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Concentrations of brominated flame retardants (PBDEs, HBCDD, HBB, PBEB, BTBPE, and DBDPE) in blood serum from first-time mothers in Uppsala, Sweden: results from 2022-2023, and temporal trends for the time period 1996-2023

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Rapporttitel
Concentrations of brominated flame retardants (PBDEs, HBCDD HBB, PBEB, BTBPE, and DBDPE) in blood serum from first-time mothers in Uppsala, Sweden: results from 2022-2023, and temporal trends for the time period 1996-2023

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Tidpunkt för insamling av underlagsdata
1996-2023

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) polybromerade difenyletrar (PBDE), hexabromcyklododekan (HBCDD) samt hexabrombensen (HBB), pentabrometylbensen (PBEB) och 1,2-bis(2,4,6-tribromfenoxy)etan (BTBPE) i samlingsprover av serum (3 samlingsprover per provtagningsår) insamlade 2022-2023. Dessutom har tidstrenden för åren 1996-2023 uppdaterats.

Samtliga halter för BDE 153 låg över kvantifieringsgränsen (LOQ) och medelhalten (±SD) för perioden 2022-2023 var 0.8 (±0.2) ng/g fett. För BDE 47, BDE 209 och BTBPE var inga pooler >LOQ men en, sex respektive två pooler låg över detektionsgränsen (LOD) och hade medelkoncentrationerna 0.3 (±0.4), 0.6 (±0.08) och 0.1 (±0.03) ng/g fett. Halterna av BDE 28, BDE 66, BDE 99, BDE 100, BDE 154, BDE 183, HBCDD, HBB och PBEB låg under LOD i samtliga prover från 2022-2023. För hela studieperioden, 1996-2023, hade BDE 209 den högsta medianhalten (0.87 ng/g fett) följt av BDE 153 (0.85 ng/g fett) och BDE 47 (0.69 ng/g fett).

Den uppdaterade tidstrenden för åren 1996-2023 visade en fortsatt minskning med 3% per år för BDE 209. Halterna av BDE 47, BDE 99 och BDE 100 har sjunkit med ca 7-10 % per år under studieperioden. Även halterna av HBCDD visade på en signifikant minskande tidstrend med ca 5 % per år. Den uppdaterade tidstrenden för BDE 153 visade att den tidigare svaga ökning som setts nu inte är signifikant. Fortsatta mätningar är viktiga för att följa tidstrenderna av BFR och för att utreda om BDE 153 i serum nu håller på att börja minska hos förstföderskor i Uppsala, på liknande sätt som ses i bröstmjölk i POPUP och i Livsmedelsverkets matkorgsundersökningar.

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) 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, 2023). 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 (Hedvall Kallerman, *et al.*, 2024). However, since some BFRs are less lipid-soluble and not easily transferred from the blood of the mother to mother´s milk it is necessary to measure them in blood serum.

The first temporal trend study of PBDEs and HBCDD in pooled blood serum samples from the POPUP-cohort was published in 2011 (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. For the first time, serum samples from the POPUP mothers showed a decrease in concentrations of BDE 209 during the time-period 1996-2017 (Ålander et al., 2019).

There are many emerging BFRs that at least partially have been introduced as substitute chemicals for PBDEs and HBCDDs. 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 so-called emerging BFRs, hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and decabromodiphenylethane (DBDPE) (Due to uncertainty with the DBDPE standard, this analyte could not be analysed.), in blood serum, sampled 2022-2023 (according to agreements 215-21-003 and 215-24-005). The new data are used to establish updated temporal trends for the period 1996-2023 adding two more years of data to the latest report (Hedvall Kallerman, *et al.*, 2023).

MATERIALS AND METHODS

Recruitment and sampling

In the POPUP study, over 1100 first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2023. 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.

In the present study, we used pooled serum samples from the participants for analysis of BFRs. The composition of the 75 pools from 1996-2023 is given in Table 1. The total number of individual samples included in all pools was 802.

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 (21-38)
1998	74	3	24-25	29 (22-36)
1999	17	3	5-6	27 (22-32)
2000	20	2	10	30 (21-37)
2001	9	1	9	29 (23-36)
2002	31	3	10-11	30 (24-37)
2004	32	3	10-11	29 (21-35)
2006	30	3	10	30 (19-40)
2007	29	3	9-10	30 (21-39)
2008	30	3	10	29 (20-36)
2009	30	3	10	29 (22-40)
2010	30	3	10	30 (21-41)
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 (25-37)
2017	30	3	10	29 (22-34)
2018	30	3	10	31 (24-40)
2019	30	3	10	30 (25-38)
2020	30	3	10	31 (26-43)
2021	30	3	10	32 (27-40)
2022	30	3	10	32 (26-38)
2023	30	3	10	32 (24-46)

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

Analytical method for determination of PBDEs, HBCDD and emerging BFRs in blood serum

Sample preparation

The extraction and clean-up method used has been described earlier (Darnerud, *et al.*, 2015; Lignell, *et al.*, 2015; Lignell, *et al.*, 2011).

