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Levels of persistent halogenated organic pollutants (POP) in mother's milk from first-time mothers in Uppsala, Sweden: results from year 2014 and temporal trends for the time period 1996-2014

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Sammanfattning
Sedan 1996 har Livsmedelsverket regelbundet samlat in modersmjölk från förstföderskor i Uppsala för analys av persistenta halogenerade organiska miljöföroreningar (POP). I följande rapport redovisas halterna av industrikemikalien PCB (mono-, di- och non-*orto* PCB), oavsiktligt bildade dioxiner och furaner (PCDD/F) samt bromerade flamskyddsmedel (PBDE, HBCD) i 30 modersmjölksprover insamlade 2014. Dessa nya data används också för att uppdatera tidstrenderna för dessa ämnen.

Bland PCB var medelkoncentrationen i modersmjölk (2014) högst för PCB 153 (27 ng/g fett). Medelhalten för PCDD TEQ (2,1 pg/g fett) var högre än för PCDF TEQ (1,7 pg/g fett). Bland de polybromerade difenyletrarna (PBDE) uppvisade BDE-153 (0,55 ng/g fett) och BDE-47 (0,36 ng/g fett) högst medelhalter.

Utvärdering av tidstrender för perioden 1996-2014 (multipel linjär regression) visade att halterna av di-*orto* PCBer, mono-*orto* PCB TEQ och non-*orto* PCB TEQ har minskat med ca 6-7% per år. Halterna av PCDD TEQ har minskat fortare än halterna av PCDF TEQ (7% respektive 4% per år). Dessa resultat stämmer överens med de trender som tidigare observerats för perioden 1996-2012. En uppdelning av studieperioden i två delar visade en tendens till att haltminskningarna för PCB och PCDD/F varit snabbare under perioden 1996-2004 än under perioden 2005-2014. Fortsatta undersökningar av dessa ämnen i modersmjölk kan ge svar på om halterna håller på att stabiliseras på nuvarande nivåer.

Resultaten för PBDEer stämmer överens med det som rapporterats tidigare för perioden 1996-2012, dvs. halterna av BDE-47, -99 och -100 har minskat (5-12% per år). Resultaten antyder att minskningarna varit snabbare 2005-2012 än under 1996-2004. Nivåerna av BDE-153 ökade under 1996-2004 men har därefter minskat med 3 % per år. BDE-209 har bara analyserats i modersmjölk sedan 2009 och hittills kan inte någon tidstrend observeras. Trenden för HBCD är osäker eftersom halterna ligger under analysmetodens kvantifieringsgräns (LOQ) i många prover. En utvärdering av resultat från år då de flesta resultat låg över LOQ (2002-14) visade dock en signifikant haltminskning för HBCD på 5% per år. Fler datapunkter behövs för BDE-209 och HBCD innan några säkra slutsatser kan dras om tidstrender.

En mer omfattande statistisk utvärdering av trenderna av POP i modersmjölk kommer att genomföras och redovisas till Naturvårdsverket under 2016 (avtalsnummer 2215 15 001).. Denna utvärdering kommer att ge svar på om det finns icke-linjära trender i materialet och också ge underlag för hur trendstudien ska genomföras och utvärderas i framtiden.

INTRODUCTION

With funding from the Swedish Environmental Protection Agency (EPA), the Swedish National Food Agency (NFA) has made recurrent measurements of persistent halogenated organic pollutants (POP) in mother's milk from primiparous women in Uppsala since 1996. The study is called POPUP (Persistent Organic Pollutants in Uppsala Primiparas), and the aim is to estimate the body burdens of POP among pregnant and nursing women and to estimate temporal trends of the exposure of fetuses and breast-fed infants. Temporal trends of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), chlorinated pesticides (e.g. DDT-compounds) and brominated flame retardants (e.g. polybrominated diphenylethers (PBDE)) between 1996 and 2012 have been published earlier (Glynn et al. 2007a, Lignell et al. 2008, Lignell et al. 2009a, Lignell et al. 2009b, Lignell et al. 2012, Lignell et al. 2014).

The following report presents results of analysis of di-*ortho* PCBs, mono-*ortho* PCBs, non-*ortho* PCBs, PCDD/Fs, PBDEs and hexabromocyclododecane (HBCD) in mother's milk sampled in 2014 (according to agreement 215 1214). The new data is used to establish updated temporal trends for the period 1996-2014.

MATERIALS AND METHODS

Recruitment and sampling

Mothers were randomly recruited among primiparas who were Swedish by birth and delivered at Uppsala University Hospital from January to November 2014 (N=30). The participating rate was 52%.

