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Concentrations of four new brominated flame retardants (HBB, PBEB, BTBPE, DBDPE), PBDEs and HBCD in blood serum from first-time mothers in Uppsala 1996-2015

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Sammanfattning
Sedan 1996 har Livsmedelsverket regelbundet samlat in prover från förstföderskor i Uppsala för analys av persistenta halogenerade organiska miljöföroreningar (POP). I följande rapport redovisas halterna av de bromerade flamskyddsmedlen (BFR) PBDE och HBCD i samlingsprover av serum (3 samlingsprover per provtagningsår) insamlade 1996-2015. Halter och trender för perioden 1996-2014 har rapporterats tidigare, och i denna rapport har data från prover insamlade 2015 lagts till. Dessutom har halterna av 4 nya BFR analyserats i proverna 1996-2015, det vill säga hexabrombensen (HBB), pentabrometylbensen (PBEB), 1,2-bis(2,4,6-tribromfenoxy)etan (BTBPE) and dekabromdifenyletan (DBDPE). Halterna av HBB, PBEB och DBDPE i de årliga samlingsproverna låg alla under detektionsgränsen (LOD), utom i enstaka prover. För HBB och PBEB låg LOD på 0,13 pg/g serum och för DBDPE på 6 pg/g serum. För BTBPE hade 8 årspooler halter (7,1-32 pg/g serum) över LOQ, och 23 prover detekterbara halter (0,19-4,4 pg/g serum) över LOD. Medianhalten för hela perioden 1996-2015 uppskattades till 0,50 pg/g serum, och halterna tycktes inte förändras under perioden. Bland PBDE förekom BDE-209 i högst halter under studieperioden (median 1,1 ng/g fett), följt av BDE-153 (0,83 ng/g fett), BDE-47 (0,69 ng/g fett), BDE-99 (0,34 ng/g fett), HBCD (0,32 ng/g fett) och BDE-100 (0,29 ng/g fett). Den uppdaterade tidstrenden för BDE-209 antydde inte någon förändring av halterna under studieperioden. Även för HBCD detekterades ingen statistiskt säkerställd tidstrend. Koncentrationerna av BDE-153 ökade med 2 % per år. BDE-47, -99 och -100 har sjunkit med 6-11 % per år mellan 1996 och 2015.

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are brominated flame retardants (BFRs) that have been, or are in the process of being, regulated because of their persistence, bioaccumulative properties and toxicity (KemI 2016). The more lipid-soluble so–called legacy BFRs are found in mother´s milk from Swedish mothers, fortunately at decreasing concentrations for the most common ones (Lignell et al. 2015a). However, some BFRs are less lipid-soluble and are not easily transferred from the blood of the mother to mother´s milk making it necessary to measure them in blood serum.

A temporal trend study of PBDEs and HBCD in pooled blood serum samples from POPUP-cohort has been initiated (Lignell et al. 2011). The main reason to start monitoring blood serum was to initiate a time series for the deca-brominated congener (BDE-209), that is poorly transferred to mother´s milk. During the time-period 1996-2014 concentrations of BDE-209 in serum has not changed significantly, suggesting that there has been a more or less constant human exposure to this PBDE for almost 2 decades (Darnerud et al. 2015).

There are many emerging BFRs that at least partially have been introduced as substitute chemicals for PBDEs and HBCDs. As a consequence there may be a risk that human exposure to these substitutes may have increased in parallel with decreasing concentrations of legacy BFRs. Here we present temporal trend results for serum concentrations of hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and decabromodiphenylethane DBDPE during the period 1996-2015 in POPUP mothers. These BFRs have previously been found in previous Swedish EPA screening of biota (Brorström-Lundén et al. 2013), and/or have been identified as emerging BFRs by the European Food Safety Authority (EFSA 2012).

In addition, the temporal trends for legacy PBDEs and HBCD has been updated covering the period 1996-2015. This is an interim report and the final report including validation of the new developed analytical serum method will be presented in 2017.

MATERIALS AND METHODS

Recruitment and sampling

In the POPUP study, almost 600 first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2015. The participants donated a blood sample three 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.

