ANALYSIS OF ORGANOCHLORINE COMPOUNDS IN HUMAN MILK

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Introduction

Analysis of organochlorine compounds in Swedish human milk has been carried out since the late 1960s (1). The aim has been to monitor the mothers' body burden and the infants' intake of these environmental pollutants. The analytical data have subsequently been used as a basis in the risk assessment of organochlorines for these risk groups. In order to update and expand the data base regarding exposure of pregnant women and infants to organochlorines in Sweden, a project was initiated in 1997 in which levels of these compounds were measured in mother's milk and blood. Within this project, we present a small-scale method for the determination of PCBs and pesticides in human milk and preliminary results from a study of 187 samples of breast milk from primiparous mothers in Uppsala county.

Material and Methods

The human milk was obtained from primiparous mothers recruited in an ongoing study on persistent organic pollutants in blood and milk from mothers in Uppsala county. The participating mothers were between 22 and 41 years old with a mean age of 28 years. The milk samples were collected using a breast pump provided specifically for the purpose. The samples (30-500 ml) were frozen immediately and stored at -20 °C until analysis. The samples were analysed for the PCB congeners 28, 52, 101, 105, 118, 138, 153, 156, 167 and 180 and the chlorinated pesticides hexachlorobenzene (HCB), hexachloro-cyclohexane (α-, β- and γ-HCH), chlordane (oxychlordane and *trans*-nonachlor) and DDT (*p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDE and *o,p'*-DDT).

The analytical methodology used for the analysis of the chlorinated pesticides, mono-*ortho*- and di-*ortho*-substituted PCBs is a small scale version of the extraction method described by Slorach and Vaz (2) and an analytical technique described by Atuma and Aune (3). The thawed milk samples were homogenised and then 3 g of milk was extracted twice with a mixture of n-hexane / acetone (1:1). After addition of 1 ml of ethanol (99.5 %) to the combined extracts, the solvents were evaporated and the lipid content was determined gravimetrically. The fat was then re-dissolved in n-hexane and treated with sulphuric acid. The PCBs were separated from the bulk of the chlorinated pesticides over a silica gel column (4.5 g of 3 % water-deactivated silica gel). Elution of the column with about 30 ml of n-hexane gave a fraction (F1) containing the PCB congeners, HCB and *p,p*-DDE. A second fraction (F2), containing mainly the chlorinated pesticides, was eluted with 40 ml of a n-hexane / diethyl ether mixture (3:1).

Further fractionation of the silica gel fraction F1 was performed for some samples on HPLC in order to confirm the levels of the PCB congeners (4). After appropriate reduction in volume, F1

was injected into a HPLC system, equipped with a hypercarb column. n-Hexane was used as the mobile phase at a flow rate of 1 ml/min. Two fractions were collected at 0-5 ml and 5-25 ml containing the di-*ortho* and mono-*ortho* congeners, respectively. A third fraction containing the planar PCBs could be obtained by back flushing the system with DCM (100%) but a larger sample is needed for quantification.

All samples were fortified with the internal standards PCB 53, PCB 189 and *o,p'*-DDD prior to extraction to correct for analytical losses and to ensure quality control. The final analysis of the mono-*ortho* and di-*ortho* 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.

Results and Discussion

The recoveries of the different chlorinated compounds from fortified cows' milk ranged from 87 to 118 % (PCBs) and 79 to 106 % (pesticides). The reproducibility of the method was demonstrated by replicate determinations (n=5-9) of 4 different milk samples. The coefficients of variation were less than 12 % for the PCBs and less than 10 % for the pesticides.

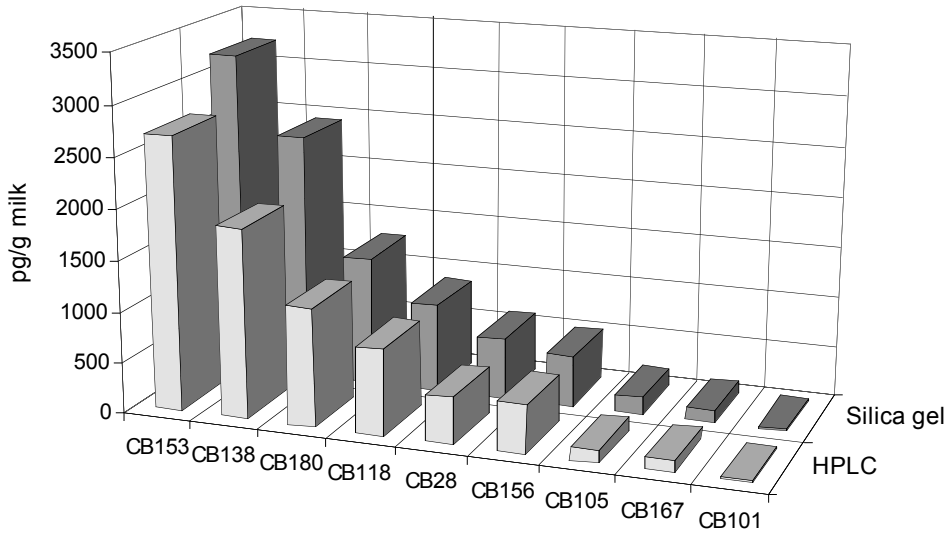

Figure 1. *Comparison between the levels of PCB congeners obtained after silica gel separation and HPLC separation, respectively*.

