Sakrapport till Naturvårdsverkets Miljöövervakning:

Polyklorerade bifenyler och klorerade bekämpningsmedel/metaboliter i bröstmjölk från förstföderskor i Uppsala, tidstrend 1996-2003.

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Sanna Lignell, Per Ola Darnerud, Marie Aune, Anna Törnkvist, Anders Glynn

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Sammanfattning

Under perioden 1996-2003 har Livsmedelsverket samlat in bröstmjölk från förstföderkor i Uppsala län. Syftet med studien är att undersöka om halten av vissa polyklorerade bifenyler (PCBer) och klorerade bekämpningmedel/metaboliter förändrats med tiden. Eftersom livsmedel är den stora källan till mödrarnas halter av PCBer och bekämpningsmedel/metaboliter kommer resultaten också att utnyttjas vid riskvärdering av miljöföroreningar i livsmedel, samt vid riskvärdering av spädbarns exponering under fosterstadiet och amning. Mellan åren 1996 och 2003 minskade medianhalten av PCB (CB 28, 105, 118, 138, 153, 156, 167, 180), hexaklorbensen (HCB), -hexaklorocyklohexan (-HCH), *trans*-nonaklor, oxyklordan, *p,p'*-DDT och *p,p´*-DDE med 5-14% per år. Minskningen var långsammast för vissa PCB-föreningar och snabbast för DDT-föreningar. En jämförelse av halveringtid för PCB-halterna i bröstmjölk mellan Uppsala-studien och en tidigare studie från Stockholm (1972-1997), visar att de uppskattade halveringtiderna är kortare i Uppsala än i Stockholm. De längre halveringstiderna i Stockholm beror troligen framförallt på att man i denna studie inte tagit hänsyn till en ökande medelålder bland kvinnorna som donerade mjölk mellan 1972 och 1997.

Report to the Swedish Environmental Protection Agency, 2004-06-01

Sanna Lignell, Per Ola Darnerud, Marie Aune, Anna Törnkvist

Polychlorinated biphenyls and chlorinated pesticdes/metabolites in breast milk from primiparae women in Uppsala County, Sweden – levels and trends 1996-2003

Introduction

Exposure estimation is an important part of risk assessment of environmental pollutants in food. Among the Swedish human population, food is the major source of exposure to persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), DDT-compounds, hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB). These compounds are lipophilic and accumulate in the lipid compartment of the body. Because of the relatively high lipid content, breast milk is a good matrix for analysis of POP levels. The POP levels in breast milk reflect the long-term exposure of the individual mother to the persistent POPs and also give information about body burden of POPs in women at the time of pregnancy and nursing.

In order to estimate the body burdens of POPs among pregnant and breast feeding women, and to estimate the intake of the compounds by breast feeding infants, the Swedish National Food Administration (NFA) has made recurrent measurements of levels of POPs in human breast milk. Another aim of this project is to establish if there are temporal trends of POP levels in breast milk. This study covers the period between 1996 to 2003, and temporal trends of PCBs, HCB, HCHs, *trans*-nonachlor, oxychlordane, and DDT-compounds are reported.

Material and methods

Recruitment

From January 1996 to May 1999 pregnant women (N=953) from the general population in Uppsala County were recruited as controls in a case-control study of risk factors for early miscarriages (Clausson et al., 2002). All primiparas (women having their first baby) recruited from early fall 1996 and onwards (N=365) were in late pregnancy asked to participate in a study of body burdens of persistent organic pollutants. Of these primiparous women, 188 agreed to donate breast milk for chemical analysis. Another 10 primiparas from the City of Uppsala, recruited for a special study of concentrations of PCB and chlorinated pesticides in serum during pregnancy, were in late pregnancy also asked to participate in the breast milk study. Of these women, 7 donated breast milk. At the prenatal clinic in Östhammar, located at the coast of the Baltic Sea, all primiparas were in late pregnancy (N=25) asked to participate in the study, and 16 of these donated breast milk (between fall 1997 and spring 1999).

