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Levels of persistent halogenated organic pollutants (POP's) in mother's milk from first-time mothers in Uppsala, Sweden: results from year 2022, chlorinated pesticides from 2020-2022, and temporal trends for the time period 1996-2022

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1996-2022

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) och bromerade flamskyddsmedel (PBDE, HBCDD) i 15 modersmjölksprover insamlade 2022. 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) har analyserats i 3 pooler per år för åren 2020-2022. Nya data har också använts för att uppdatera tidstrenderna för dessa ämnen. Bland PCBerna var medelkoncentrationen i modersmjölk (2022) högst för CB 153 (18 ng/g fett). Medelhalten för PCDD TEQ och PCDF TEQ var för båda 1,0 pg/g fett beräknade med de nya toxiska ekvivaleringsfaktorerna, 2022 WHO TEF. Bland de polybromerade difenyletrarna (PBDE) hade BDE 153 (0,36 ng/g fett) högst medelhalt. Den DDT-förening som hade högst medelhalt i de poolade proverna var *p,p'*-DDE (28 ng/g fett).

Utvärdering av tidstrender för perioden 1996-2022 visade att halterna av di-*orto* PCBer, mono-*orto* PCB TEQ och non-*orto* PCB TEQ har minskat med i medeltal 5-6 % per år. Halterna av PCDD TEQ har minskat fortare än halterna av PCDF TEQ (7 % respektive 2 % per år). När olika tidperioder studerades visade resultaten att minskningen skett snabbare i början av studien 1996-2006/2010 jämfört med den senare delen fram till 2022. För PCDF sågs ingen signifikant minskning 2008-2022. Även om modersmjölknivåerna av total TEQ minskar, hade fortfarande 4 av de 15 kvinnor som provtagits under 2022 nivåer över Efsas modellerade kritiska halt för modersmjölk (beräknat utifrån de tidigare TEFarna från 2005 eftersom Efsa ännu inte har uppdaterat sin riskvärdering med de nya TEFarna).

En jämförelse mellan summahalter av dioxinlika PCB, PCDF och PCDD beräknade med 2005 WHO TEF och 2022 WHO TEF, gjordes för hela studien. Resultaten visade att summahalterna blir lägre med de nya TEFarna från 2022, i medel 38 % för summa PCB/PCDD/F TEQ.

Halterna av BDE 47, BDE 99, BDE 100, och sumPBDE har minskat med 5-11% per år 1996-2022. När studieperioden delades upp i flera delar visar resultaten att halterna sjunker snabbare mot slutet av perioden. BDE 209 har bara analyserats kontinuerligt i modersmjölk sedan 2009 och för första gången

ses en minskning av BDE 209 med i medeltal 5% per år. För BDE 153 och HBCDD ökade halterna signifikant i bröstmjölk 1996-2005/2007 och därefter minskade halterna med i medeltal 3 respektive 8 %.

Halterna av klorpesticider i modersmjölk minskade med 5-10 % per år och för HCB sågs först en minskning för att sedan öka åren 2009-2016 och efter det minska. Resultaten stämmer överens med det som rapporterats tidigare inom POPUP. Fortsatta undersökningar av POPar i modersmjölk kan ge svar på om halterna av PCBer, PCDD/F och HCB håller på att stabiliseras på nuvarande nivåer eller om nivåerna fortsätter att minska.

INTRODUCTION

With funding from the Swedish Environmental Protection Agency (EPA), the Swedish Food Agency (SFA) 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), poly-chlorinated dibenzofurans (PCDFs), brominated flame retardants (e.g. polybrominated diphenylethers (PBDE)), and hexabromocyclododecane (HBCDD) between 1996 and 2021 and chlorinated pesticides (e.g. DDT-compounds) 1996-2019 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, Lignell et al. 2015, Gyllenhammar et al 2017, Gyllenhammar et al. 2021, Hedvall Kallerman et al. 2021, Hedvall Kallerman et al. 2023a), and this is a follow-up.

The following report presents results of analysis of di-*ortho* PCBs, mono-*ortho* PCBs, non-*ortho* PCBs, PCDD/Fs, PBDEs, and HBCDD in mother's milk sampled in 2022 and DDT-compounds (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT), hexachlorobenzene (HCB), hexachlorocyclohexane (β-HCH), chlordane (oxychlor-dane and *trans*-nonachlor) sampled 2020-2022 (according to agreement 215-21-003). The new data is used to establish updated temporal trends for the period 1996-2022. The temporal trends in breast milk are also used as one of the indicators for the environmental quality objectives "A non-toxic environment" (sverigesmiljomal.se).

New TEFs from WHO

The toxicity of dioxins, furans, and dioxin-like PCBs are expressed as a toxic equivalency factor (TEF) and are set in relation to the compound concluded the most toxic, 2,3,7,8-TCDD, which is given the TEF 1. The TEF is multiplied by the concentration of each compound to generate toxic equivalents (TEQs). Recently the TEFs have been revised and the new are referred to as 2022 WHO TEFs (DeVito et al. 2024) compared the older TEFs from 2005, 2005 WHO TEFs (Van den Berg et al. 2006). Several compounds have got lower TEFs in the updated version of WHO TEFs (DeVito et al. 2024). This is in part due to an updated database, and a different method to evaluate the data, than for the 2005 WHO TEFs. The 2005 WHO TEFs was used by the European Food Safety Authority (EFSA), in 2018, when publishing a scientific opinion on health risks of PCDD/Fs and dioxin-like PCBs in food (EFSA 2018). EFSA established a tolerable weekly intake (TWI) of 2 pg TEQ_{2005}/kg body weight/week. When establishing the TWI, a modelling of a lifetime maternal body burden before pregnancy was performed to protect children from negative health effects (taking into account pregnancy and 12 months of breastfeeding). As a result, an estimated safe breast milk level was determined to 5.9 pg TEQ_{2005}/g lipid based on 2005 WHO TEF (Van den Berg et al. 2006). As the EFSA estimated "safe breast milk level" is based on 2005 WHO TEFs, and an update based on 2022 WHO TEFs is lacking at present, comparisons with data from the POPUP-study in the present report are made on data calculated with the 2005 WHO TEFs.

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 2020 to November 2022 (n=90). The participating rate was 50% for the entire study period and 47% in 2020-2022.

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 etc. of the mothers were obtained from questionnaires (Table 1 and 2). The recruitment procedure has been described earlier (Glynn et al. 2007a, Lignell et al. 2009a). Mother's milk was sampled from a total of 909 women between 1996 and 2022 (90 women in 2020-2022). From the participants in 2022, 15 women were randomly selected for analysis. For the chlorinated pesticides, pooled samples were used 2020-2022, with 3 pools per year and 10 randomly selected individuals in each pool.

Table 1. Characteristics of the mothers in the study in 2020-2022 (n=45).

Variable		**N**	**Mean**	**Median**	**Range**
Age of the mother (yr)		45	31.6	31.5	25.5-42.7
Pre-pregnancy body mass index (BMI, kg/m^2)		43	25.1	24.1	18.9-47.3
Weight gain during pregnancy (% of initial weight)		43	18.7	20.3	0.0-37.3
Weight reduction from delivery to sampling (%)[a]		43	8.2	8.8	2.1-13.2
Variable		**N**	**%**		
Education	max 3-4 yr high school	4	9		
	1-3 yr higher education	15	33		
	>3 yr higher education	26	58		

[a]Weight reduction minus birth weight of the child in % of the mothers weight just before delivery.

Table 2. Composition of the pooled samples used for analyses of chlorinated pesticides in mother's milk sampled from primiparous women in Uppsala in 2020-2022.

Year:pool	N/pool	Age (yrs)	BMI[a] (kg/m^2)	Weight gain[b] (%)	Weight reduction[c] (%)
2020:1	10	31 (28-38)	24.4 (18.5-34.0)	18.8 (0.0-29.0)	7.2 (3.6-12.1)
2020:2	10	31 (26-37)	24.6 (20.0-30.9)	16.5 (2.4-28.1)	7.1 (3.3-10.4)
2020:3	10	31 (26-43)	22.7 (18.9-27.0)	21.6 (8.6-33.3)	8.9 (2.1-14.7)
2021:1	10	33 (27-38)	23.9 (21.5-27.4)	19.6 (9.7-29.8)	7.7 (2.8-10.8)
2021:2	10	31 (27-40)	24.2 (19.8-31.2)	21.9 (5.9-31.0)	9.0 (4.4-13.2)
2021:3	10	34 (31-38)	23.1 (18.9-33.7)	22.8 (6.1-37.1)	9.1 (4.9-11.7)
2022:1	10	32 (30-36)	23.0 (20.4-28.3)	24.2 (8.4-50.8)	9.1 (4.1-13.8)
2022:2	10	33 (29-38)	28.1 (21.8-47.3)	18.4 (4.8-20.9)	8.6 (5.2-11.4)
2022:3	10	31 (26-36)	22.3 (18.7-25.4)	25.2 (16.1-37.5)	8.7 (2.3-13.1)

Data are presented as mean and range in brackets. [a]Body Mass Index (BMI) = weight (kg)/height (m)^2. [b]Weight gain during pregnancy (% of initial weight). [c]Weight reduction from delivery to sampling minus birth weight of the child in, % of the mothers weight just before delivery.

Analysis

The compounds that were analysed in the mother's milk samples from 2020 to 2022 were 6 non-dioxin like PCBs (CB 28, 52, 101, 138, 153, 180), 8 mono-*ortho* substituted PCBs (CB 105, 114, 118, 123, 156, 157, 167, 189), 4 non-*ortho* PCBs (CB 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, 99, 100, 153, 154, 183, 209), hexabromocyclododecane (HBCDD) and the chlorinated pesticides HCB, β-HCH, oxychlordane, *trans*-nonachlor, *p,p´*-DDE, *p,p´*-DDD, *o,p´*-DDT, and *p,p´*-DDT.

All analyses of samples from 2020 to 2022 were performed at the SFA. PCBs and PCDD/Fs were analysed using a method based on Aune et al. 2012 with modifications. Briefly the determination was performed with gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) using isotopic dilution technique and single ion monitoring (SIM) at the resolution of 10 000. The clean-up and fractionation of PCBs and PCDD/Fs was performed with a Miura GO-2HT from Miura Co (Japan). PBDEs and HBCDD were analysed by gas chromatography/mass spectroscopy/electron-capture negative ionization (GC/MS/ECNI) using SIM technique (Lignell et al. 2009a). Chlorinated pesticides were analysed using gas chromatography/electron capture detection (GC-ECD).

In all analyses, internal standards were added to samples 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 according to ISO/ICE 17025 for analysis of PCDD/F, PCBs, brominated flame retardants and chlorinated pesticides 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 1996-2022. After this exclusion, a total of 651 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 (medium bound, MB) was taken as an estimated value in the calculations. PBDEs and HBCDD concentrations were adjusted for concentrations in blank samples. PBDE-levels below LOQ were available for breast milk samples from 2009-2022 (and in some samples 2002-2008) and these reported levels below LOQ (adjusted for levels in blank samples) were used instead of half of LOQ. Levels estimated to be zero or negative after blank reduction were in the statistical analyses set to the lowest estimated level found above zero for each compound.

Before the evaluation of temporal trends, POPs were grouped into di-*ortho* PCBs (sum of CB 153, 138, and 180), mono-*ortho* PCB toxic equivalents (TEQ) (sum of CB 105, 118, 156, and 167 TEQs), non-*ortho* PCB TEQ (sum of CB 77, 126, and 169 TEQs), PCDD TEQ, PCDF TEQ and sumPBDE (sum of BDE 47, 99, 100, and 153) (Table 3 and 4). For the chlorinated pesticides, pooled samples were used 2017-2022, with 3 pools per year (Table 5). In addition, temporal trends were evaluated for the single compounds CB 28, CB 153, BDE 47, BDE 99, BDE 100, BDE 153, BDE 209, and HBCDD. BDE 209 was included in the analytical method in year 2009 and has so far only been quantified in samples collected in 2009-2022, and in some samples 2002-2008. However, in the statistical analyses only data from 2009-2022 for BDE 209 were used. Calculated TEQs were based on 2022 WHO TEFs for PCB/PCDD/F (DeVito et al. 2024) but calculations based on the 2005 WHO TEFs (Van den Berg et al. 2006) were also presented in Table 3 and Figure 1 for comparison. The temporal trends were based on 2022 WHO TEFs (Table 6).

Temporal trends were investigated for the whole study period (1996-2022) using multiple linear regressions to analyse associations between concentrations of POP in mother's milk and sampling year. Logarithmically transformed POP-levels were used to present the association between sampling year and POP concentrations as percent change of concentrations per year. In addition, linear regression models with sampling year modeled with restricted cubic splines (henceforth called cubic spline models) were used to analyse associations between log-transformed concentrations and sampling year (shown as a red line in trend graphs, Figure 2-4 and Appendix). Multiple linear regressions and cubic spline models were adjusted for 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. 2011). The variables considered were age of the mother (years), pre-pregnancy body mass index (BMI) (kg/m^2), body weight gain during pregnancy (%), body weight change during the period from delivery to sampling (%), and for PCB, PCDD, PCDF, PBDE, and HBCDD also educational level (Table 1-2 and 6-8). The number of knots for the cubic spline bases were determined by fitting models with 3 to 7 knots and the model that provided the lowest Akaike information criterion (AIC) was preferred. The temporal trends expressed as mean percentage change per year in Table 6-8 were assessed using linear regression models with sampling year modeled with linear splines (henceforth called linear spline models), where the specified knots were placed to approximate the cubic spline models. The linear spline models were adjusted for the same covariates as for the cubic spline models.

