TEMPORAL TRENDS OF PBDES AND HBCDD IN MILK FROM STOCKHOLM MOTHERS, 1980-2004

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Introduction

The environmental contamination of polybrominated diphenyl ethers (PBDEs) was first reported in fish from Sweden in the early 1980's [1], and has been followed by numerous studies worldwide [2-4]. Extensive summaries on human PBDE exposure are given in recent reviews [2,5,6]. From the early 1970s to 1997, human milk, from Stockholm, Sweden, has been shown to contain increasing concentrations of PBDEs [7], while samples from 1997 to 2000 indicated a decrease [8]. The observed decrease was mainly due to a reduction of BDE-47 concentrations. A decrease of PBDE concentrations in Swedish milk was also indicated by Lind and co-workers 2003 [9]. Similar PBDE concentrations as in Sweden has been reported in Norway, Germany and in Japan [7-12]. These temporal trend studies also indicate decreasing PBDE concentrations, all driven by decreasing BDE-47 concentrations [7-10]. Several reports have indicated increasing concentration of BDE-153, at concentrations similar or even higher than BDE-47 [13-15]. Comparing the mean and median concentration of PBDE in humans, the levels are approximately 20 times higher in the United States than those hitherto reported from European countries [2,7-9,11-13,16].

Hexabromocyclododecane (HBCDD) has recently been reported at highly varying concentrations (ranging from 0.09 to 10.4 ng/g lipids) in milk from Swedish mothers living in Uppsala, Göteborg, Lund and Lycksele [17]. HBCDD has also been reported in human milk from Norway but at slightly lower concentrations, ranging from 0.25 to 2.0 ng/g lipids [12].

The objective of the present study was to extend previous studies of PBDEs in pooled human milk samples from Stockholm to cover the period 1980 to 2004 with emphasis on the last ten years, to investigate any temporal concentration changes of PBDE congeners and of HBCDD, to ascertain any temporal trend of decabromodiphenyl ether (BDE-209) and to add information on the PBDE time trend in human milk from Sweden. Further, these concentrations were compared to previous studies of PBDEs performed in pooled human milk from Sweden.

Material and Methods

Chemicals: The individual PBDE congeners (numbered according to Ballschmiter *et al.* [18]): BDE-47, BDE-77, BDE-99, BDE-100, BDE-153 were synthesized in-house [19]. HBCDD was purchased from Cambridge Isotopes Laboratories, USA and BDE-209 from Fluka Chemie, Switzerland. All solvents were of the highest commercially available grade [13].

Instruments: The PBDE analysis was performed by gas chromatography/mass spectrometry (GC/MS) using a Finnigan TSQ 700 instrument (ThermoFinnigan, Bremen, Germany) connected to a Varian 3400 gas chromatograph equipped with an AS200S CTC autosampler. All instrumentation settings are reported elsewhere [13]. The PBDE congeners were analysed with selected ion monitoring (SIM) by scanning for the negative bromide ion (isotopes m/z 79 and 81) formed by electron capture reactions at chemical ionization (ECNI) with methane as the electron thermalization buffer gas at 5.6 torr and a primary electron energy of 70 eV [13].

Samples: Milk was collected from healthy native Swedish mothers by the Mothers milk centre in Stockholm. Milk samples are purchased from the centre and banked annually. Fourteen milk samples were taken out from 1980 to 2004 for analysis. The compositions of the pools were prepared to be as comparable as possible, with 55-80% of the milk from mothers nursing their first infant. Equal amounts of milk from individual mothers were mixed, from the years 1980, 1984/85, 1988-2002, 2003 and 2004, representing milk from 116, 102, 20, 15 and 20 mothers respectively. The average age of the mothers was 27-28 years in 1980 and 1984/85, and between 29-31 years in 1988-2004.

Extraction and cleanup procedure: The extraction and cleanup procedure is a modified method for analysis of organohalogen substances (OHS) in serum and has previously been applied to PBDEs and PCBs analysis in human milk [13,20]. Surrogate standard, BDE-77 was added to the samples prior to extraction. All samples were protected from daylight during handling and storage to prevent any photochemical degradation of the brominated compounds to be analyzed.