Mothers were also recruited among primiparas who had delivered at Uppsala University Hospital from April 2000 to March 2001 and from March 2002 to February 2003. Women who delivered during the first week in every month and on randomly selected days during this week were asked to participate in the breast milk study. 2-3 primiparas were recruited every month. In 2000-2001, 67 women were asked to participate in the study and 31 donated milk. In 2002-2003, 49 women were asked to participate and 31 donated milk.

Data on age, weight, lifestyle, medical history, dietary habits etc. of the mothers were obtained via questionnaires (Table 1).

Breast milk sampling

The mothers sampled milk at home during the third week after delivery (approximately day 14-21 post partum). The milk was sampled during breastfeeding using a manual breast pump and/or a passive breast milk sampler. The women were instructed to sample milk both at the beginning and at the end of the breastfeeding session. The goal was to sample 500mL from each mother during 7 days of sampling. During the sampling week, the breast milk was stored in the home freezer, in hexane-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.

POP analysis

The PCBs and chlorinated pesticides were analysed at the NFA using metods described in Atuma & Aune (1999) and Aune et al. (1999). In brief, 3 g of thoroughly homogenized thawed milk was extracted with a mixture of n-hexane/acetone (1:1). The extracted lipid content was determined gravimetrically after evaporation of the solvents and the sample was then treated with sulphuric acid. The PCB fraction in the samples was separated from the bulk of the chlorinated pesticides by column chromatography on silica gel. The analysis of the PCB congeners and the chlorinated pesticides was performed on a gas chromatograph equipped with dual capillary columns (Ultra-2 and DB-17) and dual electron-capture detectors.

All samples were fortified with internal standards (PCB 189 and *o,p'*-DDD) prior to extraction to correct for analytical losses and to ensure quality control. A number of control samples have been analysed together with the samples to verify the accuracy and precision of the measurements.

Statistics

Lipid adjusted breast milk POP concentrations were used in the analysis. Non-adjusted concentrations are influenced by temporal changes in breast milk lipid content as well as by inter-individual differences in milk lipid content. As in the case of blood serum, the distribution of organochlorines between breast milk lipids and lipids in body tissues is a dynamic equilibrium (Phillips et al. 1989). A change in the lipid content of the breast milk alters this equilibrium. Thus, if non-adjusted breast milk concentrations are used, two women with similar body burdens of organochlorines, but with different breast milk lipid contents, may be miss-classified in different exposure categories. Therefore, lipid-adjustment of breast milk concentrations gives a better estimate of the body burden than non-adjusted concentrations.

Breast milk levels of CB 52, CB 101, -HCH, -HCH, *p,p´*-DDD, *o,p´*-DDE and *o,p'*-DDT were low (>75% below LOQ; 0.2-3.4 ng/g lipid depending on lipid content of the sample) and were therefore omitted from the statistical analysis. The distributions of the organochlorine analytical results closely followed a log-normal distribution, therefore all statistical analysis were performed on logaritmically transformed data. Simple linear regression was used to analyse associations between POP levels and sampling year (un-adjusted analysis). In order to adjust the associations between POP levels and sampling year for influences of different life-style/medical factors, multiple regression was performed with models including both continuous and categorical explanatory variables. As a consequence of the logaritmic transformation, influence of sampling year on POP levels after adjustment is presented as percent change of the median per year. The primary explanatory variables considered in this study were sampling year, the mother´s age (yr), body mass index (BMI, kg/m^2) before pregnancy, body weight change during pregnancy (% of initial body weight/week) and after delivery (% of weight directly after delivery), place of birth (Nordic or non-Nordic country), education (categories, see Table 1), and smoking (categories, see Table 1). In the final regression model only variables that were significantly associated ($p<0.05$) to the POP levels were included.

