Rapport till Naturvårdsverket för projektet "Analys av polybromerade difenyletrar (PBDE) och hexabromcyklododekan (HBCDD) i human mjölk från Stockholm – en tidstrends studie [Dnr 721-2653-05Mm]

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Temporal trends of brominated flame retardants in milk from Stockholm mothers, 1980-2004

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Summary

The objective of the present study was to assess the temporal trends of polybrominated diphenyl ethers (PBDEs), including decaBDE, and hexabromocyclododecane (HBCDD) in mothers milk from the Stockholm area. The pooled samples were covering the time period 1980 to 2004, with emphasis on samples from the last ten years. The temporal trend of PBDEs must be expressed on a congener basis since the development 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 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. It is not possible to quantify decaBDE (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. HBCDD concentrations are 2004, approximately four times the concentration in 1980 showing an increasing temporal trend until the early 2000's. It is too early to judge if the levels are decreasing or leveling off. The HBCDD concentrations are in a range between BDE-47 and BDE-99 and BDE-100.

Background

The environmental contamination of polybrominated diphenyl ethers (PBDEs) were first reported in fish from Sweden in the early 1980's (*1*), subsequently in Japan (*2*) and in human milk from Germany in 1988 (*3*). These initial studies have been followed by numerous studies worldwide in humans, fish, birds and mammals (*4,5*). Extensive summaries on human PBDE exposure are given in recent reviews, including those by e.g. Hites (*4*), Sjödin et al (*6*) and Ryan (*7*).

Swedish human milk, from Stockholm, have been shown to contain increasing concentrations of PBDEs from the early 1970s to 1997 (*8*), while samples from 1997 to 2000 indicated a decrease (*9*). The observed decrease was mainly due to a reduction of BDE-47 concentrations in the milk. A decrease of PBDE concentrations in Swedish milk has also been indicated by Lind and co-workers 2003 (*10*). In Norway, PBDE concentrations in human milk increased from 1986 to 2001 (*11*), within a similar concentration range as reported in Sweden and in Japan (*8-10,12*). These temporal trend studies all indicate decreasing PBDE concentrations, all driven by decreasing BDE-47 concentrations. Recently several reports have indicated increasing concentration of BDE-153, at concentrations similar or even higher than BDE-47 (*13-16*). In the United States, the PBDE concentrations are approximately 20 times higher in human subjects, comparing mean or median levels, than those hitherto reported in Europeans (*4,17*).

Hexabromocyclododecane (HBCDD) has recently been reported at highly varying concentrations (ranging from 0.09 to 10.4 ng/g lipids) in milk from mother's from Uppsala, Göteborg, Lund and Lycksele (*18*). 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 (*19*).

The aims of the present study were:
to extend previous studies of PBDEs in pooled human milk samples from Stockholm to cover the period 1980 to 2004
to indicate any temporal concentrations changes of PBDE congeners and of HBCDD
to determine any potential temporal trends of decabromodiphenyl ether (BDE-209)
to add information on PBDE time trend in human milk from Sweden

Further these concentrations were compared to previous studies for PBDEs performed in pooled human milk from Sweden.

Material and Methods

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 (c.f. Table 1 for the years). 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.

Chemicals: The individual PBDE congeners (numbered according to Ballschmiter *et al.* (*20*)): BDE-47, BDE-77, BDE-99, BDE-100, BDE-153 were synthesized in-house (*21*). HBCDD was purchased from Cambridge Isotope Laboratory and BDE-209 from Fluka Chemie, Switzerland. All solvents were of *pro analysis* quality. 2-Propanol from AnalaR (BDH laboratory supplies pool, England) and methyl *tert*-butyl ether (HPLC-grade; Rathburn, Walkerburn, Scotland) were glass-distilled prior to use. Silica gel (<0.063 mm) was purchased from Merck (Darmstadt, Germany) and activated (300°C, 12 h) before use.

