Sakrapport till Naturvårdsverkets Miljöövervakning:

BDE-209 i blodserum från förstföderskor i Uppsala – tidstrend 1996-2010

Avtalsnummer:	**215 0906**
Utförare:	**Livsmedelsverket**
Programområde:	**Hälsorelaterad miljöövervakning**
Delprogram:	**Biologiska mätdata – organiska ämnen**
Undersökningar/uppdrag:	**1. Utveckling och validering av en metod för analys av BDE-209 i humant blodserum.** **2. Tidsserie för BDE-209: Analys av BDE-209 i poolade serumprover från förstföderskor i Uppsala 1996-2010**

LIVSMEDELS VERKET

Sanna Lignell, Marie Aune, Martin Isaksson, Johan Redeby, Per Ola Darnerud, Anders Glynn
2011-03-31

Report to the Swedish Environmental Protection Agency, 2011-03-31
Sanna Lignell, Marie Aune, Martin Isaksson, Johan Redeby, Per Ola Darnerud, Anders Glynn

BDE-209 in blood serum from first-time mothers in Uppsala – temporal trend 1996-2010

Background

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are brominated flame retardants that have been found in breast milk from Swedish first-time mothers. In contrast to the clear decrease for most organochlorinated compounds that were simultaneously studied (Lignell et al., 2009a; Lignell et al., 2009b), temporal trends for the PBDE group during the years 1996-2006/08 did not follow any consistent pattern. BDE-47, BDE-99 and BDE-100 decreased significantly during this time period, whereas BDE-153 levels significantly increased in breast milk. The analyses of PBDEs in breast milk did not include the deca-brominated congener (BDE-209), and the aim of this study was to develop a method for analysis of BDE-209 in blood serum and to start a time series for BDE-209. Tri- to hepta-brominated PBDE congeners, i.e. BDE-28, BDE-47, BDE-66, BDE-99, BDE-100, BDE-138, BDE-153, BDE-154 and BDE-183, as well as HBCD were also included in the analytical method. In the present report we examine temporal trends for BDE-47, BDE-99, BDE-100, BDE-153, BDE-209 and HBCD.

In 2003, strict bans were put on the global use of commercial penta- and octaBDE mixtures, making decaBDE (BDE-209) the remaining PBDE for commercial use. This may have resulted in an increased demand for the decaBDE mixture for some years thereafter, but also the decaBDE is now under ban by the EU for use in electrical and electronic applications (since 2008), and large-scale producers of decaBDE state that they will discontinue their production and sale by the end of 2013.

The decaBDE is used as an industrial flame retardant with similar field of application as the lower brominated PBDEs, i.e. for use in electric, electronic and textile products. However, biological properties of decaBDE are different from the lower brominated PBDEs by being less persistent, and the half-time in animals and in man is estimated to be short. Calculations give decaBDE a half-life of only 15 days in occupationally exposed workers (Thuresson et al., 2006), whereas the half-life of the heptabrominated BDE-183 and hexabrominated BDE-153 are reported to be 94 and 2380 days, respectively (Geyer et al.,

2004; Thuresson et al., 2006). Although low persistence would seem to be a beneficial property for an industrial chemical, the breakdown of the decaBDE molecule may result in lower brominated PBDEs, or other products, with increased toxic potential. These issues have been discussed by e.g. U.S. Environmental Protection Agency (US-EPA, 2008).

The reason to monitor blood serum samples instead of breast milk for BDE-209 is because this congener is reported to be poorly transferred to milk (Fängström et al., 2008). In a study from Japan, lactating mothers from four regions were sampled for both breast milk and blood, and the matched samples (N=89) were analysed for a number of PBDE (and PCB) congeners (Inoue et al., 2006). The (geometric) mean of serum BDE-209 levels was 1.20 ng/g fat (geometric SD 2.05) and the corresponding mean in breast milk was 0.12 ng/g fat (geometric SD 2.61). Although the standard deviations were large, these results suggest that the levels of BDE-209 are about 10 times higher in serum compared to breast milk, on a fat basis. This is in contrast to the relative distribution of lower brominated (tetra-hexa) BDE congeners in serum and milk, where the levels in the two matrices were rather similar (Inoue et al., 2006). The partitioning ratio between serum and breast milk was also studied in matched samples from 29 women in Texas, USA (Schecter et al., 2010). In this study, the mean blood serum to breast milk ratio of BDE-47 (a tetraBDE) was 0.6, whereas the ratio gradually increased with bromine content and was around 1 for the hexabrominated BDE-153 (Schecter et al., 2010). BDE-209 was not included in the study by Schecter et al.

