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Levels of persistent halogenated organic pollutants (POP) in mother's milk from first-time mothers in Uppsala, Sweden – results from 2008/2010 and temporal trends 1996-2010

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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). Tidstrender för POP i modersmjölk mellan 1996 och 2006/2008 har rapporterats tidigare (Glynn et al. 2007a, Lignell et al. 2008, Lignell et al. 2009a). I följande rapport redovisas halterna av mono-, di- och non-*orto* PCBer, dioxiner och furaner (PCDD/F) samt bromerade flamskyddsmedel (PBDE, HBCD) i modersmjölk från 2008 och 2010 (enligt överenskommelse 215 0906 och 215 0615). Nya data används också för att uppdatera tidstrenderna för dessa ämnen.

Bland PCBerna var mediankoncentrationen i modersmjölk (2008/2010) högst för PCB 153 (32/24 ng/g fett), följt av PCB 180 (16/13 ng/g fett) och PCB 138 (17/14 ng/g fett). PCB 126 var den non-*orto* PCB som hade högst medianhalt (21/19 pg/g fett) och bidrog mest till totalhalten non-*orto* PCB TEQ. Medianhalten för PCDD TEQ (2,4/1,8 pg/g fett) var högre än för PCDF TEQ (1,4/1,1 pg/g fett). Bland de polybromerade difenyletrarna (PBDE) uppvisade BDE-47 (0,76/0,27 ng/g fett) och BDE-153 (0,57/0,45 ng/g fett) de högsta medianhalterna.

Utvärdering av tidstrender för perioden 1996-2010 (multipel linjär regression) visade att halterna av PCB 28 har minskat med i medeltal 4% per år, medan halterna av di-*orto* PCBer, mono-*orto* PCB TEQ och non-*orto* PCB TEQ har minskat med 7-8% per år. Halterna av PCDD TEQ har minskat fortare än halterna av PCDF TEQ (8 respektive 5% per år). De beräknade minskningshastigheterna stämmer överens med de trender som tidigare observerats för perioden 1996-2006/2008 (Lignell et al. 2009a & 2009b).

Resultaten för PBDEer stämmer också överens med det som rapporterats tidigare för perioden 1996-2006/8 (Lignell et al. 2009a & 2009b), dvs. halterna av BDE-47, -99 och -100 har minskat, medan nivåerna av BDE-153 har ökat. Resultaten tyder dock på att minskningen nu går något fortare för BDE-47, -99 och -100 och att halterna av BDE-153 har börjat vända neråt. Trenden för HBCD är osäker eftersom halterna ligger under analysmetodens kvantifieringsgräns i många prover. En utvärdering av tidsperioden 2002-2010 indikerar ändå att halterna har minskat med ca 4% per år. Fler datapunkter på tidstrendskurvorna krävs för att bekräfta trenderna för BDE-153 och HBCD.

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 2006 have been published earlier (Glynn et al. 2007a, Lignell et al. 2008, Lignell et al. 2009a). The trends for PCBs, chlorinated pesticides and PBDEs have been updated with data for 2008 (Lignell et al. 2009b).

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 2010 (according to agreement 215 0906). Data on PCBs and PCDD/Fs in samples from 2008 are also reported (according to agreement 215 0615). The new data is used to establish updated temporal trends for the period 1996-2010. The Swedish EPA also financed sampling of mother's milk in 2009. NFA financed analyses of mono- and di-*ortho* PCBs, PBDEs and HBCD in these samples, and the results are included in the temporal trends.

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 2008 (N=31), 2009 (N=30) and 2010 (N=30). The participating rate was 57, 59 and 42% in 2008, 2009 and 2010 respectively.

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 1996-2006 (N=335) has been described earlier (Glynn et al. 2007a, Lignell et al. 2009a). Mother's milk was sampled from a total of 426 women between 1996 and 2010.

Table 1. Characteristics of the mothers donating mother's milk in 2008, 2009 and 2010.

