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Concentrations of brominated flame retardants (HBB, PBEB, BTBPE, DBDPE, PBDEs and HBCD) in blood serum from first-time mothers in Uppsala 1996-2017

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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 2016-2017. Dessutom har halterna av fyra nya BFR analyserats i proverna 2016-2017, det vill säga hexabrombensen (HBB), pentabrometylbensen (PBEB), 1,2-bis(2,4,6-tribromfenoxy)etan (BTBPE) and dekabromdifenyletan (DBDPE). De nya data används också för att uppdatera tidstrender för samtliga BFR för åren 1996-2017.

Halterna av HBB, PBEB, BTBPE och DBDPE i de årliga samlingsproverna låg alla under detektionsgränsen (LOD). För HBB, BTBPE och PBEB låg LOD på 0,15 ng/g fett och för DBDPE på 3,1 ng/g fett.

För BTBPE hade 8 årspooler halter över LOQ (1,2-6,5 ng/g fett), och 23 årspooler detekterbara halter (0,04-0,98 ng/g fett) under tidsperioden 1996-2017. Medianhalten för hela perioden 1996-2017 uppskattades till 0,46 ng/g fett, och halterna tycktes inte förändras under perioden.

Bland PBDE förekom BDE-209 i högst halter under hela studieperioden (median 1,0 ng/g fett), följt av BDE-153 (0,85 ng/g fett), BDE-47 (0,70 ng/g fett), BDE-99 (0,40 ng/g fett), HBCD (0,35 ng/g fett) och BDE-100 (0,29 ng/g fett). Den uppdaterade tidstrenden för BDE-209 visar på en statistiskt signifikant minskning av halterna under studieperioden med 2,5 % per år. Koncentrationerna av BDE-153 ökade med 2 % per år. BDE-47, -99 och -100 har sjunkit med 6-8 % per år mellan 1996 och 2017. Även för HBCD detekterades en statistiskt säkerställd tidstrend med 4 % minskning per år.

För år 2015-2017 har också BFR-halter i poolade prover jämförts med halter i de enskilda prover som ingår i poolerna. Resultaten visade att 22 av 90 individer hade halter som var 3 gånger högre än värdet för poolen för en eller flera BFR. Det finns således en risk att individer med hög exponering inte noteras om enbart poolade prover analyseras.

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 (Kemikalieinspektionen, 2018). 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 (Gyllenhammar, *et al.*, 2017) However, some BFRs are less lipid-soluble and 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 1996-2015 concentrations of BDE-209 in serum did not change significantly, suggesting that there has been a more or less constant exposure to this PBDE for almost two decades in young Swedish women (Darnerud, *et al.*, 2015; Gyllenhammar, *et al.*, 2016).

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.

The following report presents results of analysis of BFRs, including four emerging BFRs, in blood serum sampled in 2016 and 2017 (according to agreement 2215-15-001). The new data is used to establish updated temporal trends for the period 1996-2017.

In addition, a comparison of BFR levels in pooled and the individual samples included in the pools, for the years 2015-2017 has been made, in order to investigate whether there are any differences between the two methods due to dilution effects when pooling the samples.

MATERIALS AND METHODS

Recruitment and sampling

In the POPUP study, more than 600 first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2017. 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 BFRs.

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)
2016	30	3	10	30 (24-36)
2017	30	3	10	29 (21-34)

[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 57 pools from 1996-2017 is given in Table 1. The total number of individual samples included in all pools was 622.

Method for determination of PBDEs, HBCD and emerging BFRs in blood serum

Sample preparation

The extraction and clean-up method used and described earlier (Darnerud, *et al.*, 2015; Lignell, *et al.*, 2015a; Lignell, *et al.*, 2011) 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.*, 2015a). 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 method has varied during the studied time but are now determined as LOD plus five times the standard deviation of the blanks (LOD + 5 SD_{blank}). The LOD, LOQ and Measurement uncertainty are listed in Table 3, in brackets previous LOQ are shown.

Table 3. Limit of detection (LOD), limit of quantification (LOQ) and measurement uncertainty (MU) for the analytical method, assuming a lipid content in 4 g serum of 0.4 %. Previous LOQ are in brackets.