The participating mothers sampled milk at home during the third week after delivery (day 14-21 post partum). Milk was sampled during nursing using a manual mother's milk pump and/or a passive mother's milk sampler. The women were instructed to sample milk both at the beginning and at the end of the breast-feeding sessions. The goal was to sample 500 mL from each mother during 7 days of sampling. During the sampling week, the milk was stored in the home freezer in acetone-washed bottles. Newly sampled milk was poured on top of the frozen milk. At the end of the sampling week, a midwife visited the mother to collect the bottles. Data on age, weight, length, lifestyle, medical history, food habits etc. of the mothers were obtained from questionnaires (Table 1). The recruitment during the period 1996-2012 has been described earlier (Glynn et al. 2007a, Lignell et al.

2009a, Lignell et al. 2012, Lignell et al. 2014). Mother's milk was sampled from a total of 486 women between 1996 and 2014 (30 women in 2014).

Table 1. Characteristics of the mothers donating mother's milk in 2014 (N=30).

Variable		**N**	**Mean**	**Median**	**Range**
Age of the mother (yr)		30	29.6	29.9	20-38
Pre-pregnancy body mass index (BMI, kg/m^2)		30	22.9	22.5	19-30
Weight gain during pregnancy (% of initial weight)		30	22.8	22.3	5.3-43
Weight reduction from delivery to sampling (%)[a]		30	8.4	8.5	2.2-13
Variable		**N**	**%**		
Education	max 3-4 yr high school	4	13		
	1-3 yr higher education	7	23		
	>3 yr higher education	19	63		
Smoking[b]	Non-smoker	22	73		
	Former smoker	6	20		
	Smoker	2	7		

[a]Weight reduction minus birth weight of the child in % of weight just before delivery.
[b]Women who stopped smoking before pregnancy are considered to be former smokers. Women who smoked during pregnancy, even if they stopped smoking during the first or second month of pregnancy, are considered to be smokers.

Analysis

The compounds that were analysed in the mother's milk samples from 2014 were 6 non-dioxin like PCBs (PCB 28, 52, 101, 138, 153, 180), 8 mono-*ortho* substituted PCBs (PCB 105, 114, 118, 123, 156, 157, 167, 189), 4 non-*ortho* PCBs (PCB 77, 81, 126, 169), 7 tetra- to octa-chlorinated PCDD congeners, 10 tetra- to octa-chlorinated PCDF congeners, 10 tri- to deca-brominated PBDE-congeners (BDE-28, -47, -66, -100, -99, -154, -153, -138, -183, -209) and hexabromocyclododecane (HBCD).

All analyses of samples from 2014 were performed at the NFA. PCBs and PCDD/Fs were analysed using a method based on gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) (Aune et al. 2012). The clean-up and fractionations were performed with a PowerPrep^{TM}-system from Fluid Management Systems (MA, USA). The final analyses of chlorinated pesticides were performed on a gas chromatograph with dual capillary columns of different polarity and dual electron-capture detectors. PBDEs and HBCD were analysed by gas chromatography/mass spectroscopy/electron-capture negative ionization (GC/MS/ECNI) and detected by single ion monitoring technique (Lignell et al. 2009a).

In all analyses, samples were fortified with internal standards prior to extraction to correct for analytical losses and to ensure quality control. A number of control samples were analysed together with the samples to verify the accuracy and precision of the measurements. The laboratory is accredited for analysis of PCBs, chlorinated pesticides and brominated flame retardants in human milk.

Calculations and statistics

A few mothers recruited in the beginning of the study were not Swedish by birth, and mothers who were born in non-Nordic countries (N=13) were excluded before the statistical analysis of temporal trends 1996-2014. After this exclusion, a total of 473 women were included in the data set. Mother's milk concentrations of POP were lipid-adjusted and when the concentrations were below the limit of quantification (LOQ), half of LOQ was taken as an estimated value in the calculations. PBDE-levels below LOQ were available for breast milk samples from 2009-2014. In this case, reported levels below LOQ (adjusted for levels in blank samples) were used in the statistical analyses of temporal trends instead of half the LOQ-value.

Before the evaluation of temporal trends, POPs were grouped into di-*ortho* PCBs (sum of PCB 153, 138 and 180), mono-*ortho* PCB TEQ (sum of PCB 105, 118, 156 and 167 TEQs), non-*ortho* PCB TEQ (sum of PCB 77, 126 and 169 TEQs), PCDD TEQ, PCDF TEQ and sumPBDE (sum of BDE-47, -99, -100 and -153) (Table 2 and 3). In addition, temporal trends were evaluated for the single compounds PCB 28, PCB 153, BDE-47, BDE-99, BDE-100, BDE-153, BDE-209 and HBCD. BDE-209 was included in the analytical method in year 2009, and has so far only been quantified in samples collected in 2009, 2010, 2012 and 2014. Calculated TEQs were based on 2005 WHO TEFs (Van den Berg et al. 2006).