Results and discussion

<u>POP-levels in breast milk</u>

Among the PCB congeners, the di-*ortho* congeners CB 138, CB 153 and CB 153 showed the highest median concentrations (>30 ng/g lipid) (Table 2), whereas the median concentrations among the congeners of lower chlorination (CB 28, 52, 101 and 105) were close to or lower than the LOQ. Among the mono-*ortho* congeners CB 167 had the lowest median concentration and CB 118 the highest. CB 28 showed the largest variation in levels (>100-fold). The levels found in the Uppsala women were similar to levels reported for breast milk from mothers living in Stockholm (eg. median CB 153 level Uppsala: 57 ng/g lipid; CB 153 level in pooled sample Stockholm 1997: 73 ng/g lipid) (Norén & Meironýte, 2000).

Table 1. Characteristics of the participating women.

	N	Mean	Median	Range
Age of the mother	272	28.6	28.7	21-41
BMI (kg/m^2)	267	22.7	21.9	16.2-37.7
Weight gain during pregnancy (% of initial wt/week)	267	0.63	0.61	0.03-1.54
Weight reduction from delivery to sampling (%)[a]	258	9.5	9.2	-1.7-21

		N	%
Country of birth	Nordic	234	96
	Non-Nordic	10	4
Education	Max 3-4 yr high school	120	44
	1-3 yr higher education	62	23
	>3 yr higher education	89	33
Smoking during pregnancy[b]	Non-smoker	189	70
	Former smoker	37	14
	Smoker	45	16

[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.

p,p´-DDE was the compound with the overall highest median concentration (>100 ng/g lipid). The median concentrations of the other chlorinated pesticides/metabolites were low (<10 ng/g lipid), with medians of -HCH, -HCH, *p,p´*-DDD, *o,p'*-DDE and *o,p´*-DDT below LOQ. The largest variation in concentrations was found among the DDT-compounds (>35-fold). As in the case of PCBs, the levels in Uppsala were similar to levels reported from Stockholm 1997 (eg. median *p,p´*-DDE level Uppsala: 102 ng/g lipid; level in pooled sample Stockholm 1997: 129 ng/g lipid) (Norén & Meironýte, 2000).

Table 2. Concentrations of PCBs and chlorinated pesticides/metabolites in breast milk (ng/g lipid).

Substance	N	Mean (SD)	Median	Min	Max	N<LOQ[a]
CB 28	273	2.9 (3.9)	1.8	0.3	307.0	32
CB 105	273	1.4 (1.4)	1.1	0.2	14.8	82
CB 118	273	12.2 (6.8)	10.6	3.8	64.1	0
CB 138	273	30.9 (13.6)	28.1	5.3	93.5	0
CB 153	273	62.0 (28.0)	56.5	11.4	186.0	0
CB 156	273	4.7 (2.7)	4.1	0.4	23.6	2
CB 167	273	1.4 (0.8)	1.3	0.2	5.7	55
CB 180	273	29.4 (13.3)	27.3	4.8	83.8	0
Mono-ortho TEQ (pg/g lipid)	273	3.7 (2.0)	3.3	0.7	17.9	
SumPCB	273	145.8 (63.3)	135.1	30.1	401.9	
HCB	273	15.2 (5.2)	14.5	6.3	31.2	0
-HCH	273	14.1 (11.5)	11.5	3.7	127.0	0
Oxy-chlordane	273	4.4 (2.2)	3.8	1.1	21.2	0
trans-Nonachlor	273	7.5 (4.2)	6.6	1.0	30.9	0
p,p´-DDE	273	273 (115)	102	24	894	0
p,p´-DDT	273	9.1 (16.1)	6.1	1.7	240	0

[a]The limit of quantification (LOQ) varied between 0.2-3.4 ng/g lipids depending of the lipid content of the samples. Concentrations <LOQ were set to 1/2 LOQ.

Temporal trends

Simple linear regression showed that the levels of all POP, except CB 105 and CB 167, decreased significantly during the time period (Table 3, Fig. 1 and 2). The average POP levels decreased between 6-12% per year. The simple regression model, with sampling year as an explanatory variable, in most cases explained only a small fraction of the variation in POP-levels, as shown by the low R^2 (Table 3).