Variable			**N**	**Mean**	**Median**	**Range**
Age of the mothers (yr)		2008	31	28.6	29.3	17.4-35.8
		2009	30	29.1	29.4	22.2-39.9
		2010	30	30.1	30.2	20.5-41.3
Pre-pregnancy body mass index (BMI, kg/m^2)		2008	31	23.3	22.4	17.8-33.9
		2009	30	23.7	22.8	18.9-39.7
		2010	30	23.3	22.7	18.6-32.2
Weight gain during pregnancy (% of initial weight)		2008	31	24.5	22.6	12.2-44.6
		2009	30	21.5	20.8	12.5-41.3
		2010	30	23.7	25.0	-5.9-37.9
Weight reduction from delivery to sampling (%)[a]		2008	31	9.3	9.4	2.1-14.6
		2009	30	9.7	9.3	1.3-24.6
		2010	30	10.4	10.4	2.6-16.9
			N	**%**		
Education	max 3-4 yr high school	2008	10	32		
	1-3 yr higher education	2008	3	10		
	>3 yr higher education	2008	18	58		
	max 3-4 yr high school	2009	6	20		
	1-3 yr higher education	2009	4	13		
	>3 yr higher education	2009	20	67		
	max 3-4 yr high school	2010	3	10		
	1-3 yr higher education	2010	9	30		
	>3 yr higher education	2010	18	60		
Smoking[b]	Non-smoker	2008	20	64		
	Former smoker	2008	8	26		
	Smoker	2008	3	10		
	Non-smoker	2009	25	83		
	Former smoker	2009	5	17		
	Smoker	2009	0	0		
	Non-smoker	2010	23	77		
	Former smoker	2010	5	17		
	Smoker	2010	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, and women who 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 2008 and 2010 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, 9 tri- to hepta brominated PBDE-congeners (BDE-28, BDE-47, BDE-66, BDE-100, BDE-99, BDE-154, BDE-153, BDE-138, BDE-183), BDE-209 (deca-BDE) (in samples from 2010) and hexabromocyclododecane (HBCD).

PCBs and PCDD/Fs were analysed at the NFA using a newly developed method based on gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) (Aune et al. 2012). In the samples from 2008, mono- and di-*ortho* PCBs were also analysed earlier according to an older method based on gas chromatography with dual electron-capture detectors (Lignell et al. 2009a). Since the limit of quantification (LOQ) is lower with the new method, the new results of the 2008-samples are presented here.

Results obtained with the new method for analysis of PCBs and PCDD/Fs at the NFA have been compared with results obtained earlier on the same samples (Aune et al 2012). The results of this calibration study showed good agreement between previous and new results. However, the new NFA method generated somewhat higher concentrations of non-dioxin like PCBs and mono-*ortho* PCBs and lower levels of PCDFs (see section "Calculations and statistics").

PBDEs and HBCD were analysed at the NFA by gas chromatography/mass spectroscopy/electron-capture negative ionization (GC/MS/ECNI) and detected by the single ion monitoring technique (Lignell et al. 2009a). An updated version of the method was used from 2009, and included BDE-209. LOQs were generally lower with the new method.

In all analyses performed at the NFA, 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 413 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 and 2010. 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).

In a calibration study, where PCBs and PCDD/Fs were analysed in the same samples (N=20 and 14 for PCBs and PCDD/Fs respectively) with both the new and old method, the new method generated somewhat higher concentrations of non-dioxin like PCBs and mono-*ortho* PCBs and lower levels of PCDFs (Aune et al. 2012). More specifically, a paired t-test showed significant differences for PCB 28 (q=median quotient between result with the new and old method=1.2), PCB 105 (q=1.4), PCB 156 (q=1.3), PCB 167 (q=1.2), PCB 28 (q=1.2), PCB 138 (q=1.4), PCB 153 (q=1.3), PCB180 (q=1.2) and PCDF TEQ (q=0.9). The levels of these substances were corrected according to the median quotients between new and old method, and the statistical analyses of temporal of temporal trends were performed with both corrected and uncorrected data.