Material and methods

Recruitment and sampling

In the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas), first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2006 (N=335) (Lignell et al. 2009). Additional women were recruited in 2007, 2008, 2009 and 2010 (N=29-30 every year). The participants donated breast milk and a blood sample 3 weeks after delivery. Blood sampling was done using 9 ml Vacutainer® or Vacuette® serum tubes and serum was stored at -20°C. The study was approved by the local ethics committee of Uppsala University, and the participating women gave informed consent prior to the inclusion in the study.

In this study we used pooled serum samples from the participants for analysis of PBDEs and HBCD. Samples from mothers born in non-Nordic countries were not included in the pools (N=10). From about 30 women, there was no serum left or the volume was too

small to allow inclusion in the pools. The total number of individual samples included in the pools was 413. An effort was made to produce 3 pooled serum samples for each sampling year. 5-25 individual samples were included in each pool (Table 1)

Table 1. Composition of the pooled serum samples used for analyses of PBDEs.

Sampling year	N[a]	No of pools	N in each pool	Age (yrs)[b] mean (range)
1996	19	3	6-7	30 (21-41)
1997	62	3	20-21	28 (21-37)
1998	74	3	24-25	29 (21-35)
1999	17	3	5-6	27 (21-31)
2000	20	2	10	30 (21-37)
2001	9	1	9	29 (22-35)
2002	31	3	10-11	30 (24-37)
2004	32	3	10-11	29 (20-34)
2006	30	3	10	30 (19-40)
2007	29	3	9-10	30 (21-39)
2008	30	3	10	29 (20-35)
2009	30	3	10	29 (22-39)
2010	30	3	10	30 (20-41)

[a]Total number of serum samples from the specific sampling year.
[b]Mean age of the women donating blood during the specific sampling year.

Method for determination of brominated flame retardants in human blood serum

Extraction and clean-up

Thawed serum (4 g) was mixed with methanol (4 ml) by vortexing in a 16 ml test tube. A mixture of diethyl ether and n-hexane (5 ml, 1+1 (V/V)) and 100 µl each of the internal surrogate standards, BDE-85 (2 pg/µl) and ^{13}C12 BDE-209 (1 pg/µl) were added. The sample was extracted on a rotary mixer for 15 min and then centrifuged at 2500 rpm for 10 min. The top, organic, layer was transferred to a pre-weighed test tube. The extraction was repeated twice and the organic layers were combined. The solvent was evaporated using a gentle stream of nitrogen and the lipid weight was determined gravimetrically.

A chemical blank (4 g of MilliQ water) was included in each extraction series to monitor background levels. To avoid possible analyte loss, the chemical blank was not evaporated until dryness. A spiked in-house control sample was also included in each extraction series.

In order to remove the lipids and other polar materials the lipid extract was re-dissolved in 2 ml of n-hexane using ultra-sonication and then treated with 2 ml of sulphuric acid. After separation (centrifugation at 2000 rpm for 15 min) and collection of the organic

layer the procedure was repeated once by adding 1 ml of n-hexane to the acidic layer. The organic phases were combined and the volume reduced to 0.5 ml by using a gentle stream of nitrogen.

The lipid-free extract was transferred to a pre-washed open silica gel column (8 mm id, 4.5 g of 3 % deactivated silica gel). Most of the PCBs were separated from the PBDEs by elution with 20 ml n-hexane (waste). A second fraction, containing the brominated flame retardants, was eluted with 20 ml of dichloromethane. The second fraction was reduced to 1 ml using a rotary evaporator and after transfer to a test tube, the solvent was changed to n-hexane. The final volume of the sample was adjusted to 100 µl using a gentle stream of nitrogen and then kept in an amber GC vial until analysis.

Analysis on GC-LRMS

The quantification of the analytes was performed using capillary gas chromatography and mass selective detection in electron capture negative ionization and selected ion monitoring modes (GC/LRMS/ECNI-SIM). The system used for quantification consisted of an Agilent 6890N GC equipped with an Agilent 5973N MS.

