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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 2012 and temporal trends for the time period 1996-2012

Sanna Lignell, Marie Aune, Anders Glynn, Tatiana Cantillana, Ulrika Fridén

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Rapportförfattare
Sanna Lignell, Livsmedelsverket
Marie Aune, Livsmedelsverket
Anders Glynn, Livsmedelsverket
Tatiana Cantillana, Livsmedelsverket
Ulrika Fridén, Livsmedelsverket

Utgivare
Livsmedelsverket

Postadress
Box 622, 751 26 Uppsala

Telefon
018-175500

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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), de klorerade pesticiderna DDT (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT), hexaklorbensen (HCB), hexaklorcyklohexan (β-HCH) och klordan (oxyklordan och transnonaklor) samt bromerade flamskyddsmedel (PBDE, HBCD) i 30 modersmjölksprover insamlade 2012. Nya data används också för att uppdatera tidstrenderna för dessa ämnen.

Bland PCBerna var medelkoncentrationen i modersmjölk (2012) högst för PCB 153 (22 ng/g fett). Medelhalten för PCDD TEQ (1,9 pg/g fett) var högre än för PCDF TEQ (1,3 pg/g fett). Den DDT-förening som hade högst medelhalt var *p,p'*-DDE (39 ng/g fett). Bland de polybromerade difenyletrarna (PBDE) uppvisade BDE-47 (0,84 ng/g fett) och BDE-153 (0,74 ng/g fett) de högsta medelhalterna.

Utvärdering av tidstrender för perioden 1996-2012 (multipel linjär regression) visade att halterna av di-*orto* PCBer, mono-*orto* PCB TEQ och non-*orto* PCB TEQ har minskat med ca 7% per år. Halterna av PCDD TEQ har minskat fortare än halterna av PCDF TEQ (8 respektive 5% per år). Dessa resultat stämmer överens med de trender som tidigare observerats för perioden 1996-2010. En uppdelning av studieperioden i två delar visade att haltminskningarna för PCB och PCDD/F var ungefär lika under perioden 1996-2003 som under 2004-2012. Halterna av *p,p'*-DDE och HCB i modersmjölk minskade med 7 respektive 6% per år, vilket stämmer överens med de minskningshastigheter som rapporterats för 1996-2008. Haltminskningarna var snabbare under 1996-2003 än under 2004-2012.

Resultaten för PBDEer stämmer också överens med det som rapporterats tidigare för perioden 1996-2010, dvs. halterna av BDE-47, -99 och -100 har minskat (5-10% per år), medan nivåerna av BDE-153 har ökat något (1,5% per år). Resultaten antyder att minskningarna för BDE-47, -99 och -100 varit snabbare 2004-2012 än under 1996-2003 och att halterna av BDE-153 ökade under 1996-2003 men att de därefter har minskat. BDE-209 har analyserats i modersmjölk sedan 2009 och det behövs mer data innan en tidstrend kan utvärderas. Trenden för HBCD är osäker eftersom halterna ligger under analysmetodens kvantifieringsgräns i många prover. En utvärdering av tidsperioden 2002-2012 visade en icke signifikant haltminskning för HBCD på 2,5% per år, men fler datapunkter krävs för att bekräfta denna trend.

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 primipare 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 2008/10 have been published earlier (Glynn et al. 2007a, Lignell et al. 2008, Lignell et al. 2009a, Lignell et al. 2009b, Lignell et al. 2012).

The following report presents results of analysis of di-*ortho* PCBs, mono-*ortho* PCBs, non-*ortho* PCBs, PCDD/Fs, DDT-compounds (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT), hexachlorobenzene (HCB), hexachlorocyclohexane (β-HCH), chlordane (oxychlordane and *trans*-nonachlor), PBDEs and hexabromocyclododecane (HBCD) in mother's milk sampled in 2012 (according to agreement 215 1214). The new data is used to establish updated temporal trends for the period 1996-2012.

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 December 2012 (N=30). The participating rate was 58 %.

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, lifestyle, medical history etc. of the mothers were obtained from questionnaires (Table 1). The recruitment during the period 1996-2010

(N=426) has been described earlier (Glynn et al. 2007a, Lignell et al. 2009a, Lignell et al. 2012). Mother's milk was sampled from a total of 456 women between 1996 and 2012.