A comparison was made between the levels of PCB congeners obtained after silica gel fractionation and the levels obtained after separation of the mono- and di-*ortho* on a hypercarb column (fig. 1). The levels of the congeners were in most cases lower (74-106 %) after HPLC fractionation, but this was also the case for injected standard solutions (74-104 %). After

compensation for the HPLC yields, the levels of the PCB congeners were in good agreement with those obtained by analysis directly after silica gel separation. Since the HPLC separation seemed unnecessary in this case it was only applied to a small number of samples.

The concentrations of ΣPCB, PCB153, HCB, β-HCH, chlordane, *p,p'*-DDE and ΣDDT are listed in table 1. The concentrations of ΣPCB and ΣDDT were generally higher than those of HCB, β-HCH and chlordane. The present results show a decrease in levels compared to the levels obtained in human milk samples from Uppsala 1994 (5). For instance, the mean concentration of PCB 153 has declined from 103 ng/g fat in 1994 to 66 ng/g fat in this study.

Table 1. *Concentrations (ng/g fat) of PCB and chlorinated pesticides in human milk from primiparous women in Uppsala county, Sweden (n=187).*

	ΣPCB[a]	PCB 153	HCB	β-HCH	Chlordane[b]	*p,p'*-DDE	ΣDDT[c]
Mean	156	66	16.5	15.5	13.2	145	157
Median	148	62	15.8	12.9	12.1	114	127
Min	51.4	19.4	7.5	4.83	3.7	23.9	27.2
Max	402	186	32.1	110	41.8	848	870

[a] PCB congeners 28, 52, 101, 118, 153, 105, 138, 167, 156, 180
[b] Oxychlordane and *trans*-nonachlor
[c] *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT

The concentration of PCB 153 is strongly correlated to the ΣPCB concentration (r^2=0.953) (fig. 2A), and this congener may be used as an indicator substance for total PCB in future studies. In fig. 2B the concentration of PCB 153 is shown for the different ages (r^2=0.363). Oxychlordane resulted in a similar degree of age-to-concentration correlation (r2=0.347), whereas the other studied compounds gave lower correlations with age (e.g. *p,p'*-DDE: r^2=0.177). *p,p'*-DDE contributed 92 % (±6 %) to the ΣDDT in the milk. An explanation to the age-related uptake in milk could, apart from the fact that the compounds are accumulated in the body fat with time, also be caused by a continuous decrease in organochlorine levels in the environment.

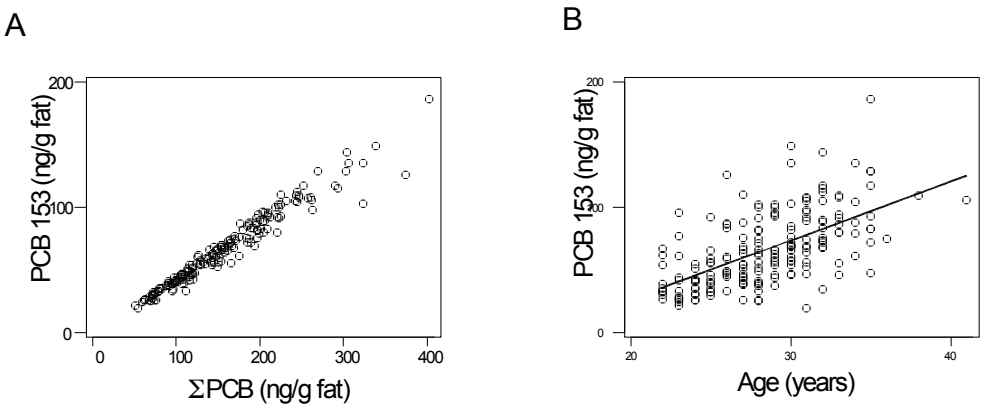

Figure 2. *(A) Correlation between the concentrations of PCB 153 and ΣPCB in human milk from primiparous mothers from Sweden (r^2=0.953, n=187). (B) Concentrations of PCB 153 in human milk from primiparous mothers versus age (r^2=0.363, n=187).*

A number of these milk samples have also been analysed for polybrominated diphenyl ethers (PBDEs), non-*ortho* PCBs, dioxins and furans. The results of those analysis will be published elsewhere.

In conclusion, this simple small-scale method works well for the analysis of PCB congeners and chlorinated pesticides in human milk. The result, showing decreased levels of organochlorines in the milk, is positive from risk assessment aspect, as considerable amounts of these compounds could be transported to the infant during the lactational period.

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