The specified knots in the linear spline models were placed at the year after the year with the highest geometric mean levels compared to 1996 (when two or more years had the same level the last year of the two were chosen). For BDE 209, the year 2009 was used for comparison of geometric mean instead of 1996, since data was available only from 2009. If the geometric mean was decreasing throughout the time period, a change in size of the decrease between years were considered to set the specified knots, together with how the trend line in the trend graph was outlined. For all the chlorinated pesticides the last knot was placed at the year 2017 to separate concentrations analysed in pooled serum samples (2017-2022) from the years based on individual serum samples. Statistical analyses were performed using the software package STATA version 17.0.

RESULTS AND DISCUSSION

PCB, dioxin and furan concentrations in mother's milk

Levels of POPs in milk samples collected in 2022 are shown in Tables 3, 4, and 5. In Table 3 concentrations of PCBs, PCDDs, and PCDFs were calculated with the 2022 WHO TEFs with the 2005 WHO TEFs in parentheses. Among the PCBs, the di-*ortho* congener CB 153 showed the highest mean concentration (18 ng/g lipid) followed by CB 180 (10.0 ng/g lipid) and CB 138 (8.5 ng/g lipid) (Table 3). All PCB-congeners could be quantified in all samples, except of one sample for CB 52 and seven samples for CB 123, although the levels of some congeners were very low (e.g. CB 52, 101, 105, 114, 123, 157, 167, 189). CB 126 was the non-*ortho* congener with the highest concentration followed by CB 169, and these two contributed most to the non-*ortho* PCB TEQ_{2022}. Among the PCDD/Fs in Table 3, 1,2,3,4,7,8-HxCDF and 2,3,4,7,8-PeCDF contributed most to the total PCDD/F TEQ_{2022} concentration (19% and 17% respectively), followed by 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD (16% for both) (data not shown). The mean total-TEQ_{2022} level was 3.1 pg/g lipid and PCDFs, and PCDDs, contributed equally to this level (mean 1.0 pg TEQ/g lipid) followed by non-*ortho* PCBs (0.94 pg TEQ/g lipid) and mono-*ortho* PCBs (0.18 pg TEQ/g lipid) (Table 3).

In year 2022, 27% of the women (4 out of 15 women) had breast milk levels above the EFSA estimated safe level of 5.9 pg TEQ_{2005}/g lipid (calculated with 2005 WHO TEFs). Whereas for the entire study period, 1996-2022 it was estimated that 66% of the women had levels above the safe level.

Comparison of PCB/PCDD/F concentrations between 2005 and 2022 WHO TEFs

In Figure 1, mean concentrations of PCB TEQ, PCDD TEQ, and PCDF TEQ (1996-2022) are presented as TEQ_{2005} and TEQ_{2022}. The new 2022 TEFs, results in lower sum TEQs with the largest decrease for PCB TEQ, in mean 48%, followed by PCDF TEQ (38%) and PCDF TEQ (27%). For the total TEQ, the 2022 TEFs results in mean 38% lower breast milk levels compared to the 2005 TEFs. This is in line with the 40-42% lower total TEQ_{2022} in human milk compared to total TEQ_{2005} presented by DeVito at al. (DeVito et al. 2024).

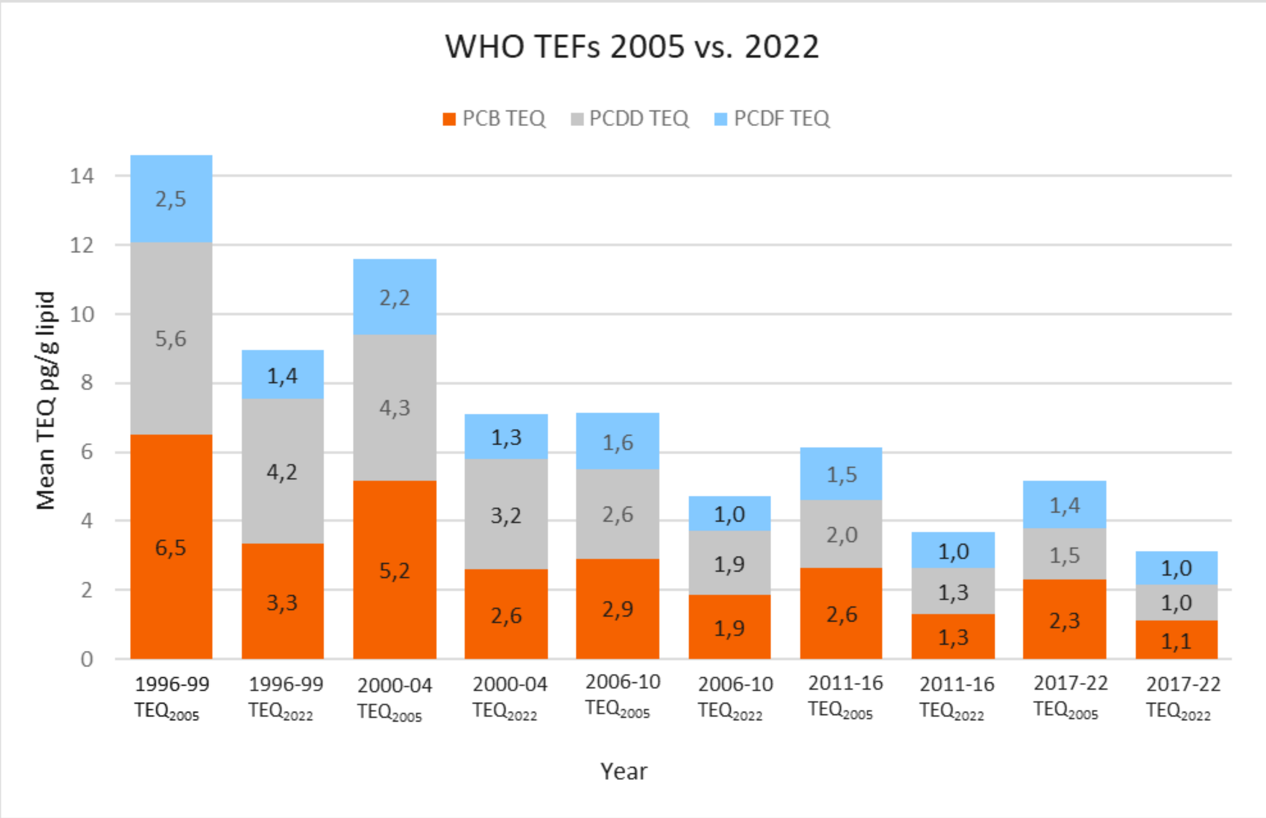


Figure 1. Comparison of mean concentrations of PCB TEQ, PCDD TEQ, and PCDF TEQ using WHO TEFs from 2005 and 2022, from the study period 1996-2022.

Table 3. Concentrations of PCBs and PCDD/Fs in mother's milk sampled from primiparous women in Uppsala in 2022 (n=15). Values <LOQ were set to ½LOQ in the calculations of means, medians and TEQs.

Compound	Mean	Median	Min	Max	n<LOQ
PCBs (ng/g lipid)					
CB 28	1.25	0.66	0.34	8.6	0
CB 52	0.51	0.09	0.04	6.0	1
CB 101	0.20	0.16	0.05	0.55	0
CB 138	8.5	8.1	4.4	12	0
CB 153	18	17	8.1	30	0
CB 180	10	10	4.1	18	0
di-*ortho* PCB[a]	37	36	17	60	-
CB 105	0.61	0.60	0.44	0.89	0
CB 114	0.13	0.13	0.06	0.22	0
CB 118	3.0	2.8	1.9	4.3	0
CB 123	0.03	0.03	<0.03	0.05	7
CB 156	2.0	1.8	0.77	3.9	0
CB 157	0.33	0.30	0.12	0.65	0
CB 167	0.45	0.44	0.22	0.80	0
CB 189	0.19	0.20	0.08	0.38	0
mono-*ortho* PCB $TEQ_{2022\ \&\ 2005}$[b] (pg/g lipid)	0.18	0.18	0.10	0.29	-
non-*ortho* PCBs (pg/g lipid)					
CB 77	1.1	0.92	0.6	2.0	0
CB 81	0.60	0.57	<0.05	1.1	1
CB 126	18	16	11	31	0
CB 169	12	12	4.5	22	0
non-*ortho* PCB $TEQ_{2022\ (2005)}$[c]	0.94 (2.1)	0.88 (2.0)	0.56 (1.2)	1.6 (3.4)	-
PCDDs (pg/g lipid)					
2,3,7,8-TCDD	0.31	0.30	0.16	0.48	0
1,2,3,7,8-PeCDD	0.79	0.85	<0.11	1.4	2
1,2,3,4,7,8-HxCDD	0.36	0.35	0.17	0.61	0
1,2,3,6,7,8-HxCDD	1.8	1.7	0.82	3.2	0
1,2,3,7,8,9-HxCDD	0.51	0.49	0.28	0.88	0
1,2,3,4,6,7,8-HpCDD	3.5	2.2	1.2	7.5	0
OctaCDD	17	14	6.3	30	0
PCDD $TEQ_{2022\ (2005)}$	1.0 (1.4)	1.1 (1.5)	0.45 (0.47)	1.6 (2.3)	-
PCDFs (pg/g lipid)					
2,3,7,8-TCDF	0.33	0.27	0.12	0.79	0
1,2,3,7,8-PeCDF	0.24	0.12	<0.06	0.66	6
2,3,4,7,8-PeCDF	3.5	3.2	1.8	6.0	0
1,2,3,4,7,8-HxCDF	1.3	1.3	0.55	2.1	0
1,2,3,6,7,8-HxCDF	1.3	1.3	0.53	2.4	0
1,2,3,7,8,9-HxCDF	0.09	0.10	<0.07	0.13	14
2,3,4,6,7,8-HxCDF	0.76	0.61	<0.06	2.5	2
1,2,3,4,6,7,8-HpCDF	0.94	0.70	0.19	2.1	0
1,2,3,4,7,8,9-HpCDF	0.05	0.03	<0.02	0.18	9
OctaCDF	0.05	0.02	<0.01	0.34	11
PCDF $TEQ_{2022\ (2005)}$	1.0 (1.4)	0.96 (1.4)	0.43 (0.67)	1.8 (2.6)	-
PCDD/F $TEQ_{2022\ (2005)}$[d] (pg/g lipid)	2.0 (2.8)	1.9 (2.9)	1.1 (1.4)	3.4 (4.8)	-
TOTAL-$TEQ_{2022\ (2005)}$[e] (pg/g lipid)	3.1 (5.2)	3.0 (4.9)	1.9 (3.1)	4.7 (8.0)	-

[a]The sum of CB 153, 138 and 180. [b]The sum of CB 105, 118, 156, 167 TEQs (TEF_{2005} and TEF_{2022} have the same factors). [c]The sum of CB 77, 126, 169 TEQs. [d]The sum of PCDD TEQ and PCDF TEQ. [e]The sum of mono-*ortho* PCB TEQ, non-*ortho* PCB TEQ, PCDD TEQ and PCDF TEQ.

PBDE and HBCDD concentrations in mother's milk

Among the PBDEs, BDE 153 showed the highest mean concentration (0.36 ng/g lipids) followed by BDE 209 (0.32 ng/g lipids), BDE 47 (0.19 ng/g lipids), and BDE 99 (0.12 ng/g lipids) (Table 4). However, the levels of BDE 100, BDE 153, BDE 154, and HBCDD were below LOQ in 9, 3, 6, and 10 of the analysed samples, respectively. The level of BDE 28, and BDE 47 were also below LOQ in most samples whereas BDE 66, BDE 99, BDE 183, and BDE 209 were all below LOQ. Estimated PBDEs and HBCDD concentrations below LOQ, adjusted for concentrations in blank samples, are presented in brackets in Table 4 and were used in the analyses of temporal trends.

Table 4. Concentrations of PBDEs and HBCDD (ng/g lipid) in mother's milk sampled from primiparous women in Uppsala in 2022 (n=15). 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 [0.02]	0.02	<0.04 [0]	0.07	13 [0]
BDE 47	0.19 [0.17]	0.16 [0.17]	<0.3 [0.03]	0.54	14 [0]
BDE 66	0.02 [0.01]	0.02 [0.01]	<0.03 [0.004]	0.03 [0.02]	15 [0]
BDE 99	0.12 [0.03]	0.12 [0.03]	<0.19 [0.01]	0.15 [0.06]	15 [0]
BDE 100	0.05	0.03 [0.04]	<0.04 [0.01]	0.11	9 [0]
BDE 153	0.36 [0.37]	0.31	<0.13 [0.09]	0.99	3 [0]
BDE 154	0.04 [0.05]	0.05	<0.04 [0.02]	0.06	6 [0]
BDE 183	0.02 [0.01]	0.02 [0.01]	<0.03 [0.001]	0.03	15 [0]
BDE 209	0.32 [0.04]	0.33 [0.03]	<0.1 [0.001]	0.42 [0.10]	15 [0]
sumPBDE(4)[a]	0.72 [0.62]	0.65 [0.59]	0.44 [0.22]	1.4	-
HBCDD	0.07 [0.08]	0.05 [0.08]	<0.07 [0.05]	0.12	10 [0]

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

Chlorinated pesticide concentrations in mother's milk

For the chlorinated pesticides, the highest levels were found for *p,p'*-DDE in the pooled samples (range 19-36 ng/g lipids), followed by HCB with a level that was approximately 1/4 of the *p,p'*-DDE level (Table 5). The levels of *p,p'*-DDT, *p,p'*-DDE, oxychlordane and *trans*-nonachlor were lower but above LOQ in all samples, except for one pool from 2022, for β-HCH. Levels of *p,p'*-DDD and *o,p'*-DDT were below LOQ for all samples.