Temporal trends were investigated for the whole study period (1996-2014), but the period was also divided into two parts (1996-2004 and 2005-2014) in an attempt to study if the trends differ between the early and late parts of the study. Multiple linear regressions (MINITAB 15® Statistical Software for Windows) were used to analyse associations between concentrations of POP in mother's milk and sampling year. Logarithmically transformed POP-levels were used, since the distribution of data closely followed a log-normal distribution. Independent variables (life-style factors) that have been shown to influence POP levels in serum and mother's milk (Glynn et al. 2007b, Lignell et al. 2011a) were included as explanatory variables in the model. The variables considered were age of the mother (years),

pre-pregnancy body mass index (BMI) (kg/m^2), body weight gain during pregnancy (%), and body weight change during the period from delivery to sampling (%) (Table 1). As a consequence of the logarithmic transformation, the associations between sampling year and POP concentrations are presented as percent change of concentrations per year, and not as change in absolute levels.

RESULTS AND DISCUSSION

POP concentrations in mother's milk

Levels of POPs in milk samples collected in 2014 are shown in Tables 2 and 3. Among the PCBs, the di-*ortho* congener PCB 153 showed the highest mean concentration (27 ng/g lipid) followed by PCB 138 (14 ng/g lipid) and PCB 180 (14 ng/g lipid) (Table 2). All PCB-congeners could be quantified in all samples although the levels of some congeners were very low (e.g. PCB 52, 101, 114, 123, 157, 189). PCB 126 was the non-*ortho* congener with the highest concentration and contributed most to the non-*ortho* PCB TEQ. Among the PCDD/Fs, 1,2,3,7,8-PeCDD contributed most to the total PCDD/F TEQ concentration (34%), followed by 2,3,4,7,8-PeCDF (28%), 2,3,7,8-TCDD (13%) and 1,2,3,6,7,8-HxCDD (11%) (Table 2). The mean total-TEQ level was 6.8 pg/g lipid and non-*ortho* PCBs contributed most to this level (mean 2.7 pg TEQ/g lipid) followed by PCDDs (2.1 pg TEQ/g lipid), PCDFs (1.7 pg TEQ/g lipid) and mono-*ortho* PCBs (0.27 pg TEQ/g lipid).

Among the PBDEs, BDE-153 and BDE-47 showed the highest mean concentrations (0.55 and 0.36 ng/g lipids, respectively) followed by BDE-99, BDE-100 and BDE-209 with mean levels that were ca 3-5 times lower (Table 3). However, the levels of BDE-47, BDE-99 and BDE-209 were below LOQ in 19, 28 and 24 of the analysed samples, respectively. The levels of BDE-28, BDE-66, BDE-138 and BDE-183 were also below LOQ in most samples. Estimated PBDE-levels below LOQ are presented in brackets in Table 3 and were used in the analyses of temporal trends.

Table 2. Concentrations of PCBs and PCDD/Fs in mother's milk sampled from primiparous women in Uppsala in 2014 (N=30). Values below the LOQ were set to ½LOQ in the calculations of means, medians and TEQs.

Compound	**Mean**	**Median**	**Min**	**Max**	**N<LOQ**
PCBs (ng/g lipid)					
PCB 28	1.8	1.2	0.32	17	0
PCB 52	0.18	0.16	0.09	0.51	0
PCB 101	0.29	0.24	0.06	0.78	0
PCB 105	0.99	0.82	0.24	3.2	0
PCB 114	0.22	0.18	0.05	0.80	0
PCB 118	4.5	3.5	1.4	13	0
PCB 123	0.04	0.03	0.01	0.13	0
PCB 138	14	14	4.6	29	0
PCB 153	27	24	7.5	60	0
PCB 156	2.9	2.5	0.66	7.0	0
PCB 157	0.49	0.40	0.10	1.1	0
PCB 167	0.69	0.58	0.21	1.7	0
PCB 180	14	12	3.1	35	0
PCB 189	0.25	0.22	0.06	0.62	0
di-*ortho* PCB[a]	56	51	15	124	-
mono-*ortho* PCB TEQ[b] (pg/g lipid)	0.27	0.23	0.08	0.63	-
non-*ortho* PCBs (pg/g lipid)					
PCB 77	2.4	2.4	1.0	4.5	0
PCB 81	0.95	0.88	0.29	2.7	0
PCB 126	23	19	6.8	48	0
PCB 169	14	14	4.5	31	0
non-*ortho* PCB TEQ[c]	2.7	2.2	0.87	5.5	-
PCDDs (pg/g lipid)					
2,3,7,8-TCDD	0.41	0.35	0.17	1.2	0
1,2,3,7,8-PeCDD	1.2	1.1	0.46	2.6	0
1,2,3,4,7,8-HxCDD	0.41	0.38	0.18	0.74	0
1,2,3,6,7,8-HxCDD	2.8	2.7	1.3	6.1	0
1,2,3,7,8,9-HxCDD	0.73	0.70	0.26	1.5	0
1,2,3,4,6,7,8-HpCDD	4.5	4.2	0.78	14	0
OctaCDD	27	25	7.7	44	0
PCDD TEQ	2.1	2.0	0.91	4.5	-
PCDFs (pg/g lipid)					
2,3,7,8-TCDF	0.43	0.37	0.07	1.4	0
1,2,3,7,8-PeCDF	0.29	0.20	0.06	1.4	0
2,3,4,7,8-PeCDF	4.3	3.8	1.6	14	0
1,2,3,4,7,8-HxCDF	1.5	1.2	0.67	6.1	0
1,2,3,6,7,8-HxCDF	1.5	1.2	0.59	5.8	0
1,2,3,7,8,9-HxCDF	0.06	0.04	<0.02	0.36	13
2,3,4,6,7,8-HxCDF	0.82	0.64	0.18	3.6	0
1,2,3,4,6,7,8-HpCDF	1.9	1.1	0.43	9.4	0
1,2,3,4,7,8,9-HpCDF	0.10	0.05	<0.02	0.59	6
OctaCDF	0.18	0.11	<0.06	0.83	4
PCDF TEQ	1.7	1.5	0.66	5.0	-
PCDD/F TEQ[d] (pg/g lipid)	3.8	3.6	1.7	9.5	-
TOTAL-TEQ[e] (pg/g lipid)	6.8	6.2	2.6	15	-