A large sample volume, 10 µl (5x2 µl), was injected in solvent vent mode using a programmable temperature vaporizing (PTV) injector with an initial temperature of 70°C followed by rapid heating to 300°C after solvent evaporation. The analytes were separated on a 15 m x 0.25 mm x 0.10 µm DB-5MS (J&W Scientific) capillary column with a 5 m Duraguard section at a constant carrier gas (helium) flow of 1.3 ml/min. The oven temperature was programmed from 60°C to 310°C including several ramps. The mass fragments m/z 79 and 81 were monitored for BDE-28, -47, -66, -85, -99, -100, -116 and -154; m/z 79, 81 and 160 for BDE-138, -153 and -183 as well as for HBCD; m/z 486.6 /484.6 and 494.6/496.6 (target/qualifier) were monitored for BDE-209 and 13C12 BDE-209, respectively. Methane was used as reaction gas and the ion source, quadrupole and transfer line temperatures were kept at 210°C, 110°C and 310°C, respectively.

Calibration standard solutions corresponding to a level range (fresh weight) in serum of 1.25-100 ng/kg for BDE-28 through BDE-183 (2.5-200 ng/kg for BDE-47 and HBCD) and 0.625-750 ng/kg for BDE-209 were included in the run. N-hexane was injected in between sample and calibration standard series to make sure there were no memory effects.

The different analytes were identified by their retention times relative to the internal standards. The samples were quantified using calibration curves created from the calibration standards analysed in the same run. Quadratic regression with the inverse square

of concentration was used for the calibration curves. The internal surrogate standard used for the quantification of BDE-28, -47, -66, -100, -99, -154, -153, -138 and -183 as well as HBCD was BDE-85. ^{13}C12 BDE-209 was used for the quantification of BDE-209 (isotope dilution technique).

Quality Assurance / Quality Control

All solvents used were tested for trace amounts of analytes. The glassware was either rinsed with acetone or heated in an oven at 420°C for at least 2 hours before use. Due to possible UV induced degradation of the analytes, particularly for BDE-209, all sample extracts and standard solutions were stored in amber glassware. For each batch of samples, the corresponding blank sample levels were subtracted from the sample levels. The limit of quantification (LOQ) was determined as three times the standard deviation of the blanks analysed together with the samples or the lowest calibration level. The LOQ varied between 1.25 and 10.7 ng/kg fresh weight, depending on the blank sample levels of the different analytes. Levels below LOQ were also reported in order to improve the statistical analysis. The coefficient of variation ranged from 2.6 to 10 % for the different analytes in the spiked in-house control sample.

Calculations and statistics

The analysis of temporal trends was performed in MINITAB® for Windows 14 using simple linear regression. Lipid adjusted blood serum levels of PBDEs and HBCD were used in the statistical analysis. When the serum concentrations were below LOQ, the actual determined concentration was used in the statistical analysis. In a first step, all results (N=36) were used in the regression analyses. Thereafter, a few outliers (with standardized residuals ≥3) were omitted and the regression analyses were performed again.

Results and Discussion

Concentrations of BDE-47, BDE-99, BDE-100, BDE-153, BDE-209 and HBCD in the serum pools are shown in table 2. Levels <LOQ are reported in the table since these were used in the statistical analyses of temporal trends. The concentrations of BDE-47, BDE-99, BDE-100 and HBCD were <LOQ in more than 70% of the samples while BDE-153 and BDE-209 could be quantified in all samples. LOQ for BDE-47 was higher than for the other compounds due to

Table 2. Levels of PBDEs and HBCD in 36 pooled samples of blood serum and in individual samples of mother's milk from first-time mothers in Uppsala (ng/g lipid weight). For levels <LOQ (limit of quantification) in serum, the actual determined value is given *in italics*.