Table 5. Concentrations of chlorinated pesticides (ng/g lipid) in pooled samples of mother's milk from primiparous women in Uppsala in 2020-2022. Each pool includes 10 individual samples.

Year:pool	*p,p'*-DDT	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDT	HCB	β-HCH	Oxy-chlordane	*trans*-nonachlor
2020:1	1.6	32	<0.79	<0.79	7.0	1.4	1.2	2.1
2020:2	1.2	30	<0.74	<0.74	6.7	1.2	1.1	2.1
2020:3	1.2	29	<0.99	<0.99	7.6	1.4	1.4	2.8
2021:1	1.1	25	<0.78	<0.78	5.9	1.5	1.2	2.4
2021:2	1.2	31	<0.64	<0.64	7.6	1.3	1.2	2.4
2021:3	1.2	36	<0.85	<0.85	7.3	1.6	1.2	2.6
2022:1	1.3	24	<1.2	<1.2	7.0	1.4	1.1	2.0
2022:2	1.7	28	<1.1	<1.1	6.3	1.2	0.85	1.9
2022:3	1.1	19	<1.0	<1.0	5.7	<1.0	0.77	1.8

Temporal trends

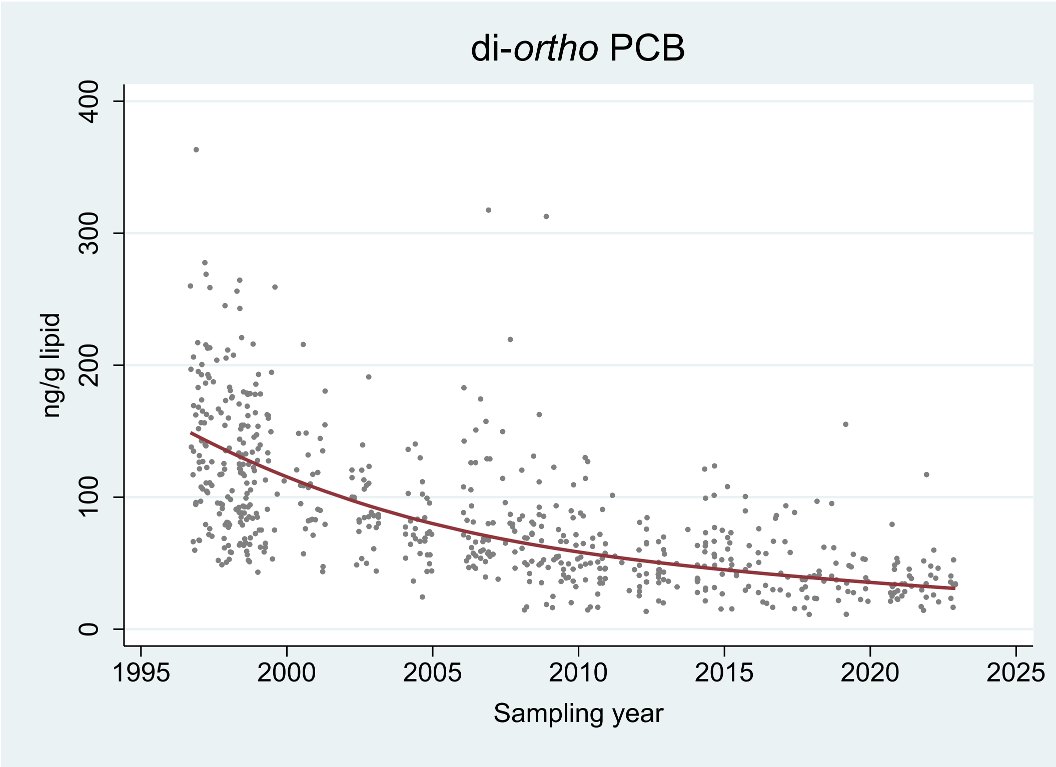
Multiple linear regressions showed that the adjusted mean decrease in concentrations of PCBs were around 5-6% per year during 1996-2022, (Table 6, Figure 2, and Appendix). The mean decrease in levels of PCDD TEQ and PCDF TEQ were 6.6 % and 2.3% respectively, showing a faster declining rate for PCDD TEQ than for PCDF TEQ (Table 6, Appendix). These results are in agreement with previous observed declining trends between 1996 and 2021 within the POPUP study (Hedvall Kallerman et al. 2023a).

When PCBs and PCDD/Fs were analysed before and after specified years using linear spline models, slower decreasing rates were observed towards the end of the study period for all compounds, except of CB 28, in comparison to the entire study period and the beginning of the study. For PCDF TEQ there was no significant change in levels after the year 2007, implying that breast milk levels have not decreased in the POPUP study between 2008-2022 (Table 6, Figure 2, and Appendix). Furthermore, the decline in breast milk levels of PCBs and PCDD/Fs between 1996 and 2022 is in agreement with results from four Swedish market basket studies performed 1999, 2005, 2010, and 2015, showing an annual decline of around 4.5% in the calculated per capita intake of CB 153 and PCDD/Fs from food (National Food Agency 2017). It is important to continue with the temporal trend studies of POPs in breast milk to investigate whether concentrations continue to decrease or are stabilizing at current levels.

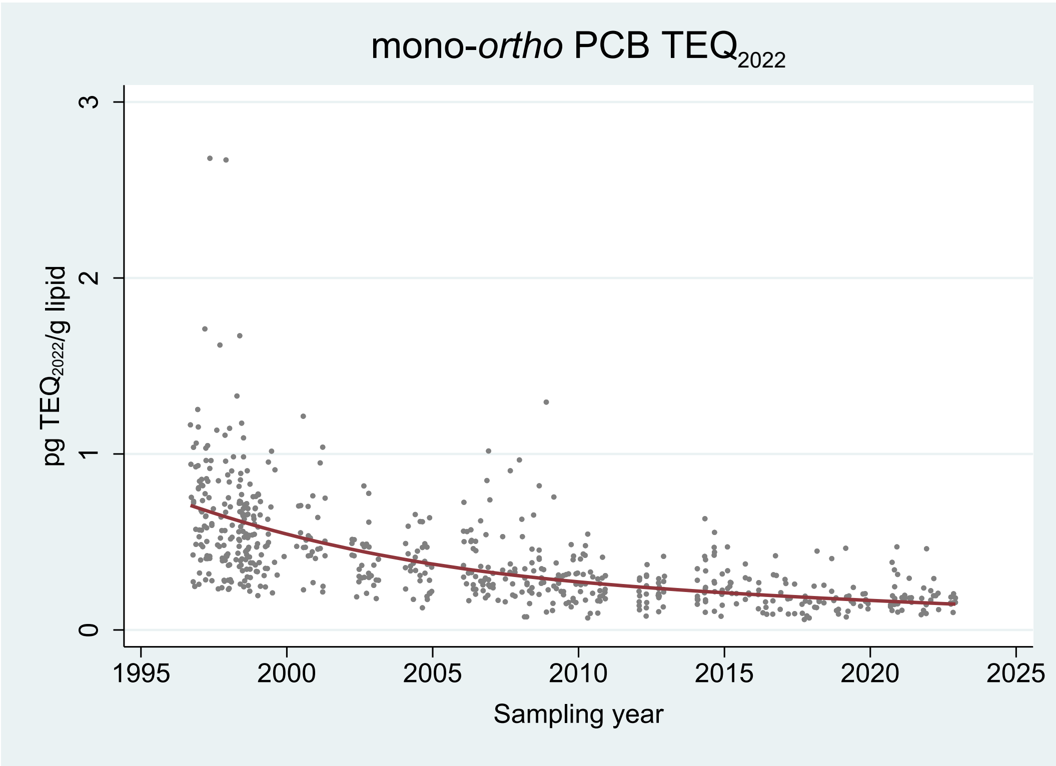
Table 6. Percent change in concentrations of PCBs and PCDD/Fs per year in mother's milk from primiparous women in Uppsala 1996-2022 using linear regression models. The change in concentration per year is presented before and after specified years using linear spline models. Both models are adjusted for age of the mother, pre-pregnancy BMI, weight gain during pregnancy, weight loss after delivery and educational level. Concentrations <LOQ was recalculated to ½LOQ.

Compound	**n**	**Years**	**Mean change per year %**	**95% CI**	**p**	**Years**	**Mean change per year %**	**95% CI**	**p**	**R^{2a}**
CB 28	594	1996-2022	-4.5	-5.2/-3.7	<0.001					22
		1996-2009	-4.1	-5.4/-2.7	<0.001	2010-2022	-5.0	-6.8/-3.3	<0.001	22
CB 153	593	1996-2022	-6.1	-6.5/-5.7	<0.001					70
		1996-2007	-7.4	-8.1/-6.6	<0.001	2008-2022	-4.7	-5.5/-3.9	<0.001	70
di-*ortho* PCB[b]	549	1996-2022	-5.9	-6.3/-5.6	<0.001					70
		1996-2007	-6.8	-7.6/-6.1	<0.001	2008-2022	-4.9	-5.7/-4.1	<0.001	71
mono-*ortho* PCB TEQ[c]	582	1996-2022	-5.9	-6.3/-5.6	<0.001					69
		1996-2005	-7.2	-8.2/-6.3	<0.001	2006-2022	-5.0	-5.7/-4.4	<0.001	70
non-*ortho* PCB TEQ[d]	466	1996-2022	-5.2	-5.7/-4.8	<0.001					60
		1996-2010	-6.9	-7.7/-6.1	<0.001	2011-2022	-2.2	-3.3/-1.1	<0.001	63
PCDD TEQ	431	1996-2022	-6.6	-7.0/-6.3	<0.001					79
		1996-2007	-8.5	-9.2/-7.8	<0.001	2008-2022	-5.0	-5.7/-4.3	<0.001	80
PCDF TEQ	432	1996-2022	-2.3	-2.8/-1.9	<0.001					33
		1996-2007	-4.3	-5.2/-3.4	<0.001	*2008-2022*	*-0.6*	*-1.5/0.3*	*ns*	37
PCDD/F TEQ[e]	431	1996-2022	-5.0	-5.4/-4.7	<0.001					67
		1996-2006	-7.3	-8.2/-6.5	<0.001	2007-2022	-3.4	-4.1/-2.8	<0.001	70
Total-TEQ[f]	430	1996-2022	-5.1	-5.5/-4.8	<0.001					69
		1996-2006	-7.3	-8.1/-6.4	<0.001	2007-2022	-3.6	-4.3/-3.0	<0.001	72

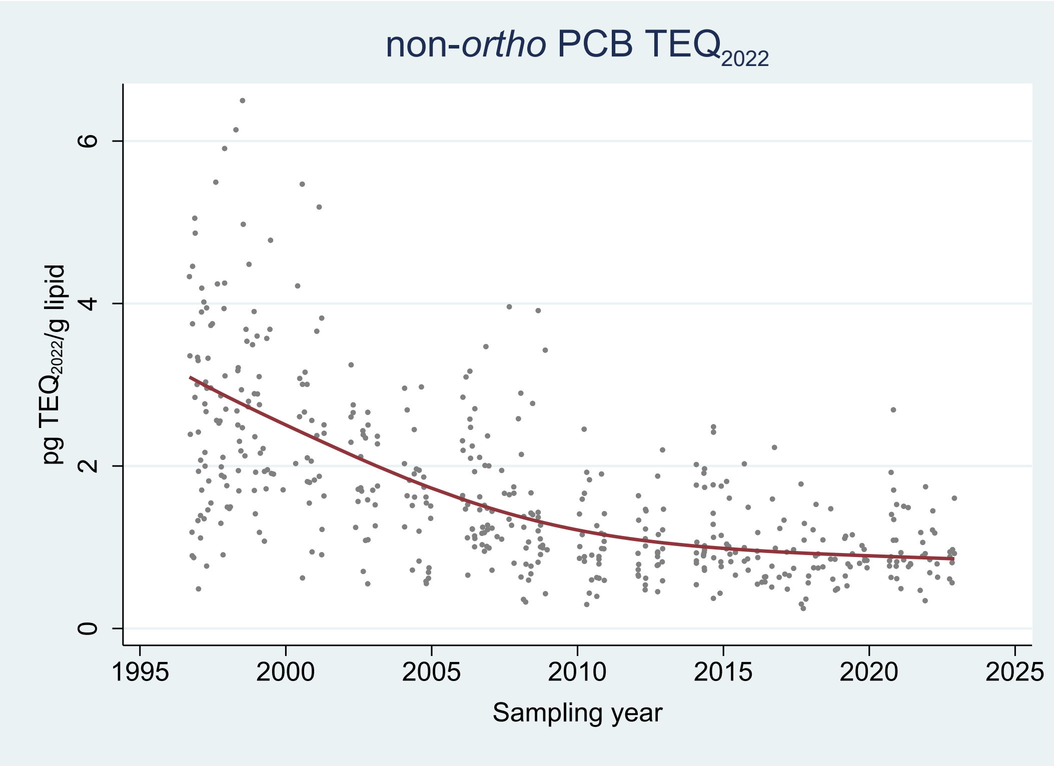
[a]Coefficient of determination for the regression model. [b]sum of CB 153, 138, 180. [c]sum of CB 105, 118, 156, 167 TEQs based on 2022 WHO TEFs (DeVito et al. 2024). [d]sum of CB 77, 126, 169 TEQs based on 2022 WHO TEFs (DeVito et al. 2024). [e]sum of PCDD TEQ and PCDF TEQ. [f]sum of mono-*ortho* PCB TEQ, non-*ortho* PCB TEQ, PCDD TEQ and PCDF TEQ