[a]sum of PCB 153, 138 and 180. [b]sum of PCB 105, 118, 156, 167 TEQs. [c]sum of PCB 77, 126, 169 TEQs. [d]sum of PCDD TEQ and PCDF TEQ. [e]sum of mono-*ortho* PCB TEQ, non-*ortho* PCB TEQ, PCDD TEQ and PCDF TEQ.

Table 3. Concentrations (ng/g lipid) of PBDEs and HBCD in mother's milk sampled from primipara women in Uppsala in 2014 (N=30). Values below the LOQ were set to ½LOQ in the calculations of means, medians and sumPBDE. Levels below LOQ were also reported and calculated results using these levels (adjusted for levels in blank samples) are presented in brackets ([ ]).

Compound	Mean	Median	Min	Max	N<LOQ[b] [N=0][c]
BDE-28	0.03 [0.03]	0.03 [0.03]	<0.03 [0]	0.06	22 [2]
BDE-47	0.36 [0.37]	0.22 [0.24]	<0.21 [0.04]	1.7	19 [0]
BDE-66	0.02 [0.004]	0.02 [0.003]	<0.02 [0]	0.04	30 [9]
BDE-99	0.13 [0.08]	0.12 [0.05]	<0.16 [0]	0.40	28 [3]
BDE-100	0.17 [0.17]	0.10 [0.10]	<0.04 [0.02]	2.1	2 [0]
BDE-138	0.02 [0.0006]	0.02 [0]	<0.02 [0]	<0.08 [0.01]	30 [26]
BDE-153	0.55 [0.55]	0.46 [0.46]	0.23 [0.23]	3.0	0 [0]
BDE-154	0.06 [0.06]	0.06 [0.06]	<0.03 [0.02]	0.13	9 [0]
BDE-183	0.02 [0.01]	0.02 [0.01]	<0.02 [0]	0.09	30 [5]
BDE-209	0.11 [0.10]	0.08 [0.07]	<0.07 [0.002]	0.50	24 [0]
sumPBDE(4)[a]	1.2 [1.2]	0.92 [0.87]	0.57 [0.39]	6.8	-
HBCD	0.25 [0.24]	0.19 [0.19]	<0.04 [0]	1.3	5 [2]

[a]sum of BDE-47, -99, -100 and -153. [b]number of samles with levels below LOQ [c]number of samples with levels estimated to be zero after adjustment for blank levels.

Temporal trends

Multiple linear regressions showed that the adjusted mean decrease in concentrations of PCB 28 was 3.5% per year, while the concentrations of PCB 153, di-*ortho* PCB, mono-*ortho* PCB TEQ and non-*ortho* PCB TEQ decreased with about 6-7% per year (Table 4, Figure 1). These results are in agreement with earlier observed declining trends between 1996 and 2012 (Lignell et al. 2014). The decreases in levels of PCDD TEQs and PCDF TEQs (Table 4) are also in agreement with earlier results (Lignell et al. 2014), showing a faster declining rate for PCDD TEQs than for PCDF TEQs. For PCBs as well as for PCDD/Fs, there was a tendency of faster declining rates during the early part of the study (1996-2004) than during the later part (2005-2014), although the confidence intervals overlap. This was especially pronounced for PCDF TEQs, with a significant downward trend in 1996-2004 (-5.4% per year) but no trend in 2005-2014. The concentrations of PCBs and PCDD/Fs in the samples from 2014 were similar to or even higher than levels in samples from 2010 and 2012. This may be due to chance, but it is also possible that the concentrations of these chemicals are stabilizing at current levels.