Analyte	LOD pg/g f.w.	LOD ng/g l.w.	LOQ pg/g f.w.	LOQ ng/g l.w.	MU %
BDE-28	0.60	0.15	1.1 (1.3-1.4)	0.30 (0.33-0.35)	40
BDE-47	1.3	0.30	6.6 (6.1-10)	1.7 (1.5-2.0)	50
BDE-66	0.60	0.15	0.6 (1.0-1.3)	0.15 (0.25-0.33)	30
BDE-99	0.60	0.15	9.3 (4.4-9.7)	2.3 (1.2-2.4)	60
BDE-100	0.60	0.15	2.2 (1.4-2.4)	0.55 (0.35-0.60)	40
BDE-153	0.60	0.15	2.8 (1.0)	0.70 (0.25)	50
BDE-154	0.60	0.15	2.4 (1.0-1.3)	0.60 (0.25-0.33)	30
BDE-183	0.60	0.15	0.60 (0.3-1.3)	0.15 (0.08-0.33)	30
BDE-209	1.3	0.30	20 (3.3-16)	5.0 (0.83-4.0)	30
HBCD	1.3	0.30	2.5 (2.0-2.5)	0.63 (0.50-0.63)	50
HBB	0.60	0.15	0.6 0	0.15	30
PBEB	0.60	0.15	0.60	0.15	50
BTBPE	0.60	0.15	1.1 (6.6)	0.28	40
DBDPE	12.5	3.1	20 (12)	5.0 (3.0)	50

