Levels and trends of PCB 28, PCB 153 and DDE in breast milk from primiparae women in Uppsala County, Sweden

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Introduction

Breast milk is vital for the little child, in most cases being the single nutriment during an important developmental period in life. However, it has become obvious that breast milk may be more or less contaminated with environmental pollutants, and that these chemicals may be a potential risk for the baby. Because of this, measurements of persistent organic pollutants (POPs) in breast milk have been performed in many countries and a large number of studies have been presented. Advantages with breasts milk sampling for exposure analyses are the non-invasive sampling technique and the fact that lipid levels are high enough to facilitate analyses of lipid-soluble compounds. Also, the levels of POPs in breast milk estimate the body burden of the compounds during pregnancy, when the critical exposure of the foetus occurs. In Sweden, levels of POPs, including PCBs and dioxins (PCDD/DFs), in breast milk have been measured since the beginning of the 1970's [1], and a declining trend has been noted for most of these compounds since this time. For instance, on pooled milk samples from Stockholm women, a more than 3-fold decline in PCB and dioxin levels was observed between 1972 and 1997.

Since 1996, the Swedish NFA has made recurrent measurements of levels of selected POPs, chiefly PCBs, dioxins and persistent pesticides (e.g. DDTs), in human breast milk. The analyses were made on individual basis, which offer possibility to adjust the time trends in POP levels for differences among the participating women in life-style or other factors that could affect the levels in breast milk. The ambition with the NFA sampling is to follow changes in the levels of these environmental contaminants in human breast milk and to continue the Swedish time trend measurements that was started in the 1970s. The measured levels will be used as base for evaluation of possible health risks for the mother and in particular for the breastfed infant.

This report presents breast milk results from 1996 to 2003, concentrating on the selected PCB congeners (PCB 28 and 153) and p,p'-DDE. The reason for selecting these compounds are the data showing differences in e.g. sources and persistence, which could make them interesting type substances for larger groups of compounds.

Materials and Methods

Recruitment

From January 1996 to May 1999 pregnant women from the general population in Uppsala County were recruited as controls in a case-control study of risk factors for early miscarriages [2]. All primiparas (women giving birth to their first baby) recruited from early fall 1996 and onwards were in late pregnancy asked to participate in a study of body burdens of POPs. Of these women 188 agreed to donate breast milk for chemical analysis. Another 7 recruited primiparas from the City of Uppsala and 16 from the prenatal clinic in Östhammar, located at the coast of the Baltic Sea, also agreed to donated breast milk.

Later, mothers were recruited among primiparas who delivered at Uppsala University Hospital from April 2000 to March 2001 and from March 2002 to February 2003. Two or three primiparas were recruited every month. At each occasion (2000-2001, 2002-2003) breast milk was obtained from 31 women.

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

Breast milk sampling

The mothers sampled their 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 500 mL from each mother during 7 days of sampling. During the sampling week, the breast milk was stored cold, preferably in a freezer, in acetone-washed bottles and 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.

Analysis

The PCB and DDT congeners were analysed at the NFA, using methods earlier described [3]. A number of additional analyses have been performed on the breast milk samples, including other PCB congeners, chlorinated pesticides, and PBDEs, but they will be, and have to some extent already been [4,5], presented elsewhere.

Statistics

Statistical analysis was performed using simple or multiple linear regression analysis. The distribution of the POP analytical results closely followed a log-normal distribution; therefore all statistical analysis was performed on logarithmically transformed data. The following explanatory variables was first included in the regression models: ‘sampling year’, ‘BMI before pregnancy’, ‘weight gain during pregnancy’, ‘weight loss after delivery’, ‘age of the mother at sampling’, ‘country of birth’, ‘education level of the mother’, and ‘smoking’. In the final regression analysis only ‘sampling year’ and variables that were significantly associated to the POP-levels ($p<0.05$) were included in the regression model. As a first step the regression analysis was performed with all participating women included. In the second step individuals with standardised residuals >3 were excluded as outliers in the analysis. The results are presented as % decline in OCC level/year and as half-life of OCC.

Results and Discussion

Table 1 gives a summary of basic data for the women participating in the study (age, body mass index before pregnancy, weight reduction from delivery to sampling etc.). The results on the mothers' age at milk sampling and their body mass index (BMI) are similar to the national reference values reported earlier [5], i.e. a primiparous birth age of 27.3 years, and a BMI before pregnancy of 23.7.

Table 1. Data on age, BMI (body mass index), weight gain during pregnancy, weight reduction from delivery to sampling, birth weight of the child, smoking habits, breast-feeding and education for the women participating in the study.