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, -153 and -154) (Table 2&3). In addition, temporal trends were evaluated for the single compounds PCB 28, BDE-47, BDE-99, BDE-100, BDE-153 and HBCD. PCB 52 and PCB 101 was not included in the sum of di-*ortho* PCBs since the levels of these congeners were below LOQ in most samples, at least in the beginning of the study when LOQs were higher. For the same reason, PCB 114 was not included in mono-*ortho* PCB TEQ and BDE-28, BDE-66, BDE-138 and BDE-183 were not included in sumPBDE. In addition, PCB 123, PCB 157 and PCB 189 were not included in mono-*ortho* PCB TEQ, PCB 81 was not included in non-*ortho* PCB TEQ and BDE-209 was not included in sumPBDE since these congeners only have been analysed in samples from the last few years 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). In the regression analyses, a few outliers (with standardized residuals ≥ 3) were excluded due to their large influence on the results. 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 PCBs, PCDD/Fs, PBDEs and HBCD in milk samples collected in 2008 and 2010 are shown in table 2 and 3.

Among the PCBs, the di-*ortho* congener PCB 153 showed the highest mean and median concentration followed by PCB 138 and PCB 180. The LOQs with the new analytical method are lower than with the previously used method, and the levels of PCB 52, 101, 114 and 157 could be quantified in all samples although the levels were low. The new method quantified PCB 123 and PCB 189 in all samples, but the levels of these congeners were also low. PCB 126 was the non-*ortho* congener with the highest concentration and contributed most to the non-*ortho* PCB TEQ.

Among the PBDEs, BDE-47 and BDE-153 showed the highest mean concentrations. BDE-47 was highest in samples from 2008 while BDE-153 was highest in samples from 2010. In the samples from 2008, the levels of BDE-28, BDE-66, BDE-99, BDE-138, BDE-154, BDE-183 and HBCD were below LOQ in 87-100% of the samples. An updated analytical method, with lower LOQs (Table 3), was used for the samples in 2010. This resulted in fewer samples with levels of BDE-28, BDE-100, BDE-154 and HBCD below LOQ. The considerably lower mean/median levels of BDE-28, BDE-47, BDE-66, BDE-100,

Table 2. Concentrations of PCBs and PCDD/Fs in mother's milk sampled from primipara women in Uppsala during 2008 and 2010. Values below the LOQ were set to ½LOQ in the calculations of means, medians and TEQs. Results for PCBs (not non-*ortho* PCBs) in samples from 2008 have been reported earlier (Lignell et al 2009b), but the results presented here were obtained with a new analytical method.