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. The transfer line temperature was set to 290°C and the ion source temperature maintained at 200°C. Injections were made using a septum-equipped temperature programmable injector (SPI) fitted with a high performance insert directly connected to a DB-5 HT capillary column (15 m x 0.25 mm i.d., 0.1 µm film thickness; J&W Scientific) with helium as carrier gas at a head pressure of 3 psi. The injector temperature was programmed from 60°C to 320°C at 150°C/min and the oven from 80°C (1 min), 15°C/min to 300°C (16 min). 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 (5.0, AGA, Stockholm, Sweden) as the electron

thermalization buffer gas at 5.6 torr and a primary electron energy of 70 eV. All chromatographic data were collected, analysed and quantified using the proprietary ICIS2 software from Thermofinnigan. The linear relationship of the GC/MS system was determined and the quantifications were performed using a single point external standard within the concentration range of the linear relationship.

Extraction and cleanup procedure: The extraction and cleanup procedure for the milk samples is a modified version of a method for serum analysis first described and validated by Hovander and co-workers (*22*). In the modified version of the method, formic acid and diethyl ether are used instead of hydrochloric acid and methyl *tert*-butyl ether (*13*). Surrogate standard, BDE-77 was added to the samples prior to extraction. In short, formic acid (1 ml) and 2-propanol (6 ml) were added to a milk sample (5 g), subsequently extracted with a mixture of n-hexane/diethyl ether (1:1, 6 ml), and re-extracted once (3 ml). The lipid content was determined gravimetrically after gentle evaporation of the solvent. The bulk of lipids was removed with concentrated sulfuric acid and the samples were pooled 2 x 5 g. Additional cleanup was performed on two subsequently applied sulfuric acid/silica gel columns, according to Hovander *et al.* (*22*). Finally the sample was fractionated on a column of activated silica gel (0.7 g). Most of the PCB congener and major traditional organochlorine pesticide interferences were eluted with hexane (3 ml) and the PBDEs were eluted with hexane:DCM (1:1, 8 ml). The solvent in the PBDE fraction were changed to hexane and reduced to 50-100 µl before GC/MS analysis. All samples were protected from daylight during handling and storage to prevent any photochemical degradation of the brominated compounds to be analyzed.

Recovery experiment: A recovery study for the PBDE congeners, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-209 was performed on 5g of cow milk with a lipid content at 3% to validate the recovery of the method at two different spike levels (0.1 ng/sample and 1 ng/sample) (*13*).

Analysis: Four PBDE congeners, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-209 were analysed with GC/MS (ECNI), as specified above, were quantified in the duplicate samples (A/B) with a total sample volume at 10g with the SS, BDE-77.

Table 1. Concentrations of PBDE congeners (in ng/g lipid and pmol/g lipid) in pooled human milk samples from Sweden, 1980-2004

År	BDE-47		BDE-99		BDE-100		BDE-153		HBCDD	
	ng/g lipid	pmol/g lipid	ng/g lipid	pmol/g lipid	ng/g lipid	pmol/g lipid	ng/g lipid	pmol/g lipid	ng/g lipid	pmol/g lipid
1980	0.12	0.25	0.060	0.11	0.044	0.078	0.037	0.058	0.084	0.13
1984	0.26	0.53	0.043	0.076	0.073	0.13	0.082	0.13	0.096	0.15
1988	0.57	1.2	0.26	0.46	0.16	0.29	0.24	0.38	0.24	0.38
1990[a] (Pool 1)	0.55	1.1	0.24	0.42	0.17	0.30	0.17	0.26	0.22	0.34
1990[a] (Pool 2)	1.0	2.1	0.62	1.1	0.28	0.49	0.28	0.44	0.20	0.31
1992	1.4	2.9	0.48	0.84	0.31	0.55	0.48	0.75	0.29	0.44
1994	1.7	3.6	0.84	1.5	0.31	0.54	0.45	0.70	0.38	0.59
1995	2.1	4.3	0.75	1.3	0.51	0.90	0.67	1.0	0.51	0.80
1996	2.0	4.0	0.74	1.3	0.43	0.77	0.53	0.82	0.33	0.52
1997	1.6	3.3	0.69	1.2	0.36	0.64	0.65	1.0	0.29	0.45
1999	2.1	4.4	0.53	0.94	0.29	0.52	0.82	1.3	0.37	0.57
2001	1.8	3.6	0.57	1.0	0.63	1.1	1.3	2.0	0.54	0.83
2002	1.4	2.9	0.33	0.59	0.27	0.48	0.72	1.1	0.60	0.93
2003	1.2	2.5	0.29	0.51	0.31	0.55	1.1	1.8	0.49	0.77
2004	0.93	1.9	0.26	0.47	0.29	0.51	0.92	1.4	0.39	0.60

a) Two different pools from the same year, Pool 1; n=20, mean age = 30, 65% of the mothers nursing their first infant, Pool 2; n=20, mean age = 29, 55% of the mothers nursing their first infant.