Year	BDE-47		BDE-99		BDE-100		BDE-153	BDE-209	HBCD	
1996	<2.18	*1.4*	<0.90	*0.67*	<0.49	*0.45*	0.73	1.49	<0.51	*0.49*
1996	<1.95	*1.1*	<0.80	*0.40*	<0.44	*0.24*	0.53	1.42	0.47	
1996	6.00		1.62		1.79		1.85	1.15	0.69	
1997	<1.98	*1.2*	<0.81	*0.43*	<0.44	*0.35*	0.70	0.85	<0.46	*0.11*
1997	<1.98	*1.9*	1.11		0.56		0.74	0.57	<0.46	*0.24*
1997	<1.95	*1.0*	<0.80	*0.45*	<0.44	*0.31*	0.62	0.64	<0.45	*0.24*
1998	<2.06	*1.8*	0.92		0.56		0.75	0.85	0.50	
1998	<1.91	*1.4*	<0.79	*0.41*	<0.43	*0.41*	0.75	0.71	<0.45	*0.14*
1998	<1.91	*0.79*	<0.79	*0.38*	<0.43	*0.30*	0.75	2.43	<0.45	*0.20*
1999	<2.18	*1.3*	<0.90	*0.39*	<0.49	*0.43*	0.73	1.37	<0.51	*0.49*
1999	<1.84	*1.1*	<0.76	*0.31*	<0.41	*0.40*	0.59	4.03	<0.43	*0.38*
1999	<2.28	*1.1*	<0.94	*0.34*	<0.51	*0.36*	0.85	1.26	<0.53	*0.43*
2000	<2.14	*0.44*	<0.88	*0.88*	<0.48	*0.38*	0.62	2.46	<0.50	*0.30*
2000	<2.06	*0.31*	0.98		0.60		0.48	2.48	<0.48	*0.35*
2001	<1.95	*0.07*	0.93		<0.44	*0*	0.60	1.13	<0.45	*0.29*
2002	<1.84	*0.69*	0.93		<0.41	*0.28*	0.74	1.71	<0.43	*0.40*
2002	<2.02	*0.28*	<0.83	*0.72*	0.45		0.81	1.72	<0.47	*0.36*
2002	<2.23	*0.56*	0.98		0.54		1.08	1.44	<0.52	*0.48*
2004	<2.18	*0.63*	<0.90	*0.14*	1.35		1.59	0.80	0.78	
2004	<2.18	*1.1*	<0.90	*0.31*	0.98		1.04	0.53	<0.51	*0.45*
2004	<2.28	*0*	<0.94	*0.34*	<0.51	*0.49*	0.74	0.74	<0.53	*0.38*
2006	<2.14	*0.42*	<0.88	*0.06*	<0.48	*0.26*	0.86	0.74	<0.50	*0.38*
2006	<2.02	*0.25*	<0.83	*0*	<0.45	*0.15*	1.09	0.83	<0.47	*0.40*
2006	<2.02	*0.17*	<0.83	*0.08*	<0.45	*0.19*	0.92	1.11	<0.47	*0.38*
2007	<1.88	*1.0*	<0.77	*0.51*	<0.42	*0.35*	0.84	1.12	<0.44	*0.23*
2007	3.30		1.33		0.80		0.85	1.26	<0.46	*0.22*
2007	<1.78	*0.60*	<0.73	*0.20*	<0.40	*0.27*	0.85	1.27	<0.42	*0.30*
2008	<2.14	*1.7*	<0.88	*0.42*	0.54		1.76	0.86	<0.50	*0.14*
2008	<1.91	*0.66*	<0.79	*0.21*	<0.43	*0.25*	0.88	0.88	<0.45	*0.32*
2008	<2.06	*0.87*	<0.85	*0.35*	<0.46	*0.29*	0.88	0.98	<0.48	*0.27*
2009	<1.98	*0.76*	<0.81	*0.17*	<0.44	*0.22*	0.56	1.50	<0.46	*0.13*
2009	<1.98	*0.54*	<0.81	*0.22*	<0.44	*0.20*	0.80	1.50	<0.46	*0.13*
2009	<2.02	*0.57*	<0.83	*0.04*	<0.45	*0.26*	0.83	0.96	<0.47	*0.17*
2010	<1.88	*0.26*	<0.77	*0.02*	<0.42	*0.14*	0.77	1.12	<0.44	*0*
2010	<2.14	*0.24*	<0.88	*0*	<0.48	*0.08*	0.58	0.88	<0.50	*0.06*
2010	<2.10	*0.49*	<0.86	*0.18*	<0.47	*0.27*	1.02	0.84	<0.49	*0*
Mean	1.22[a]	*1.00*	0.57[a]	*0.48*	0.39[a]	*0.43*	0.86	1.27	0.28[a]	*0.31*
N <LOQ	34		28		26		0	0	32	
Breast milk[b]										
Mean	1.9[a]		0.43[a]		0.36[a]		0.66[a]	-	0.38[a]	

[a]Levels <LOQ were set to ½LOQ in the calculations of mean

[b]Mean levels in individual breast milk samples from the mother's participating in the study in 1996-2008. N=307 for PBDEs and N=208 for HBCD.

problems with high blank values. BDE-209 showed the highest mean concentration, followed by BDE-47, BDE-153, BDE-99, BDE-100 and HBCD.