	2008 (PCB N=31; PCDD/F N=30)					**2010 (N=30)**				
Compound	**Mean**	**Median**	**Min**	**Max**	**N<LOQ**	**Mean**	**Median**	**Min**	**Max**	**N<LOQ**
PCBs (ng/g lipid)										
PCB 28	1.9	1.2	0.50	14	0	1.4	0.98	0.39	7.1	0
PCB 52	0.16	0.12	0.06	0.38	0	0.16	0.13	0.06	0.40	0
PCB 101	0.35	0.31	0.11	1.1	0	0.40	0.33	0.11	1.4	0
PCB 105	1.3	1.0	0.25	4.9	0	0.95	0.90	0.19	1.9	0
PCB 114	0.28	0.21	0.05	1.3	0	0.20	0.20	0.04	0.38	0
PCB 118	6.1	5.1	1.4	20	0	4.6	4.3	1.3	8.8	0
PCB 123	0.05	0.04	0.008	0.16	0	0.04	0.03	0.005	0.25	0
PCB 138	21	17	4.9	85	0	16	14	4.5	38	0
PCB 153	36	32	7.1	157	0	27	24	7.0	62	0
PCB 156	3.6	3.1	0.63	15	0	2.7	2.6	0.58	6.2	0
PCB 157	0.63	0.49	0.10	2.9	0	0.45	0.43	0.09	1.0	0
PCB 167	0.94	0.81	0.18	3.2	0	0.67	0.62	0.18	1.5	0
PCB 180	18	16	2.6	71	0	22	13	3.1	245	0
PCB 189	0.32	0.27	0.04	1.5	0	0.24	0.20	0.05	0.63	0
di-*ortho* PCB[a]	76	69	15	313	-	65	50	15	335	-
mono-*ortho* PCB TEQ[b] (pg/g lipid)	0.36	0.30	0.08	1.3	-	0.27	0.26	0.07	0.54	-
non-*ortho* PCBs (pg/g lipid)										
PCB 77	4.1	2.6	1.2	16	0	4.2	1.9	0.26	54	1
PCB 81	0.92	0.71	0.26	2.8	0	0.95	0.86	0.28	3.2	1
PCB 126	25	21	6.2	75	0	20	19	5.7	47	0
PCB 169	17	14	3.2	87	0	13	12	2.6	25	0
non-*ortho* PCB TEQ[c]	3.0	2.4	0.72	8.6	-	2.4	2.1	0.64	5.3	-
PCDDs (pg/g lipid)										
2,3,7,8-TCDD	0.49	0.48	0.14	1.3	0	0.40	0.40	0.13	0.71	0
1,2,3,7,8-PeCDD	1.3	1.3	0.37	3.8	0	1.0	0.97	0.33	2.0	0
1,2,3,4,7,8-HxCDD	0.46	0.42	0.11	1.3	0	0.37	0.37	0.10	0.64	1
1,2,3,6,7,8-HxCDD	3.9	3.8	1.1	11	0	2.9	2.8	0.67	5.9	0
1,2,3,7,8,9-HxCDD	0.84	0.69	0.33	2.3	0	0.59	0.57	0.32	0.96	0
1,2,3,4,6,7,8-HpCDD	6.1	4.8	1.8	16	0	5.2	4.8	1.5	12	0
OctaCDD	42	36	13	111	0	33	25	9.6	86	0
PCDD TEQ	2.4	2.4	0.71	5.9	-	1.9	1.8	0.60	3.6	-
PCDFs (pg/g lipid)										
2,3,7,8-TCDF	0.37	0.31	0.07	1.1	0	0.35	0.36	0.07	0.63	0
1,2,3,7,8-PeCDF	0.21	0.17	0.06	0.59	0	0.19	0.16	0.03	0.61	4
2,3,4,7,8-PeCDF	4.3	3.4	1.1	19	0	3.2	3.1	0.75	6.1	0
1,2,3,4,7,8-HxCDF	1.0	0.91	0.34	2.6	0	0.80	0.78	0.30	1.5	0
1,2,3,6,7,8-HxCDF	0.97	0.85	0.29	2.4	0	0.77	0.73	0.21	1.4	0
1,2,3,7,8,9-HxCDF	0.05	0.04	0.01	0.42	5	0.05	0.04	0.01	0.10	19
2,3,4,6,7,8-HxCDF	0.56	0.44	0.11	1.7	0	0.45	0.42	0.14	0.89	0
1,2,3,4,6,7,8-HpCDF	2.9	1.1	0.45	51	0	1.1	0.97	0.25	3.0	0
1,2,3,4,7,8,9-HpCDF	0.08	0.05	0.01	0.7	1	0.09	0.06	0.02	0.47	17
OctaCDF	0.15	0.12	0.04	0.54	0	0.20	0.13	0.03	1.1	15
PCDF TEQ	1.6	1.4	0.40	6.1	-	1.2	1.1	0.30	2.2	-
PCDD/F TEQ[d] (pg/g lipid)	4.0	3.9	1.1	12	-	3.1	3.0	0.90	5.6	-
TOTAL-TEQ[e] (pg/g lipid)	7.4	6.7	2.0	22	-	5.8	5.2	1.6	10	-

[a]sum of PCB 153, 138 and 180

[b]sum of PCB 105, 118, 156, 167 TEQs based on 2005 WHO TEFs (Van den Berg et al. 2006).