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

Sampling year	N[a]	No of pools	N in each pool	Age (years)[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)
2011	29	3	9-10	30 (21-38)
2012	30	3	10	29 (21-38)
2013	30	3	10	29 (22-39)
2014	30	3	10	30 (20-38)
2015	30	3	10	30 (22-38)

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

In this study we used pooled serum samples from the participants for analysis of BFRs. The composition of the 51 pools from 1996-2015 is given in Table 1. The total number of individual samples included in all pools was 562.

Method for determination of PBDEs, HBCD and NBFRs in blood serum

Sample preparation

The extraction and clean-up method used and described earlier (Darnerud et al. 2015, Lignell et al. 2011; 2015b) has been modified as new analytes have been included in the method. The new analytes are hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and decabromodiphenylethane (DBDPE). In addition, the internal standard (BDE-85) has been replaced by 13C-BDE-155 and BDE-138 is no longer included in the analysis.

Briefly, serum was extracted with methanol and a diethyl ether/n-hexane mixture. The organic phase was washed twice with aqueous potassium chloride (1% w/w) and transferred to a pre-weighed test tube. The lipid weight was determined gravimetrically. In order to remove lipids and other polar materials the lipid extract was re-dissolved in n-hexane and treated with concentrated sulphuric acid and the sample was transferred to an impregnated silica/sulphuric acid gel column and eluted with a mixture of dichloromethane/n-hexane. The lipid-free extract was transferred to a pre-washed alumina/silica gel column and eluted with n-hexane (fraction 1) and dichloromethane/n-hexane (fraction 2). The volume of the second fraction was adjusted to 100 µl using a gentle stream of nitrogen and then kept in an amber GC vial until analysis.

GC/MS analysis

The quantification of the analytes was performed with minor modifications of previously described method (Lignell et al. 2015). The analytes were quantified 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. The sample (2 x3 µl), was injected (pulsed splitless) using a programmable temperature vaporizing (PTV) injector with an initial temperature of 70°C followed by rapid heating to 300°C. The analytes were separated on a DB-5MS capillary column (15m x 0.25 mm id, 0.1 µm, J&W Scientific) using a ramped carrier gas flow and the oven temperature was programmed from 60°C to 325°C. 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.

The mass fragments monitored for PBDEs, emerging BFRs, HBCD and internal standards are described in Table 2. 13C-BDE-155 was used as internal surrogate standard for the quantification of BDE-28, -47, -66, -100, -99, -154, -153 and -183 as well as HBB, PBEB, BTBPE and HBCD. 13C-BDE-209 was used for the quantification of BDE-209 (isotope dilution technique) and DBDPE.

Calibration standard solutions corresponding to a level range in serum of 0.625-125 ng/kg fresh weight for PBDEs, HBB, PBEB and BTBPE, 1.25-250 ng/kg for BDE-47, BDE-209 and HBCD and 12.5-625 ng/kg for DBDPE were included in the run. 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.

Table 2. Negative ions monitored (m/z) for PBDEs, HBCD and emerging BFR

Analyte	m/z
BDE-28, -47, -66, -100, -99- 154, -153, -183	79, 81
HBCD	79, 81, 160
HBB	79, 81, 551.5
PBEB	79, 81
BTBPE	79, 81
DBDPE	79, 81
BDE-209	484.6
13C-BDE-155 (IS)	334.8
13C-BDE-209 (IS)	496.6

Quality assurance

All solvents used were tested for trace amounts of analytes. The glassware was either rinsed with acetone or heated in an oven at 450°C for at least 3 hours before use. Silica and alumina gel was heated at 450°C overnight to eliminate PBDE residuals and lower the background levels of the blanks. Silica gel was deactivated with 3% MilliQ water and both silica and alumina gel was washed with n-hexane before used.

Due to possible UV induced degradation of the analytes, particularly for BDE-209, all sample extracts and standard solutions were stored in amber glassware and all the steps were performed in a UV-free environment.