Table 1. Characteristics of the mothers donating mother's milk in 2012 (N=30).

Variable		**N**	**Mean**	**Median**	**Range**
Age of the mother (yr)		30	29	29	20-38
Pre-pregnancy body mass index (BMI, kg/m^2)		30	24.6	24.6	17-33
Weight gain during pregnancy (% of initial weight)		30	23.9	24.6	7.9-44
Weight reduction from delivery to sampling (%)[a]		30	9.1	8.8	2.4-15
Variable		**N**	**%**		
Education	max 3-4 yr high school	10	33		
	1-3 yr higher education	6	20		
	>3 yr higher education	14	47		
Smoking[b]	Non-smoker	15	50		
	Former smoker	13	43		
	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 2012 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, 8 chlorinated pesticides and their metabolites (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, HCB, β-HCH, oxychlordane, *trans*-nonachlor), 9 tri- to hepta-brominated PBDE-congeners (BDE-28, -47, -66, -100, -99, -154, -153, -138, -183), BDE-209 (deca-BDE) and hexabromo-cyclododecane (HBCD).

All analyses of samples from 2012 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 the 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

Mothers who were born in non-Nordic countries (N=13) were excluded before the statistical analysis of temporal trends. After this exclusion, a total of 443 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 2009-2012. In this case, reported levels below LOQ (adjusted for levels in blank samples) were used in the statistical analyses of temporal trends. Calculated TEQs were based on 2005 WHO TEFs (Van den Berg et al. 2006).

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, BDE-47, BDE-99, BDE-100, BDE-153 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 and 2012. More data points are needed before an evaluation of a temporal trend is possible.

Temporal trends were investigated for the whole study period (1996-2012), but the period was also divided into two parts (1996-2003 and 2004-2012) in a first preliminary 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 2012 are shown in table 2 and 3. Among the PCBs, the di-*ortho* congener PCB 153 showed the highest mean concentration (22 ng/g lipids) followed by PCB 138 (13 ng/g lipids) and PCB 180 (12 ng/g lipids) (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 (Table 2), 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 2,3,4,7,8-PeCDF contributed most to the PCDD/F TEQ concentration (mean total contribution was 86%). The mean total-TEQ level was 5.7 pg/g lipids and non-*ortho* PCBs contributed most to this level (mean 2.3 pg TEQ/g lipids) followed by PCDDs (1.9 pg TEQ/g lipids), PCDFs (1.3 pg TEQ/g lipids) and mono-*ortho* PCBs (0.22 pg TEQ/g lipids).

For the chlorinated pesticides, the highest mean level was found for *p,p'*-DDE (39 ng/g lipids), followed by HCB with a mean level that was approximately 1/5 of the mean *p,p'*-DDE level (Table 3). The mean levels of *p,p'*-DDT, *p,p'*-DDE, β-HCH, oxychlordane and *trans*-nonachlor were lower but above LOQ in all samples. Levels of *p,p'*-DDD and *o,p'*-DDT were below LOQ.