Analysis: To make the results as reliable as possible duplicate analyses were performed of the pools, control samples and blank samples were run in parallel throughout the whole procedure. A recovery study was performed with cow's milk (3% fat content). The overall recovery for BDE-47, BDE-99, BDE-100 and BDE-153 were about 90% within a range of 79-107% [13].

Limit of quantification (LOQ) for the PBDEs were defined in direct relation to the amount of interference of PBDEs in the blank samples. The PBDE concentrations in the sample had to be three times higher than the PBDE amount in the blank to be considered as a quantifiable. The average amount in blank samples was subtracted from the amount in the milk samples. Laboratory reference material was run in parallel to the analyzed samples.

Results and Discussion

The BDE-47, BDE-153 and HBCDD concentrations are presented in Figure 1. This study stresses the results from previous studies on PBDE in human milk from Stockholm, where the concentrations of the lower brominated PBDEs were shown to decrease from the middle of 1990's, after increasing concentrations from the 1970 to 1980's (Figure 1). The BDE-153 concentration seems to increase (Figure 1). This is in accordance to previous studies of human milk from Sweden where the influence of BDE-153 was shown to increase and BDE-47 to decrease [7,8]. The ratio of BDE-153/BDE-47 (ng/g lipid) in 1980 to 2004 is changed from 30 % to 99 %.

The concentrations of HBCDD in the Stockholm human milk show a fluctuating increase over time (Figure 1). From 1980 the concentration has increased from 0.13 pmol/g lipid to 0.60 pmol/g lipid in 2004. During the last 10 years the concentrations have varied between 0.60 and 0.93 pmol/g lipid. These concentrations are in a similar range as BDE-99, BDE-100 and BDE-153 and even higher during the last few years than for BDE-99 and BDE-100. In a recently reported study on regional differences of PBDEs in human milk from Sweden, similar HBCDD concentrations were reported, ranging from 0.09 to 10.4 ng/g lipid [17].

For BDE-209 it is not possible to see any time trend in the milk samples due to low and similar concentrations over the time period studied. All concentrations were above limit of detection (LOD) but below the quantification limit (LOQ) (0.01-0.10 pmol/g lipid). Human milk might not be as good indicator matrix for BDE-209 as it has been proved to be for other OHS. The short half-life of BDE-209 in human blood (15 days) [21] may strongly influence the concentrations also in the milk. Further, we do not know how efficient the transfer of BDE-209 is from blood to milk. We may speculate that the transfer is less efficient for higher brominated

diphenyl ethers than for the lower brominated diphenyl ethers. A previous study on paired samples of human milk and blood plasma showed that the BDE-153/BDE-47 ratio was lower in milk compare to blood, 28% and 67% respectively [22]. This difference between milk and blood for BDE-153 has also been seen in Norway, where the relative amount of BDE-153 was lower in breast milk compared to serum [15]. Therefore, blood seems to be a more suitable matrix for assessing human exposure to higher brominated diphenyl ethers i.e. those with six bromine atoms or more. Concentration of up to 3.4 pmol/g lipid have been reported for BDE-209 in Faroese mother's milk [13] confirming that this compound can be transferred to the milk.
Higher concentrations of BDE-153 compared to BDE-47 have been seen in humans from the U.S.A., Norway, The Netherlands and the Faroe Islands [13-15,23,24]. The reason for this is still not explained but could possibly be due to a higher persistence of BDE-153 than of BDE-47. Possibly the change is influenced by the fact that PBDE products containing the lower brominated diphenyl ethers (PentaBDE and OctaBDE) are phased out. Moreover it is too early to dismiss the hypothesis of BDE-153 being formed abiotically or via metabolism from BDE-209.

The PBDE concentrations in the pooled human milk samples in the present study are in close agreement with concentrations reported in previous studies on human milk from Sweden [7-9] c f. Figure 1. To some extend the same pools were used in the present study as in previous studies by Meironyté and co-workers [7,8] and the agreement between the studies is good although the extraction method, standards, analytical instrumentation and time for the analyses differ.