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 as previously described (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, HBCDD 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 HBCDD. 13C-BDE 209 was used for the quantification of BDE 209 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 HBCDD 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. The calibration curve for DBDPE could not be calculated and hence no concentrations could be determined. The instrumental response for DBDPE in the samples was however under the lowest calibration point indicating that the concentrations were <LOD.

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

Analyte	m/z
BDE 28, 47, 66, 100, 99, 154, 153, 183	79, 81
HBCDD	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 were 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 + $5\times SD_{blank}$). 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[1] %
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
HBCDD	1.3	0.30	2.5 (2.0-2.5)	0.63 (0.50-0.63)	50
HBB	0.60	0.15	0.60	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

[1] The given measurement uncertainty is calculated by a coverage factor 2 for an approximate level of confidence of 95 %).

Calculations and statistics

In order to improve the statistical power of the statistical analyses, reported levels below LOD (corrected for blank sample concentrations) were used, although they are less accurate and with higher uncertainty than concentrations above LOD/LOQ. Linear regressions were used to analyze associations between BFR serum concentrations and sampling year for the whole period, 1996-2023, using the software package STATA version 17.0. In the linear regression analyses, reported concentrations of zero were replaced with the lowest value for each compound during the years 1996-2023. Due to non-normal distributions BFR concentrations were ln-transformed (The Shapiro-wilks test). In a first step, all data were used in the linear regression analyses. Thereafter, a few outliers (with standardized residuals >3) were omitted and the regression analyses were performed again. There were two outliers for BDE 47, and one for BDE 209. As a consequence of the ln-transformation, the associations between sampling year and BFR concentrations are presented as percent change of concentrations per year, and not as change in absolute levels.

RESULTS AND DISCUSSION

Serum concentrations of BFRs in pooled samples from the period 2022-2023 (n=6) are presented in Table 4. BFR concentrations in serum pools from 1996-2021 can be found in previous reports from the POPUP study (Ålander *et al.*, 2019 Hedvall Kallerman *et al.* 2023).

In the present report BDE 153 had all concentrations >LOQ with the mean value (±SD) of 0.83 (±0.25) ng/g lipid. For BDE 47, BDE 209, and BTBPE all concentrations were <LOQ but in one, all six, and two pools respectively, concentrations were >LOD. Mean concentrations (±SD) were 0.3 (±0.4), 0.6 (±0.08), and 0.1 (±0.03) respectively. BDE 154, HBCDD, and PBEB were all <LOD with mean concentrations 0.09 (±0.03), 0.1 (±0.04), and 0.02 (±0.01) respectively for the period 2022-2023 (Table 4).

The concentrations of HBB, BDE 28, BDE 66, BDE 99, BDE 100, and BDE 183, were all <LOD and/or had non-detectable concentrations and are therefore not shown in Table 4.

Taking all concentrations into account during the study period 1996-2023, BDE 209 showed the highest median concentration (0.87 ng/g lipid), followed by BDE 153 (0.85 ng/g lipid), BDE 47 (0.69 ng/g lipid), BDE 99 (0.40 ng/g lipid), BDE 100 (0.27 ng/g lipid), HBCDD (0.24 ng/g lipid) and BTBPE (0.04 ng/g lipid).

Table 4. Concentrations of PBDE, HBCDD, PBEB, and BTBPE in pooled serum samples from first-time mothers in Uppsala (ng/g lipid weight), sampled 2022-2023. Concentrations ≥LOQ (limit of quantification) in **bold**, ≥LOD (limit of detection) in ***bold italics***. Reported concentrations <LOD are presented in *italics.*