Among the PBDEs, BDE-47 and BDE-153 showed the highest mean concentrations (0.84 and 0.74 ng/g lipids, respectively) followed by BDE-99, BDE-100 and BDE-209 with mean levels that were 3-5 times lower (Table 3). However, the levels of BDE-99 and BDE-209 were below LOQ in 27 and 21 of the analysed samples, respectively. The levels of 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 primipara women in Uppsala in 2012 (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	1.6	0.96	0.49	16	0
PCB 52	0.15	0.14	0.08	0.24	0
PCB 101	0.33	0.34	0.15	0.51	0
PCB 105	0.84	0.82	0.35	1.4	0
PCB 114	0.17	0.17	0.04	0.35	0
PCB 118	3.9	3.8	1.7	6.9	0
PCB 123	0.04	0.04	0.02	0.08	0
PCB 138	13	12	4.7	24	0
PCB 153	22	21	6.5	41	0
PCB 156	2.1	1.9	0.42	4.5	0
PCB 157	0.38	0.35	0.08	0.69	0
PCB 167	0.58	0.58	0.17	1.1	0
PCB 180	12	10	2.2	23	0
PCB 189	0.19	0.16	0.03	0.40	0
di-*ortho* PCB[a]	47	43	13	845	-
mono-*ortho* PCB TEQ[b] (pg/g lipid)	0.22	0.22	0.08	0.42	-
non-*ortho* PCBs (pg/g lipid)					
PCB 77	3.2	3.0	2.0	7.1	0
PCB 81	0.93	0.83	0.45	2.4	0
PCB 126	19	18	8.5	42	0
PCB 169	11	10	2.9	20	0
non-*ortho* PCB TEQ[c]	2.3	2.1	1.0	4.8	-
PCDDs (pg/g lipid)					
2,3,7,8-TCDD	0.38	0.36	0.17	0.61	0
1,2,3,7,8-PeCDD	1.1	1.0	0.44	1.6	0
1,2,3,4,7,8-HxCDD	0.34	0.32	0.16	0.56	0
1,2,3,6,7,8-HxCDD	2.8	2.6	0.93	5.3	0
1,2,3,7,8,9-HxCDD	0.64	0.55	0.30	1.4	0
1,2,3,4,6,7,8-HpCDD	4.3	4.1	1.3	8.6	0
OctaCDD	28	29	10	48	0
PCDD TEQ	1.9	1.8	0.82	2.8	-
PCDFs (pg/g lipid)					
2,3,7,8-TCDF	0.38	0.35	0.15	0.79	0
1,2,3,7,8-PeCDF	0.22	0.19	0.07	0.52	0
2,3,4,7,8-PeCDF	3.2	3.2	1.2	5.2	0
1,2,3,4,7,8-HxCDF	1.1	1.0	0.46	2.0	0
1,2,3,6,7,8-HxCDF	1.0	0.96	0.40	1.9	0
1,2,3,7,8,9-HxCDF	0.04	0.04	<0.03	0.08	4
2,3,4,6,7,8-HxCDF	0.62	0.58	0.20	1.3	0
1,2,3,4,6,7,8-HpCDF	3.3	1.1	0.42	30	0
1,2,3,4,7,8,9-HpCDF	0.07	0.07	<0.03	0.18	3
OctaCDF	0.13	0.11	<0.05	0.37	1
PCDF TEQ	1.3	1.4	0.49	2.2	-
PCDD/F TEQ[d] (pg/g lipid)	3.2	3.4	1.3	4.7	-
TOTAL-TEQ[e] (pg/g lipid)	5.7	5.5	2.4	9.3	-

[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 chlorinated pesticides, PBDEs and HBCD in mother's milk sampled from primipara women in Uppsala in 2012 (N=30). Values below the LOQ were set to ½LOQ in the calculations of means, medians and sumPBDE. For PBDEs, estimated levels below LOQ were reported and calculated results using these levels are presented in brackets ([ ]).

Compound	Mean	Median	Min	Max	N<LOQ [N=0]
p,p'-DDT	2.2	1.8	0.98	10	0
p,p'-DDE	39	34	14	77	0
p,p'-DDD	0.34	0.32	<0.4	<1.5	30
o,p'-DDT	0.36	0.32	<0.4	0.65	29
HCB	7.2	7.0	4.4	10	0
β-HCH	2.8	2.5	1.4	5.2	0
oxychlordane	1.5	1.4	0.48	2.9	0
trans-nonachlor	2.9	2.4	0.43	6.2	0
BDE-28	0.06 [0.06]	0.04 [0.04]	<0.03 [0.01]	0.29	13 [0]
BDE-47	0.84 [0.82]	0.43 [0.42]	<0.24 [0.06]	7.1	9 [0]
BDE-66	0.02 [0.01]	0.02 [0.01]	<0.03 [0]	0.07	28 [4]
BDE-99	0.22 [0.16]	0.12 [0.07]	<0.14 [0]	1.6	27 [6]
BDE-100	0.22 [0.22]	0.10 [0.10]	<0.04 [0.01]	2.3	7 [0]
BDE-138	0.02 [0.003]	0.02 [0.001]	<0.03 [0]	<0.1 [0.02]	30 [12]
BDE-153	0.74 [0.74]	0.54 [0.54]	0.22 [0.22]	2.9	0 [0]
BDE-154	0.06 [0.06]	0.04 [0.04]	<0.03 [0.02]	0.23	12 [0]
BDE-183	0.02 [0.02]	0.02 [0.02]	<0.03 [0.01]	0.04 [0.06]	27 [0]
BDE-209	0.16 [0.13]	0.08 [0.05]	<0.08 [0]	0.65 [0.65]	21 [7]
sumPBDE(4)[a]	2.0 [1.9]	1.3 [1.2]	0.61 [0.43]	13 [13]	-
HBCD	0.40 [0.40]	0.33 [0.33]	<0.20 [0.11]	1.5 [1.5]	1 [0]