The continuous decline in breast milk levels of PCBs and PCDD/Fs is in agreement with results from three Swedish market basket studies performed between 1999 and 2010 (National Food Agency 2012a) showing declining exposure to PCBs and PCDD/Fs

from food. In addition, results from the Swedish control of contaminants in food show that levels of PCB 153 have decreased in rainbow trout, bovine fat, egg and milk from the late 1990s up to year 2010 (National Food Agency 2012b). However, exposure to PCBs and PCDD/Fs from food, as estimated in the market basket study, seems to have decreased at a slower rate at the end of the study period 1999-2010 (National Food Agency 2012a), supporting our results indicating that the decline in mother's milk levels has slowed down.

Table 4. Percent change in concentrations of PCBs and PCDD/Fs per year in mother's milk from primiparous women in Uppsala 1996-2014. Adjusted for age of the mother, pre-pregnancy BMI, weight gain during pregnancy and weight loss after delivery.

Compound	**Period**	**Change/year (%)[a]**		**half-time[b]**	**R^{2d}**	**N**	**P**
		Mean	**95% CI**	**(years)**			
PCB 28	1996-2014	-3.5	-4.7/-2.3	19	9	458	<0.001
	1996-2004	-4.0	-7.9/0.1	17	3	278	0.06
	2005-2014	-2.3	-5.9/1.5	30	10	180	0.23
PCB 153	1996-2014	-6.8	-7.3/-6.3	10	69	458	<0.001
	1996-2004	-8.3	-9.6/-6.9	8	61	278	<0.001
	2005-2014	-4.9	-7.0/-2.7	14	49	180	<0.001
di-*ortho* PCB[e]	1996-2014	-6.5	-6.9/-6.0	10	69	458	<0.001
	1996-2004	-8.0	-9.2/-6.7	8	64	278	<0.001
	2005-2014	-5.2	-7.2/-3.1	13	44	180	<0.001
mono-*ortho* PCB TEQ[f]	1996-2014	-6.3	-6.9/-5.8	11	63	458	<0.001
	1996-2004	-8.3	-9.8/-6.7	8	52	278	<0.001
	2005-2014	-4.8	-6.8/-2.8	14	43	181	<0.001
non-*ortho* PCB TEQ[g]	1996-2014	-5.9	-6.6/-5.2	11	58	329	<0.001
	1996-2004	-6.9	-9.0/-4.8	10	36	178	<0.001
	2005-2014	-4.1	-6.1/-2.1	16	45	151	<0.001
PCDD TEQ	1996-2014	-7.1	-7.6/-6.6	9	76	295	<0.001
	1996-2004	-6.9	-8.5/-5.3	10	56	145	<0.001
	2005-2014	-5.3	-6.9/-3.6	13	47	150	<0.001
PCDF TEQ	1996-2014	-3.9	-4.6/-3.3	17	46	295	<0.001
	1996-2004	-5.4	-7.4/-3.4	12	45	145	<0.001
	2005-2014	-1.6	-3.9/0.6	42	24	150	0.15
PCDD/DF TEQ[h]	1996-2014	-5.9	-6.5/-5.4	11	68	295	<0.001
	1996-2004	-6.4	-7.9/-4.8	11	56	145	<0.001
	2005-2014	-3.8	-5.6/-1.9	18	37	150	<0.001
Total-TEQ[i]	1996-2014	-6.0	-6.5/-5.4	11	69	294	<0.001
	1996-2004	-6.7	-8.4/-5.0	10	55	144	<0.001
	2005-2014	-3.9	-5.7/-2.0	18	44	150	<0.001

[a]Percent change (decrease (-) or increase (+)) of the concentrations per year. [b]The estimated time it takes for the concentrations to be *halved* in the population. [c]Estimated time for the concentrations to be *doubled* in the population. [d]Coefficient of determination for the regression model. [e]sum of PCB 153, 138 and 180. [f]sum of PCB 105, 118, 156, 167 TEQs based on 2005 WHO TEFs (Van den Berg et al. 2006). [g]sum of PCB 77, 126, 169 TEQs based on 2005 WHO TEFs (Van den Berg et al. 2006). [h]sum of PCDD TEQ and PCDF TEQ. [i]sum of mono-*ortho* PCB TEQ, non-*ortho* PCB TEQ, PCDD TEQ and PCDF TEQ.