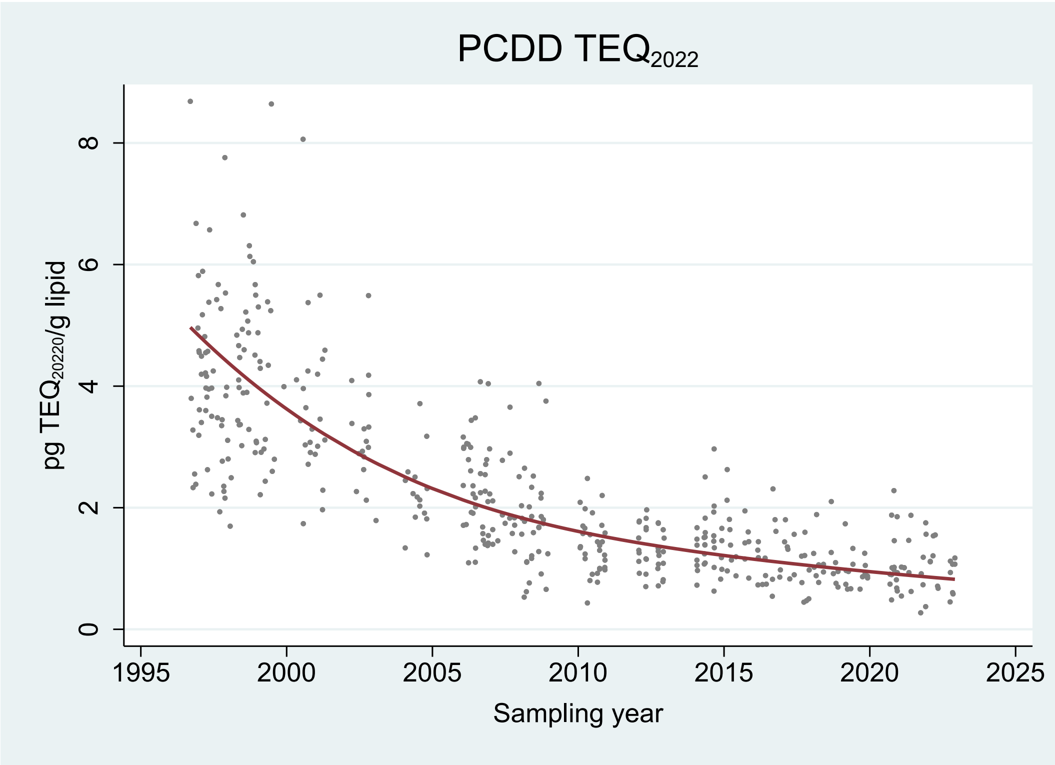


di-*ortho* PCB
ng/g lipid
0
100
200
300
400
1995
2000
2005
2010
2015
2020
2025
Sampling year



mono-*ortho* PCB TEQ2022
pg TEQ2022/g lipid
0
1
2
3
1995
2000
2005
2010
2015
2020
2025
Sampling year


non-ortho PCB TEQ2022
pg TEQ2022/g lipid
0
2
4
6
1995
2000
2005
2010
2015
2020
2025
Sampling year


PCDD TEQ2022
pg TEQ20220/g lipid
0
2
4
6
8
1995
2000
2005
2010
2015
2020
2025
Sampling year

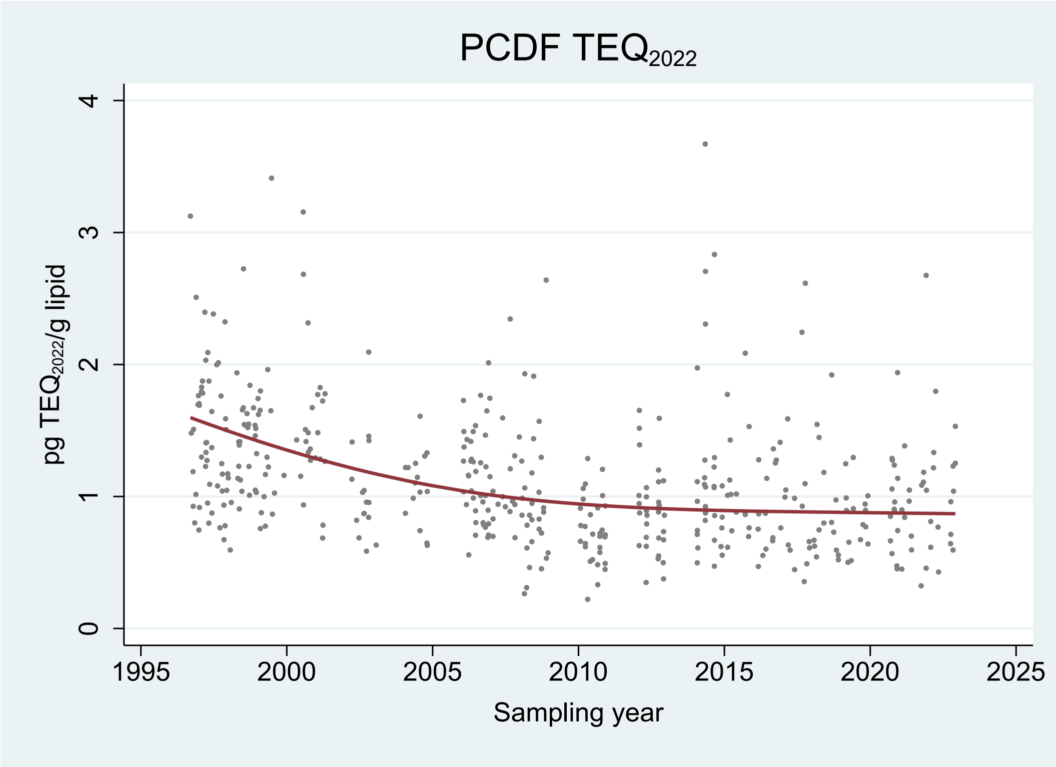


Figure 2. Levels of di-*ortho* PCBs (n=549), mono-*ortho* PCB TEQ_{2022} (n=582), non-*ortho* PCB TEQ_{2022} (n=466), PCDD TEQ_{2022} (n=431), and PCDF TEQ_{2022} (n=432) in mother's milk from first-time mothers in Uppsala, Sweden sampled in 1996-2022. Each point corresponds to the concentration in a milk sample from an individual woman. Concentrations <LOQ were recalculated to LOQ/2. The red lines show the temporal trend from the cubic spline models adjusted for maternal age, pre-pregnancy BMI, body weight gain during pregnancy, body weight change during the period from delivery to sampling, and educational level.

The levels of BDE 47, BDE 99, BDE 100, sumPBDE, and HBCDD have decreased with similar rates in mean 5-11 % per year, as were previously reported for the period 1996-2021 (Hedvall Kallerman et al. 2023a) (Table 7). For the first time, there is a significant decrease for BDE 209 with about 5% per year (2009-2022). This is in agreement with a recent study of BDE 209 in pooled blood serum samples from women in the POPUP-study that showed a significant decreasing temporal trend between 1996 and 2021 (Hedvall Kallerman et al. 2023b).

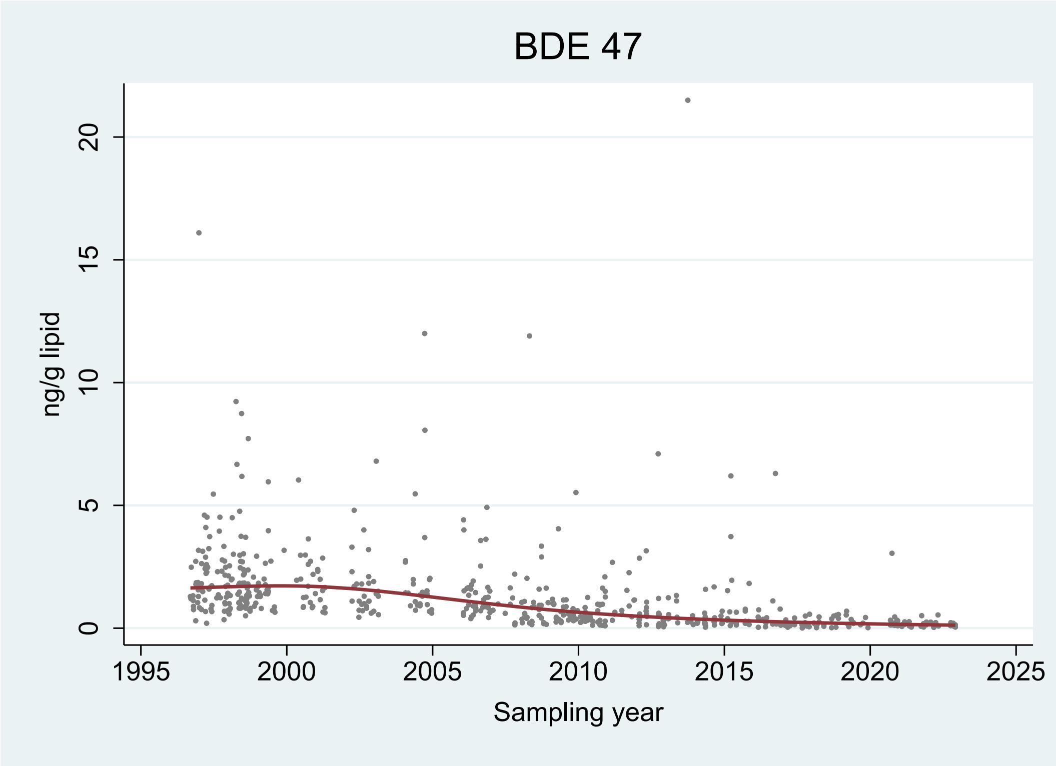
In addition, when PBDEs were studied before and after specified years using linear spline models, faster declining trends were observed towards the end of the study period compared with the entire study period and the beginning of the study (Table 7, Figure 3, and Appendix). For BDE 47 and BDE 99 no significant change in levels were observed in the beginning of the study (1996-2000/2001) but after 2006 the levels decreased with in mean 9-15% per year (Table 7). Also, for BDE 100 the first period of the study, 1996-2005, showed no significant change in levels, but after that a decrease around 12% per year was observed. Similar results were shown for BDE 209, starting at year 2009, with non-significant change during the first years (2009-2013) and a decrease around 9% per year thereafter. For BDE 153 and HBCDD a small increase in levels were seen in the beginning of the study, 1996-2005/2006, however after that the trends were decreasing with about 2 and 8% respectively which was slightly higher than in the latest report (Hedvall Kallerman et al. 2023a).

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 2008 (European Parliament 2011). In agreement with our results, Swedish market basket studies performed between 1999 and 2015, showed that the mean intake of BDE 47 and BDE 99 has decreased around 10% per year during the study period and for HBCDD a 3-fold decrease in median intake between 2010 and 2015 was observed (National Food Agency 2017).

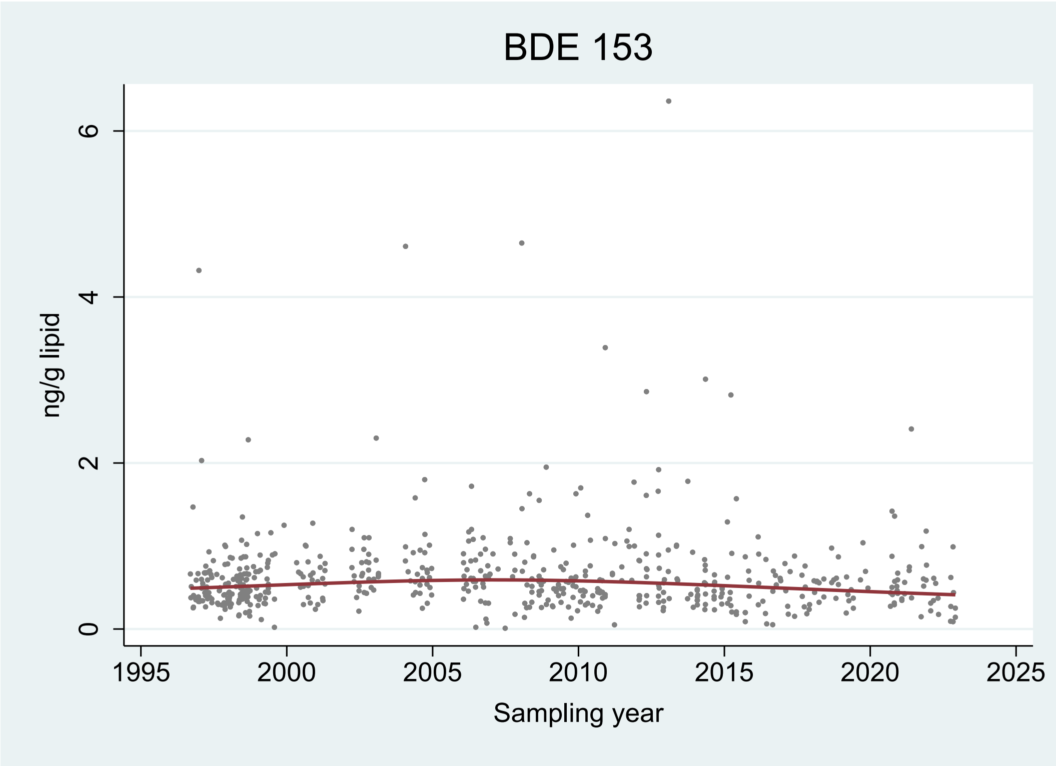
Table 7. Percent change in concentrations of PBDEs and HBCDD per year in mother's milk from primiparous women in Uppsala 1996-2022 using linear regression models. Thereafter the change in concentration per year is presented before and after specified years using linear spline models. Adjusted for age of the mother, pre-pregnancy BMI, weight gain during pregnancy, weight loss after delivery, and educational level. Reported PBDE and HBCDD concentrations, adjusted for concentrations in blank samples, were used instead of LOQ/2 when available.