[a]sum of BDE-47, -99, -100 and -153

Temporal trends

Multiple linear regressions showed that the adjusted mean decrease in concentrations of PCB 28 was 4% per year, while the levels of PCB 153, di-*ortho* PCB, mono-*ortho* PCB TEQ and non-*ortho* PCB TEQ decreased with about 7% per year (Table 4, Figure 1). These results are in agreement with earlier observed declining trends between 1996 and 2010 (Lignell et al. 2012). There were some differences in declining rates between the early (1996-2003) and the late (2004-2012) parts of the study period, but the differences are small and confidence intervals for the declining rates overlap. The largest difference was observed for mono-*ortho* PCB TEQ, where a faster declining rate was observed during the early part of the study. The decreases in levels of PCDD TEQs and PCDF TEQs (Table 4) are also in agreement with earlier results (Lignell et al. 2012) showing a faster declining rate for PCDD TEQs than for PCDF TEQs. There were only small differences in declining rates between the early and late parts of the study. A tendency to a faster declining rate in 2004-2012 than in 1996-2003 was

however observed for PCDD TEQ. The continuous decline in breast milk levels of PCBs and PCDD/Fs is in agreement with results from Swedish market basket studies performed in 1999, 2005 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).

Table 4. Percent change in concentrations of PCBs and PCDD/Fs per year in mother's milk from primiparous women in Uppsala 1996-2012. 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	1996-2012	-3.9	-5.2/-2.5	18	8	428	<0.001
	1996-2003	-7.9	-14/-1.8	8	4	247	0.01
	2004-2012	-5.2	-8.6/-1.8	13	9	181	0.004
PCB 153	1996-2012	-7.3	-7.8/-6.7	9	69	428	<0.001
	1996-2003	-8.3	-10/-6.2	8	56	247	<0.001
	2004-2012	-6.4	-8.3/-4.4	10	49	181	<0.001
di-*ortho* PCB[e]	1996-2012	-6.9	-7.4/-6.3	10	70	428	<0.001
	1996-2003	-8.1	-10/-6.3	8	59	247	<0.001
	2004-2012	-6.2	-8.1/-4.3	11	50	181	<0.001
mono-*ortho* PCB TEQ[f]	1996-2012	-6.8	-7.4/-6.2	10	63	428	<0.001
	1996-2003	-9.6	-12/-7.3	7	48	247	<0.001
	2004-2012	-6.2	-8.0/-4.3	11	48	181	<0.001
non-*ortho* PCB TEQ[g]	1996-2012	-6.6	-7.4/-5.8	10	58	299	<0.001
	1996-2003	-5.4	-8.4/-2.2	13	31	152	0.001
	2004-2012	-6.2	-8.3/-4.1	11	46	147	<0.001
PCDD TEQ	1996-2012	-7.8	-8.3/-7.2	9	77	265	<0.001
	1996-2003	-4.6	-6.7/-2.3	15	49	130	<0.001
	2004-2012	-7.4	-9.2/-5.6	9	56	135	<0.001
PCDF TEQ	1996-2012	-4.9	-5.6/-4.2	14	55	265	<0.001
	1996-2003	-6.1	-8.9/-3.3	11	45	130	<0.001
	2004-2012	-5.1	-7.2/-3.0	13	38	135	<0.001
PCDD/DF TEQ[h]	1996-2012	-6.8	-7.3/-6.2	10	73	265	<0.001
	1996-2003	-5.0	-7.2/-2.8	13	51	130	<0.001
	2004-2012	-6.5	-8.4/-4.7	10	50	135	<0.001
Total-TEQ[i]	1996-2012	-6.8	-7.3/-6.2	10	72	264	<0.001
	1996-2003	-5.8	-8.1/-3.4	12	52	129	<0.001
	2004-2012	-6.5	-8.4/-4.6	10	52	135	<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.