[c]sum of PCB 77, 126, 169 TEQs based on 2005 WHO TEFs (Van den Berg et al. 2006).

[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 2008 and 2010. Values below the LOQ were set to ½LOQ in the calculations of means, medians and sumPBDE. Results for the samples from 2008 have been reported earlier (Lignell et al 2009b). Samples from 2010 were analysed with an updated version of the analytical method with lower LOQs. Levels below LOQ were reported for the samples from 2010 and calculated results using these levels are presented in brackets ([ ]).

Compound	**Mean**	**Median**	**Min**	**Max**	**N<LOQ [N=0]**
2008 (N=31)					
BDE-28	0.16	0.13	<0.14	0.96	29
BDE-47	1.2	0.76	<0.29	12	13
BDE-66	0.13	0.13	<0.14	<0.44	31
BDE-99	0.23	0.14	<0.14	1.4	28
BDE-100	0.31	0.14	<0.14	2.8	22
BDE-138	0.13	0.13	<0.14	<0.44	31
BDE-153	0.80	0.57	<0.28	4.7	1
BDE-154	0.13	0.13	<0.14	<0.44	31
BDE-183	0.13	0.13	<0.14	<0.44	31
BDE-209	na	na	na	na	na
sumPBDE[a]	2.7	1.8	0.68	17	-
HBCD	0.33	0.26	<0.29	1.5	27
2010 (N=30)					
BDE-28	0.06 [0.07]	0.03 [0.04]	<0.03 [0.01]	0.47	19 [0]
BDE-47	0.58 [0.64]	0.27 [0.46]	<0.22 [0.10]	2.1	17 [0]
BDE-66	0.02 [0.01]	0.02 [0.006]	<0.03 [0]	<0.09 [0.05]	30 [9]
BDE-99	0.15 [0.11]	0.13 [0.08]	<0.16 [0]	0.48	27 [1]
BDE-100	0.20 [0.20]	0.13 [0.13]	<0.06 [0.03]	1.4	6 [0]
BDE-138	0.02 [0.003]	0.02 [0.002]	<0.03 [0]	<0.08 [0.01]	30 [19]
BDE-153	0.65 [0.65]	0.45 [0.45]	0.21 [0.21]	3.4	0 [0]
BDE-154	0.05 [0.05]	0.04 [0.05]	<0.03 [0.01]	0.11	12 [0]
BDE-183	0.02 [0.01]	0.02 [0.01]	<0.03 [0]	<0.08 [0.04]	30 [1]
BDE-209	0.08 [0.07]	0.07 [0.06]	<0.09 [0.02]	0.18	29 [0]
sumPBDE[a]	1.6 [1.7]	1.2 [1.4]	0.55 [0.47]	6.4	-
HBCD	0.26 [0.27]	0.22 [0.22]	0.07 [0.07]	1.0	1 [0]

[a]sum of BDE-47, -99, -100, -153 and -154; na= not analysed

BDE-138 and BDE-154 in samples from 2010 compared with samples from 2008 is probably mainly because of differences in LOQs between years. Since a large proportion of the analytical results are below LOQ, the reduction of LOQs between 2008 and 2010 has a large influence on the calculated means since ½LOQ was used as an estimated value. PBDE-levels below LOQ were available for breast milk samples from 2010. These reported levels were used in the statistical analyses of temporal trends and are also presented in table 3 (in brackets). The updated analytical method used for samples collected in 2010 included the deca-brominated congener BDE-209. The levels were however low and only quantifiable in

one sample. In contrast, BDE-209 was the PBDE congener with highest mean level in pooled blood serum samples from women in the POPUP-study (1.3 ng/g lipid) (Lignell et al. 2011b). This points out that the partitioning of BDE-209 to mother's milk is limited and that blood serum is a better matrix for analysis of this compound.