Calculations and statistics

The analyses of temporal trends were performed in MINITAB 15® Statistical Software for Windows 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 between LOD and LOQ 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 55 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	HBB		PBEB		BTBPE		DBDPE	
	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD
1996	<0.15	<0.03	<0.15	<0.03	<1.6	<0.03	<2.9	<1.4
1996	<0.13	<0.03	<0.13	<0.03	<1.3	***0.46***	<2.4	<1.2
1996	<0.18	<0.04	<0.18	<0.04	**2.1**		<3.4	<1.7
1997	<0.12	<0.02	<0.12	<0.02	<1.2	<0.02	<2.2	<1.1
1997	<0.14	<0.03	<0.14	<0.03	<1.5	***0.13***	<2.7	<1.4
1997	<0.13	<0.03	<0.13	<0.03	<1.3	<0.03	<2.4	<1.2
1998	<0.14	<0.03	<0.14	<0.03	<1.4	<0.03	<2.6	<1.3
1998	<0.12	<0.02	<0.12	<0.02	<1.2	***0.08***	<2.3	<1.1
1998	<0.13	<0.03	<0.13	<0.03	<1.4	***0.77***	<2.5	<1.2
1999	<0.14	<0.03	<0.14	<0.03	<1.5	***0.80***	<2.7	<1.4
1999	<0.15	<0.03	<0.15	<0.03	**2.1**		<2.9	<1.4
2000	<0.12	<0.03	<0.12	<0.03	<1.3	<0.03	<2.3	<1.2
2000	<0.13	<0.03	<0.13	***0.03***	<1.4	<0.03	<2.5	<1.3
2001	<0.13	<0.03	<0.13	<0.03	<1.4	<0.03	<2.5	<1.2
2002	<0.12	<0.03	<0.12	<0.03	<1.3	***0.21***	<2.3	<1.1
2002	<0.12	<0.02	<0.12	<0.02	<1.2	***0.09***	<2.3	<1.1
2002	<0.14	<0.03	<0.14	<0.03	<1.5	***0.22***	<2.7	<1.4
2004	<0.15	<0.03	<0.15	<0.03	<1.6	***0.21***	<2.9	<1.4
2004	<0.14	<0.03	<0.14	<0.03	<1.5	<0.03	<2.7	<1.4
2004	<0.17	<0.04	<0.17	<0.04	<1.8	***0.13***	<3.2	<1.6
2006	<0.15	<0.03	<0.15	<0.03	<1.6	<0.03	<2.9	<1.4
2006	<0.15	<0.03	<0.15	<0.03	<1.6	<0.03	<2.9	<1.5
2007	<0.14	<0.03	<0.14	<0.03	<1.5	<0.03	<2.7	<1.4
2007	<0.17	<0.04	<0.17	<0.04	<1.8	***0.18***	<3.2	<1.6
2007	<0.13	<0.03	<0.13	<0.03	<1.4	***0.46***	<2.5	<1.2
2008	<0.09	<0.02	<0.09	<0.02	**1.2**		<1.7	<0.87
2008	<0.12	<0.03	<0.12	***0.06***	<1.3	***0.07***	<2.3	<1.2
2008	<0.14	<0.03	<0.14	<0.03	<1.5	***0.19***	<2.7	<1.3
2009	<0.14	***0.04***	<0.14	<0.03	<1.5	***0.93***	<2.7	<1.3
2009	<0.15	<0.03	<0.15	<0.03	**3.6**		<2.8	<1.4
2009	<0.14	<0.03	<0.14	<0.03	<1.5	***0.04***	<2.7	<1.4
2010	<0.12	<0.03	<0.12	<0.03	<1.3	***0.06***	<2.3	<1.2
2010	<0.14	<0.03	<0.14	<0.03	<1.4	<0.03	<2.6	<1.3
2010	<0.14	<0.03	<0.14	<0.03	<1.5	***0.71***	<2.7	<1.3
2011	<0.13	<0.03	<0.13	<0.03	<1.3	<0.03	<2.4	<1.2
2011	<0.13	<0.03	<0.13	<0.03	<1.4	<0.03	<2.5	<1.3
2011	<0.13	<0.03	<0.13	<0.03	**6.5**		<2.4	<1.2
2012	<0.12	<0.03	<0.12	<0.03	<1.3	***0.19***	<2.3	<1.1
2012	<0.14	<0.03	<0.14	<0.03	<1.4	***0.06***	<2.6	<1.3
2012	<0.12	<0.03	<0.12	<0.03	<1.3	***0.11***	<2.3	<1.1
2013	<0.13	<0.03	<0.13	<0.03	<1.3	<0.03	<2.4	<1.2
2013	<0.13	<0.03	<0.13	<0.03	**1.5**		<2.5	<1.3
2013	<0.12	<0.03	<0.12	<0.03	<1.3	<0.03	<2.3	<1.1
2014	<0.15	<0.03	<0.15	<0.03	<1.6	***0.46***	<2.9	***1.6***
2014	<0.13	<0.03	<0.13	<0.03	<1.3	<0.03	<2.4	<1.2
2014	<0.11	<0.02	<0.11	<0.02	<1.2	<0.02	<2.1	<1.1
2015	<0.14	<0.03	<0.14	<0.03	<1.5	***0.98***	<2.7	<1.3
2015	<0.18	<0.04	<0.18	<0.04	**2.0**		<3.3	<1.7
2015	<0.15	<0.03	<0.15	<0.03	**4.9**		<2.9	<1.5
2016	<0.12	<0.12	<0.12	<0.12	<0.22	<0.12	<4.0	<2.5
2016	<0.13	<0.13	<0.13	<0.13	<0.23	<0.13	<4.3	<2.7
2016	<0.13	<0.13	<0.13	<0.13	<0.23	<0.13	<4.3	<2.7
2017	<0.11	<0.11	<0.11	<0.11	<0.20	<0.11	<3.6	<2.3
2017	<0.12	<0.12	<0.12	<0.12	<0.21	<0.12	<3.8	<2.4
2017	<0.13	<0.13	<0.13	<0.13	<0.23	<0.13	<4.2	<2.6
Median	<0.13	<0.03	<0.13	<0.03	<1.40	0.46	<2.67	<1.33

RESULTS AND DISCUSSION

HBB, PBEB, BTBPE, and DBDPE concentrations could only be quantified in a few samples (Table 4). HBB concentrations were low and not detectable 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 (Table 4), and only two samples had concentrations above LOD. 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 0.48 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 fresh weight) 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 corresponding mean concentration of 0.17 pg/g, resulting in a slightly 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, and only one sample had a concentration above LOD. As a comparison the screening study (Haglund, *et al.*, 2016) reported all concentrations below a LOQ of 20 pg/g serum. DBDPE was detected in our blanks with mean concentrations of 0.35 pg/g.