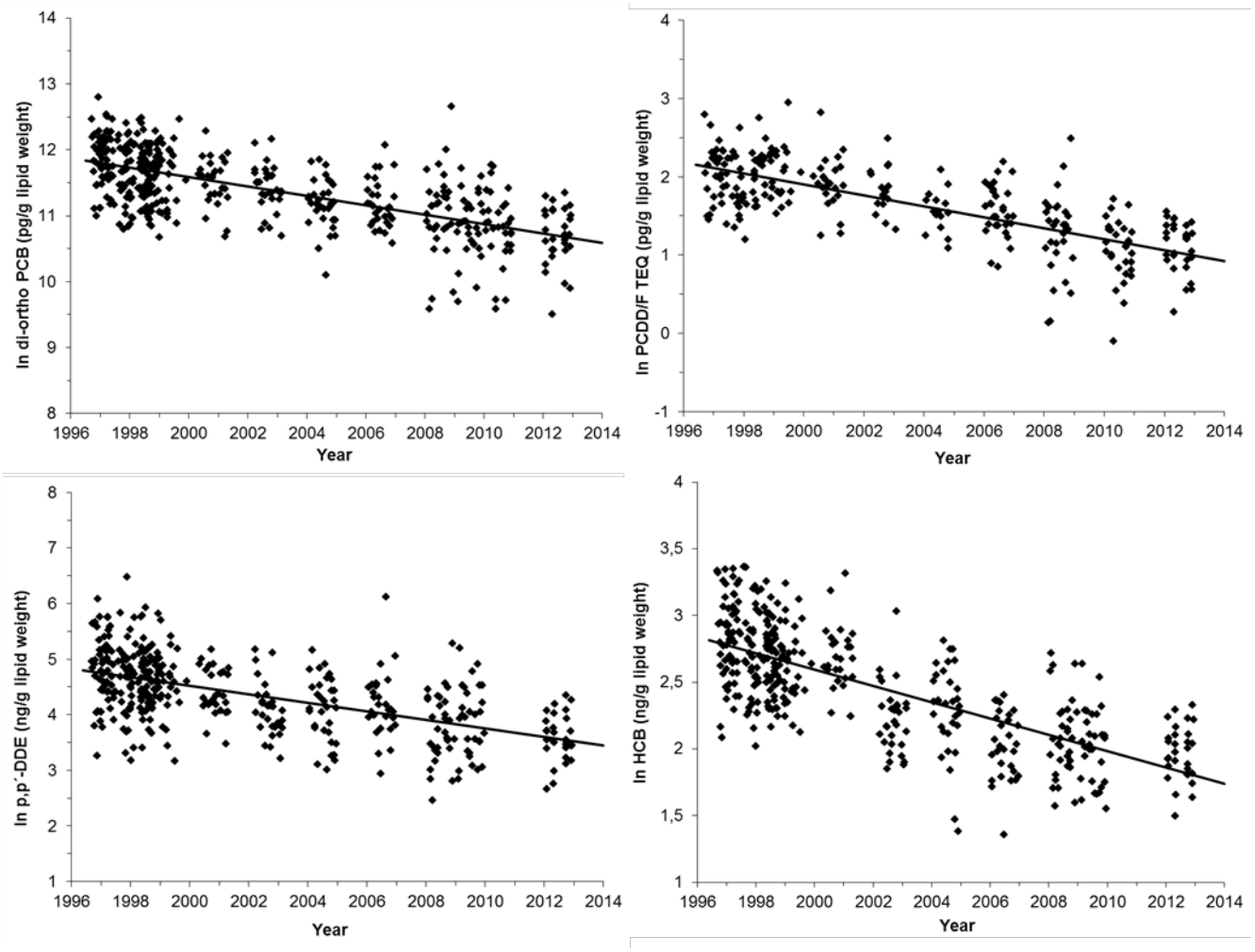

Figure 1. Levels of di-*ortho* PCBs (N=428), PCDD/F TEQ (N=265), *p,p'*-DDE (N=398) and HCB (N=398) in mother's milk from first-time mothers in Uppsala, Sweden in 1996-2012. Each point corresponds to the concentration in a milk sample from an individual woman. The lines represent regression lines obtained from multiple regression analysis including important life-style factors in the model. All temporal trends are significant ($p \leq 0.05$).