Temporal trends

Multiple linear regressions showed that the adjusted mean decrease in concentrations of PCB 28 was 4% per year, while the levels of di-*ortho* PCB, mono-*ortho* PCB TEQ and non-*ortho* PCB TEQ decreased with 7-8% per year (Table 4, Figure 1). These results show that earlier observed declining trends (1996 to 2006/2008) (Lignell et al 2009a & 2009b) have continued after 2006. The decrease in levels of PCDDs and PCDFs is also in agreement with earlier results (Lignell et al. 2009a), and the decline in levels of PCDDs is still faster than the decline in levels of PCDFs. 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 2012) showing declining exposure to PCBs and PCDD/Fs from food.

The new analytical method generated somewhat higher levels of PCB 28, di-*ortho* PCBs and mono-*ortho* PCBs and lower levels of PCDFs than the previously used methods. However, there were no major differences between the temporal trends calculated with data that were or were not corrected for these analytical differences (Table 4). As expected, uncorrected data generated a slower decrease of PCB 28, di-*ortho* PCBs and mono-*ortho* PCB TEQ and a faster decrease of PCDF TEQ than corrected data.

In agreement with earlier results (Lignell et al 2009a and 2009b), the levels of BDE-47, BDE-99, BDE-100 and sumPBDE have decreased during the study period (Table 4, Figure 1). However, the calculated declines are somewhat faster now than previously. For example, the yearly decrease in levels of sumPBDE between 1996 and 2008 was 3.5% (Lignell et al 2009b), and after adding data for 2009-2010 the decrease is now 5% per year. The more rapid declining rates are however difficult to interpret since the analytical method has been improved during the years with decreased LOQs as a result. More data-points are needed to confirm the observed trends.

The previously observed increase in levels of BDE-153 seems to be leveling out. Looking at the whole study period, there is still a significant increase with 1% per year (Table 4, Figure 1). Since the plotted data indicated that the levels increased up to about

2004, multiple regression analysis was also performed on data from 1996 to 2004 and 2004 to 2010 separately. The results showed that there was a significant increase between 1996 and 2004, while the levels have decreased thereafter. It is important to continue following the trend of BDE-153 in breast milk in order to confirm this decreasing trend.

Table 4. Percent change in concentrations of POPs per year in mother's milk from primiparous women in Uppsala 1996-2010. Adjusted for age of the mother, pre-pregnancy BMI, weight gain during pregnancy and weight loss after delivery.

Compound	**Change/year (%)[a]**		**half-time[b]**	**R^{2c}**	**N**	**P**
	Mean	**SE**	**(years)**			
PCB 28	-3.6	0.7	19	10	398	<0.001
PCB 28[corr]	-4.4	0.7	16	12		<0.001
di-*ortho* PCB[e]	-7.0	0.3	10	72	398	<0.001
di-*ortho* PCB[e, corr]	-8.2	0.3	8	75		<0.001
mono-*ortho* PCB TEQ[f]	-6.8	0.3	10	67	398	<0.001
mono-*ortho* PCB TEQ[f, corr]	-7.2	0.3	9	69		<0.001
non-*ortho* PCB TEQ[g]	-7.4	0.4	9	63	269	<0.001
PCDD TEQ	-8.2	0.3	8	77	235	<0.001
PCDF TEQ	-5.4	0.4	12	58	235	<0.001
PCDF TEQ[corr]	-4.7	0.4	14	54		<0.001
PCDD/DF TEQ[h]	-7.3	0.3	9	74	235	<0.001
PCDD/DF TEQ[h, corr]	-7.1	0.3	9	73		<0.001
Total-TEQ[i]	-7.2	0.3	9	73	234	<0.001
Total-[TEQi, corr]	-7.1	0.3	9	73		<0.001
BDE-47	-9.6	0.7	7	32	353	<0.001
BDE-47[incl <LOQ values]	-8.6	0.7	8	31		<0.001
BDE-99	-7.9	0.6	8	32	353	<0.001
BDE-99[incl <LOQ values]	-9.7	0.7	7	35		<0.001
BDE-100	-5.1	0.7	13	14	353	<0.001
BDE-100[incl <LOQ values]	-4.8	0.7	14	13		<0.001
BDE-153	+1.1	0.5	-64[d]	22	353	0.021
BDE-153 1996-2004	+6.3	1.1	-11[d]	30	233	<0.001
BDE-153 2004-2010	-7.0	1.5	9	34	145	<0.001
sumPBDE[j]	-5.5	0.5	12	24	353	<0.001
sumPBDE[k, incl <LOQ values]	-5.3	0.5	13	23		<0.001
HBCD[k]	-4.4	1.7	15	7.6	114	0.011
HBCD[k, incl <LOQ values]	-4.2	1.6	16	5.9		0.012