In the multiple regression, when only the explanatory variables showing a significant association to the POP levels were included in the model, the % decline per year with a few exceptions increased, compared to the estimate from the simple regression. Moreover, the R^2 increased considerable, showing that it is important to include lifestyle/medical factors that are associated with POP levels in the analysis of temporal trends. This is further illustrated by the comparison of half-times of PCB levels in breast milk between our study of mothers from Uppsala and the study of mothers from Stockholm (Norén & Meironýte 2000) (Table 4). The estimated half-times were considerably longer in the Stockholm study.

Table 3. Changes in levels of PCBs and chlorinated pesticides/metabolites in breast milk from primiparous women living in Uppsala County 1996-2003 (mean (95% confidence interval)).

Compound	Simple regression[a]		Multiple regression[b]		
	change/yr	R^{2c}	change/yr	R^{2c}	Sign. variables[d]
CB 28	-8.9 (5.4)	4%	-8.3 (5.6)	10%	Weight gain/loss
CB 105	-4.1 (5.3)	0.8%	-6.7 (4.9)	24%	Age, weight gain
CB 118	-7.9 (2.9)	10%	-9.0 (2.2)	50%	Age, BMI, weight gain/loss
CB 138	-6.1 (2.7)	7%	-7.8 (2.0)	53%	Age, BMI, weight gain/loss
CB 153	-6.6 (2.9)	7%	-8.0 (1.9)	63%	Age, BMI, weight gain/loss
CB 156	-8.6 (3.2)	10%	-10.4 (2.0)	70%	Age, BMI, weight gain/loss, country, smoking
CB 167	-2.3 (4.5)	0.4%	-4.6 (3.6)	40%	Age, weight gain
CB 180	-6.5 (2.9)	7%	-8.4 (1.6)	76%	Age, BMI, weight gain/loss
Mono TEQ	-8.7 (3.0)	11%	-10.2 (1.8)	71%	Age, BMI, weight gain/loss
HCB	-9.3 (1.9)	27%	-9.9 (1.6)	53%	Age, weight gain, weight loss
-HCH	-10.6 (2.7)	20%	-11.9 (2.0)	66%	Age, weight gain, country
Oxychlordane	-6.5 (2.7)	8%	-8.3 (2.2)	56%	Age, BMI, weight gain/loss, country, education
trans-Nonachlor	-5.3 (3.1)	4%	-7.2 (2.3)	56%	Age, BMI, weight gain/loss, country, education
p,p´-DDE	-12.1 (3.9)	13%	-13.7 (3.3)	59%	Age, country, education
p,p´-DDT	-11.3 (3.4)	15%	-13.3 (3.2)	33%	Age, weight gain, education

[a]Simple linear regression of the association between ln-transformed POP levels and year of sampling.
[b]Multiple linear regression. Association between ln-transformed POP levels and year of sampling adjusted for differences in lifestyle/medical variables among the women.
[c]Coefficient of determination.
[d]Variables used for adjustment in the multiple regression. Only variables that showed a statistically significant association to the POP levels in the multiple regression ($p<0.05$) were used in the final regression model.

There are probably several reasons behind this difference. One reason could be the difference in time span of the studies (Stockholm: late 60s/early 70s to late 90s; Uppsala:1996-2003). It may be possible that the rate of decline in PCB levels were slower in the early part of Stockholm study, when direct exposure from products containing PCB was still a reality.