Within the health-related environmental monitoring program, temporal trends of PCBs and PCDD/Fs have also been investigated in pooled samples of breast milk from the Stockholm region. The latest update shows that levels of dioxin-like PCBs and PCDD/Fs (TEQs) have decreased with 6-7% per year during the period 1972-2011 (Fång et al. 2013). Especially for PCDDs and dioxin-like PCBs, the trends were faster in the last decade of the study (2002-2011), with declining rates of 10 and 12% per year, respectively. In agreement with our results, the declining rate for PCDD TEQ (10% per year) was faster than for PCDF TEQ (7% per year) in 2002-2011 in the Stockholm study (Fång et al. 2013).

The last update of temporal trends for chlorinated pesticides (1996-2008) in the POPUP cohort showed decreasing levels of *p,p'*-DDE and HCB in breast milk (Lignell et al. 2009). Other compounds were not evaluated at that time. The present results show similar declining rates for the period 1996-2012 as was previously reported for 1996-2008 (Table 5, Figure 1). Levels of *p,p'*-DDE and HCB declined with 7.7 and 6.1% per year, respectively. For both compounds, the declining rates were faster during the first part of the study (1996-2003) than during the second part (2004-2012). This was especially pronounced for HCB. Levels of *p,p'*-DDT, β-HCH, oxychlordane and *trans*-nonachlor also decreased with 6-11% per year during the study period (Table 5), and there were only small differences in declining rates between 1996-2003 and 2004-2012.

Table 5. Percent change in concentrations of chlorinated pesticides per year in mother's milk from primiparous women in Uppsala 1996-2012. 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)**			
p,p'-DDT	1996-2012	-9.5	-11/-8.4	7	46	398	<0.001
p,p'-DDT	1996-2003	-13	-17/-9.4	5	23	247	<0.001
p,p'-DDT	2004-2012	-8.9	-12/-5.6	7	22	151	<0.001
p,p'-DDE	1996-2012	-7.4	-8.3/-6.5	9	47	398	<0.001
p,p'-DDE	1996-2003	-12	-15/-9.3	5	36	247	<0.001
p,p'-DDE	2004-2012	-6.7	-9.6/-3.8	10	27	151	<0.001
HCB	1996-2012	-5.9	-6.4/-5.5	11	66	398	<0.001
HCB	1996-2003	-9.1	-11/-7.5	7	47	247	<0.001
HCB	2004-2012	-3.5	-4.8/-2.1	20	35	151	<0.001
β-HCH	1996-2012	-11	-11/-9.8	6	73	398	<0.001
β-HCH	1996-2003	-12	-14/-9.7	5	45	247	<0.001
β-HCH	2004-2012	-12	-14/-10	6	55	151	<0.001
oxychlordane	1996-2012	-6.7	-7.3/-6.1	10	67	398	<0.001
oxychlordane	1996-2003	-7.2	-9.1/-5.2	9	52	247	<0.001
oxychlordane	2004-2012	-8.1	-10/-6.1	8	56	151	<0.001
trans-nonachlor	1996-2012	-6.4	-7.2/-5.7	10	61	398	<0.001
trans-nonachlor	1996-2003	-6.3	-8.6/-4.0	11	48	247	<0.001
trans-nonachlor	2004-2012	-7.6	-10/-5.0	9	50	151	<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

Decreasing body burdens of *p,p'*-DDE and HCB is supported by decreasing levels in rainbow trout, bovine fat, egg and milk analysed within the Swedish control of contaminants in food from the 1990s up to 2010 (National Food Agency 2012b). Also in agreement with the results for breast milk, levels of *p,p'*-DDE in bovine fat decreased faster in 1991-2000 than in 2001-

2010. In addition, HCB levels in bovine fat decreased between 1991 and 2000 but increased slightly between 2001 and 2010 (National Food Agency 2012b). Taken together, the results indicate that exposure to these chlorinated compounds from food is stabilizing at current levels and that this can be observed as slower downward trends in human body burdens (breast milk levels). Future analyses of breast milk are needed to verify this tendency.

