Bull. Environ. Contam. Toxicol. (1997) 59:407-413

Bulletin of Environmental Contamination and Toxicology

Effect of Age and Number of Deliveries on Mean Concentration of Organochlorine Compounds in Human Breast Milk in Poland

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Received: 25 April 1997/Accepted: 15 May 1997

Organochlorine compounds (OCs) characterized by environmental stability and strong lipophilic properties tend to accumulate in animal organisms by entering into a network of alimentary interrelations. Animals from the upper levels of the trophic pyramid gather such compounds in greater concentrations in their tissues. As man constitutes the last link of the food chain, it is man's body that accumulates the highest concentrations of organochlorine compounds (Dewailly *et al.* 1993, Góralczyk and Strucinski 1996). According to some authors (Czaja *et al.* 1997, Noren 1983, Spicer and Keren 1993), one of the most important means of excreting these compounds from the man's body may be lactation. On the other hand it has been shown in our previous studies (Ludwicki and Góralczyk 1994) that the age may considerably contribute in OCs concentration in human adipose tissues.

Assuming that lactation may be an important factor in disposing organochlorine compounds from the woman's body, an attempt has been made to answer how the number of deliveries and the following lactations affect the organochlorine compound levels excreted in the mother's milk. The results from this study may also constitute a contribution to an overall discussion on infant exposure to these compounds.

MATERIALS AND METHODS

The relationship between age, number of deliveries and the concentrations of OCs in mothers' milk was to be identified by examining the findings from the analyses of 253 samples of human breast milk. Subjected to analysis were 108 milk samples from primiparous and 145 samples from multiparous females who had had from 2 to 7 deliveries. The assessment did not take into consideration women whose previous deliveries were not followed by lactation (e.g., stillborns).

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Samples were collected in a lactarium, maternity clinic in Warsaw, and from donors in selected regions of the country. Each milk sample was accompanied by necessary data concerning age, number of previous deliveries or stillborns and the general health status of the milk donors.

The milk samples (10 ml) were kept frozen at -18°C until analysis. After defrosting, the samples were deproteinized with acetone and extracted with n-hexane, and the resulting extract was cleaned up using concentrated sulphuric acid. The purified extracts were then analyzed by GLC-ECD for organochlorine insecticides and HCB.

Following the detection of organochlorine insecticides and HCB, further analysis focused on detecting polychlorinated biphenyls. The hexane extracts collected were concentrated to a 1 ml volume and dehydrochlorinated using a 2.5% potassium hydroxide solution in a 96% ethanol. Following extraction with hexane, the samples were oxidized using an oxidizing agent (1.6 g of potassium dichromate, 18.5 ml of distilled water, and 100 g of concentrated H_2SO_4). PCBs were detected in the hexane layer by GLC-ECD.

Gas chromatography analysis was done on a Pye-Unicam 104, equipped with ^{63}Ni electron capture detector (ECD). Glass chromatographic column (1.8 m, 4 mm internal diameter) was packed with 7.5% QF-1 and 3% OV-17 (9:1) on Gas Chrom Q (80-100 mesh). The operating conditions were: injection port and column temperature: 200°C, detector temperature 22° C; the carrier gas was nitrogen at a flow rate of 40 ml per minute and sample volume 5 µ1.

RESULTS AND DISCUSSION

The comparison of mean concentrations of the compounds examined in the milk of primiparous and multiparous females is shown in Table 1.

No decline was found in the mean concentrations of organochlorine compounds in multiparous females as compared to primiparous. This may stem from the age of the women studied. Mean HCB and Σ HCH concentrations were similar for both groups of donors, and mean p,p'-DDT and PCBs concentrations were even higher for multiparous females (statistically significant only for PCBs; $p \leq 0.05$).

Women with the highest number of deliveries (over four) were reported to have the highest DDT, its metabolites, and PCB levels (Fig. 1). Also, it must be noticed that the average age of those donors was also the highest and amounted to 33 years.

Table 1. Mean concentration of chlorinated hydrocarbons in the milk of primiparous and multiparous females (mg/l).

