Analysis of polybrominated diphenyl ethers in human milk

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in resins and polymers, predominantly used in electronic equipment and textiles. Since they are not chemically bounded, they can migrate from the products and pollute the environment. Due to their lipophilic and persistent character, these compounds can accumulate in the body. Occurrence of PBDEs in the environment was first reported in sediments in USA (1) and in fish from Sweden (2). Since then, several investigations of PBDEs in sediments and fish from different water areas have been performed in Sweden (3,4,5). PBDEs have also been found in human blood plasma (6), adipose tissue (7) and milk (8).

The aim of this study was to adopt the method previously used for organochlorine compounds for analysis of PBDEs in human milk, to determinate the levels of these compounds in the milk and investigate the temporal trends during the course of 1972-1997.

Materials and Methods

Samples

Pooled samples of milk were analysed. The milk was collected during different periods from 1972 to 1997 and supplied by the Mothers' Milk Centre in Stockholm. The pools contained equal amounts of milk from each mother, although the number of mothers in the pools differed. The investigations of milk from the years 1972, 1976, 1980, 1984, 1990, 1994, 1996 and 1997 represent milk from 75, 78, 116, 26, 20, 20, 20 and 40 mothers, respectively. The average age of mothers donating milk was 27-28 years in 1972 - 1984, 29 years in 1994 and 30 - 31 years in 1990, 1996 and 1997. Of the donators 55-75% were nursing their first infant.

Standards

The standards 2,2',4-triBDE (BDE-17), 2,4,4'-triBDE (BDE-28), 2,2',4,4'-tetraBDE (BDE-47), 2,3',4,4'-tetraBDE (BDE-66), 2,2',4,4',6-pentaBDE (BDE-100) and 2,2',4,4',5,6'-hexaBDE (BDE-154) were synthesised as described elsewhere (9, 10). 2,2',3,4,4'-pentaBDE (BDE-85),

2,2',4,4',5-pentaBDE (BDE-99), 2,2',4,4',5,5'-hexaBDE (BDE-153) and internal standard C^{13}-3,3',4,4'-tetraBDE (BDE-77) were from CIL (Andover, MA, USA).

Method

In the present study a previously described method for analysis of organochlorine compounds in human milk (11) was slightly modified for analysis of PBDEs. The scheme of analytical procedure is shown in the Figure 1.

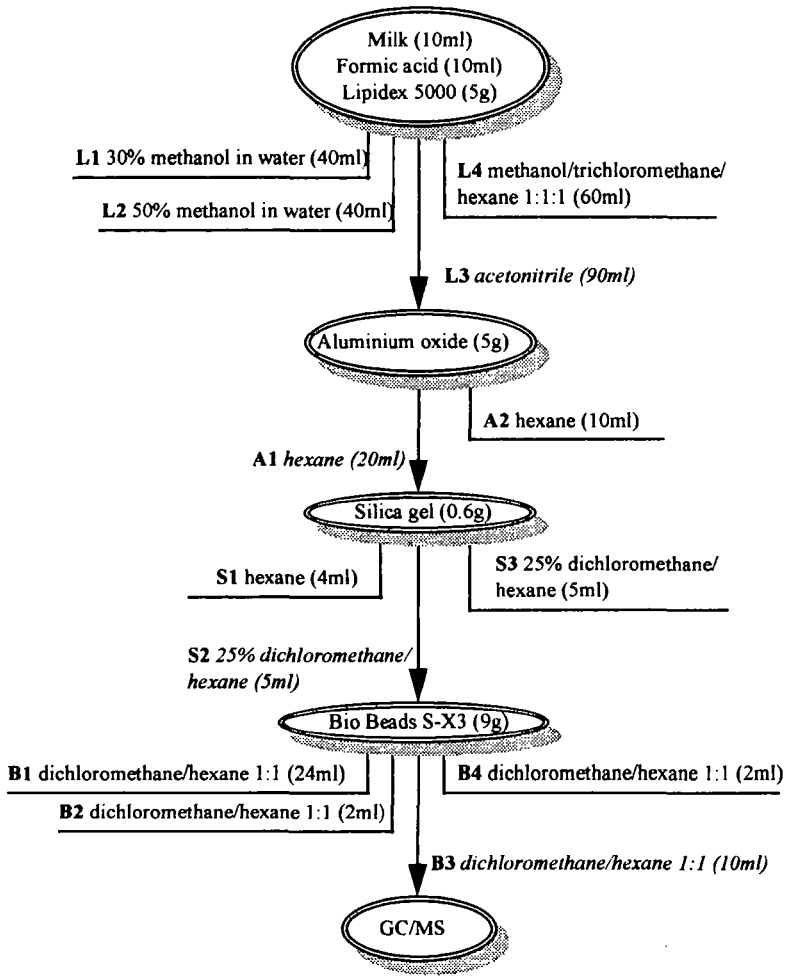

Figure 1. The scheme of the method for determination of PBDEs in mothers milk.