The levels of BDE-47, BDE-99, BDE-100 and sumPBDE decreased with similar rates as were previously reported for the period 1996-2010 (Lignell et al. 2012) (Table 6, Figure 2). The declining rates for BDE-47, BDE-100 and sumPBDE were faster during the latter part of the study (2004-2012). For BDE-153, there is still a significant increase with 1.5% per year (Table 6, Figure 2), but dividing the study period in two parts showed that there was a significant increase between 1996 and 2003, while the levels decreased thereafter.

Table 6. Percent change in concentrations of PBDEs and HBCD per year in mother's milk from primiparous women in Uppsala 1996-2012. 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	1996-2012	-8.6	-9.9/-7.2	8	28	383	<0.001
BDE-47	1996-2003	-2.6	-7.3/+2.3	-	0.2	205	0.3
BDE-47	2004-2012	-17	-21/-12	4	22	178	<0.001
BDE-99	1996-2012	-10	-12/-8.9	6	34	376	<0.001
BDE-99	1996-2003	-16	-20/-11	4	24	205	<0.001
BDE-99	2004-2012	-20	-25/-16	3	26	171	<0.001
BDE-100	1996-2012	-4.8	-6.2/-3.4	14	12	383	<0.001
BDE-100	1996-2003	-2.4	-7.3/+2.7	-	2	205	0.3
BDE-100	2004-2012	-13	-17/-8.4	5	14	178	<0.001
BDE-153	1996-2012	+1.5	+0.5/+2.4	-48[c]	16	383	0.003
BDE-153	1996-2003	+6.2	+2.8/+9.8	-11[c]	19	205	<0.005
BDE-153	2004-2012	-3.3	-6.2/-0.3	21	17	178	0.03
sumPBDE[e]	1996-2012	-5.2	-6.3/-4.1	13	20	383	<0.001
sumPBDE[e]	1996-2003	-2.4	-6.3/+1.7	-	2	205	0.2
sumPBDE[e]	2004-2012	-11	-15/-8	6	19	178	<0.001
HBCD[f]	2002-2012	-2.5	-5.4/+0.4	-	2	144	0.1

[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 2009-2012 were included

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). BDE-209 has only been analysed in samples collected in 2009, 2010 and 2012 and more data points are needed before an evaluation of a temporal trend is possible. However, levels of BDE-209 in breast milk are shown in 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).