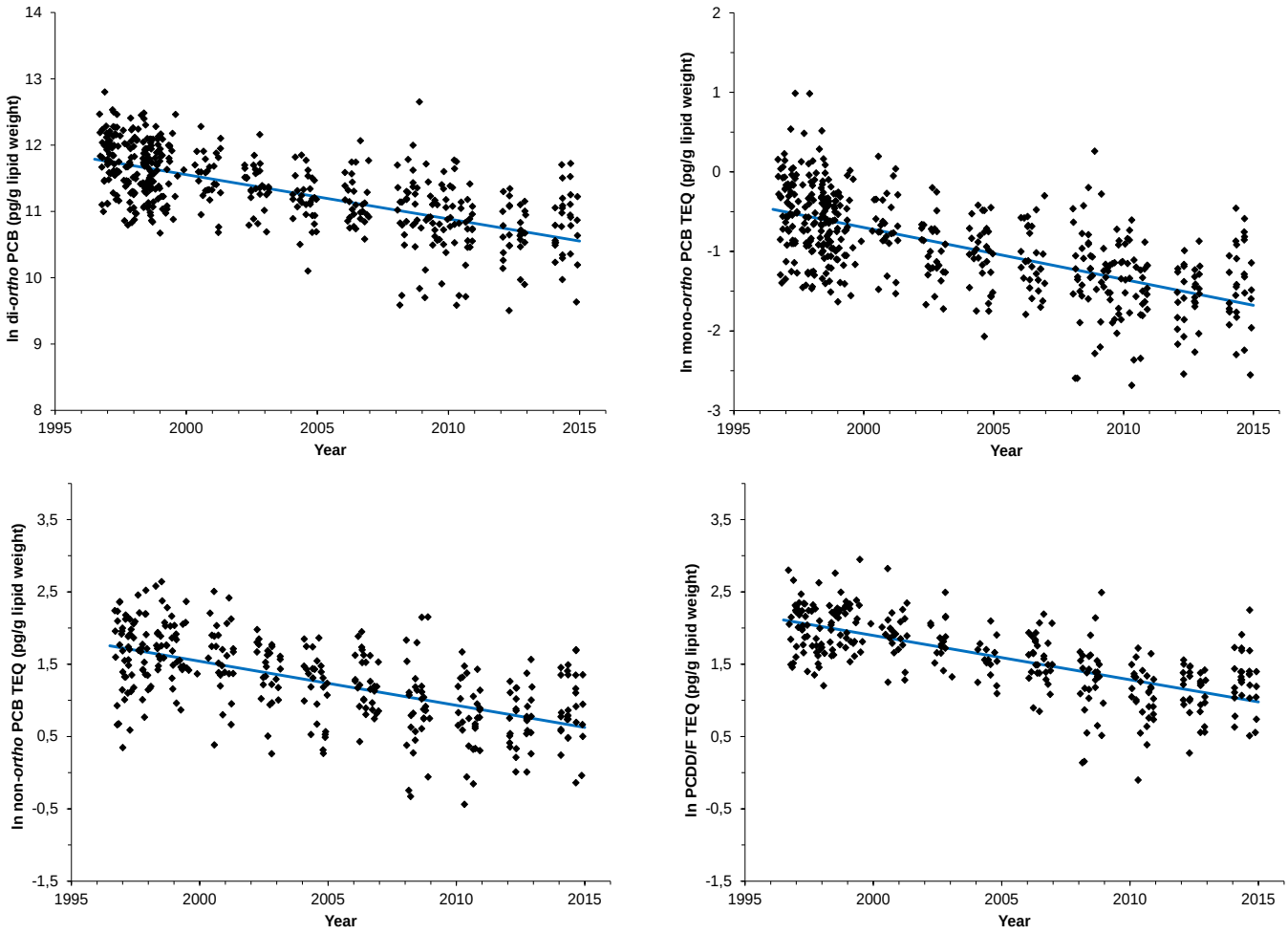

Figure 1. Levels of di-*ortho* PCBs (N=428), mono-*ortho* PCB TEQs (N=458), non-*ortho* PCB TEQs (N=329) and PCDD/F TEQs (N=295) in mother's milk from first-time mothers in Uppsala, Sweden in 1996-2014. Each point corresponds to the concentration in a milk sample from an individual woman. The blue lines represent regression lines obtained from multiple regression analysis including important life-style factors in the model. All temporal trends are significant ($p \leq 0.001$).

The levels of BDE-47, BDE-99, BDE-100 and sumPBDE decreased with similar rates as were previously reported for the period 1996-2012 (Lignell et al. 2014) (Table 6, Figure 2). Declining rates were faster during the latter part of the study (2005-2014) and for BDE-47, BDE-100 and sumPBDE there were no significant trends during the earlier part (1996-2004). Previously, a significant positive trend has been observed for BDE-153 (Lignell et al. 2014). However, when data for 2014 was added there was no longer any significant trend (Table 6, Figure 2). Dividing the study period in two parts showed that there was a significant increase between 1996 and 2004, while the levels decreased thereafter. Decreasing levels of PBDEs in humans and faster declining rates during the latter part of the study are expected since the use of lower brominated congeners has been voluntarily reduced since the 1990s and the use of PBDEs in electric and electronic products has been restricted by law since 2006. In agreement with our results, Swedish market basket studies performed in 1999, 2005 and 2010 showed that exposure to BDE-47 and BDE-99 from food was significantly lower in 2010

than in 1999, and that exposure to BDE-47, BDE-99 and BDE-100 from fish decreased between 1999 and 2010 (National Food Agency 2012a).