Compound	n	Years	Mean change per year %	95% CI	p	Years	Mean change per year %	95% CI	p	Years	Mean change per year %	95% CI	p	R^{2a}
BDE 47	561	1996-2022	-10.0	-10.9/-9.1	<0.001									47
		1996-2000	*2.6*	*-3.0/8.5*	*ns*	2001-2010	-11.5	-13.8/-9.1	<0.001	2011-2022	-12.6	-15.1/-10.1	<0.001	50
BDE 99	561	1996-2022	-11.2	-12.3/-10.0	<0.001									40
		1996-2001	*-4.7*	*-9.6/0.5*	*ns*	2002-2010	-15.0	-18.6/-11.3	<0.001	2011-2022	-8.9	-12.3/-5.4	<0.001	41
BDE 100	561	1996-2022	-7.4	-8.3/-6.5	<0.001									33
		1996-2005	*-0.5*	*-2.6/1.7*	*ns*	2006-2022	-11.6	-13.1/-10.1	<0.001					39
BDE 153	561	*1996-2022*	*-0.4*	*-1.1/0.3*	*ns*									8
		1996-2007	1.5	0.1/2.9	<0.05	2008-2022	-2.4	-3.9/-0.8	<0.01					10
BDE 209	262	2009-2022	-4.7	-8.5/-0.9	<0.05									1
		2009-2013	*8.9*	*-1.8/22.8*	*ns*	2014-2022	-9.1	-15.0/-2.7	<0.01					4
sumPBDE[b]	561	1996-2022	-5.8	-6.5/-5.1	<0.001									36
		1996-2006	-3.7	-5.2/-2.2	<0.001	2007-2022	-7.5	-8.8/-6.2	<0.001					37
HBCDD	471	1996-2022	-4.5	-5.4/-3.5	<0.001									15
		1996-2005	2.5	0.2/4.8	<0.05	2006-2022	-7.7	-9.2/-6.3	<0.001					21

[a]Coefficient of determination for the regression model. [b]Sum of BDE 47, 99, 100, 153.



BDE 47
ng/g lipid
0
5
10
15
20
1995
2000
2005
2010
2015
2020
2025
Sampling year



BDE 153
ng/g lipid
0
2
4
6
1995
2000
2005
2010
2015
2020
2025
Sampling year

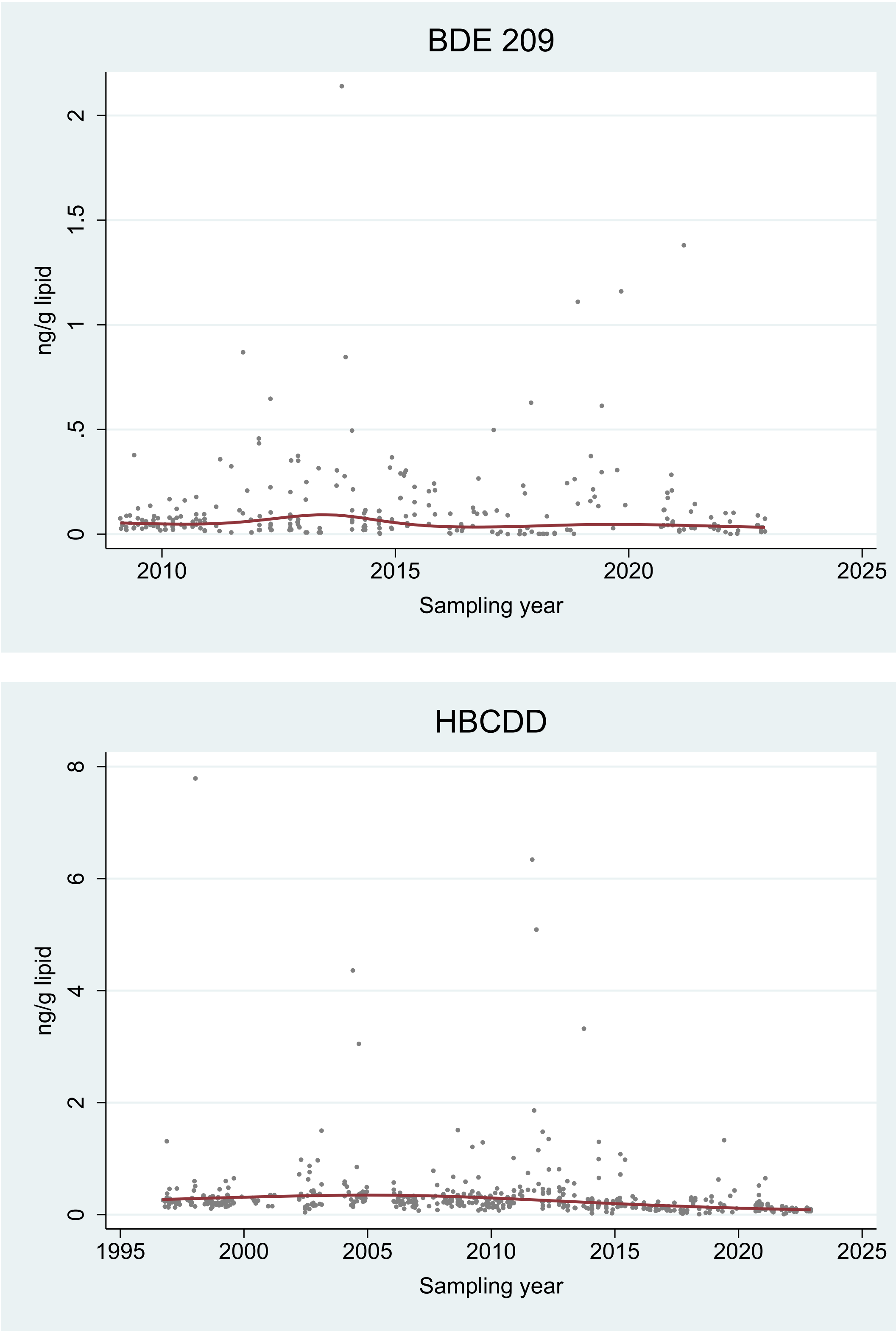


Figure 3. Levels of BDE 47 (n=561), BDE 153 (n=561), BDE 209 (n=262), and HBCDD (n=471) in mother's milk from first-time mothers in Uppsala, Sweden 1996-2022 (2009-2022 for BDE 209). Each point corresponds to the concentration in a milk sample from an individual woman. Reported PBDE and HBCDD concentrations, adjusted for concentrations in blank samples, were used instead of LOQ/2 when available. The red lines show the temporal trend from the cubic spline models adjusted for maternal age, pre-pregnancy BMI, body weight gain during pregnancy, body weight change during the period from delivery to sampling, and educational level.

Significant declining temporal trends were seen for all evaluated chlorinated pesticides during the period 1996-2022 (Table 8). Levels of *p,p'*-DDE and HCB declined with in mean 7 and 5% per year, respectively. Levels of *p,p'*-DDT, β-HCH, oxychlordane and *trans*-nonachlor also decreased with 6-10% per year during the study period. The results are similar as previously reported temporal trends 1996-2019 (Hedvall Kallerman et al. 2021).

The linear spline models showed faster declining trends in the beginning of the study compared to the entire study period and towards the end of the study. Since chlorinated pesticides were analysed in pooled samples from the year 2017 the last part of the study, 2017-2022 were chosen in the models for all compounds. Overall, the yearly declining rates during the latter part of the study are slowing down for all compounds, except for β-HCH that was decreasing around 10% during all studied periods (Table 8). For HCB large differences in rates were observed during the study period, with a decrease around 7% in the beginning of the study (1996-2008), a yearly increase of about 2% between 2009-2016, and after that a decrease of 5% per year. From 2017, the declining rates were not statistically significant for *p,p'*-DDT, oxychlordane, and *trans*-nonachlor (Table 8).

Decreasing body burdens of HCB and *p,p'*-DDE between 1996 and 2022 is supported by the reported mean decline in intake of these substances in the Swedish market basket studies performed between 1999 and 2015, where the mean yearly decline was estimated to 1.5% for HCB and 4.8% for *p,p'*-DDE (National Food Agency 2017), although the overall decrease in the present study is slightly higher for these compounds.

Table 8. Percent change in concentrations of chlorinated pesticides per year in mother's milk from primiparous women in Uppsala 1996-2022 using linear regression models[a]. Thereafter the change in concentration per year is presented before and after specified years using linear spline models. Adjusted for age of the mother, pre-pregnancy BMI, weight gain during pregnancy, and weight loss after delivery. Concentrations <LOQ were recalculated to LOQ/2.

Compound	**N**	**Years**	**Mean change per year %**	**95% CI**	**p**	**Years**	**Mean change per year %**	**95% CI**	**p**	**Years**	**Mean change per year %**	**95% CI**	**p**	**R^2[b]**
p,p'-DDT	504	1996-2022	-8.5	-9.2/-7.7	<0.001									49
		1996-2004	-9.4	-11.4/-7.4	<0.001	2005-2016	-7.7	-9.7-5.8	<0.001	*2017-2022*	*-4.2*	*-9.5/1.3*	*ns*	49
p,p'-DDE	504	1996-2022	-6.7	-7.3/-6.0	<0.001									50
		1996-2002	-8.5	-10.7/-6.2	<0.001	2003-2016	-5.7	-7.1/-4.4	<0.001	2017-2022	-4.7	-9.0/-0.2	<0.05	51
HCB	504	1996-2022	-4.6	-5.0/-4.1	<0.001									46
		1996-2008	-6.7	-7.5/-5.9	<0.001	2009-2016	1.7	0.3/3.1	<0.05	2017-2022	-5.0	-7.4/-2.6	<0.001	53
β-HCH	504	1996-2022	-10.4	-10.9/-10.0	<0.001									81
		1996-2005	-10.4	-11.3/-9.4	<0.001	2006-2016	-10.3	-11.6/-9.0	<0.001	2017-2022	-9.9	-15.5/-3.9	<0.01	80
Oxychlordane	504	1996-2022	-6.5	-7.1/-6.0	<0.001									62
		1996-2009	-7.0	-7.7/-6.2	<0.001	2010-2016	-4.9	-7.6/-2.1	<0.01	*2017-2022*	*-5.2*	*-10.5/0.4*	*ns*	62
Trans-	504	1996-2022	-6.3	-7.0/-5.6	<0.001									47
nonachlor		1996-2005	-7.5	-9.0/-6.0	<0.001	2006-2016	-5.0	-7.3/-2.6	<0.001	*2017-2022*	*-2.8*	*-8.6/3.5*	*ns*	48

[a]Analyses included individual samples until 2016. From 2017 analyses included three pooled samples each year where each pool consisted of 10 randomly selected individual samples. [b]Coefficient of determination for the regression model.