Table 5.Concentrations of PBDE and HBCD in 57 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	
	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOQ	LOD	LOQ	LOD
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	**6.0**		**1.6**		**1.8**		**1.9**	**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	***1.1***	<0.34	***0.31***	**1.2**	<3.9	***1.4***	<0.49	*<0.06*
2016	<1.3	*<0.26*	<1.9	***0.17***	<0.44	*<0.12*	**0.86**	<4.0	***0.65***	<0.50	*<0.26*
2016	<1.4	***0.62***	<2.0	***0.53***	<0.47	***0.25***	**0.89**	<4.3	***0.78***	<0.53	*<0.26*
2016	<1.4	*<0.26*	<2.0	***0.86***	<0.47	***0.25***	**1.2**	<4.3	***0.34***	<0.53	*<0.26*
2017	<1.2	*<0.26*	<1.7	***0.74***	<0.40	***0.31***	**0.81**	<3.6	***0.56***	<0.45	*<0.26*
2017	<1.3	*<0.26*	<1.8	*<0.12*	<0.42	*<0.12*	**1.3**	<3.8	***0.55***	<0.48	*<0.26*
2017	<1.4	*<0.26*	<1.9	*<0.12*	<0.46	*<0.12*	**1.0**	<4.2	***0.67***	<0.52	*<0.26*
Median		0.70		0.40		0.29	0.85		1.0		0.35

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 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-209 showed the highest median concentration (1.0 ng/g lipid), followed by BDE-153 (0.85 ng/g lipid), BDE-47 (0.70 ng/g lipid), BDE-99 (0.40 ng/g lipid), HBCD (0.35 ng/g lipid), and BDE-100 (0.29 ng/g lipid). Concentrations of BDE-28, BDE-154 and BDE-183 were all below LOQ in all serum pools from 1996-2017; BDE-66 had one sample above LOQ (year 2017, 0.12 pg/g lipid) out of 57 samples (results not shown).

Table 6. Annual change in levels of PBDE and HBCD in pooled blood serum 1996–2017.

	N	**Change per year (%)[a]**		**R^2 (%)**	**P**
		Mean	95 % CI		
BDE-47	57	-6.1	-2.6/-9.5	17.3	0.001
BDE-99	57	-7.6	-3.1/-12.0	16.0	0.002
BDE-100	57	-6.6	-3.5/-9.6	23.4	<0.001
BDE-100[b]	56	-7.3	-4.5/-10.0	31.9	<0.001
BDE-153	57	1.5	0.4/2.6	11.6	0.01
BDE-153[b]	53	1.9	1.1/2.7	31.4	<0.001
BDE-209	57	-2.0	-0.1/4.1	6.1	0.065
BDE-209[b]	56	-2.5	0.6/4.3	10.9	0.013
HBCD	57	-3.6	-0.5/-6.5	8.6	0.027
HBCD[b]	56	-4.0	-1.3/-6.7	13.0	0.006

[a] Percent change (decrease (-) or increase (+)) of the concentration per year.
[b] A few (1-4) outliers with high standardized residuals (≥3) were omitted in the regression analysis.

Temporal trends

Log-linear regression analyses showed a statistically significant decrease in the temporal trend of BDE-209 during the period 1996-2017 (Table 6 and figure 1). This is the first time that a temporal trend was significant for serum BDE-209 in the POPUP women, indicating that exposures of young women in Uppsala has decreased enough to be detected using our study design.

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 and figure 1). The mean decrease was estimated to 6-8 % per year, which is quite similar to the estimated decrease in mother´s milk 1996-2016 (5-12% per year) (Gyllenhammar, *et al.*, 2017; Lignell, *et al.*, 2015b). In mother´s milk non-linear temporal trends were observed, with a faster decline the last decade (Glynn, *et al.*, 2016; Gyllenhammar, *et al.*, 2017). These results are also in

agreement with findings in the Swedish Market Basket Survey, where the calculated per capita intake of BDE-47 and BDE-99 decreased during the years 2000 to 2015 with approximately 10 % per year (Glynn, *et al.*, 2017).

Concentrations of BDE-153 increased with about 2% per year in serum from the POPUP mothers (Table 6 and figure 1). 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; Gyllenhammar, *et al.*, 2017). A statistically significant decrease of BDE-153 was also seen in the Swedish Market Basket Survey when comparing the calculated per capita intake for the year 2010 with the year 2015 (Glynn, *et al.*, 2017).

For HBCD, the decreasing trend of 4% per year between 1996 and 2017 was statistically significant (Table 6 and figure 1). In mother´s milk a declining trend has been observed since year 2000 (Glynn, *et al.*, 2016; Gyllenhammar, *et al.*, 2017) and a decrease was evident in the Swedish Market Basket Survey when comparing the calculated per capita intake for the year 2010 with the year 2015 (Glynn, *et al.*, 2017).