	HCB	α-HCH	β-HCH	γ-HCH	DDT	DDD	DDE	PCBs
Primaras (N=108) (Mean age of 24)								
X	0.0020	0.0007	0.0037	0.0006	0.0019	0.0005	0.0244	0.0437
SD	0.0028	0.0022	0.0091	0.0021	0.0045	0.0008	0.0241	0.0551
Min	0.0002	0.0002	0.0004	0.0002	0.0008	0.0005	0.0017	0.0010
Max	0.0143	0.0155	0.0835	0.0156	0.0290	0.0056	0.1850	0.2180
Multiparous females - 2-7 children (N=145) (Mean age of 29)								
X	0.0020	0.0005	0.0036	0.0003	0.0038	0.0006	0.0261	0.0618
SD	0.0025	0.0016	0.0055	0.0009	0.0123	0.0035	0.0228	0.0941
Min	0.0002	0.0002	0.0004	0.0002	0.0008	0.0005	0.0009	0.0010
Max	0.0152	0.0092	0.0440	0.0067	0.1355	0.0381	0.1117	0.6590

X - Mean concentration
SD - Standard deviation

Figure 1 shows a comparison of p,p'-DDT and PCBs contents in the milk collected from three groups of women: primiparous, multiparous females with 2 to 3 deliveries, and multiparous females with over 3 deliveries. The mean age of the women in each group was 24, 28, and 33 years, respectively. The study indicates that while lactation may indeed be one of the means of excreting the compounds examined, it is age rather than the number of deliveries that seems to affect the concentration of chlorinated hydrocarbon compounds in human milk.

This proves that deposits of the compounds examined in the tissues of post-lactation women are rapidly supplemented. To verify that, 108 primiparous were divided into two age groups: donors aged 15 to 24 and donors aged 25 to 35 (Fig. 2). Higher concentrations of p,p'-DDT and its metabolites and PCBs in older women were identified.

Regardless of the number of deliveries, the donors examined were found to have their mean concentrations of p,p'-DDT, p,p'-DDE, and PCBs (statistically significant only for PCBs; $p \leq 0.05$) rising with age. However, no statistically significant variance in HCB and HCH isomer levels was identified. The findings are presented in Table 2.

Some authors have reported higher concentrations of chlorinated

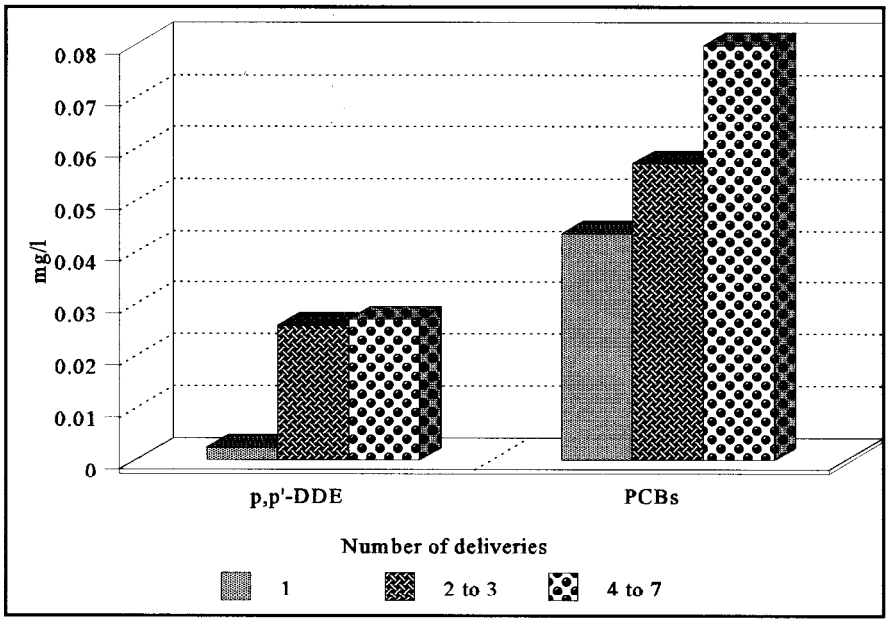

Figure 1. Comparison of p,p'-DDE & PCBs content in milk by the number of deliveries.