N-hexane was injected in between sample and calibration standard series to make sure there were no memory effects. A chemical blank was included in each extraction series to monitor background levels. A spiked in-house control sample was also included in each extraction series. For each batch of samples, the corresponding blank sample levels were subtracted from the sample levels. The limit of quantification (LOD) is derived from the lowest standard level injected giving a S/N of at least 6. The limit of quantification (LOQ) for the analysed serum samples was determined as six times the standard deviation of the blanks or the lowest calibration level, the higher of the two was selected. The LOQ varied between 0.625 and 16 ng/kg fresh weight, see Table 3. The method is still under validation so no measurement uncertainty has been determined yet but the relative standard deviation is below

20%. Both LOD and LOQ are going to be revised according to a guidance document on the estimation of LOD and LOQ for measurements in the field of contaminants in food given by the European Union reference laboratory (EU 2016).

Table 3. Limit of quantification (LOQ) and limit of detection (LOD) in ng/kg fresh weight and in ng/g lipid weight assuming a lipid content in 4 g serum of 0.4 %

Analyte	LOQ f.w.	LOQ l.w.	LOD f.w.	LOD l.w.
BDE-28	1.4	0.35	0.13	0.03
BDE-47	8.0	2.0	0.25	0.06
BDE-66	1.0	0.25	0.13	0.03
BDE-99	6.8	1.7	0.13	0.03
BDE-100	1.4	0.35	0.13	0.03
BDE-153	1.0	0.25	0.13	0,03
BDE-154	1.0	0.25	0.13	0.03
BDE-183	0.63	0.16	0.13	0.03
BDE-209	16	4.0	0.63	0.16
HBCD	2.0	0.5	0.25	0.06
HBB	0.63	0.16	0.13	0.03
PBEB	0.63	0.16	0.13	0.03
BTBPE	6.6	1.7	0.13	0.03
DBDPE	12	3.0	6.0	1.6

Calculations and statistics

The analyses of temporal trends were performed in MINITAB® for Windows 14 using simple linear regression. The serum BFR concentrations were ln-transformed due to log-normal distributions of the data. Concentrations below LOD were replaced with LOD/√2. In a first step, all results were used in the regression analyses. Thereafter, a few outliers (with standardized residuals ≥3) were omitted and the regression analyses were performed again. In order to improve the statistical power of the statistical analyses data, not only data with levels above LOQ but also data above LOD were used, although they are less accurate and with higher uncertainty than concentrations above LOQ. Concentrations were corrected for blank sample concentrations.

Table 4. Concentrations of HBB, PBEB, BTBPE and DBDPE in 49 pooled samples of serum from first-time mothers in Uppsala (pg/g fresh weight). Concentrations ≥LOQ (limit of quantification) in **bold**, and ≥LOD (limit of detection) in ***bold italics***.

Year	HBB		PBEB		BTBPE		DBDPE	
1996	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
1996	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***2.3***	<12	*<6.0*
1996	<0.63	*<0.13*	<0.63	*<0.13*	**7.5**		<12	*<6.0*
1997	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
1997	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.57***	<12	*<6.0*
1997	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
1998	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
1998	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.44***	<12	*<6.0*
1998	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***3.7***	<12	*<6.0*
1999	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***3.5***	<12	*<6.0*
1999	*	*	*	*	*	*	*	*
1999	<0.63	*<0.13*	<0.63	*<0.13*	**8.8**		<12	*<6.0*
2000	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2000	<0.63	*<0.13*	<0.63	***0.14***	<6.6	*<0.13*	<12	*<6.0*
2001	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2002	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***1.1***	<12	*<6.0*
2002	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.50***	<12	*<6.0*
2002	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.95***	<12	*<6.0*
2004	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.87***	<12	*<6.0*
2004	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2004	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.48***	<12	*<6.0*
2006	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2006	*	*	*	*	*	*	*	*
2006	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2007	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2007	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.65***	<12	*<6.0*
2007	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***2.2***	<12	*<6.0*
2008	<0.63	*<0.13*	<0.63	*<0.13*	**8.1**		<12	*<6.0*
2008	<0.63	*<0.13*	<0.63	***0.30***	<6.6	***0.38***	<12	*<6.0*
2008	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.87***	<12	*<6.0*
2009	<0.63	***0.16***	<0.63	*<0.13*	<6.6	***4.2***	<12	*<6.0*
2009	<0.63	*<0.13*	<0.63	*<0.13*	**15.6**		<12	*<6.0*
2009	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.19***	<12	*<6.0*
2010	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*0.31*	<12	*<6.0*
2010	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2010	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***3.2***	<12	*<6.0*
2011	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2011	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2011	<0.63	*<0.13*	<0.63	*<0.13*	**32**		<12	*<6.0*
2012	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***1.0***	<12	*<6.0*
2012	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.27***	<12	*<6.0*
2012	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***0.55***	<12	*<6.0*
2013	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2013	<0.63	*<0.13*	<0.63	*<0.13*	**7.1**		<12	*<6.0*
2013	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2014	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***1.9***	<12	***6.5***
2014	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2014	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*<0.13*	<12	*<6.0*
2015	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	***4.4***	<12	*<6.0*
2015	<0.63	*<0.13*	<0.63	*<0.13*	**7.2**		<12	*<6.0*
2015	<0.63	*<0.13*	<0.63	*<0.13*	**20**		<12	*<6.0*
Median	<0.63	*<0.13*	<0.63	*<0.13*	<6.6	*0.50*	<12	*<6.6*