	N	mean	median	range
Age at the milk sampling occasion (years)	272	28.6	28.7	21 - 41
BMI) before pregnancy (kg/m^2)	267	22.7	21.9	16.2 - 37.7
Weight gain during pregnancy (%/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
Birth weight of the child (g)	268	3592	3505	2430 - 5110
		N	%	
Smoking habits during pregnancy [b]		271		
Non-smokers		189	70	
Former smokers		37	14	
Smokers		45	17	
Education		271		
Maximum 3-4 years in upper secondary school or similar		120	44	
Maximum 1-3 years of university studies or similar		62	23	
More than 3 years of university studies or similar		89	33	

[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

In Table 2, the concentrations of the studied PCB congeners and DDE, compilated for the total sampling period 1996-2003, are given. LOD (limit of detection) was 20 pg/g milk, which corresponds to 0.3-1.3 ng/g milk fat. Values below the LOD were set to half the LOD in the calculations of mean and median for the individual congeners. Of the three congeners, p,p'-DDE shows the highest levels and has also a much wider range in levels than has PCB 153. However, if sumPCB and sumDDT were compared the mean/median levels of the two groups were similar (data not shown).

Table 2. Concentrations of PCB 28, PCB 153 and p,p'-DDE (all in ng/g fat) in breast milk from primiparae women from Uppsala County, using data from the whole sampling period 1996-2003.

substance	N	mean	median	min	max
PCB 28	273	2.86	1.84	0.25	30.7
PCB 153	273	62.0	56.5	11.4	186
p,p'-DDE	273	130	102	23.8	894

Figures 1a-c show the levels of PCB 28, PCB 153 and p,p'-DDE in relation to the time-point of sampling, from start of the study (1996-01-01). In case of both PCB 28 and p,p'-DDE some high outliers were observed; seven of the PCB 28 analyses were above 15 ng/g fat, and seven of the DDE values were above 500 ng/g fat. Thus, for the purpose of clarity, the diagrams are presented with logarithmic compound level scales (y axis).

The time-related decrease in breast milk levels, before and after adjustment for relevant parameters, is presented in Table 3. Note that adjustments were performed on different variables for the different compounds, a consequence of the result from the multiple regression analyses.

Table 3. Changes in levels of PCB congeners and p,p'-DDE in breast milk from primiparae women living in Uppsala County 1996-2003.

Compound	% change/year		Half-life		Adjustment [c]	R^{2} [d]
	Unadjusted [a]	Adjusted [b]	Unadj [a]	Adj [b]		
CB 28	-8.9 (5.4)	-8.3 (5.6)	7.5	8.0	Weight gain during pregnancy Weight loss after delivery	0.10
CB 153	-6.6 (2.9)	-8.0 (1.9)	10.1	8.3	BMI Weight gain during pregnancy Weight loss after delivery Age of the mother	0.63
p,p´-DDE	-12.1 (3.9)	-13.7 (3.3)	5.4	4.7	Age of the mother Place of birth (Nordic or not) Education level	0.59

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

The results of this study clearly show that the levels of the actual POPs (PCB 28 and 153, and p,p'-DDE) have been decreasing in the sampled breast milk from Uppsala County, during the period 1996-2003. In the unadjusted data set the decrease per year was between 6.6 and 12.1%. However, multiple regression showed that some tested variables showed significant association to the POP levels, and if these parameters were included in the final regression model, the yearly level changes could be adjusted. Regarding PCB 153 and p,p'-DDE the adjustment resulted in increases in percent yearly changes, whereas the PCB 28 change after adjustment became smaller. However, in all cases the adjusted values should better describe the changes in levels compared to the unadjusted values. The variables used in adjustment of PCB 153 and p,p'-DDE levels could explain about 60 % of the total variation, whereas in case of PCB 28 the variables used only explained 10%. Interestingly, the age of the mother did not seem to be an important factor for explaining the

PCB 28-levels found in breast milk, but weight-related change during and after pregnancy was. This finding may be a consequence of that PCB 28 emanates from other possible sources than the more persistent PCB 153 and p,p'-DDE do. In case of p,p'-DDE the levels depend, except for the age of the mother, also on the place of birth and the education level of the mother. Possible explanations in these cases are the heavier DDT exposure that has occurred in many non-Nordic countries, and the fact that a higher education level could be related to a higher consumption of fruit and vegetables, food items that could contain DDTs.