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 (figure 2). Nevertheless, the distribution of concentrations was skewed due to the many data below LOD, and log-linear regression analysis could not be performed.

For the year 2015 – 2017 we have also done a comparison of pooled and the individual samples included in the pooled samples for all analytes, in order to examine if there are some discrepancies between the two methods. E.g., one or more individual samples with detectable levels of BFR that will be missed in the pooled samples due to dilution effects (Table 7, 8, and 9).

For HBB, PBEB and DBDPE all analyses were under LOD in both the pooled and individual samples (not shown).

BDE-28 and BDE-183 were also under LOD except for one individual sample of each BFR during 2017, however not from the same individual, and the levels of the two BFRs were close to the LOD for the pooled samples (not shown).

Regarding the other BFRs analysed, all but a few were quite similar when comparing the mean value of the individual samples with the pooled samples (table 7-9). It is also evident that there was dilution effects, as the maximum value in the individual samples quite often was 2-3 times higher than the reported levels in the pooled samples, in some cases as much as 7 to 11 times higher (BDE-209, table 9 and BDE-47, table 8, respectively). When comparing the

individual samples with the pooled sample that includes the individual (recalculating <LOD to LOD/√2 for the pooled sample) and with an arbitrary cut-off set to 3 times or higher the pooled analysis levels for “high exposed” individuals, a total of 22 individuals out of 90 can be classified as such (not shown). Thus, there is a risk that one might miss individuals with a high exposure using only pooled samples.

There is also the risk that, when pooling individual samples into a pooled sample, it is difficult to avoid the influence of extreme values in individual samples. It has previously been concluded that this might be a problem concerning the BFRs, as some individuals may have levels that are approximately 20 times higher than the median at a specific year's sampling (Glynn, *et al.*, 2016).