RESULTS AND DISCUSSION

HBB, PBEB, BTBPE and DBDPE concentrations could not be quantified, with a few exceptions (Table 4). HBB concentrations were low and not detectable (LOD <0.13 pg/g serum) except for one sample (0.16 pg/g serum), suggesting that the current human exposure causes low HBB accumulation in serum. A recent screening study of BFRs in serum, with samples from 15 subjects, did not report HBB concentrations due to high concentrations in the blanks (10 pg/g serum) (Haglund et al. 2016). In our study no blank concentrations of HBB were detected.

All PBEB concentrations were below LOQ (0.63 pg/g serum) (Table 4), and only two samples had concentrations above LOD (0.13 pg/g serum). In the study by Haglund et al. the LOQ was reported as 0.2 pg/g serum, and 50% of the participants had concentrations above LOQ in the range of 0.11-1.4 ng/g serum. No LOD was reported and no information was given why some concentrations below the LOQ were reported (Haglund et al. 2016). In our pooled samples, individual samples with concentrations below LOD most probably caused dilution of individual samples with concentrations higher than LOD, resulting in pooled sample concentrations below LOD. No blank concentrations of PBEB were detected.

Eight pooled samples had quantifiable concentrations of BTBPE (7.1-32 pg/g serum) and 23 samples had detectable concentrations between (0.19-4.4 g/g serum). The median concentration, taking all data into account, was 0.50 pg/g serum. In the screening study (Haglund et al. 2016), BTBPE concentrations in the 15 individual samples were all quantifiable (3.4-779 pg/g fresh weight), i.e. in many cases more than an order of magnitude higher (mean concentration 12 pg/g) than in our study. Comparisons of our data with the data in the screening study are complicated by the use of different analytical methods, sampling of different populations, and the use of pooled vs individual samples. The reasons behind the differences in detected concentrations between the two studies have to be further evaluated. In our study BTBPE was detected in the blanks with a mean concentration of 0.96 pg/g serum, resulting in a higher LOQ compared to HBB and PBEB. Blank concentrations are always a complicated issue when low concentration samples are analysed.

Concentrations of DBDPE were all below LOQ (12 pg/g serum), and only one sample had a concentration above LOD (6 pg/g serum). As a comparison the screening study (Haglund et al. 2016) reported all concentrations below a LOQ of 20 pg/g serum. DBDPE were detected in our blanks with mean concentrations of 2.6 pg/g serum.

Table 5.Concentrations of PBDE and HBCD in 51 pooled samples of serum from first-time mothers in Uppsala (ng/g lipid weight). Concentrations ≥LOQ (limit of quantification) in **bold**, and ≥LOD (limit of detection) in ***bold italics***.