The extractions were made with the lipophilic gel Lipidex 5000. The analytes and lipids were incorporated into the gel by shaking the mixture of milk, formic acid and Lipidex 5000 at

35°C for 2.5 h. The mixture was transferred to a glass column and gel was eluted with solvents of different polarity. Halogenated compounds were eluted with acetonitrile (fraction L3). Fractions L3 and L4 were taken to near dryness under reduced pressure at 35°C, and the residues were dried to constant weight in a desiccator. The sum of residues of fractions L3 and L4 was used for calculation of the lipid content.

The residue from fraction L3 was quantitatively transferred with small volumes of hexane to the aluminium oxide column and PBDEs were eluted with hexane in the first fraction (A1). This fraction was concentrated and the bulk of organochlorine compounds were removed by chromatography on silica gel. The PBDEs were eluted in the second fraction (S2) with 25% dichloromethane in hexane. Additional purification was performed by gel permeation chromatography using Bio-Beads S-X3. PBDEs were quantitatively recovered in the 26 - 36 ml fraction, flow rate 1 ml/min. The identifications and quantifications were made by GC/MS, selected ion recording. The instrument used was a VG 70-250 mass spectrometer equipped with an HP 5890A gas chromatograph and a VG-250 data system. A fused silica SE-54 capillary column was used for separation. The oven temperature was 190°C for 0.1 min, programmed to 230°C at 5°C/min, hold for 0.2 min, programmed to 235°C at 1°C/min, hold for 0.2 min, programmed to 270°C at 3.5°C/min, and hold for 8 min. EI was performed in an "EI-only" ion source at the electron energy of 31 eV and the trap current of 500 μA.

Results and Discussions

The recovery studies of BDE congeners were performed by adding the standard substances to the samples before extraction. The average recoveries ranged 86-102% (n=5). The recovery of the internal standard (C^{13}-BDE-77) ranged 70-111% (n=5). The concentrations of the BDE congeners in milk sampled during different time periods are shown in Figure 2.

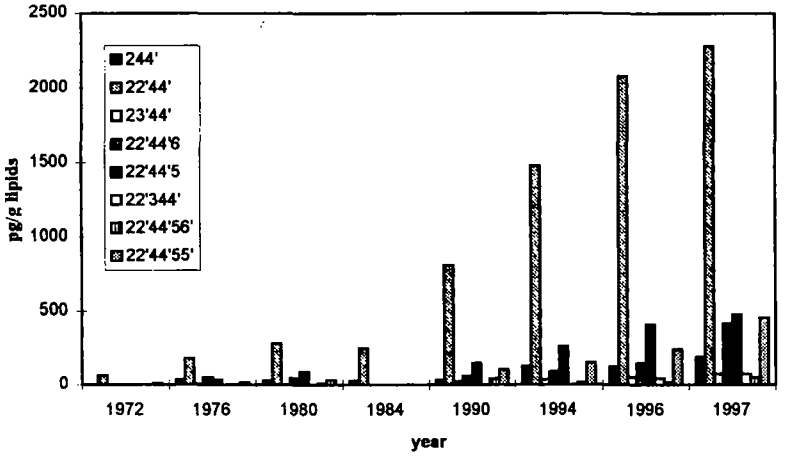

Figure 2. The concentration profile of BDEs in the human milk from Sweden collected during 1972 - 1997.

A continuos increase in the levels is demonstrated from 1972 to 1997. The sum of the concentrations of BDE congeners increased from 72 to 4010 pg/g lipids during the last 25 years. BDE-17 was not found in the samples. BDE-47 was the predominant congener in all samples and constituted during the periods 1976-1997 about 60-70% of the total amount of BDEs. This is in accordance with the previously reported levels in the blood (6). The concentrations in milk sampled during the nineties were also similar to the levels in Swedish blood plasma, 2.1±1.4 ng/g lipids (6).

Considering the stability of compounds, their accumulation in the body and increasing levels in human milk, these compounds may have important implications for health, especially for the development of infants. Therefore, further research and follow up studies are needed.

It can be assumed that the levels of BDEs in human milk reflect the total increase in PBDE pollution, a tendency which can be turned only by stopping the usage of this class of compounds.

Acknowledgements

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