The most likely explanations, however, is that the Stockholm study lacked in control of life-style/medical factors that influence breast milk levels of POP. For instance, both primiparous and multiparous women were included in the Stockholm study (55-75% primiparas). It is well known that breast feeding is a major pathway of POP excretion in women, and consequently the POP levels are usually higher in breast milk sampled after the first child is born than in breast milk sampled after subsequent deliveries (Vaz et *al*. 1993). In our study this problem was taken care of by only recruiting primiparous mothers. Another factor, probably the most important one, is the increase in average age of the women donating breast milk in the Stockholm study between 1972-85 (27-28 years) and 1996-97 (30-31 years). In our study the half-time was age-adjusted, thus taking care of possible differences in average age of the women between sampling years. PCB levels in breast milk increase with increasing age of the mothers, if the number of children the mother have nursed is accounted for in the analysis.

Table 4. A comparison of estimated half-times of POP levels in breast milk from mothers living in Stockholm (1967-1997) and Uppsala (1996-2003).

Compound	Stockholm[a] (yr)	Uppsala[b] (yr)
CB 28		8.0
CB 105		10.1
CB 118	11	7.3
CB 138	14	8.5
CB 153	17	8.3
CB 156		6.3
CB 167		14,8
CB 180		7.9
Mono-*ortho* TEQ		6.4
HCB	6	6.6
-HCH		5.5
trans-Nonachlor		9.3
Oxychlordane		8.0
p,p´-DDE	6	4.7
p,p'-DDT	4	4.9

[a]Norén & Meirunýte (2000). No adjustment was made for possible influence of lifestyle/medical factors. Both primiparous and multiparous mothers donated breast milk.
[b]Adjusted half-time.

Our study is probably the first study that seriously addresses the need for control of lifstyle/medical factors in time-trend studies of POP levels in Swedish breast milk. For some of the PCB congeners (CB 153, CB 156, CB 180 and mono-ortho TEQ) the regression models including the most important explanatory factors explained over 60% of the variation in breast milk levels. There are, however, still explanatory factors that have to be accounted for in order to increase the future precision of the estimated changes in POP levels over time. This is illustrated by the low R^2 of the regression model describing the variation in levels of CB 52, CB 105 and *p,p´*-DDT (<40%).

A closer look at the results shows that the decline in average level was similar among the PCBs, ranging from 5-10% per year. The degree of certainty of the estimates varies between the congeners, as indicated above, which makes comparisons of rates of decline difficult.

Among the chlorinated pesticides/metabolites the estimated decline in levels varied between 7-14%, indicating that the rate of decline is faster for some pesticides/metabolites.

The half-times of HCB, *p,p'*-DDT and *p,p´*-DDE estimated in the Stockholm study were more in line with our estimated half-times. The reason for this discrepancy between PCBs and chlorinated pesticides/metabolites is difficult to explain. The explanatory factors studied by us did not change the estimated decline of HCB, *p,p'*-DDT and *p,p´*-DDE levels to the same degree (6-17%) as they altered the estimated decrease of CB 138, CB 153 and CB 180 levels (21-29%). Moreover, the influence of age was more pronounced among the PCBs than among the pesticides/metabolites, as indicated by simple linear regression between POP levels and age (R^2 for PCBs 33-50%; for HCB, *p,p'*-DDT and *p,p´*-DDE 8-17%). Thus, the lack of control the influence of age on POP levels in the Stockholm study had less effect on the estimated rate of decline of pesticide/metabolites than on decline of PCBs.

Conclusions

Among primiparas living in Uppsala County, the breast milk levels of PCBs (CBs 28, 105, 118, 138, 153, 156, 167, 180), HCB, -HCH, *trans*-nonachlor, oxychlordane, *p,p'*-DDT and *p,p´*-DDE declined 5-14% per year during the period 1996 to 2003. For certain PCB congeners the decline was faster than the decline estimated in a study of mothers from Stockholm 1972-1997. The most likely reason for the slower decline in the Stockholm study is lack of control of lifestyle/medical factors that may influence the POP levels in breast milk, especially age of the women.

