBDEs was based on retention time and correct isotope ratio found in both mass traces. The quantification was performed by means of internal and external standards.
Reduction and control of blank data is a very 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. All kind of plastic equipment was avoided. For quality control a laboratory blank was run with each batch of ten samples. Quantification was only done if sample data was at very least 40 percent higher than the blank data.

Results and Discussion
The PBDE congeners BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153 and BDE-154 were quantified in archived whole blood samples. The resulting concentration profiles of these PBDE congeners (Fig. 1) are similar to the pattern reported for Swedish human milk[5]. The congener BDE-47 occured at the highest level, followed by BDE-153, BDE-99 and BDE-100. These compounds contributed approximately 65, 13, 8 and 7%, respectively, to the sum ofPBDEs in the blood samples.

The results of BDE-47 concentrations and the sum of the concentrations of congeners analyzed (ΣPBDE) in blood samples are presented in Table 1. Data are given in terms of median, means and selected percentiles for the overall groups studied as well as stratified according to gender. The difference between both year of collection and gender groupings for ΣPBDE are statistically significant ($p<0.05$, Kruskal-Wallis test).

During the time period 1985 to 1999 median PBDE concentrations in blood increased from 3075 to 4687 pg/g lipid. In all studied groups lower levels of PBDEs were observed in women compared to man. In 1985, 1995 and 1999, respectively, median concentrations for females were approximately 20% below the values for males. However, in 1990 this difference was even more pronounced, female blood showed a 75% lower contamination with PBDEs than male blood.

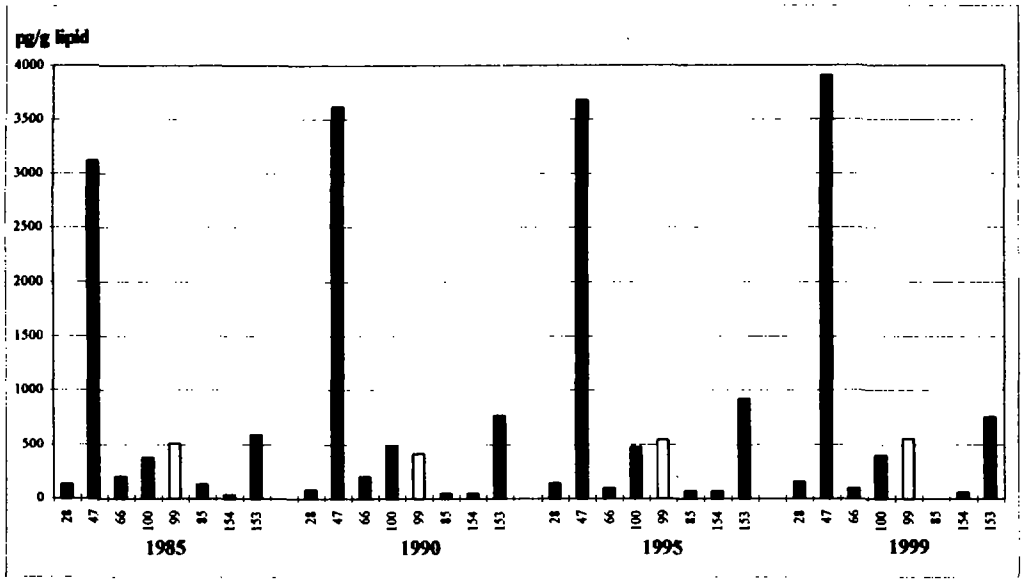

Figure 1. Mean concentrations (pg/g lipid) of polybrominated diphenyl ether congeners in human blood samples from 1985, 1990, 1995 and 1999

The PBDE values obtained in our study groups seem to be generally higher than in Swedish specimen. In Swedish human plasma samples mean PBDE concentrations (sum of congeners BDE-28, BDE-47, BDE-66, BDE-99, BDE-100 and BDE-153) of 2.1±1.4 ng/g lipid were determined[6]. Considerably higher mean PBDE concentrations (3,9-5,6 ng/g lipid) were found in German blood over the period 1985 to 1999.

Median concentrations of BDE-47 in blood plasma of Swedish workers at a computer disassembly plant, workers in a computerized office and cleaners were published recently[7]. In blood of cleaners (20 females) and computer clerks (20 females) BDE-47 concentrations of 1,6 and 1,5 ng/g lipid, respectively, were detected. The median BDE-47 concentrations in German female blood were 2,2 ng/g lipid in 1995 and 2,8 ng/g lipid in 1999.

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Table 1. Basic statistical data on BDE-47 levels and ΣPBDE levels (pg/g lipid) in human blood

	n	Max	25%	Median	Arithmetic Mean	Geometric Mean	75%
BDE-47							
1985							
all	16	9800	1650	2400	3119	2502	2850
male	8	9800	2250	2600	3425	2896	3350
female	8	9800	1300	1800	2813	2160	2625
1990							
all	19	11600	1100	2800	3619	2571	5000
male	10	11600	2300	3000	4837	3645	8025
female	9	5000	875	1400	2266	1746	4100
1995							
all	19	12600	1900	2300	3684	2890	4400
male	10	12600	1800	2300	3490	2716	3750
female	9	9600	1900	2200	3900	3100	6100
1999							
all	20	10200	2525	3000	3909	3374	4650
male	10	10200	2525	3400	3997	3368	4825
female	10	10100	2475	2800	3820	3378	4100
ΣPBDE							
1985							
all	20	15721	1942	3075	3912	2864	4030
male	10	15721	2354	3360	4310	3023	4391
female	10	11946	1697	2638	3513	2711	4171
1990							
all	20	12350	1730	3881	4891	3565	6816
male	10	12350	3611	6751	6945	5896	10345
female	10	6505	1350	1748	2838	2154	4867
1995							
all	19	17560	3303	3900	5550	4592	5897
male	10	17560	3730	4287	5725	4813	6049
female	9	11717	3020	3553	5356	4359	8660
1999							
all	20	12607	3973	4687	5572	4871	7270
male	10	12607	4241	5404	5950	5009	7945
female	10	12129	3458	4310	5195	4741	5551