Solvent blank samples representing every 5 sample were cleaned up and analyzed in the same way as the other samples. 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 blank sample amount has been subtracted from the results. Laboratory reference material was run in parallel to the analyzed samples. The overall recoveries and standard deviation (SD) of the surrogate standards were 84% S.D. 5.5 for BDE-77.

Results

The PBDE congener concentrations, run as duplicate samples (A/B), are presented in Table 1. The data presented are the "mean" value of the two analysis in the pooled milk samples from 1980 to 2004. The concentrations of decabromodiphenyl ether were low, above limit of detection (LOD) but below the quantification limit (LOQ), ranging from 0.01 to 0.10 pmol/g lipid. Data on HBCDD concentrations are also presented in Table 1. The concentrations are given both on a lipid weight basis (ng/g) and on a molar basis (pmol/g) to promote direct comparison between analytes since there are substantial differences in molecular weight between the analytes.

Discussion

This study stresses the results from previous studies on PBDE in human milk from Stockholm, where the concentrations of the lower brominated PBDEs are decreasing from the middle of 1990's, after increasing concentrations were observed in milk from the 1970-1980's to the mid 1990's. 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 (*8-10*). To some extend the same pools were used in the present study as in previous studies by Meironyté and co-workers (*8,9*) and the agreement between the studies is good although the extraction method, standards, analytical instrumentation and time for the analyses differ.