[a]Percent change (decrease (-) or increase (+)) of the concentrations per year during 1996 to 2010.
[b]The estimated time it takes for the concentrations to be halved in the population.
[c]Coefficient of determination for the regression model
[d]Estimated time for the concentrations to be *doubled* in the population.
[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
[j] sum of BDE-47, -99, -100, -153 and -154.
[k]only results from 2002-03, 2004, 2009 and 2010 were included
[corr]concentrations adjusted for differences between analytical methods (see section Calculations and statistics)
[incl <LOQ values]Reported levels below LOQ (in samples from 2009 and 2010) were used in the statistical analyses (see section Calculations and statistics).

In the analytical method used for HBCD-analysis, LOQ varied during the years. To enable an evaluation of 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 2009-10. Consequently, the trend for HBCD is uncertain but showed a significant decrease of 4% per year during the period 2002-2010 (Table 4, Figure 1).

PBDE and HBCD levels below LOQ were available for breast milk samples from 2009 and 2010. Despite larger uncertainty in these measurements, it is advantageous to use them since they add information about the distribution of data below LOQ. The calculated yearly changes obtained with concentrations below LOQ included were in most cases close to the changes obtained when ½LOQ was use to estimate levels below LOQ (Table 4). However, reported data below LOQ was on average higher than ½LOQ for BDE-47 and lower than ½LOQ for BDE-99 (Table 3). This resulted in a somewhat longer calculated half-time for BDE-47 and a shorter half-time for BDE-99 when levels below LOQ were included (Table 4).

Decreasing levels of PBDEs in humans 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. The downward trend in humans is delayed in comparison with the reduced use and decreasing levels in marine biota (Swedish Museum of Natural History 2012). This may be explained by remaining PBDE-containing products on the market and in homes/workplaces and persistence of the substances in the human body. A recent replacement of old PBDE-containing consumer products on the market is a possible explanation to the increased declining rates that we observe in this study. In agreement with our results, Swedish market basket studies performed in 1999 and 2010 showed that exposure to BDE-47 and BDE-99 from food was significantly lower in 2010 than in 1999 (National Food Agency 2012). In contrast to the decreasing trend of HBCD in breast milk, the market basket study showed no difference in exposure to HBCD from food between 2005 and 2010 (National Food Agency 2012), and the trend for HBCD in the Swedish marine biota is uncertain and depending on location and species studied (Swedish Museum of Natural History 2012).