Year	BDE-47		BDE-99		BDE-100		BDE-153	BDE-209		HBCD	
1996	<2.2	***1.4***	<0.90	***0.67***	<0.49	***0.45***	**0.73**	**1.5**		<0.51	***0.49***
1996	<2.0	***1.1***	<0.80	***0.40***	<0.44	***0.24***	**0.53**	**1.4**		**0.47**	
1996	**6**		**1.6**		**1.8**		**1.85**	**1.2**		**0.69**	
1997	<2.0	***1.2***	<0.81	***0.43***	<0.44	***0.35***	**0.70**	**0.85**		<0.46	***0.11***
1997	<2.0	***1.9***	1.1		**0.56**		**0.74**	**0.57**		<0.46	***0.24***
1997	<2.0	***1.0***	<0.80	***0.45***	<0.44	***0.31***	**0.62**	**0.64**		<0.45	***0.24***
1998	<2.1	***1.8***	**0.92**		**0.56**		**0.75**	**0.85**		**0.50**	
1998	<1.9	***1.4***	<0.79	***0.41***	<0.43	***0.41***	**0.75**	**0.71**		<0.45	***0.14***
1998	<1.9	***0.79***	<0.79	***0.38***	<0.43	***0.30***	**0.75**	**2.4**		<0.45	***0.20***
1999	<2.2	***1.3***	<0.90	***0.39***	<0.49	***0.43***	**0.73**	**1.4**		<0.51	***0.49***
1999	<1.8	***1.1***	<0.76	***0.31***	<0.41	***0.40***	**0.59**	**4.0**		<0.43	***0.38***
1999	<2.3	***1.1***	<0.94	***0.34***	<0.51	***0.36***	**0.85**	**1.3**		<0.53	***0.43***
2000	<2.1	***0.44***	<0.88	***0.88***	<0.48	***0.38***	**0.62**	**2.5**		<0.50	***0.30***
2000	<2.1	***0.31***	**0.98**		**0.60**		**0.48**	**2.5**		<0.48	***0.35***
2001	<2.0	***0.07***	**0.93**		<0.44	*<0.03*	**0.60**	**1.1**		<0.45	***0.29***
2002	<1.8	***0.69***	**0.93**		<0.41	***0.28***	**0.74**	**1.7**		<0.43	***0.40***
2002	<2.0	***0.28***	<0.83	***0.72***	**0.45**		**0.81**	**1.7**		<0.47	***0.36***
2002	<2.2	***0.56***	**0.98**		**0.54**		**1.1**	**1.4**		<0.52	***0.48***
2004	<2.2	***0.63***	<0.90	***0.14***	**1.4**		**1.6**	**0.80**		**0.78**	
2004	<2.2	***1.1***	<0.90	***0.31***	**0.98**		**1.0**	**0.53**		<0.51	***0.45***
2004	<2.3	*<0.06*	<0.94	***0.34***	<0.51	***0.49***	**0.74**	**0.74**		<0.53	***0.38***
2006	<2.1	***0.42***	<0.88	***0.06***	<0.48	***0.26***	**0.86**	**0.74**		<0.50	***0.38***
2006	<2.0	***0.25***	<0.83	*<0.03*	<0.45	***0.15***	**1.1**	**0.83**		<0.47	***0.40***
2006	<2.0	***0.17***	<0.83	***0.08***	<0.45	***0.19***	**0.92**	**1.1**		<0.47	***0.38***
2007	<1.9	***1.0***	<0.77	***0.51***	<0.42	***0.35***	**0.84**	**1.1**		<0.44	***0.23***
2007	**3.3**		**1.3**		**0.80**		**0.85**	**1.3**		<0.46	***0.22***