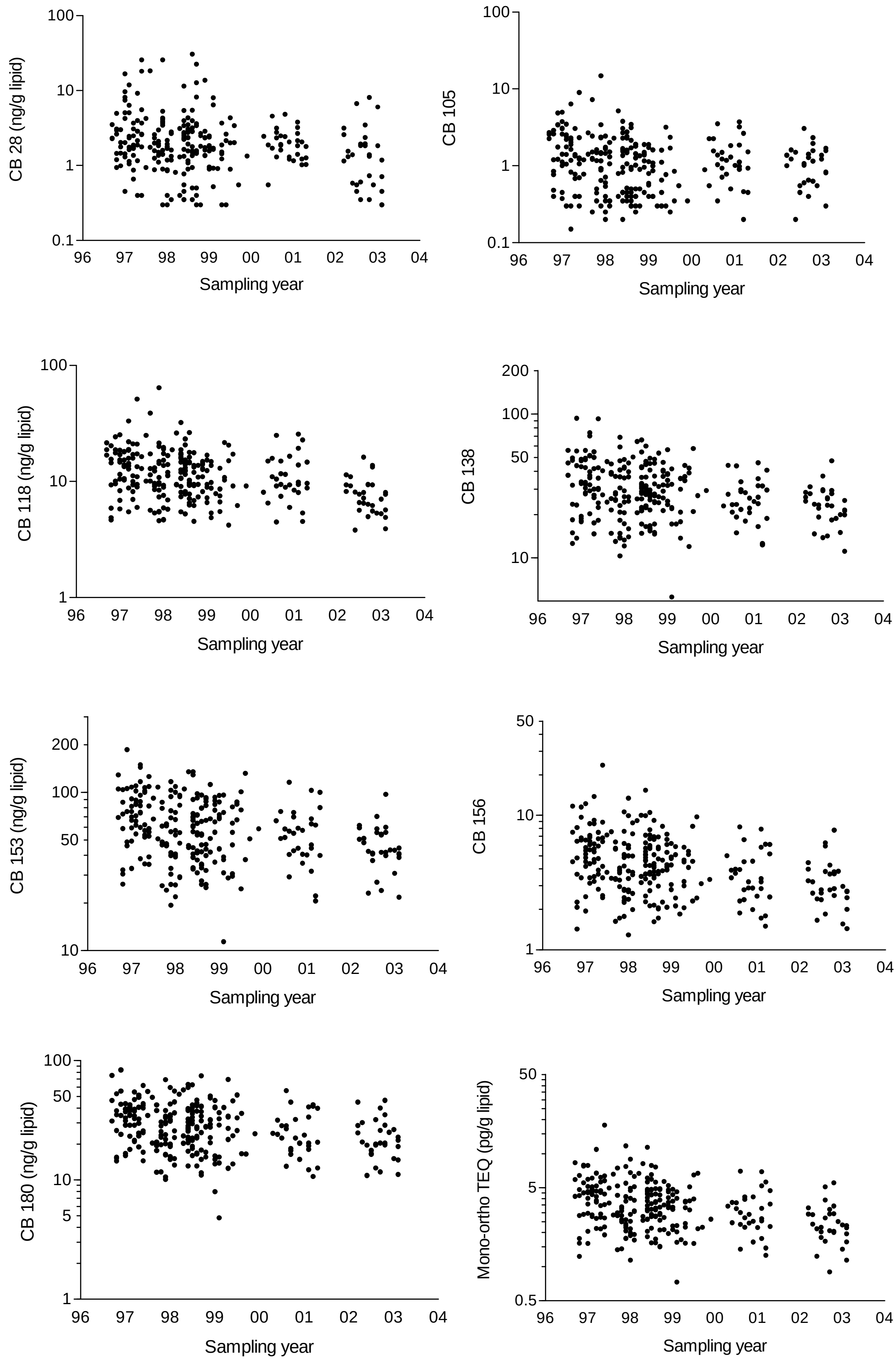

Figure 1. Temporal trends of PCB concentrations in breast milk from primiparous mothers living in Uppsala county.