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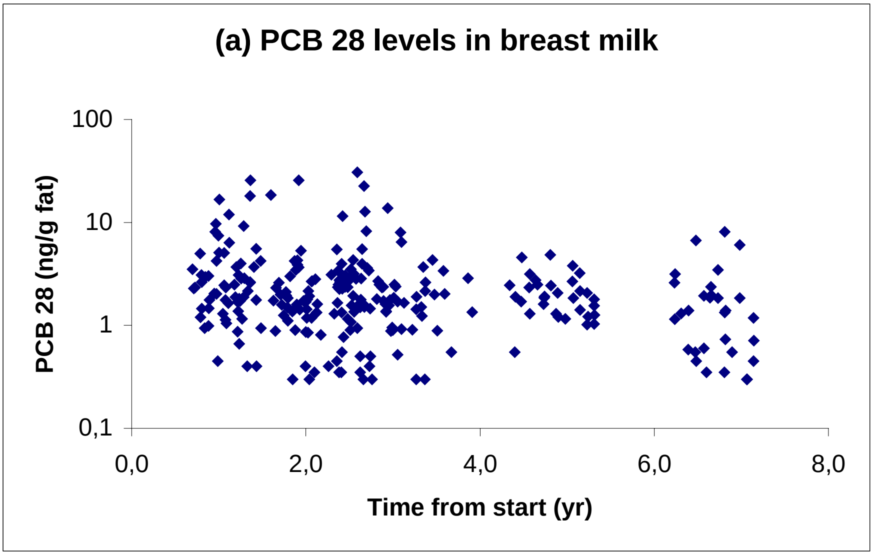
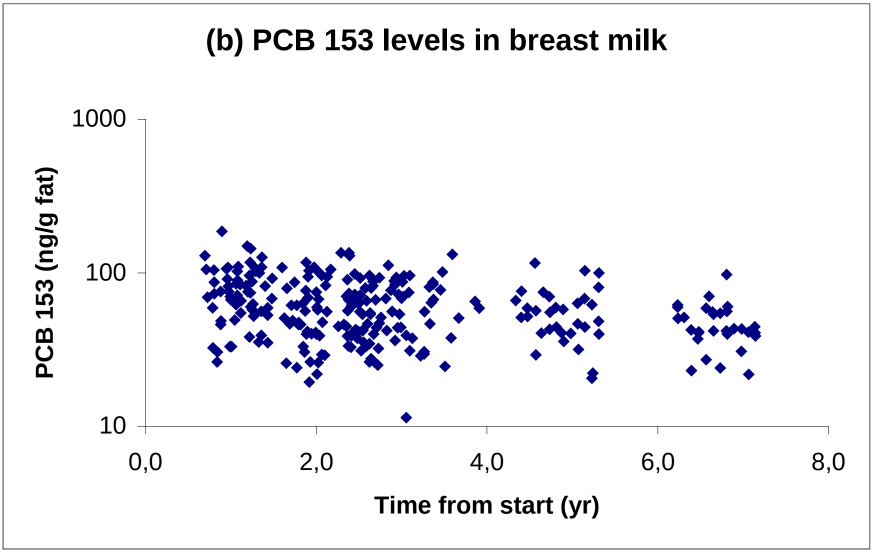
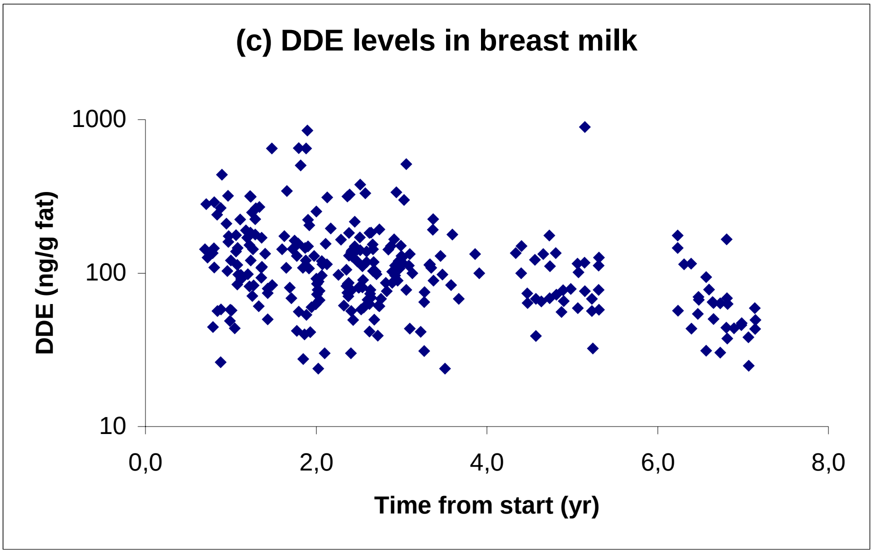
Figure 1. The figures a-c show the breast milk levels of PCB 28, PCB 153 and p,p'-DDE in relation to the time-point of sampling, beginning from the start of the study (1996-01-01). For inclusion of all data and for clarity, the y axis has a logarithmic scale.






(c) DDE levels in breast milk
1000
100
10
DDE (ng/g fat)
0,0
2,0
4,0
6,0
8,0
Time from start (yr)