The concentrations detected in blood serum are similar to the levels in breast milk from the mothers participating in the study in 1996 to 2008 (Table 2). In breast milk, the highest mean level was shown for BDE-47, followed by BDE-153, BDE-99, HBCD and BDE-100. BDE-209 was not analysed in breast milk. In support of our results, similarity in serum and breast milk levels of tetra- to hexabrominated PBDE congeners were reported by Inoue and co-workers (2006) in Japanese human samples from 2005. Also in serum and breast milk samples from Texas, USA (sampled 2007) the ratios were roughly similar, but the serum/milk ratio increased somewhat with degree of bromination from tetra to hexa PBDEs (Schecter et al., 2010).

Linear regression analyses showed that the levels of BDE-47, BDE-99 and HBCD decreased significantly in serum during the study period (Table 3, Figure 1-2). The levels of HBCD seem to have been stable up to about year 2005 and decreased thereafter. When a few outliers were omitted from the statistical analysis, a significant decreasing trend was also shown for BDE-100 and a significant increasing trend was shown for BDE-153. There was no significant trend for BDE-209. Values below LOQ were used in the statistical analysis even though these concentrations are determined with less precision than concentrations above LOQ. Despite the uncertainty in the levels <LOQ, we could still detect temporal trends.

Table 3. Annual change in concentrations of PBDEs and HBCD in blood serum, 1996–2010. Non-significant results (p>0.05) are shown in brackets.

	N	Change per year (ng/g lipid)		R^2 (%)	p
		mean	SE		
BDE-47	36	-0.08	0.036	11	0.029
BDE-47[a]	34	-0.05	0.016	22	<0.005
BDE-99	36	-0.04	0.012	26	<0.005
BDE-99[a]	35	-0.05	0.010	38	<0.0005
BDE-100	36	(-0.02)	(0.011)	(6)	(0.079)
BDE-100[a]	32	-0.01	0.005	19	0.008
BDE-153	36	(0.009)	(0.011)	(0)	(0.406)
BDE-153[a]	33	0.013	0.005	12	0.025
BDE-209	36	(-0.035)	(0.024)	(3)	(0.146)
BDE-209[a]	35	(-0.022)	(0.018)	(1)	(0.232)
HBCD	36	-0.017	0.005	20	<0.005
HBCD[a]	35	-0.018	0.005	28	<0.005

[a]A few (1-4) outliers with high standardized residuals (≥3) were omitted in the regression analysis.

The results for BDE-47, BDE-99, BDE-100 and BDE-153 are in agreement with what we have previously observed in breast milk from the participants in the study (Lignell et al., 2009a; Lignell et al., 2009b). In a study of PBDEs in mother's milk from women in Stockholm (1980-2004) (Fängström et al., 2008), decreasing levels of BDE-47 were observed from about 1995, while the levels of BDE-153 and HBCD seemed to have stabilized but not decreased after year 2000.