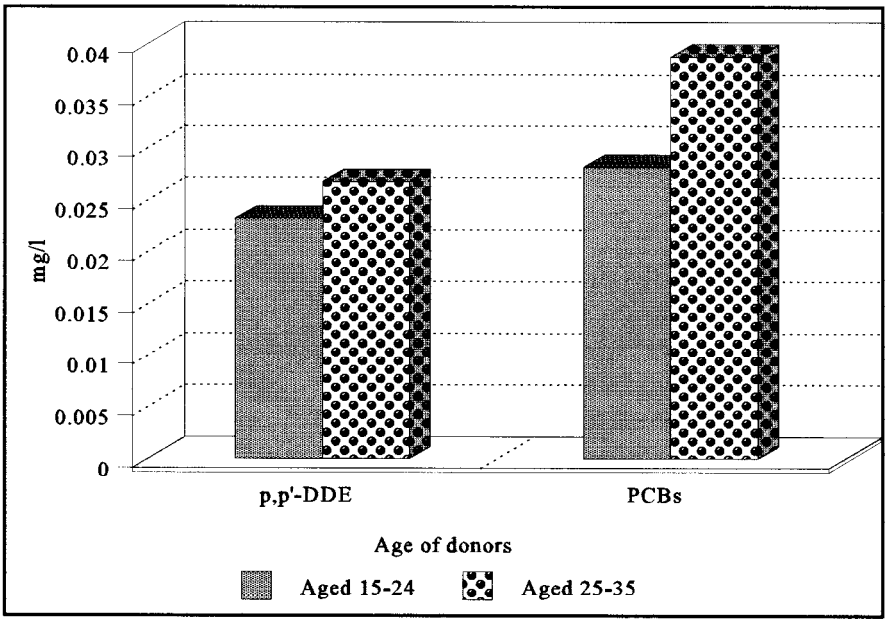

Figure 2. Comparison of p,p'-DDE & PCBs content in primiparous' milk.

hydrocarbons in primiparous (Skaare and Polder 1990), while others did not indicate any differences between both groups of donors (Krathacker 1991). An increase in organochlorine compound concentrations along with the age of donors was described by (Bates *et al.* 1994, Dillon *et al.* 1981, Gómez-Catalán *et al.* 1993, Greve and van Zoonen 1990).

Others found no correlation between the mother's age and the levels of compounds examined in human milk (Jensen and Slorach 1991).

Table 2. Mean concentration of chlorinated hydrocarbons in various age groups (mg/l).

	HCB	α-HCH	β-HCH	γ-HCH	DDT	DDD	DDE	PCBs
Up to 25 years (N=118)								
X	0.0021	0.0007	0.0034	0.0006	0.0019	0.0005	0.0227	0.0341
SD	0.0028	0.0018	0.0034	0.0019	0.0047	0.0005	0.0195	0.0418
Min	0.0002	0.0002	0.0004	0.0002	0.0008	0.0005	0.0017	0.0010
Max	0.0143	0.0108	0.0440	0.0156	0.0409	0.0025	0.1067	0.2185
25 - 30 years (N=77)								
X	0.0019	0.0004	0.0032	0.0004	0.0048	0.0011	0.0236	0.0584
SD	0.0024	0.0018	0.0043	0.0019	0.0159	0.0048	0.0194	0.0763
Min	0.0002	0.0002	0.0004	0.0002	0.0008	0.0005	0.0004	0.0010
Max	0.0152	0.0155	0.0231	0.0074	0.1355	0.0381	0.1117	0.3905
Over 30 years (N=58)								
X	0.0019	0.0009	0.0046	0.0003	0.0030	0.0005	0.0299	0.0635
SD	0.0023	0.0022	0.0112	0.0010	0.0064	0.0007	0.0259	0.0804
Min	0.0002	0.0002	0.0004	0.0002	0.0008	0.0005	0.0009	0.0013
Max	0.0146	0.0090	0.0835	0.0056	0.0385	0.0050	0.1033	0.3881

X- Mean concentration
SD - Standard deviation

The concentrations of chlorinated hydrocarbons identified in human milk are the result of two processes: bioaccumulation of such compounds in the adipose tissue and excretion of the compounds to human milk in the course of lactation. Older women may be expected to have higher concentrations of organochlorine compounds due to longer exposure thereto. On the other hand, lactation is an important means of disposing of such compounds from the

woman's body. Daily disposal during lactation is much greater than daily intake (Jensen and Slorach 1991). Thus, the concentrations of chlorinated hydrocarbons in human milk may be expected to fall as the number of deliveries increases.

Differences in the findings of studies on the correlation between mothers' parity and the concentrations of organochlorine compounds in human milk may be attributed to the simultaneous operation of many factors, such as age, number of deliveries, body weight, dietary habits, sample collection time.

Acknowledgments. The excellent technical assistance of Danuta Żurek and Grażyna Korzybska is gratefully acknowledged.

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