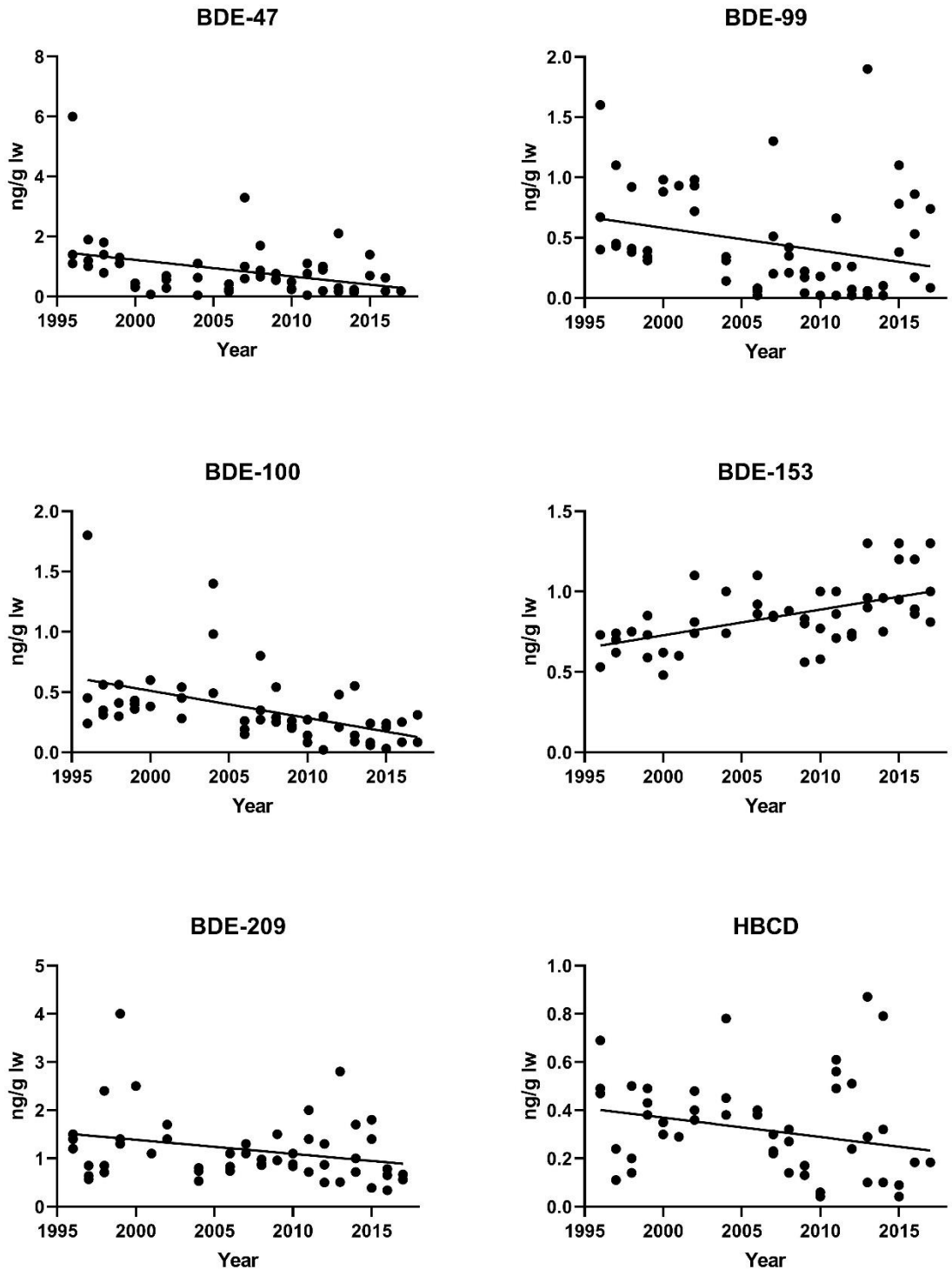

Figure 1. Temporal trends for PBDEs and HBCD in pooled blood serum (ng/g lipid weight) during the time period of 1996-2017. Outliers with high standardized residuals (≥3) from the ln-transformed regression analyses were omitted from the plots.

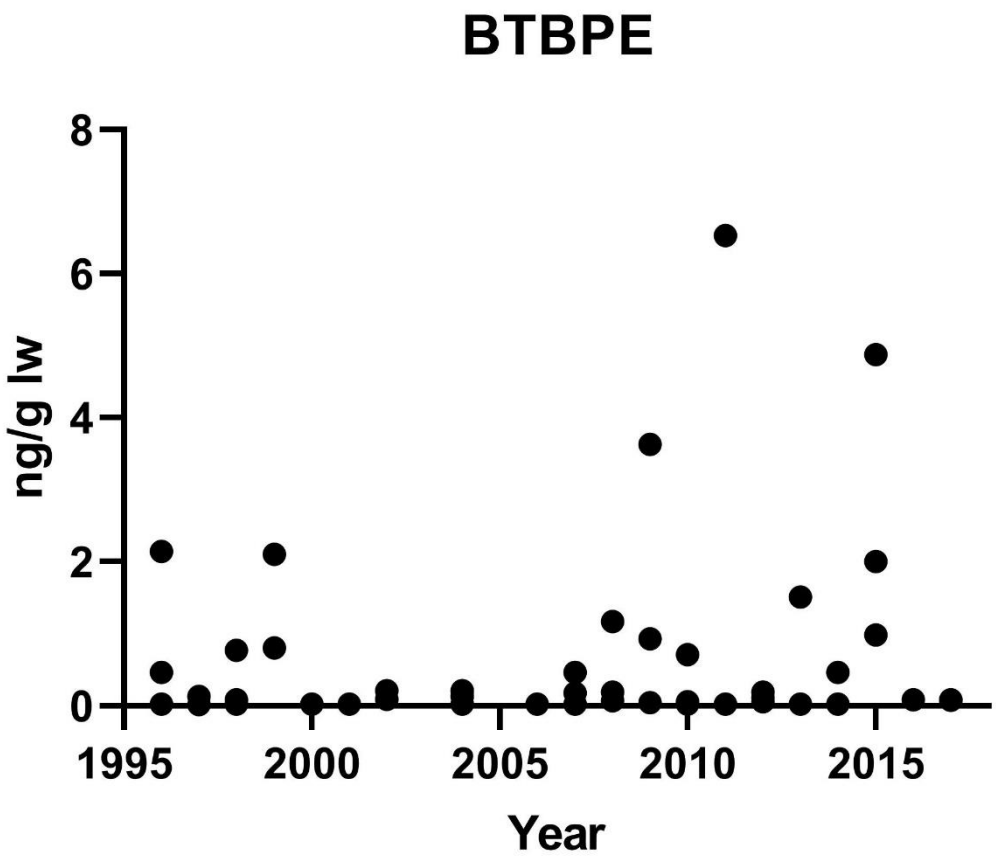

Figure 2. Levels of BTBPE in pooled blood serum (ng/g lipid weight) during the time period of 1996-2017.