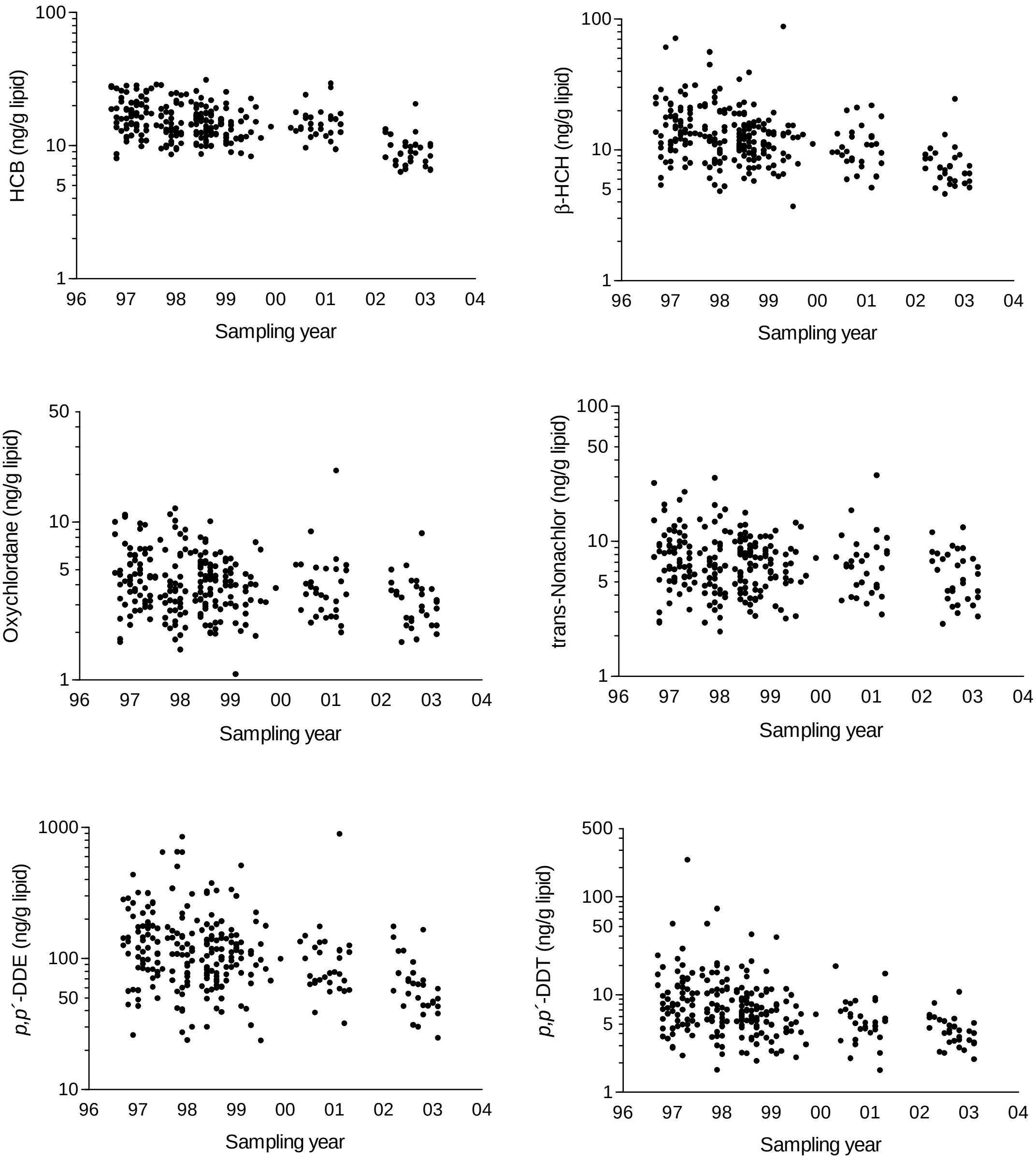

Figure 2. Temporal trends of chlorinated pesticides/metabolites in breast milk from primiparous mothers living in Uppsala County.

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