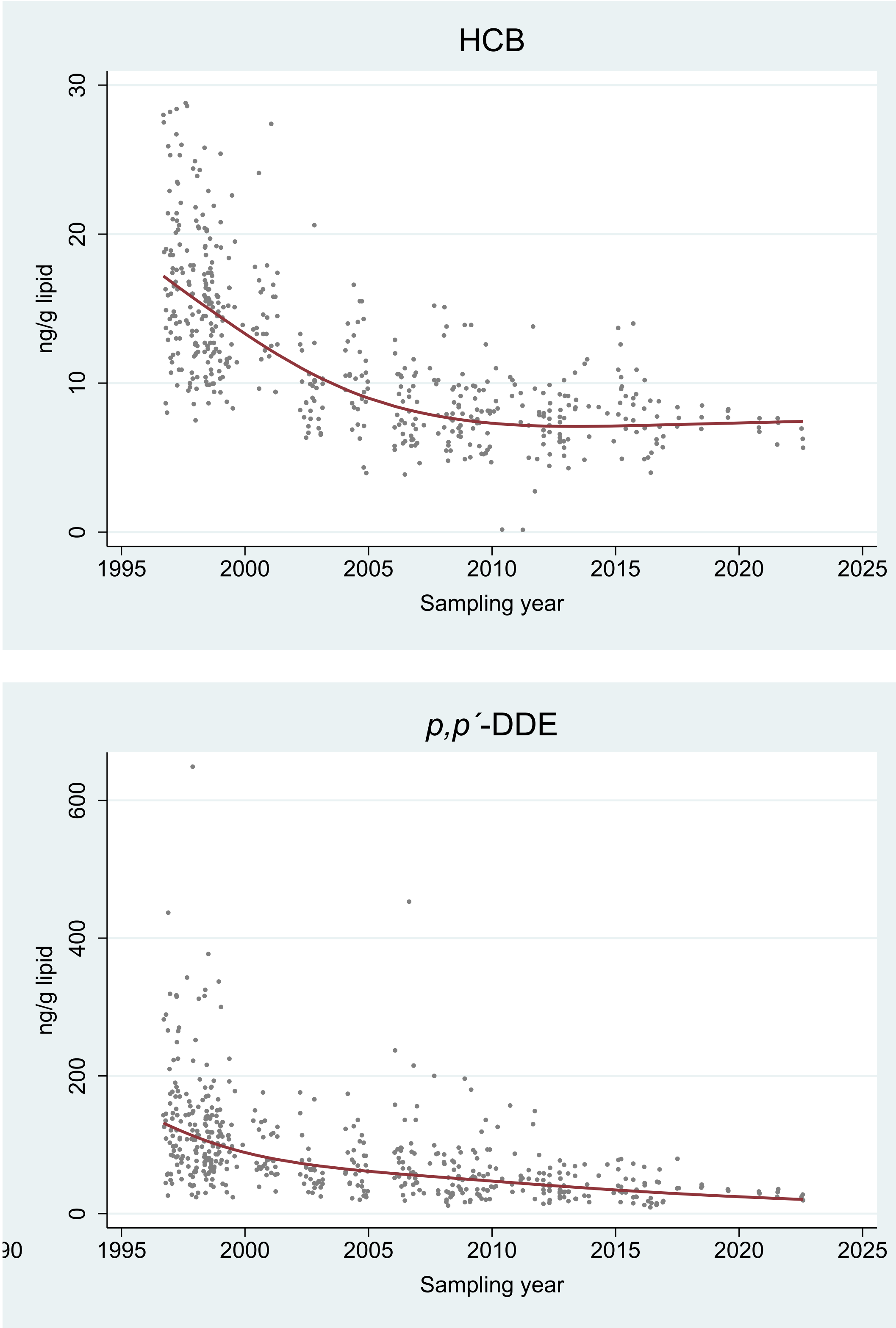


Figure 4. Levels of HCB (n=504), *p,p'*-DDE (n=504) in mother's milk from first-time mothers in Uppsala, Sweden. Each point corresponds to the concentration in a milk sample from an individual woman in 1996-2016. For 2017-2022, each point corresponds to one pool (three pools per year) containing 10 randomly selected individual samples. The red lines show the temporal trend from the cubic spline models adjusted for maternal age, pre-pregnancy BMI, body weight gain during pregnancy, and body weight change during the period from delivery to sampling.

Comparison of concentrations and temporal trends with WHO-report

In 2023, the World Health Organisation (WHO) published a report about POP levels in human milk from their global studies between 2000 and 2019 (Malisch et al. 2023). The POPUP-study has been contributing to the WHO surveys at three times; 2001, 2006 and 2019. Concentrations of most POPs in breast milk samples from POPUP (sampled 2015-2022) were generally lower compared to the last global survey from WHO (sampling period 2015-2019). However, in the WHO-report the upper bound was used for concentrations below LOQ and in the POPUP-study the medium bound was used. For PBDEs in the POPUP-study reported levels below LOQ were used instead of half of LOQ. Therefore, WHO concentrations should be slightly higher than the POPUP concentrations, solely as a result of the different usage of LOQs in the calculations.

The median concentration of dioxin-like PCBs 2015-2022 was slightly lower in the POPUP study (2.2 pg TEQ_{2005}/g lipid) compared to the WHO-report (worldwide 2.6 pg TEQ_{2005}/g lipid, Western Europe and other 3.6 pg TEQ_{2005}/g lipid). For non-dioxin-like PCBs, PCDD/PCDF TEQ_{2005}, and total TEQ_{2005} the median concentrations in the POPUP-study (38 ng/g, 2.9 pg TEQ_{2005}/g, and 5.1 pg TEQ_{2005}/g lipid) were about half of those reported for Western Europe and other (75 ng/g, 6.7 pg/g, and 10.3 pg/g lipid respectively). In the POPUP-study PCDD/PCDF TEQ_{2005} and total TEQ_{2005} were slightly lower than the worldwide concentrations (4.7 pg TEQ_{2005}/g, and 7.2 pg TEQ_{2005}/g lipid respectively) and non-dioxin-like PCBs was slightly higher than the worldwide concentrations (32 ng/g lipid) in the WHO-survey 2015-2019.

The median concentration of the sum of BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 183 in POPUP samples 2015-2022 (0.85 ng/g lipid) was in line with the level in the WHO-survey 2015-2019 for Western Europe and other (1.0 ng/g lipid) and slightly lower than worldwide (1.4 ng/g lipid). The concentration of BDE 209 in human milk from POPUP were in median below LOQ and the median from adjusted blank levels 0.06 ng/g lipid were lower compared to the median of 0.21 ng/g lipid for the sample from 39 countries sampled 2016-2019 within the WHO survey. For HBCDD the concentrations in POPUP were far lower (0.13 ng/g lipid) than in the WHO-report (worldwide median 0.5 ng/g lipid and Western Europe and other where nearly all samples were < 2.0 ng/g lipid).

The concentrations of HCB, β-HCH, and sum of DDT and DDE, in human milk sampled 2015-2022 in POPUP (median 7.7 ng/g, 1.8 ng/g, and 34 ng/g lipid respectively) were about half of the concentrations in the WHO survey 2015- 2019 for Western Europe and other 10.4 ng/g, 3.6 ng/g, and 57 ng/g lipid, respectively. Compared to the median concentrations worldwide for the same compounds (3.3 ng/g, 2.4 ng/g, 125 ng/g lipid respectively) the POPUP-study have higher concentrations of HCB but lower β-HCH and sum of DDT and DDE. Oxychlordane and *trans*-nonachlor in POPUP 2015-2022 (median 1.3 ng/g and 2.7 ng/g lipid respectively) were within what was stated as background range in the WHO-report (5 ng/g lipid) for the so called, chlordane complex, defined as the sum of *cis*-and *trans*-chlordane and oxychlordane. However, the median concentration of the chlordane complex in the WHO-report for 43 samples 2016-2019 was 0.86 ng/g lipid which was lower than in the POPUP-study during 2015-2022.

The decreasing temporal trends for POPs during 1996-2022 in the POPUP study were similar with the findings of the WHO-surveys from 2000 to 2019 and the largest differences were shown for non-dioxin-like PCBs (in mean -6% per year in POPUP compared to -9% worldwide).

CONCLUSION

The levels of PCBs and PCDD/Fs in mothers milk from the POPUP-cohort showed over all decreasing temporal trends between 1996 and 2022. The levels of PCDD TEQ have decreased faster than the levels of PCDF TEQ (mean 7% and 2% per year, respectively). When different time periods were evaluated, the results showed that the decrease in levels occurred faster at the beginning of the study 1996-2006/2010 compared to the latter part until 2022. For PCDF, no significant decrease was seen in 2008-2022. Although breast milk concentrations of total TEQ is decreasing, still 4 out of 15 women (27%), sampled 2022, had levels above the EFSA estimated safe level (using 2005 WHO TEFs as EFSA has not revised the Sceintifig opinion on dl-PCB and dioxins yet). A comparison between new and old TEFs showed that total TEQs in breast milk samples from 1996-2022 calculated with the 2022 WHO TEFs were in mean 38% lower than with the 2005 WHO TEFs. However, a lower TEQ does not mean that the risk has decreased for dl-PCBs and dioxins and an updated TWI from EFSA is warranted. Further, BDE 47, BDE 99, BDE 100, and sumPBDE showed decreasing trends 1996-2022. When the study period was divided into two or three parts, the results show that the levels decreased faster towards the end of the period compared to the beginning. The temporal trend of BDE 209 was for the first time significant with a decrease of about 5% per year 2009-2022 and with a decrease of 9% after 2014. For BDE 153 and HBCDD the levels in breast milk increased significantly from 1996-2005/2007 and then the levels decreased on average with 3 and 8% respectively. Levels of chlorinated pesticides have decreased during the study period, although slower during the latter part of the study, except for β-HCH having decreased at the same rate throughout the entire study period. The decrease of the temporal trend of HCB was interrupted by a period of an increase during 2009-2016. Breast milk levels of POPs in the POPUP-study were in general lower compared to median levels from the whole world within the surveys performed by WHO, but the decreasing trends were similar. It is important to continue monitoring concentrations of POPs in breast milk from Swedish mothers in order to follow and further investigate if the concentrations of PCBs, PCDD/Fs, and HCB are stabilizing at current levels or continue to decrease.

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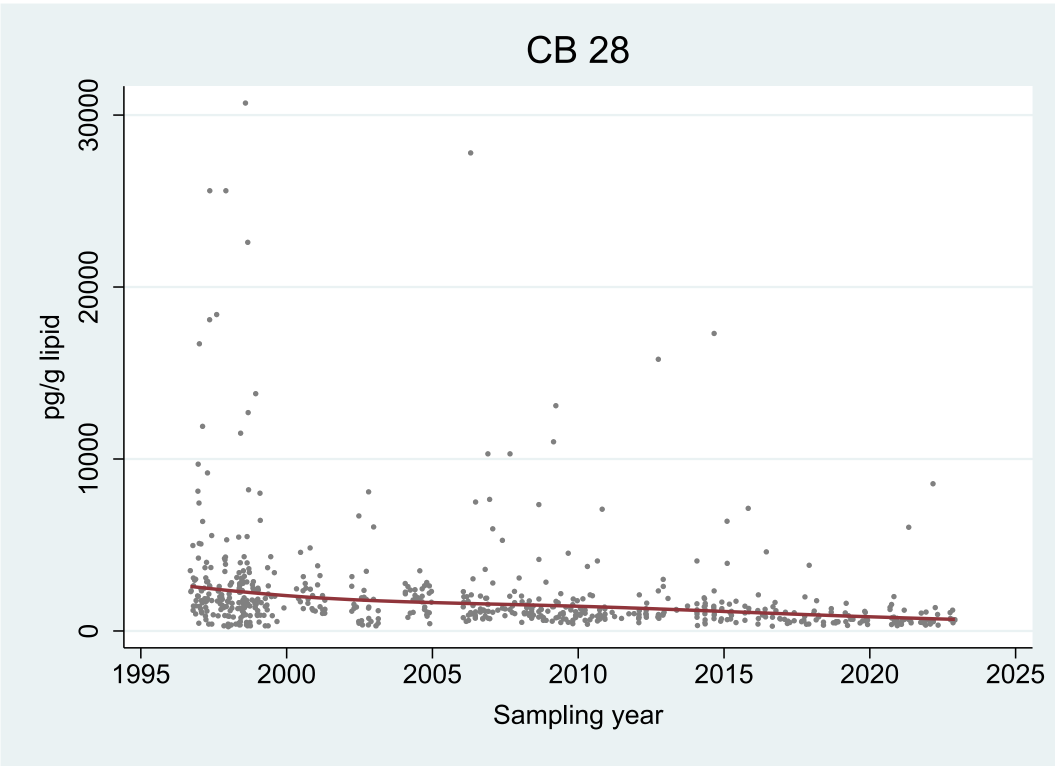
APPENDIX