Table 7. Comparison of levels of PBDE, HBCD and BTBPE (pg/g lipid weight) in blood serum in pooled and the individual samples included in the pooled samples during 2015 from first-time mothers in Uppsala. Mean values for individual data was calculated with samples above LOD only.

Year	Sample		BDE-47	BDE-66	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209	HBCD	BTBPE
2015	Pool 1		700	<130	380	240	950	<130	390	<290	970
	Individual data pool 1	Mean	910	<120	320	390	990	210	1200	<260	<120
		Min[a]	380		260	200	290	180	520		
		Max	1640		380	520	2680	250	2290		
		N>LOD	5	0	3	3	10	3	10	0	0
	Pool 2		680	<170	780	210	1300	240	1790	<360	1990
	Individual data pool 2	Mean	960	<120	380	240	1080	270	1240	<260	<120
		Min	290		260	130	500	160	500		
		Max	2050		510	400	1960	600	2340		
		N>LOD	4	0	5	5	10	6	10	0	0
	Pool 3		1390	<150	1050	310	1240	210	1360	<320	4770
	Individual data pool 3	Mean	910	<120	930	390	1080	180	1480	230	<120
		Min	260		280	150	630	130	610	210	
		Max	1180		1650	800	2030	240	3940	240	
		N>LOD	6	0	6	6	10	5	10	2	0

[a] The lowest quantified level of the analyte over LOD.

Table 8. Comparison of levels of PBDE, HBCD and BTBPE (pg/g lipid weight) in blood serum in pooled and the individual samples included in the pooled samples during 2016 from first-time mothers in Uppsala. Mean values for individual data was calculated with samples above LOD only.

Year	Sample		BDE-47	BDE-66	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209	HBCD	BTBPE
2016	Pool 1		<260	<120	170	<120	860	<120	650	<260	<120
	Individual data pool 1	Mean	950	<120	620	250	750	140	820	200	200
		Min[a]	310		440	150	410	130	310	200	110
		Max	1310		840	410	1500	150	2220	200	290
		N>LOD	3	0	3	3	10	2	8	1	2
	Pool 2		620	<120	530	250	890	220	780	<260	<120
	Individual data pool 2	Mean	2660	<120	980	450	970	410	660	590	260
		Min	850		410	340	440	190	300	590	190
		Max	7020		1910	760	1870	740	1370	590	340
		N>LOD	4	0	5	4	10	3	10	1	4
	Pool 3		<260	<120	860	250	1170	220	340	<260	<120
	Individual data pool 3	Mean	490	<120	210	<120	810	190	660	<260	<120
		Min	390		160		360	140	400		
		Max	600		290		1460	230	1000		
		N>LOD	2	0	4	0	10	2	9	0	0

[a] The lowest quantified level of the analyte over LOD.

Table 9. Comparison of levels of PBDE, HBCD and BTBPE (pg/g lipid weight) in blood serum in pooled and the individual samples included in the pooled samples during 2017 from first-time mothers in Uppsala. Mean values for individual data was calculated with samples above LOD only.

Year	Sample		BDE-47	BDE-66	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209	HBCD	BTBPE
2017	Pool 1		<260	<120	740	310	810	180	560	<260	<120
	Individual data pool 1	Mean	500	<120	300	200	780	190	870	320	<120
		Min[a]	290		300	170	300	140	240	260	
		Max	740		300	240	1460	270	1950	370	
		N>LOD	4	0	1	4	10	3	10	3	0
	Pool 2		<260	<120	<120	<120	1250	140	550	<260	<120
	Individual data pool 2	Mean	1990	<120	2760	8700	1120	200	1520	310	<120
		Min	370		280	230	500	140	210	250	
		Max	5200		5240	1520	2130	290	3970	400	
		N>LOD	3	0	2	2	10	5	10	4	0
	Pool 3		<260	<120	<120	<120	1010	130	670	<260	<120
	Individual data pool 3	Mean	2150	<120	380	250	760	190	1080	<260	<120
		Min	860		190	180	300	110	530		
		Max	3440		640	310	1610	260	1790		
		N>LOD	2	0	3	2	9	3	9	0	0

[a] The lowest quantified level of the analyte over LOD.

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