Year	**BDE 47**			**BDE 153**	**BDE 154**			**BDE 209**		**HBCDD**			**PBEB**			**BTBPE**		
	LOQ	LOD	<LOD	LOQ	LOQ	LOD	<LOD	LOQ	LOD	LOQ	LOD	<LOD	LOQ	LOD	<LOD	LOQ	LOD	<LOD
2022	<1.2	<0.24	*0.16*	**0.70**	<0,44	<0,11	*0.06*	<3.6	***0.62***	<0.45	<0.24	*0.10*	<0.11	<0.11	*0.02*	<0.20	<0.11	*0.05*
2022	<1.6	<0.32	*0.20*	**0.78**	<0.59	<0.15	*0.13*	<4.9	***0.58***	<0.61	<0.32	*0.21*	<0.15	<0.15	*0.01*	<0.27	<0.15	*0.10*
2022	<1.3	<0.27	*0.18*	**0.95**	<0.49	<0.12	*0.10*	<4.1	***0.71***	<0.51	<0.27	*0.10*	<0.12	<0.12	*0.03*	<0.22	***0.12***	
2023	<1.4	<0.28	*0.20*	**0.69**	<0.52	<0.13	*0.12*	<4.3	***0.53***	<0.54	<0.28	*0.17*	<0.13	<0.13	*0.04*	<0.24	<0.13	*0.11*
2023	<1.1	<0.22	*0.14*	**0.60**	<0.40	<0.10	*0.04*	<3.3	***0.68***	<0.42	<0.22	*0.13*	<0.10	<0.10	*0.01*	<0.18	***0.12***	
2023	<1.3	***1.1***		**1.3**	<0.46	<0.12	*0.07*	<3.8	***0.51***	<0.48	*<0.25*	*0.16*	<0.12	<0.12	*0.03*	<0.21	*<0.12*	*0.07*
Mean (±SD)	0.3 (±0.4)			0.83 (±0.25)	0.09 (±0.03)			0.6 (±0.08)		0.1 (±0.04)			0.02 (±0.01)			0.1 (±0.03)		

Table 5. Annual change in levels of PBDEs and HBCDD in pooled blood serum samples from first-time mothers in Uppsala 1996–2023.

	N	**Change per year (%)**		**R^2 (%)**	**P**
		Mean	95 % CI		
BDE 47	75	-7.0	-10.5/-3.3	15	p<0.001
BDE 47[a]	73	-7.0	-10.5/-3.3	15	p<0.001
BDE 99	75	-9.9	-13.5/-6.1	24	p<0.001
BDE 100	75	-8.4	-10.4/-6.4	45	p<0.001
BDE 153	75	0.7	-0.1/1.4	3	*0.083*
BDE 209	75	-2.9	-4.4/-1.2	13	p=0.001
BDE 209[a]	74	-3.0	-4.4/-1.5	17	p<0.001
HBCDD	75	-5.1	-7.3/-2.9	21	p<0.001

[a] A few (1-2) outliers with high standardized residuals (>3) were omitted in the regression analysis for each compound.

Temporal trends

The annual change in concentrations of PBDEs and HBCDD in pooled serum samples from the time period 1996-2023 is presented in Table 5. The annual decrease in concentrations of BDE 47, BDE 99, BDE 100, BDDE 209, and HBCDD were similar to the previous results from the POPUP study 1996-2021 (Hedvall Kallerman *et al.,* 2023) (Table 5 and Figure 1). The present results confirm a decreased exposure of young women in Uppsala by in mean 7, 10, and 3% per year for BDE 47, BDE 99, and BDE 209, respectively, which is slightly slower than the change in concentrations seen in mother´s milk from the POPUP mothers (Hedvall Kallerman *et al.*, 2024). HBCDD was decreasing around 5% per year in blood serum in the present report which is in line with recent findings in mother´s milk sampled 1996-2022 in the POPUP-study (Hedvall Kallerman, *et al*., 2024). In the present report, BDE 100 was decreasing by around 8% which is slightly faster than observed in mother´s milk from POPUP (Hedvall Kallerman, *et al*., 2024). No temporal trend in serum concentrations of BDE 153 was observed (p=0.083), which contrast with previous reports of a small annual increase of 1-1.5% per year 1996-2017/2021 (Ålander et al. 2019, Hedvall Kallerman et al 2023). This could be due to a recent decline in BDE 153 levels among first-time mothers from Uppsala. A non-significant change is also seen for BDE 153 in mother´s milk 1996-2022 (Hedvall Kallerman, *et al*., 2024). However, when analysing the time-trend in mother´s milk during the last period 2008-2022, a significantly decreasing trend of 2.4% per year was observed (Hedvall Kallerman, *et al*., 2024) which is in line with the temporal trend of per capita intake for BDE 153 presented in the Swedish Market Basket Survey during the years 1999 to 2022 (Livsmedelsverket, 2024).

Our findings of decreased concentrations of BDE 47 and HBCDD were slightly slower whereas BDE 99 and BDE 100 were slightly faster than the change in calculated per capita

intake in the Swedish Market Basket Survey during the years 1999 to 2022 (Livsmedelsverket, 2024).

Temporal trends of BDE 28, BDE 66, HBB, PBEB, and BTBPE concentrations could not be analysed due to too many concentrations <LOD and/or non-detectable concentrations during the time period 1996-2023.