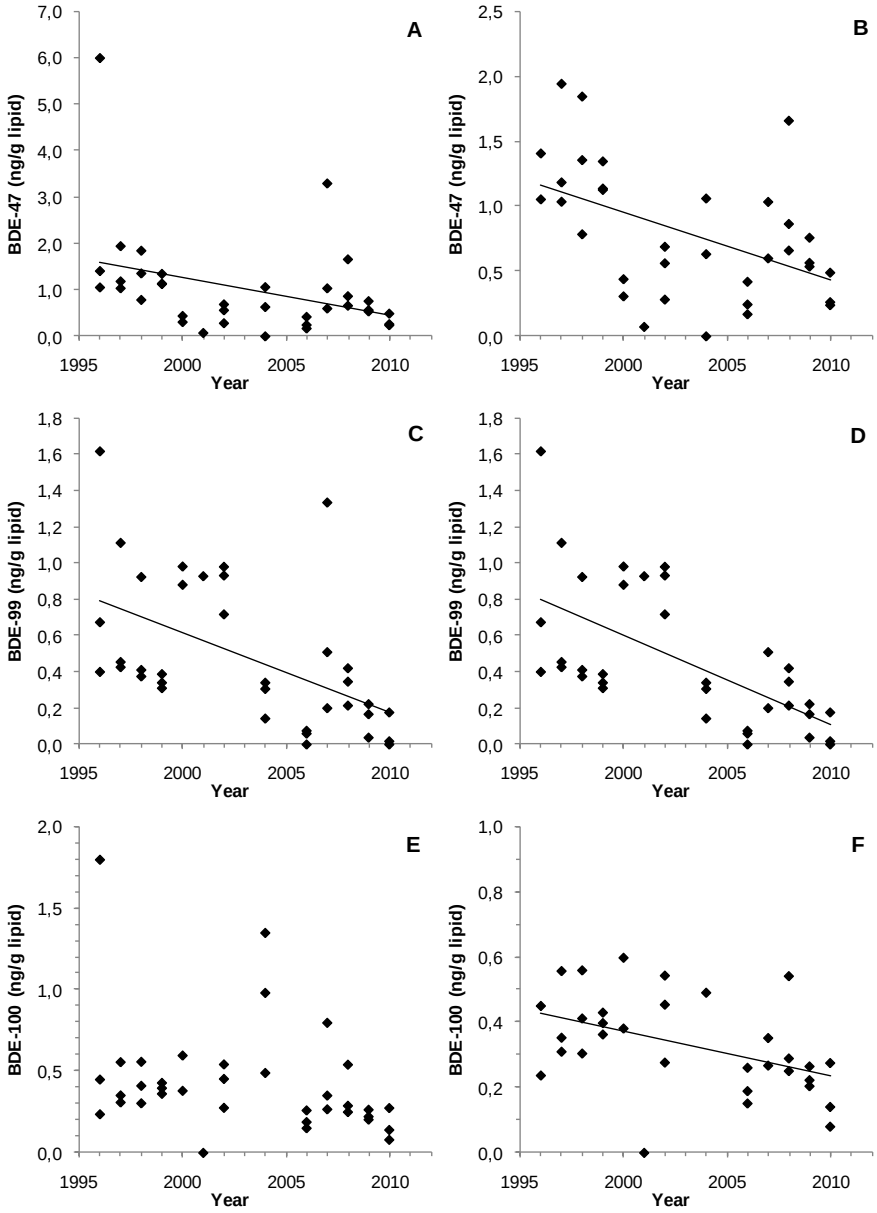

Figure 1. Concentrations of BDE-47, BDE-99 and BDE-100 in pooled samples (N=36) of blood serum from first-time mothers in Uppsala sampled between 1996 and 2010. Diagram A, C and E shows all data. In diagram B, D and F, 1-4 outliers have been omitted (see table 3). Trend lines indicate significant trends ($p<0.05$).