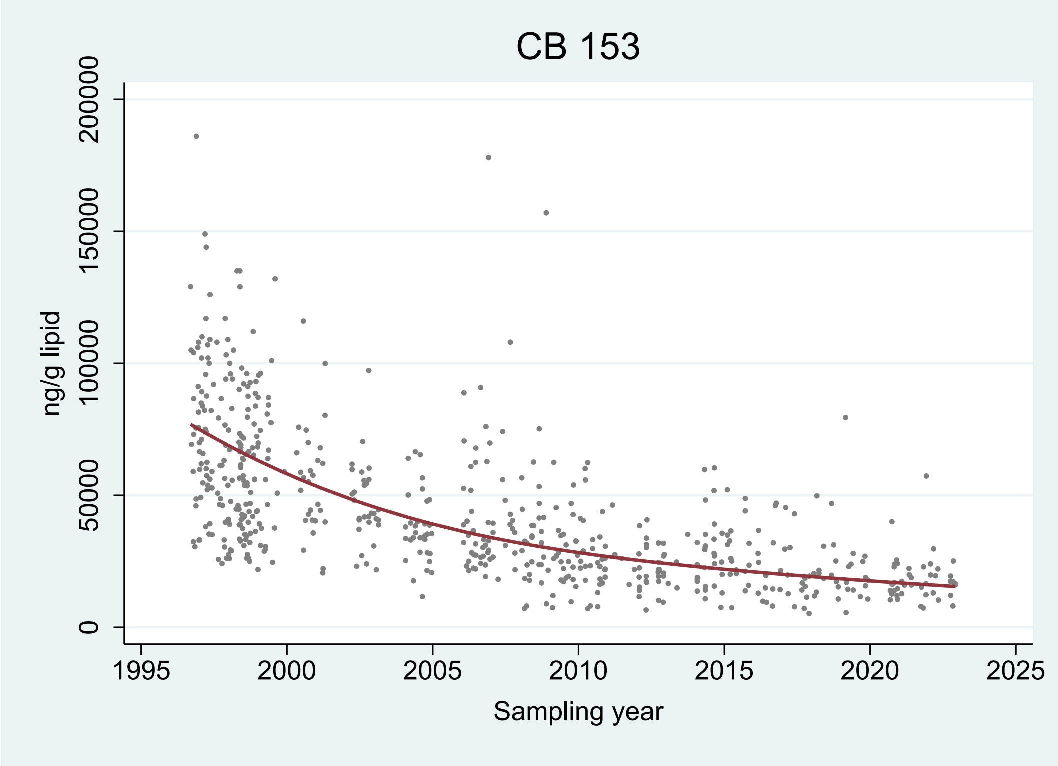
Additional figures

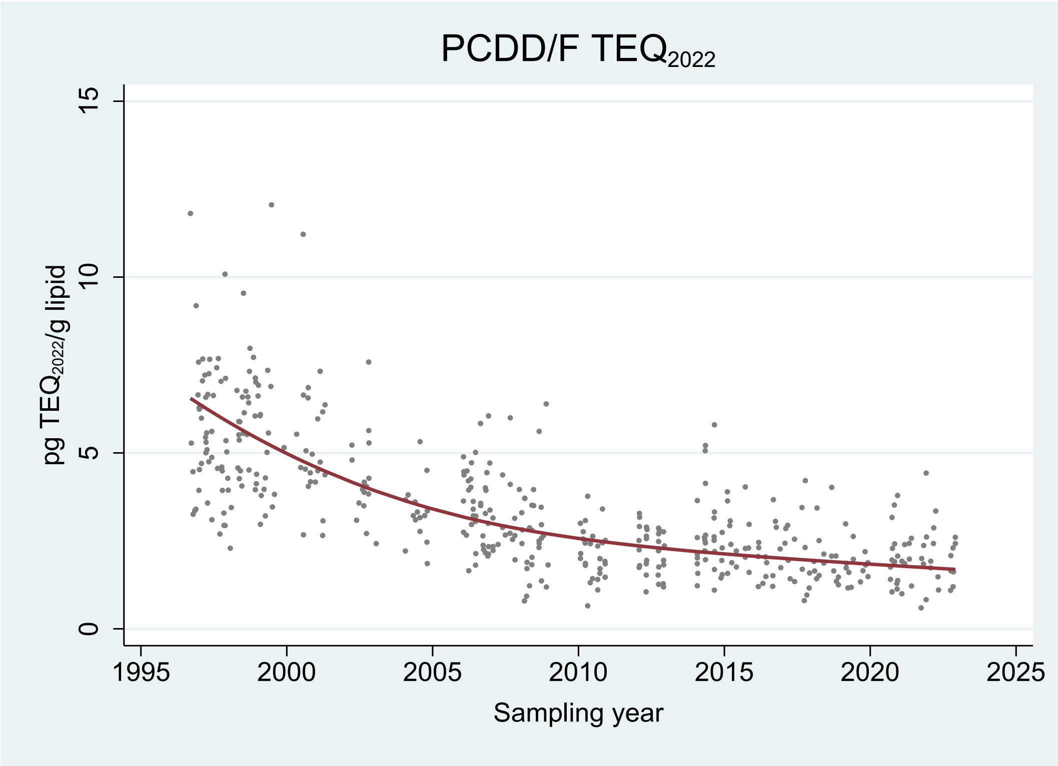
The figures below are not included in the report.

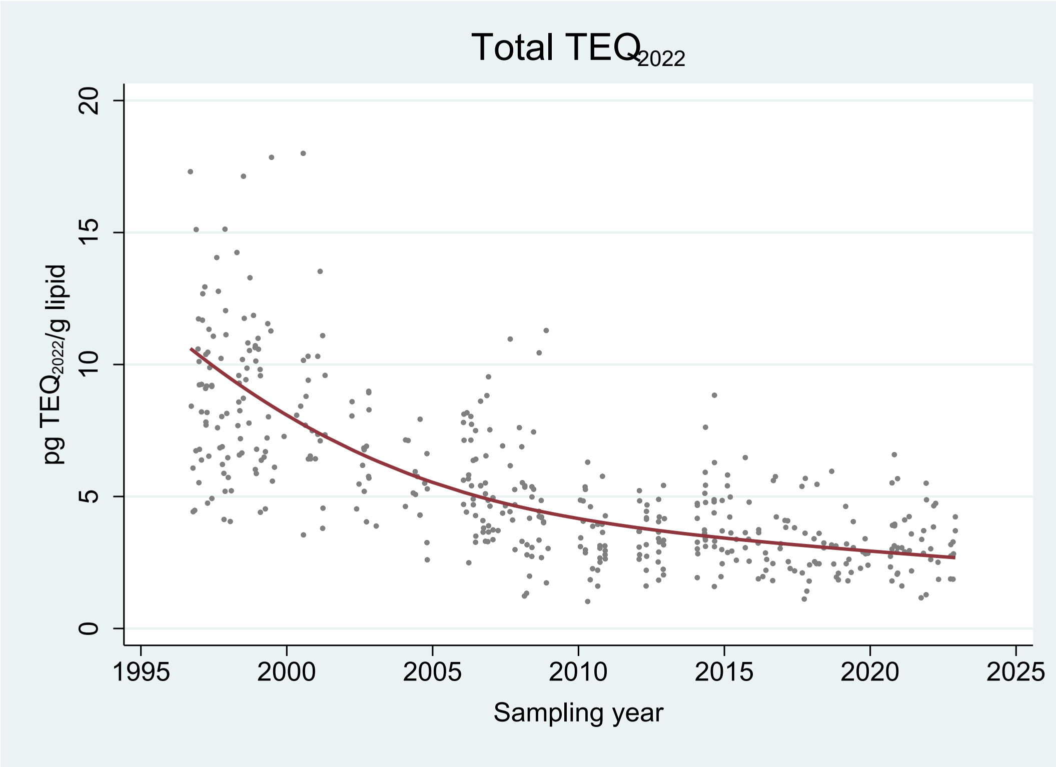
Levels of PCB 28 (n=594), PCB 153 (n=593), PCDD/F TEQ_{2022} (n=431), total TEQ_{2022} (n=430), BDE 99 (n=561), BDE 100 (n=561) and sumPBDE (the sum of BDE 47, 99, 100 and 153) (n=561) in mother's milk from first-time mothers in Uppsala, Sweden in 1996-2022. Each point corresponds to the concentration in a milk sample from an individual woman. Levels of *p,p'*-DDT (n=504), β-HCH (n=504), oxychlordane (n=504) and *trans*-nonachlor (n=504) were also from individual samples during 1996-2016. However, from 2017 three pooled samples were used for each year where each pool contains 10 randomly selected individual samples.

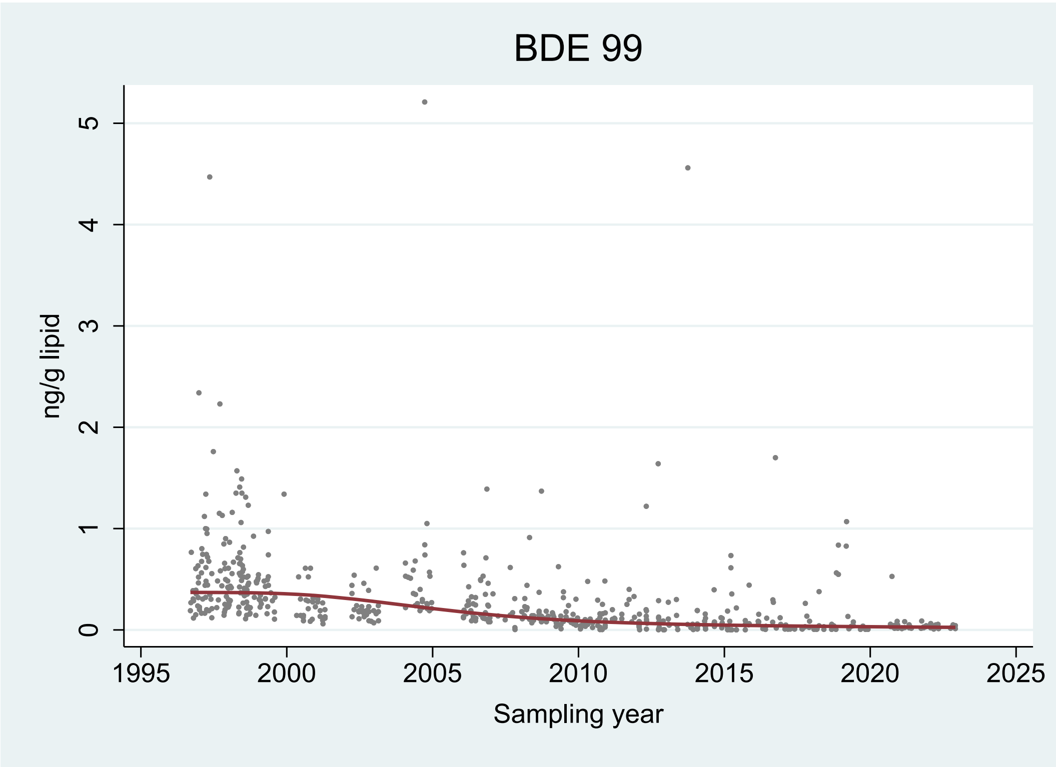
Concentrations <LOQ was recalculated to LOQ/2. PBDE-levels were adjusted for levels in blank samples. Concentrations below LOQ were available for breast milk samples from 2009-2022 (and in some samples 2002-2008) and these reported levels below LOQ (adjusted for levels in blank samples) were used instead of half of LOQ. Levels estimated to be zero or negative after blank reduction were in the statistical analyses set to the lowest estimated level found above zero. The red lines show the temporal trend from the cubic spline models adjusted for maternal age, pre-pregnancy BMI, body weight gain during pregnancy, body weight change during the period from delivery to sampling, and educational level. However, in analyses of chlorinated pesticides, educational level was not included in the model.

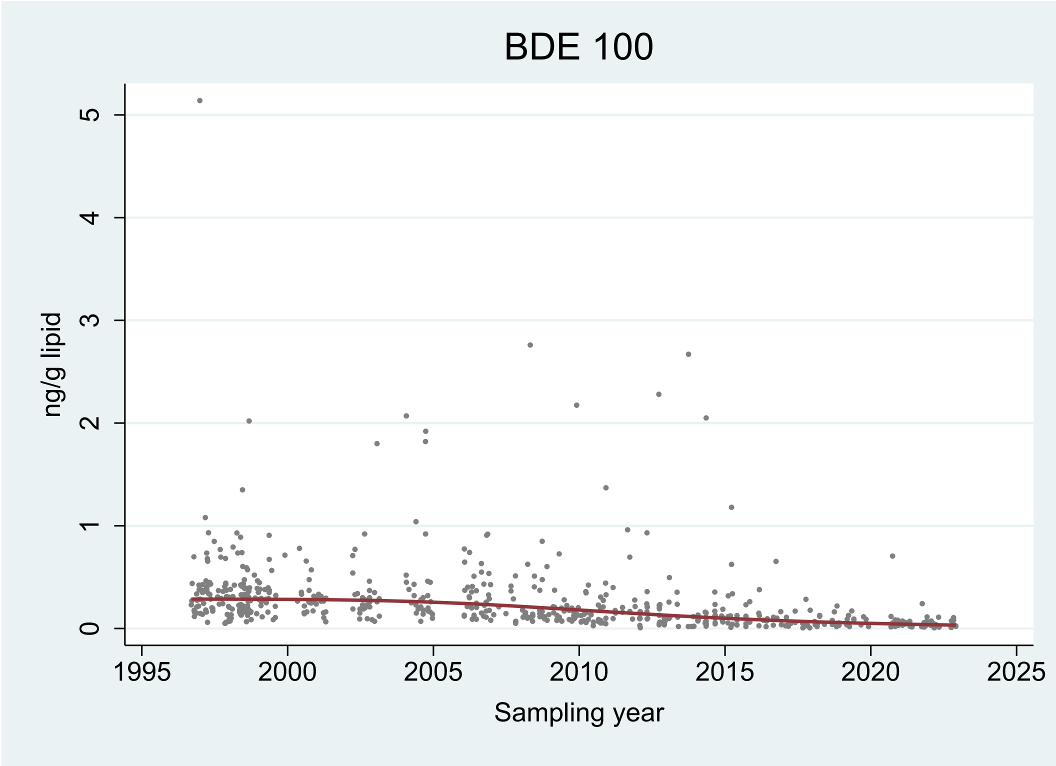


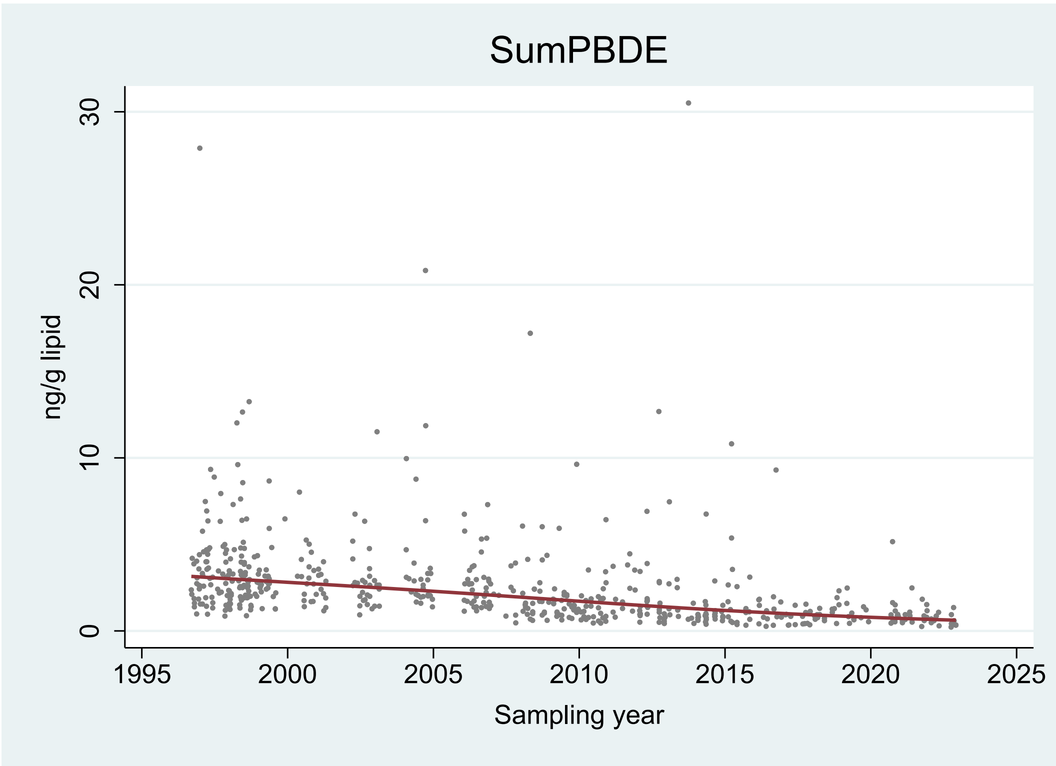

CB 153
ng/g lipid
0
50000
100000
150000
200000
1995
2000
2005
2010
2015
2020
2025
Sampling year