Table 6. Percent change in concentrations of PBDEs and HBCD per year in mother's milk from primiparous women in Uppsala 1996-2014. Adjusted for age of the mother, pre-pregnancy BMI, weight gain during pregnancy and weight loss after delivery.

Compound	Period	Change/year (%)[a]		half-time[b]	R^{2d}	N	P
		Mean	95% CI	(years)			
BDE-47	1996-2014	-9.4	-11/-8.2	7	36	413	<0.001
	1996-2004	-1.1	-4.5/-2.3	-	0.2	233	0.5
	2005-2014	-15	-19/-11	4	20	180	<0.001
BDE-99	1996-2014	-12	-14/-11	5	40	413	<0.001
	1996-2004	-6.2	-9.6/-2.6	11	9	233	0.001
	2005-2014	-20	-25/-15	3	19	180	<0.001
BDE-100	1996-2014	-5.3	-6.6/-4.1	13	15	413	<0.001
	1996-2004	+0.8	-2.8/+4.6	-	1.2	233	0.6
	2005-2014	-11	-15/-6.3	6	9	180	<0.001
BDE-153	1996-2014	+0.7	-0.2/+1.5	-	12	413	0.12
	1996-2004	+6.5	+4.1/+9.0	-11[c]	22	233	<0.001
	2005-2014	-3.4	-6.3/-0.5	20	11	180	0.02
BDE-209	2009-2014	+3.0	-5.8/13	-	0	119	0.5
sumPBDE[e]	1996-2014	-5.8	-6.7/-4.9	12	27	413	<0.001
	1996-2004	+0.1	-2.8/+3.0	-	1.8	233	0.96
	2005-2014	-9.9	-13/-6.7	7	16	180	<0.001
HBCD	1996-2014	-1.2	-2.4/0.1	59	1.5	319	0.07
	1996-2004	+5.6	+1.6/+9.8	-13[c]	6	139	0.007
	2005-2014	-3.7	-0.2/-7.0	18	2	180	0.04
	2002-2014[f]	-5.1	-7.5/-2.6	13	8	174	<0.001

[a]Percent change (decrease (-) or increase (+)) of the concentrations per year. [b]The estimated time it takes for the concentrations to be *halved* in the population. [c]Estimated time for the concentrations to be *doubled* in the population. [d]Coefficient of determination for the regression model. [e]sum of BDE-47, -99, -100 and -153. [f]only results from 2002-04 and 2009-2014 were included.

BDE-209 has only been analysed in samples collected in 2009-2014 and an evaluation of temporal trends showed no significant changes during this period (Table 6, Figure 2). An earlier study of BDE-209 in pooled blood serum samples from women in the POPUP-study showed no significant temporal trend between 1996 and 2010 (Lignell et al. 2011b). More data-points are needed before a possible temporal trend can be detected.

For HBCD, there was a small borderline significant downward trend for the whole study period (Table 6). Dividing the study in two parts resulted in significantly increasing levels in 1996-2004 and significantly decreasing levels in 2005-2014. However, the analytical method used for HBCD-analysis has been changed during the study period and periodically there have also been problems with higher blank levels. LOQ has consequently varied during the years. Because of these uncertainties in the results, we also performed a

trend analysis of trends including only results from years when LOQ was low, i.e. HBCD-levels in most samples were above LOQ. This trend analysis only included results from samples collected in 2002-2004 and 2009-14 and showed significantly decreasing levels of HBCD (Table 6, Figure 2). A downward trend was also indicated in the earlier evaluation of data from 2002-2012, although not significant (Lignell et al. 2012). More data points are needed before we can draw any firm conclusions about a temporal trend of HBCD. A study of HBCD in pooled blood serum samples from women in the POPUP-study showed a significant down-ward temporal trend between 1996 and 2010 (Lignell et al. 2011b). However, Swedish market basket studies showed no difference in exposure to HBCD from fish between 2005 and 2010 (National Food Agency 2012a).