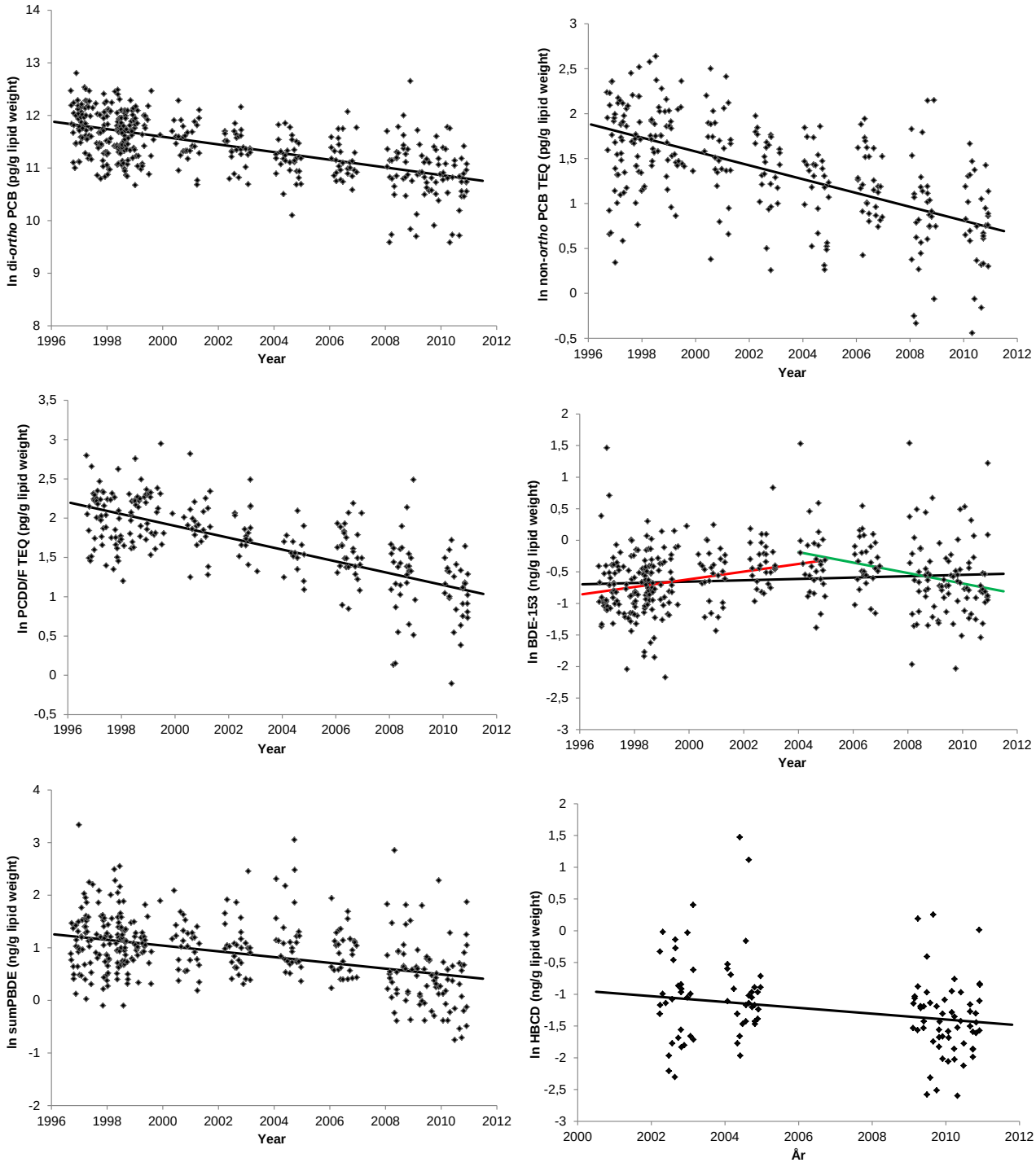

Figure 1. Temporal trends of di-*ortho* PCBs (N=398), non-*ortho* PCB TEQ (N=269), PCDD/F TEQ (N=235), BDE-153 (N=353), sumPBDE (N=353) and HBCD (N=114) in mother's milk from first-time mothers in Uppsala, Sweden. Each point corresponds to the concentration in a milk sample from an individual woman. The lines represent regression lines obtained from multiple regression analysis including important life-style factors in the model. For BDE-153, the red regression line was obtained when data from 1996 to 2004 were included, and the green regression line when data from 2004 to 2010 were used. All trends are significant ($p<0.05$).

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