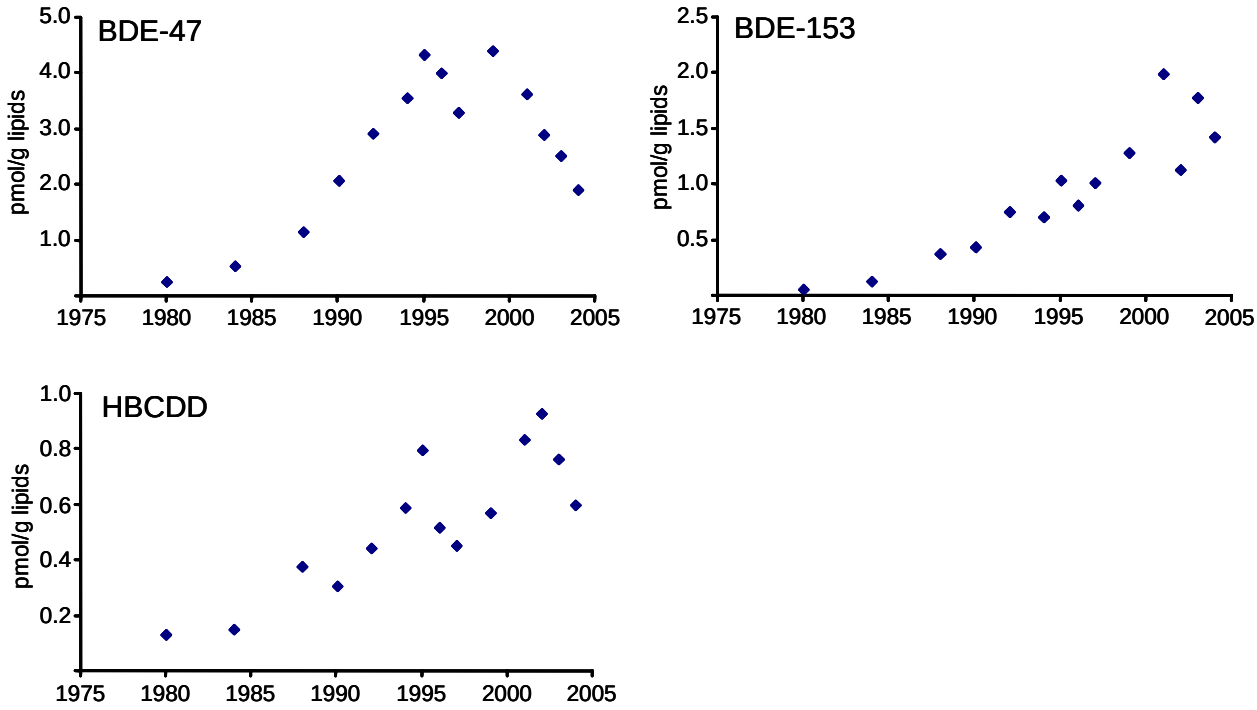

Figure 1. BDE-47, BDE-153 and HBCDD concentrations in pmol/g lipids in pooled milk samples from Sweden 1980 to 2004.

From the middle of 1990's the concentrations of the lower brominated PBDE congeners, i.e. BDE-47, BDE-99 and BDE-100, are decreasing, while the concentrations of BDE-153 are increasing (Table 1, Figure 1). This is in accordance to indications from previous studies performed in human milk from Sweden were the influence of BDE-153 have increase and BDE-47 have decreased (*8,9*). The ratio of BDE-153/BDE-47 (ng/g lipids) in 1980 to 2004 is changed from 30 % to 99 %.

It is not possible to see any time trend for BDE-209 in the milk samples due to low and similar concentrations over the time period, all below 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 organohalogen substances (OHS). The short half-life of BDE-209 in human blood (15 days) (*23*) may strongly influence the concentrations also in the milk even though we do not know the half-life of this PBDE congener in human milk. Further, we do not know how efficient the transfer of BDE-209 is to the milk. We may speculate that the transfer is less efficient than for the lower brominated diphenyl ethers since a previous study on paired samples of human milk and blood plasma where the BDE-153/BDE-47 ratio is lower in milk compare to blood, 28% and 67% respectively (*24*). This difference between milk and blood for BDE-153 has also

been seen in Norway, where the relative amount of BDE-153 is lower in breast milk compared to serum (*16*). Therefore serum seems to be a more suitable matrix for assessing human exposure to higher brominated diphenyl ethers i.e. those with six bromines or more. Concentration of up to 3.4 pmol/g lipid weight have been reported for BDE-209 in Faroese mother's milk (*13*) confirming that this compound can be transferred to the milk.

Despite the differences between milk and blood there is obviously a change in PBDE congener pattern over time, with increasing BDE-153 and decreasing BDE-47 concentrations. Higher concentrations of BDE-153 compared to BDE-47 has been seen in humans from the U.S.A., Norway, The Netherlands and the Faroe Islands (*13-16,25*). 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. It is too early to dismiss the hypothesis of BDE-153 being formed abiotically or via metabolism from BDE-209. Importantly, the present study can not confirm a decrease of BDE-153 in human milk from Stockholm.

The time trend for HBCDD in the Stockholm human milk shows a limited increase over time (Figure 1). The present situation is uncertain with no clear evidence of a decreasing tendency. From 1980 the concentrations has increased from 0.13 pmol/g lipid to 0.60 pmol/g lipid in 2004 (Table 1). 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. The result shows that HBCDD are transferred to the milk. 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 (*18*).

When pooled samples are analyzed no individual variation is taken into account, therefore the composition of the pools are very important. All pools were prepared to be as comparable as possible e.g. with 55-80% of the milk coming from mothers nursing their first infant. In the beginning of the 1980s the number of mothers in each pool was about 100, thereafter from 1988 to 2004 only 15-20 mothers are included in each pool. The average age of the mothers is 27-31 years. Despite these rather similar requirements in the pools there are differences between the pools which might have an influence on the trend e.g. pool S1 and S2 from 1990

(Table 1). However to be able to make time trend studies it is almost necessary to use pooled samples to minimize number of samples to be analyzed. To make the results as reliable as possible duplicate analyses were performed of the pools, control samples and blank samples has been run in parallel throughout the whole analysis. The method used for the PBDE analysis in human milk has previously been used for PBDEs and PCB analysis (*13,26*) and is a modified method for OHS analysis in human serum (*22*). A recovery study was performed with cow's milk (3% fat content) and the overall recovery for BDE-47, BDE-99, BDE-100 and BDE-153 were about 90% within a range of 79-107% (*13*).

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