2007	<1.8	***0.60***	<0.73	***0.20***	<0.40	***0.27***	**0.85**	**1.3**		<0.42	***0.30***
2008	<2.1	***1.7***	<0.88	***0.42***	**0.54**		**1.8**	**0.86**		<0.50	***0.14***
2008	<1.9	***0.66***	<0.79	***0.21***	<0.43	***0.25***	**0.88**	**0.88**		<0.45	***0.32***
2008	<2.1	***0.87***	<0.85	***0.35***	<0.46	***0.29***	**0.88**	**0.98**		<0.48	***0.27***
2009	<2.0	***0.76***	<0.81	***0.17***	<0.44	***0.22***	**0.56**	**1.5**		<0.46	***0.13***
2009	<2.0	***0.54***	<0.81	***0.22***	<0.44	***0.20***	**0.80**	**1.5**		<0.46	***0.13***
2009	<2.0	***0.57***	<0.83	***0.04***	<0.45	***0.26***	**0.83**	**0.96**		<0.47	***0.17***
2010	<1.9	***0.26***	<0.77	*<0.03*	<0.42	***0.14***	**0.77**	**1.1**		<0.44	*<0.06*
2010	<2.1	***0.24***	<0.88	*<0.03*	<0.48	***0.08***	**0.58**	**0.88**		<0.50	***0.06***
2010	<2.1	***0.49***	<0.86	***0.18***	<0.47	***0.27***	**1.0**	**0.84**		<0.49	*<0.06*
2011	<1.1	***1.1***	<1.7	***0.26***	<0.38	***0.30***	**1.0**	**0.72**		**0.49**	
2011	<1.2	*<0.06*	<2.0	*<0.03*	<0.43	*<0.03*	**0.71**	**2.0**		**0.61**	
2011	<1.1	***0.76***	<1.8	***0.66***	<0.38	*<0.03*	**0.86**	**1.4**		**0.56**	
2012	<1.2	***1.0***	<1.9	***0.26***	<0.41	***0.21***	**0.72**	**<0.65**	***0.50***	**0.51**	
2012	<1.2	***0.89***	<1.9	***0.07***	**0.48**		**1.7**	**0.87**		<0.48	***0.24***
2012	<1.2	***0.19***	<1.9	*<0.03*	<0.42	***0.21***	**0.74**	**1.3**		**2.6**	
2013	<1.2	***0.29***	<1.8	***0.06***	<0.40	***0.14***	**1.3**	**<0.62**	***0.51***	<0.47	***0.29***
2013	<1.3	***0.17***	<2.0	*<0.03*	<0.44	***0.09***	**0.96**	**2.8**		<0.52	***0.10***
2013	**2.1**		**1.9**		**0.55**		**0.90**	**6.2**		**0.87**	
2014	<1.3	***0.14***	<2.1	*<0.03*	<0.46	***0.08***	**0.75**	**1.7**		<0.54	***0.32***
2014	<1.1	***0.23***	<1.7	***0.10***	<0.37	***0.24***	**0.96**	**1.0**		**0.79**	
2014	<1.0	***0.21***	<1.6	*<0.03*	<0.34	***0.06***	**0.75**	**0.72**		<0.41	***0.10***
2015	<1.8	***0,70***	<1.5	***0.38***	<0.31	***0.24***	**0.95**	<3.6	***0.39***	<0.44	*<0.06*
2015	<2.2	***0.68***	<1.9	***0.78***	<0.39	***0.21***	**1.3**	<4.4	***1.8***	<0.56	***0.09***
2015	<2.0	***1.4***	<1.7	***1.05***	<0.34	***0.31***	**1.24**	<3.9	***1.4***	<0.49	*<0.06*
Median		0.69		0.34		0.29	0.83		1.1		0.32