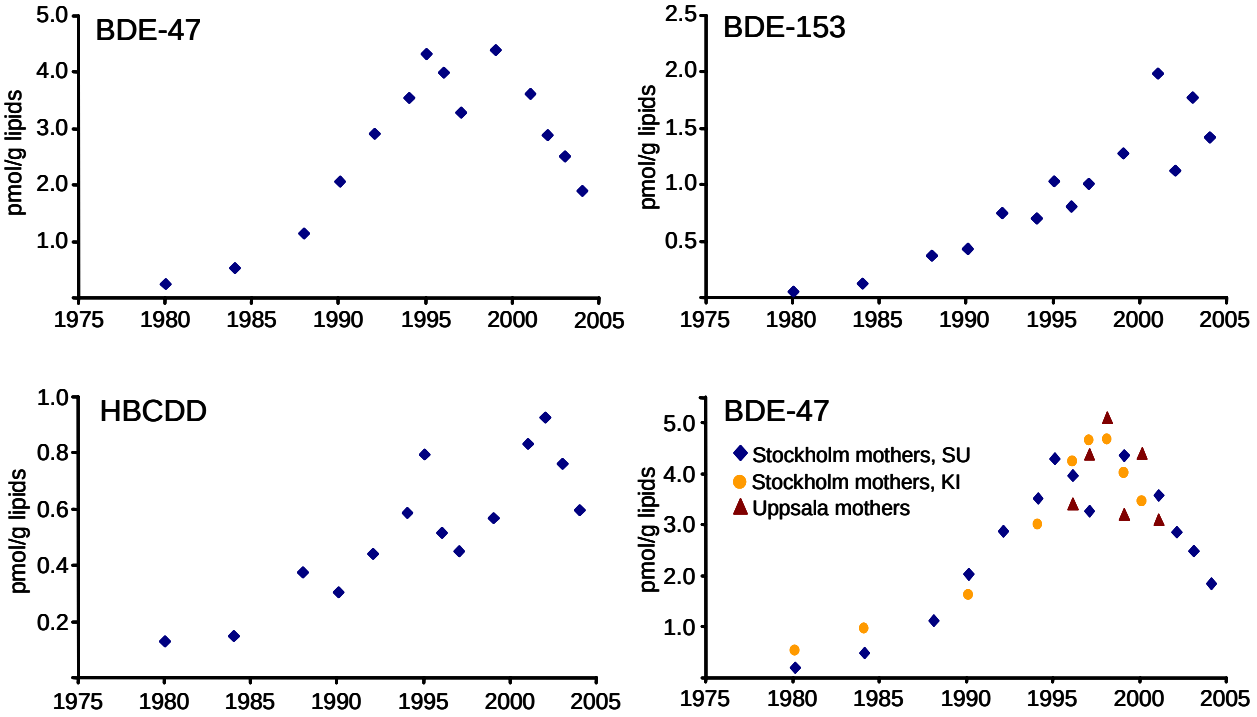

Figure 1. BDE-47, BDE-153 and HBCDD concentrations in pmol/g lipids in pooled milk samples from Sweden 1980 to 2004. BDE-47 – comparison in human milk, between present study and earlier studies performed in Sweden, Stockholm mothers, SU (Stockholm University), Stockholm mothers, KI (Karolinska Institutet) and Uppsala mothers [7-9].

In conclusion, the temporal trend of PBDEs must be expressed on a congener basis since the fate of the individual PBDE congener concentrations differ. BDE-47, -99 and -100 concentrations reached a peak in the mid 1990's and are now clearly showing decreasing levels. BDE-153 concentrations increased until the year 2000 and thereafter the concentrations may level off but it is yet not clear how the concentrations of this PBDE

congener will develop over the next few years. HBCDD concentrations in 2004, are approximately four times the concentrations in 1980 showing an increasing temporal trend. It is too early to judge if the levels are decreasing or leveling off. The HBCDD concentrations are in a range between BDE-47, BDE-99 and BDE-100. It was not possible to ascertain any temporal trend for the BDE-209 in the human milk. This may be due to poor transfer to the milk lipids but most likely it is a result of the short half-life of this compound in human blood.

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