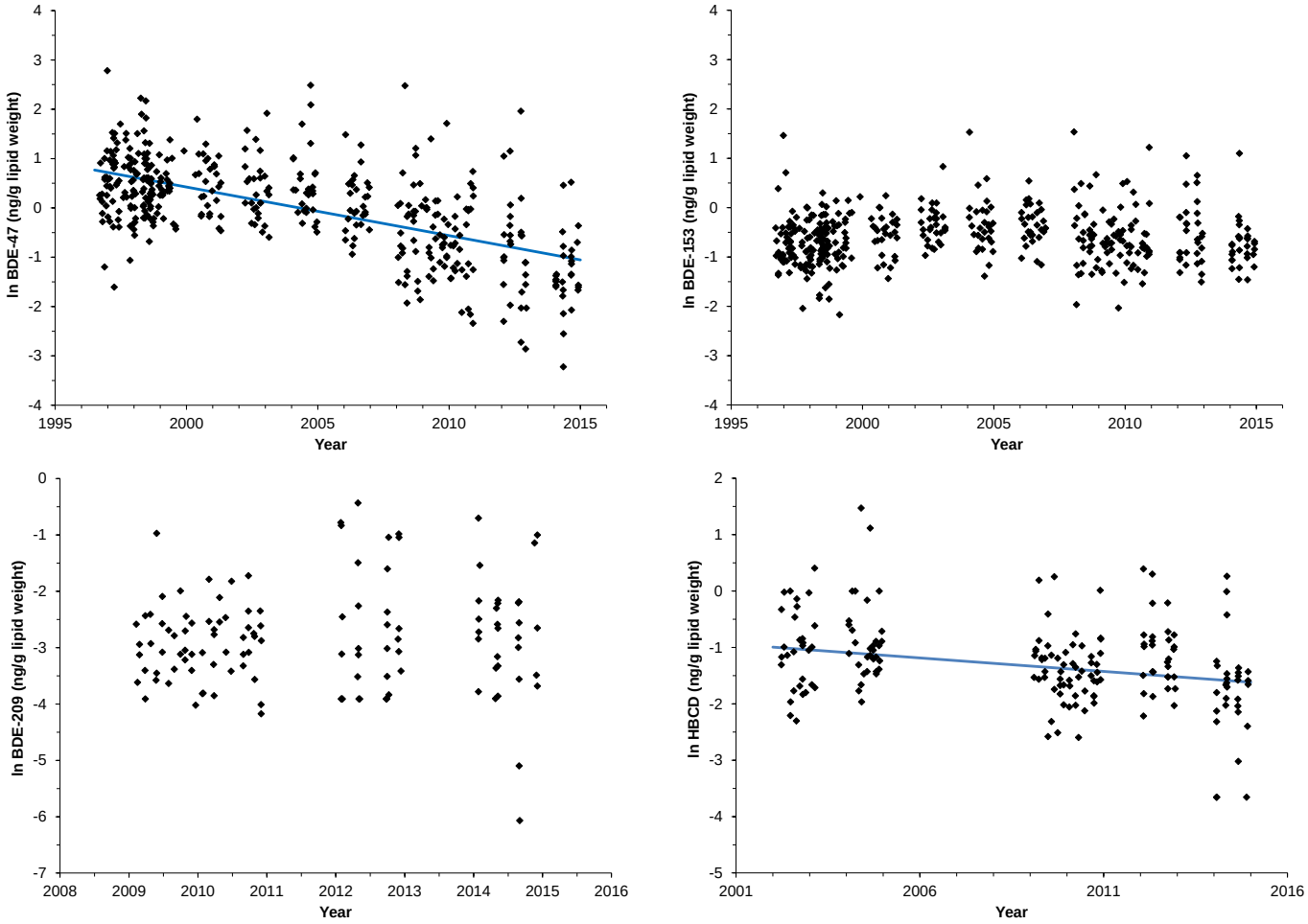

Figure 2. Levels of BDE-47 (N=413), BDE-153 (N=413), BDE-209 (N=119) and HBCD (N=174) in mother's milk from first-time mothers in Uppsala, Sweden. Each point corresponds to the concentration in a milk sample from an individual woman. The lines represent regression lines obtained from multiple regression analysis including important life-style factors in the model. There was a significant negative temporal trend for BDE-47 (1996-2014) and for HBCD (2002-2014).

CONCLUSION

The levels of PCBs and PCDD/Fs in breast milk from the POPUP-cohort show decreasing trends between 1996 and 2014, but there are indications that the declines have been slower during the latter part of the study (2005-2014) than during the earlier part (1996-2004). It is important to continue following concentrations of chlorinated POPs in breast milk from Swedish mothers in order to further investigate if the concentrations are stabilizing at current levels or continue to decrease.

Levels of PBDEs (BDE-47, BDE-99, BDE-100) show decreasing trends that, opposite to the chlorinated POPs, have been faster since 2005 than 1996-2004. Restrictions in the production and use of these compounds were initiated in the 1990s, and this is probably reflected in the more pronounced decline in mother's milk concentrations in recent years. The levels of BDE-153 showed an increasing trend in 1996-2004 but a decreasing trend thereafter. More data points are needed before we can draw any conclusions about trends regarding BDE-209 and HBCD. However, current data show no significant trend for BDE-209 (2009-2014) but a slightly decreasing trend for HBCD (1996-2014 and 2002-2014).

A more comprehensive evaluation of temporal trends of POPs in mother's milk from the POPUP study, e.g. non-linear trend elements, will be performed and reported to the EPA in 2016 (contract nr 2215 15 001). This evaluation will give information about possible non-linear trends during the study period and also give us suggestions on how to better perform and evaluate temporal trends in the future.

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