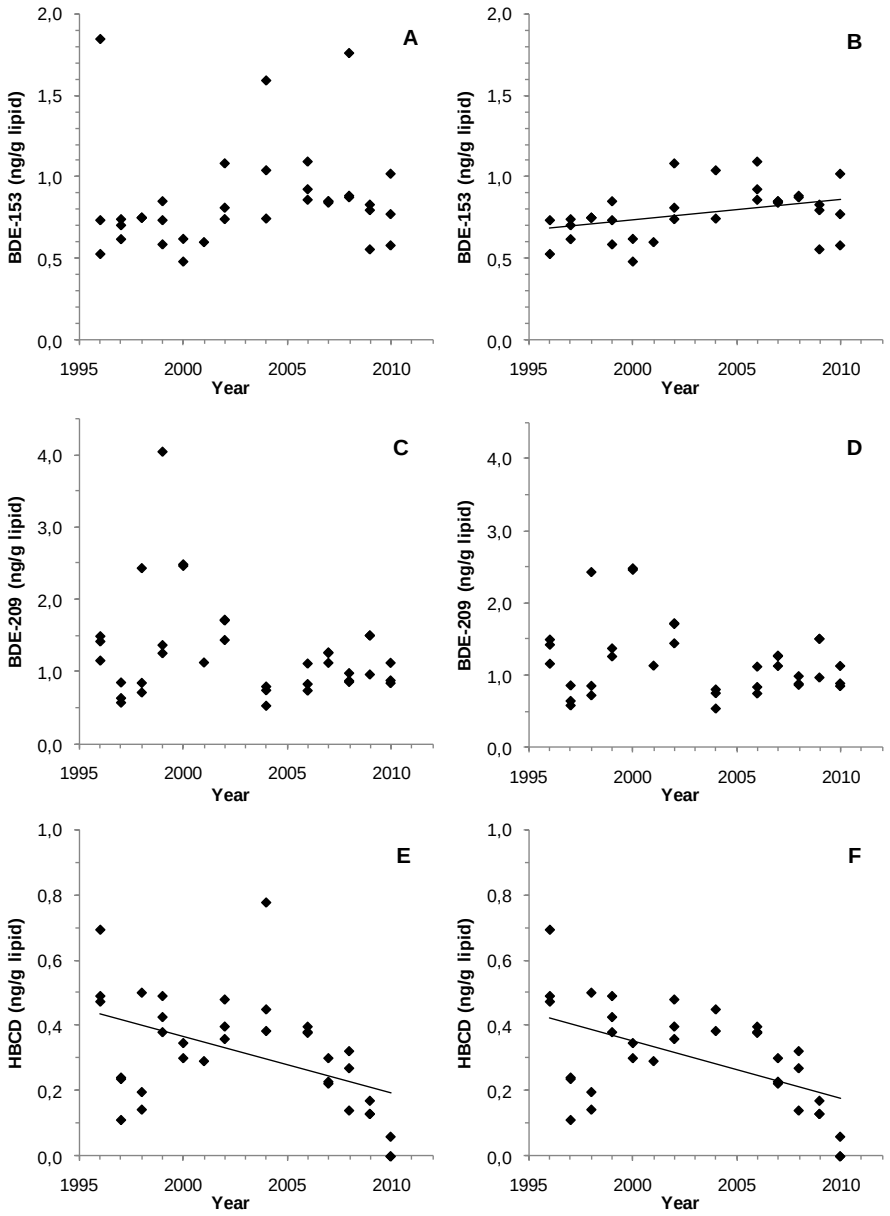

Figure 2. Concentrations of BDE-153, BDE-209 and HBCD in pooled samples (N=36) of blood serum from first-time mothers in Uppsala sampled between 1996 and 2010. Diagram A, C and E shows all data. In diagram B, D and F, 1-3 outliers have been omitted (see table 3). Trend lines indicate significant trends ($p<0.05$).

References

Fängström, B., Athanassiadis, I., Odsjo, T., Noren, K., Bergman, A., 2008. Temporal trends of polybrominated diphenyl ethers and hexabromocyclododecane in milk from Stockholm mothers, 1980-2004. Mol Nutr Food Res 52, 187-193.

Geyer, H.J., Schramm, K.W., Darnerud, P.O., Aune, M., Feicht, E.A., Fried, K.W., Henkelmann, B., Lenoir, D., Schmidt, P., McDonald, T.A., 2004. Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. Organohalogen compounds 66, 3820-3825.

Inoue, K., Harada, K., Takenaka, K., Uehara, S., Kono, M., Shimizu, T., Takasuga, T., Senthilkumar, K., Yamashita, F., Koizumi, A., 2006. Levels and concentration ratios of polychlorinated biphenyls and polybrominated diphenyl ethers in serum and breast milk in Japanese mothers. Environ Health Perspect 114, 1179-1185.

Lignell, S., Aune, M., Darnerud, P.O., Cnattingius, S., Glynn, A., 2009a. Persistent organochlorine and organobromine compounds in mother's milk from Sweden 1996-2006: compound-specific temporal trends. Environ Res 109, 760-767.

Lignell, S., Glynn, A., Törnkvist, A., Aune, M., Darnerud, P.O., 2009b. Levels of persistent halogenated organic pollutants (POP) in mother's milk from primiparae women in Uppsala, Sweden 2008. National Food Administration, Uppsala.

Schecter, A., Colacino, J., Sjodin, A., Needham, L., Birnbaum, L., 2010. Partitioning of polybrominated diphenyl ethers (PBDEs) in serum and milk from the same mothers. Chemosphere 78, 1279-1284.

Thuresson, K., Hoglund, P., Hagmar, L., Sjodin, A., Bergman, A., Jakobsson, K., 2006. Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. Environ Health Perspect 114, 176-181.

US-EPA, 2008. Toxicological review of decabromopiphenyl ether (BDE-209). CAS No. 1163-19-5. . U.S. Environmental Protection Agency, Washington D.C.