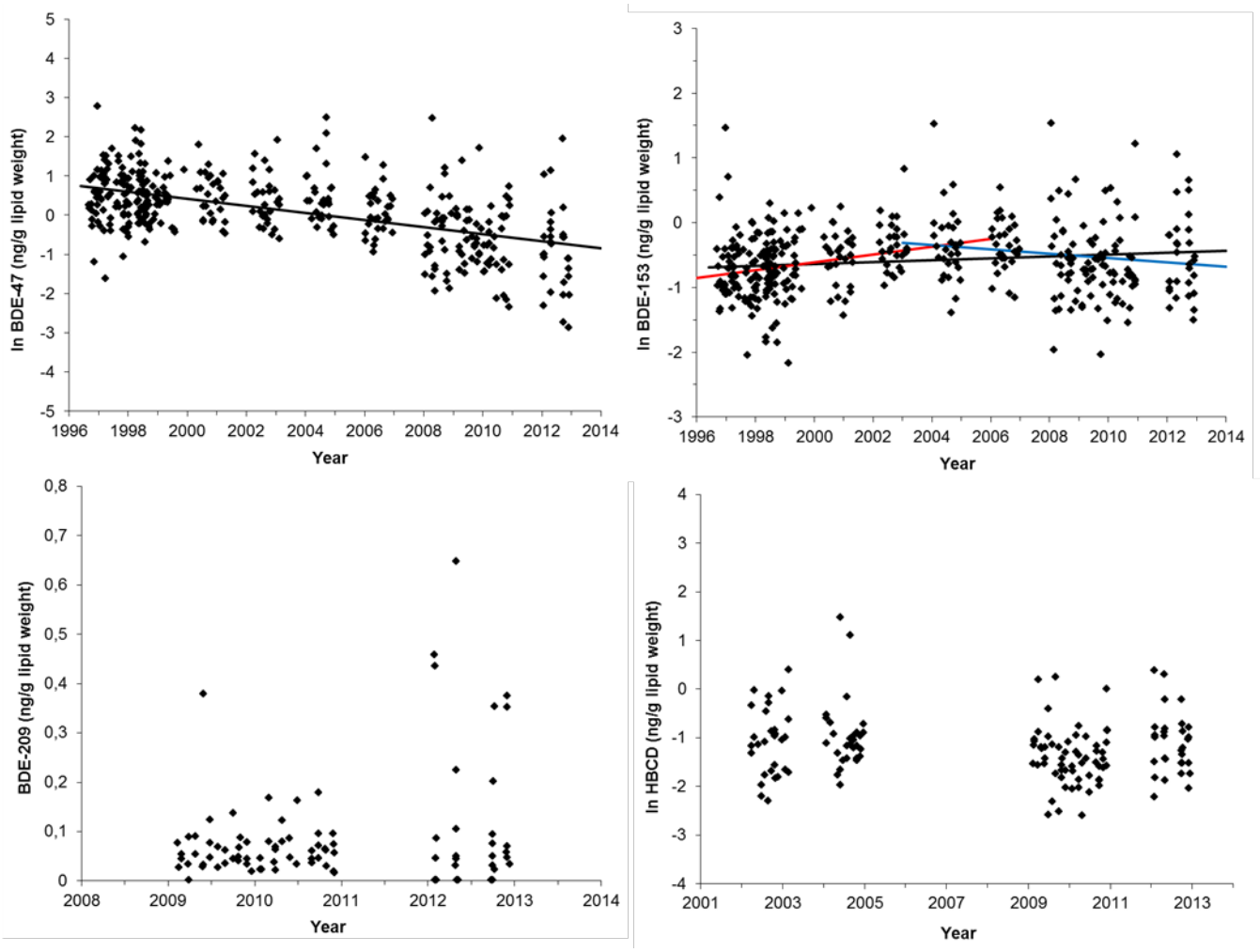

Figure 2. Levels of BDE-47 (N=383), BDE-153 (N=383), BDE-209 (N=89) and HBCD (N=144) in mother's milk from first-time mothers in Uppsala, Sweden. Each point corresponds to the concentration in a milk sample from an individual woman. Note that some of the analytical results for BDE-209 were estimated to zero, and BDE-209 levels are therefore not presented on the log-scale. The lines represent regression lines obtained from multiple regression analysis including important life-style factors in the model. There were significant ($p \leq 0.05$) temporal trends for BDE-47 (negative) and BDE-153 (positive) for the period 1996-2012. For BDE-153, the red regression line was obtained when data from 1996 to 2003 were included (significant positive trend), and the blue regression line when data from 2004 to 2012 were used (significant negative trend). No temporal trend was evaluated for BDE-209 and there was no significant trend for HBCD.

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. To enable an evaluation of a temporal trend, we only used results from years when LOQ was low, i.e. levels in most samples were above LOQ. As a result, the temporal trend analysis only includes results from samples collected in 2002-2004 and 2009-12. Consequently, the trend for HBCD is uncertain and showed a non-significant decrease of 2.5% per year during the period 2002-2012 (Table 6, Figure 2). In the earlier evaluation of data from 2002-2010, the downward trend was significant (Lignell et al. 2012) and more data points are needed to draw conclusions about a temporal trends for HBCD. A study of HBCD in pooled blood serum samples from women in the POPUP-study showed a significant downward 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).

CONCLUSION

The levels of PCBs and PCDD/Fs in breast milk from the POPUP-cohort show decreasing trends that seem to have been constant between 1996 and 2012. Levels of *p,p'*-DDE and HCB have also decreased during the study period, although the results indicate that the trends have been slower during the latter part of the study (2004-2012) than during the earlier part (1996-2003). It is important to continue following levels of chlorinated POPs in breast milk from Swedish mothers in order to further investigate if the levels are stabilizing at current levels or continue to decrease. In contrast to *p,p'*-DDE and HCB, levels of PBDEs (BDE-47, BDE-99, BDE-100) show decreasing trends that have been faster since 2004 than in 1996-2003, probably reflecting the restrictions in the use of these compounds since the 1990s. The levels of BDE-153 show a slightly increasing trend during the whole study period, but the levels have decreased from 2004. More data points are needed to verify the downward trend for BDE-153 and to enable evaluations of temporal trends for BDE-209 and HBCD.

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