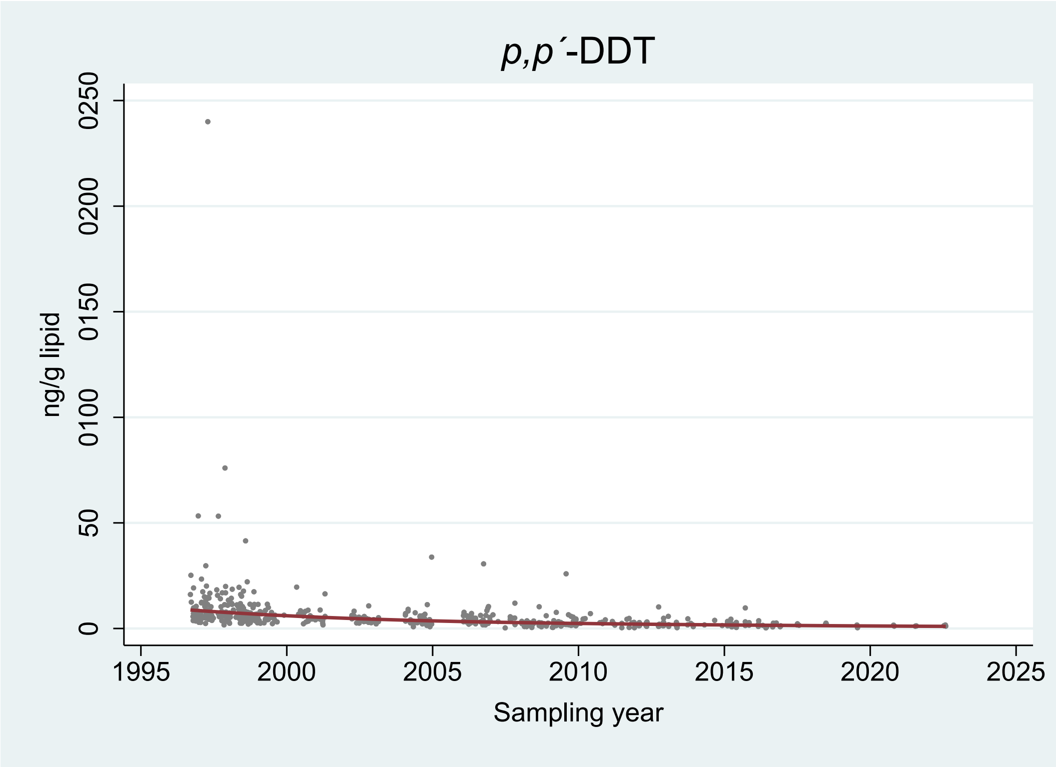

PCDD/F TEQ2022
pg TEQ2022/g lipid
0
5
10
15
1995
2000
2005
2010
2015
2020
2025
Sampling year

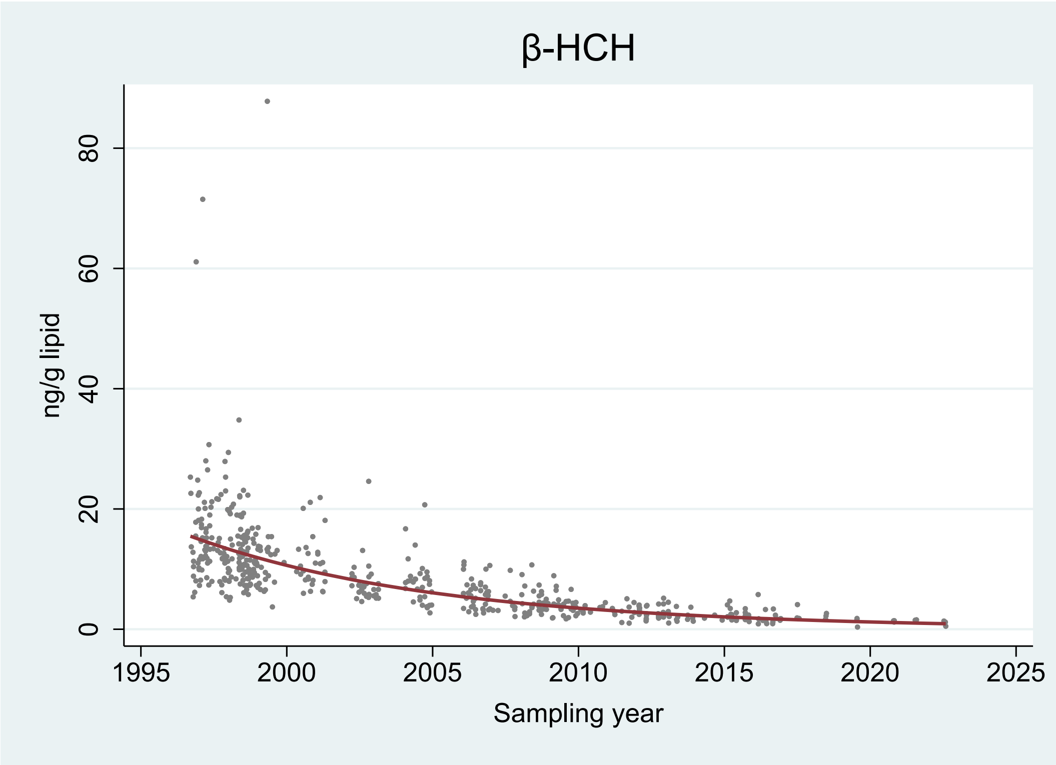

Total TEQ2022
pg TEQ2022/g lipid
0
5
10
15
20
1995
2000
2005
2010
2015
2020
2025
Sampling year

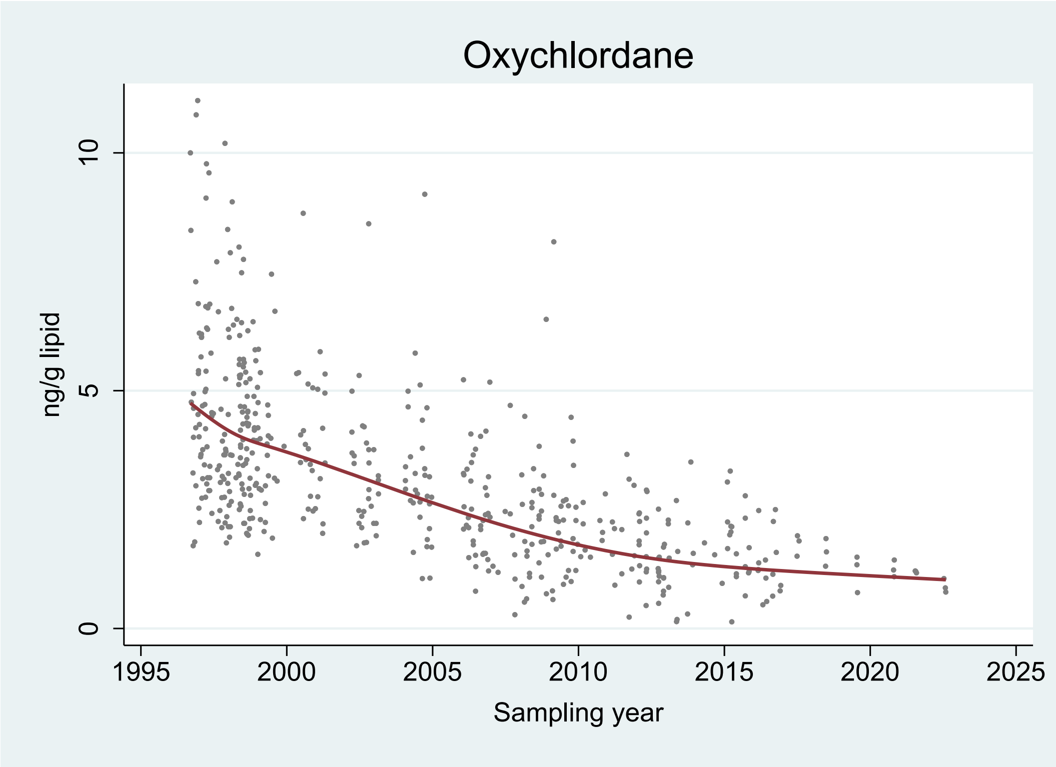

BDE 99
ng/g lipid
0
1
2
3
4
5
1995
2000
2005
2010
2015
2020
2025
Sampling year

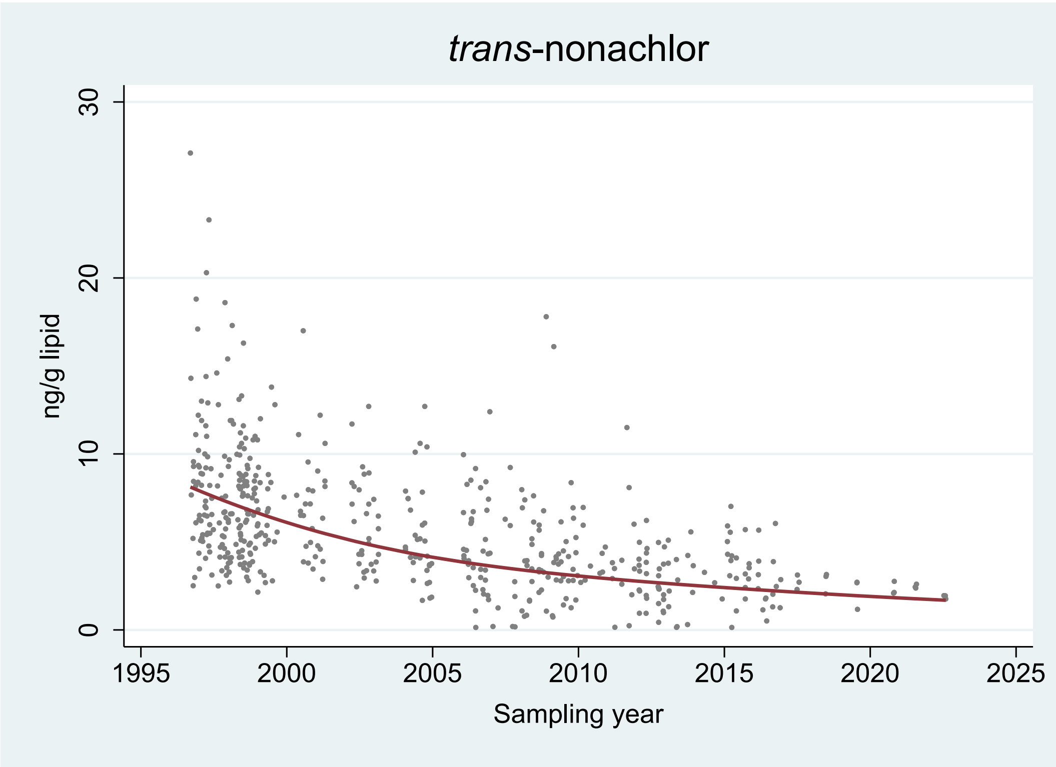

BDE 100
ng/g lipid
0
1
2
3
4
5
1995
2000
2005
2010
2015
2020
2025
Sampling year


SumPBDE
ng/g lipid
0
10
20
30
1995
2000
2005
2010
2015
2020
2025
Sampling year


p,p´-DDT
ng/g lipid
0
50
0100
0150
0200
0250
1995
2000
2005
2010
2015
2020
2025
Sampling year


β-HCH
ng/g lipid
0
20
40
60
80
1995
2000
2005
2010
2015
2020
2025
Sampling year


Oxychlordane
ng/g lipid
0
5
10
1995
2000
2005
2010
2015
2020
2025
Sampling year


trans-nonachlor
ng/g lipid
0
10
20
30
1995
2000
2005
2010
2015
2020
2025
Sampling year