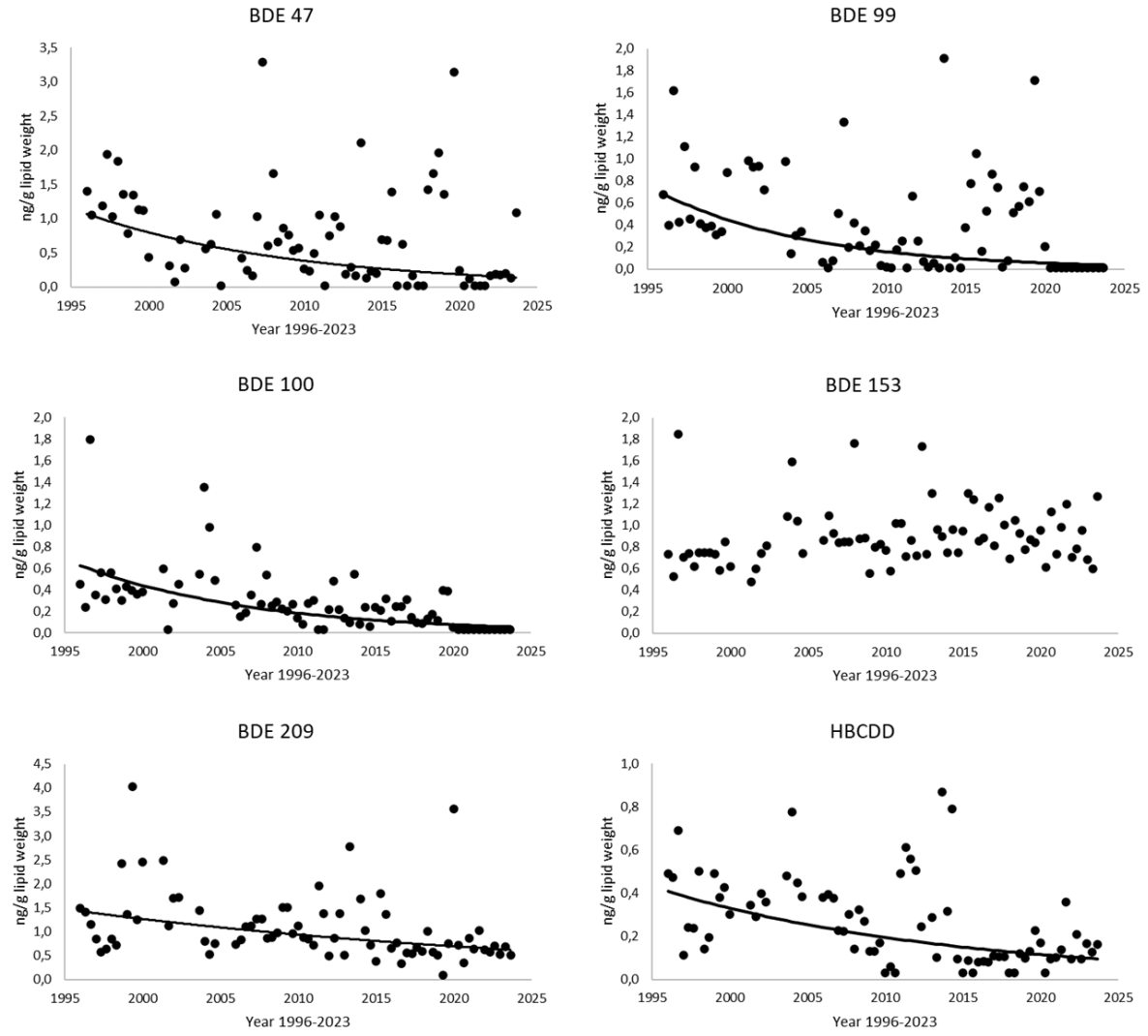

Figure 1. Temporal trends of concentrations for PBDEs, and HBCDD (ng/g lipid weight) in pooled serum samples from the time period 1996-2023. Outliers with standardized residuals >3 were omitted from the plots n=1-2.

CONCLUSIONS

The concentrations of brominated flame retardants analysed in pools from 2022-2023 in first time mothers in Uppsala were generally low. During the total study period 1996-2023, the highest median concentrations were found for BDE 209, followed by BDE 153 and BDE 47. A

decreasing temporal trend of 3-10% were seen during 1996-2023 with the fastest change for BDE 99, BDE 100, and BDE 47. The annual change in concentration of BDE 153 was non-significant in the present report compared with a small increase in the previous reports. Continued measurements are needed to investigate whether BDE 153 in serum is now beginning to decrease among first-time mothers in Uppsala, similar to what has been observed in breast milk in the POPUP study and in the Swedish Market Basket Surveys from the Swedish Food Agency.

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