[a]For LOQ and LOD see Table 3.

BDE-209 in most cases had concentrations higher than LOQ, with a median concentration of 1.1 ng/g lipid. This is in line with the screening study of BFRs in serum, with samples from adult 15 subjects, reporting a mean of 1.8 ng/g lipid (assuming 0.4% serum lipids) (Haglund et al. 2016). Concentrations of BDE-47, BDE-99, BDE-100, and HBCD in the serum pools from the Uppsala women were in most cases below LOQ, whereas BDE-153 had concentrations >LOQ. Taking concentrations >LOD into account BDE-153 showed the highest median concentration (0.83 ng/g lipid), followed by BDE-47 (0.69 ng/g lipid), BDE-99 (0.34 ng/g lipid) and BDE-100 (0.29 ng/g lipid). Concentrations of BDE-28, BDE-66, BDE-154 and BDE-183 were below LOQ in all serum pools from 1996-2015 (results not shown).

Table 6. Annual change in levels of PBDE and HBCD in pooled blood serum 1996–2015.

	N	**Change per year (%)**		**R^2 (%)**	***P***
		Mean	SE		
BDE-47	51	-5.6	2.4	8.1	0.025
BDE-47[a]	48	-5.8	1.7	18	0.002
BDE-99	51	-11	2.9	22	<0.001
BDE-100	51	-6.1	1.6	22	<0.001
BDE-153	51	1.5	0.66	8.1	0.024
BDE-153[a]	50	2.1	0.60	18	0.001
BDE-209	51	-0.59	1.2	0	0.63
BDE-209[a]	50	-1.3	1.1	0.6	0.26
HBCD	51	-4.1	2.0	5.6	0.051

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

Temporal trends

Log-linear regression analyses showed no statistically significant temporal trend of BDE-209 during the period 1996-2015 (Table 6, Fig. 1), showing that exposures of young women in Uppsala has not changed enough to be detected using our study design.

Temporal trends of HBB, PBEB and DBDPE concentrations could not be analysed due to non-detectable concentrations. BTBPE had concentrations higher than LOD in more than 50% of the samples. Nevertheless, the distribution of concentrations was very skewed due to the many data below LOD, and log-linear regression analysis could not be performed. In an alternative trend analysis, we divided the concentration data in 4 groups spanning 5 years each, 1996-2000, 2001-2005, 2006-2010 and 2011-2015. No significant differences in median concentrations were observed (Kruskal-Wallis test, p= 0.95), suggesting that exposures have not changed enough to be detected (Fig. 1).

Log-linear regression analyses showed that the concentrations of BDE-47, BDE-99 and BDE-100 decreased significantly in serum during the study period (Table 6). The mean decrease was estimated to 6-11% per year, which is similar to the estimated decrease in mother´s milk 1996-2014 (5-12% per year) (Lignell et al. 2015a). In mother´s milk non-linear temporal trends were observed, with a faster decline the last decade (Glynn et al. 2016). Concentrations of BDE-153 increased with about 2% per year in serum from the POPUP mothers (Table 6). No statistically significant linear temporal trend could be detected in mother´s milk 1996-2014. However a nonlinear trend was evident in mother´s milk, with increasing concentrations the first decade of the study and decreasing concentrations the last decade (Glynn et al. 2016). For HBCD, the decreasing trend of 4% per year between 1996 and 2015 was almost statistically significant (Table 6). In mother´s milk a declining trend has been observed since year 2000 (Glynn et al. 2016).

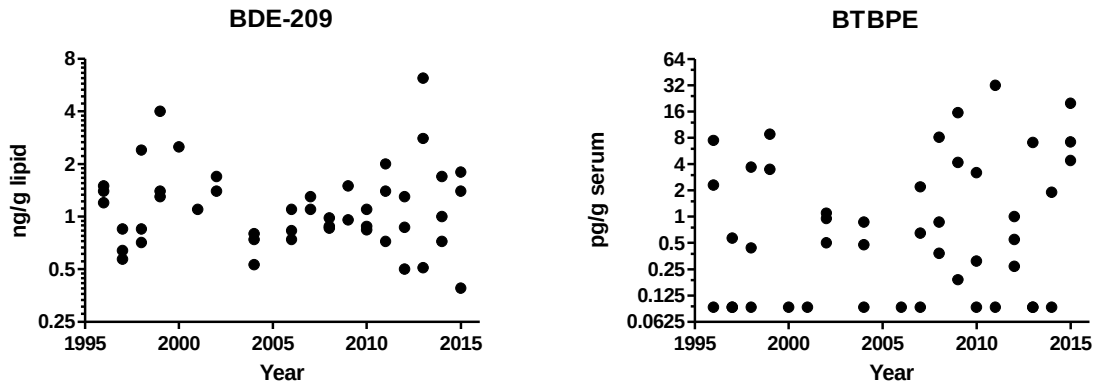

Figure 1. Concentrations of BDE-209 and BTBPE in pooled samples (N=49-51) of blood serum from first-time mothers in Uppsala sampled between 1996 and 2015**.** Log-linear regression analyses showed no significant trend for BDE-209. For BTBPE regression analysis could not be performed since data was not normally distributed even after logarithmic transformation.

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