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Concentrations of DDT, PCBs, HCB, and HCH Isomers in the Liver and Adipose Tissue of Newborn Mice Receiving an Extract of Human Milk

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Persistent organic chlorine compounds, such as DDT and its metabolites, hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs) circulate in the food chain of the ecosystem. Despite of the formal prohibition of their use in the protection of crops, the concentrations of these compounds increase in the biological materials from humans and animals. This is particularly true for HCB and PCBs introduced into the environment in such industrial products as glues, varnishes, compounds for wood impregnation and electrical equipment.

Most data on the toxicity and accumulation of organic chlorine compounds have been obtained from animal experiments after chronic or acute poisoning with marketed preparations or standards of the used components of these preparations (Allen et al. 1979 and Peters 1976). In food products of animal origin and in human milk these compounds and their metabolites are present after multiple metabolic steps in varying proportions and concentrations (Juszkiewicz et al. 1979 and Vuori et al. 1977). Their variable amounts are passed to newborns with mother's milk.

The purpose of the present study was to investigate in an experimental model of newborn mice the degree of accumulation of these compounds in the liver and adipose tissue after long-standing feeding them with an extract of human milk with added organic chlorine compounds in doses received by human newborns with milk. In the assessment of the relationship between the degree of accumulation of various compounds in the tissues of newborn mice and the daily dose concentrations were used similar to those found in human milk.

MATERIALS AND METHODS

The extract of human milk was obtained from a pool made up of 540 milk samples from the Central Lactarium in Warsaw.

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The extract contained the main organic chlorine compounds, and their metabolites, including p,p'-DDE, p,p'-DDT, p,p'-DDD, o,p-DDT, alpha-, beta- and gamma-HCH, HCB and PCBs.

In the experiment newborn mice received the doses corresponding to the daily dose obtained by human newborns with mother's milk calculated per gram of body weight, and doses ten times and hundred times greater than the first one.

The doses were established assuming that a child weights 3500g and receives 700 ml of milk daily. Therefore, the child receives 7 mg/g b.w. of milk fat containing known concentrations of organic chlorine hydrocarbons. The extract of these established concentrations was dissolved in oil of linenseed so that 0.1 ml of oil contained the required concentrations per one gram of body weight of the mouse daily (Table 1).

Table 1. Concentration of chlorinated hydrocarbons in daily dose for 1 g of body weight used in the experiment with group "1"

Compound	Concentration in ng
p,p'-DDE	35.00
p,p'-DDT	3.50
p,p'-DDD	0.21
o,p -DDT	0.35
alpha-HCH	0.14
beta -HCH	1.05
gamma-HCH	0.35
HCB	1.40
PCBs	2.80

The experiment was carried out on Balb/c mice receiving standard food for laboratory animals. The newborns remained with the mothers and were divided into four groups:
"0" - control group receiving oil of linenseed only,
"1" - group receiving the dose corresponding to the dose received by human infants calculated per gram of body weight,
"10"- group receiving the dose ten times that in group "1",
"100"- group receiving the dose one-hundred times that in group "1".

The newborn mice received the mixture five times weekly from the first day after birth to the age of six weeks. The mixture was administered through a tube into the oesophagus in volumes of 0.1 ml/g of body weight. Two weeks after the last dose the animals were killed. The liver and adipose tissue from the mesentery were taken.

The extraction of organic chlorine pesticides, including PCBs, were carried out according to the generally accepted procedures.

PCBs were separated from organic chlorine pesticides in the purified extracts by means of HPLC (Varian Model 1683 with a UV detector). The fractions containing PCBs and the remaining initially separated organic chlorine pesticides were analysed by means of gas-liquid chromatography. The Pye-Unicam gas chromatograph equipped with the electron capture detector (Ni^{63}) and the column containing 1.5% OV-17 and 1.95% OV-210 on Gas Chrom Q 80-100 mesh were used for this purpose. All standards were obtained from Poly Science Co, USA, and Institute of Organic Industry in Warsaw, Poland.

The results were expressed in mg/kg calculated for fat extract. For the statistical analysis the t test of Student and analysis of regression were used.

RESULTS AND DISCUSSION

Arranging the tested compounds according to their concentrations in human milk the highest found level was that of a highly persistent metabolite of p,p'-DDT - p,p'-DDE. The range of values of this metabolite was from 1.4 to 11.2 mg/kg and its concentrations in the liver and adipose tissues of mice in group "1" and "10" were almost equal and much lower than in human milk.

In group "100" the DDE concentration in the adipose tissue of mice was similar to that in milk, while in the liver was nearly twice as high (difference statistically significant at $p < 0.01$). The DDE presence was demonstrated also in the liver and adipose tissue of control mice.

The p,p'-DDT was present in human milk in the range from 0.10 to 1.65 mg/kg of fat. In the adipose tissue of mice in group "100" the accumulation of this compound approached the levels found in milk, but in the liver it was significantly higher ($p < 0.05$). In the remaining groups, both in mesentric adipose tissue and in the liver, the level of this compound was significantly lower than in milk.

The highest level among of all HCH isomers in milk was that of beta-HCH. This isomer was present in similar concentrations in the liver and adipose tissue in group "1" mice, and in significantly higher concentrations ($p < 0.01$ and $p < 0.001$) in groups "10" and "100". In groups "1" and "10" alpha-HCH was not detected. In group "100" in mesenteric adipose tissue and in the liver the concentration of the compound was not significantly different from that in milk. In the liver and adipose tissue of group "1" the concentrations of gamma-HCH approached those present in milk. In groups "10" and "100" the concentrations were significantly greater.

In groups "10" and "100" the concentrations of HCB in the liver and adipose tissue were significantly higher than in milk and in group "1". The hepatic concentration of this compound in group "100" was significantly higher than in mesenteric fat ($p < 0.05$).

HCB was present also in the liver and fat in group "0" animals.

PCBs - the concentrations of these compounds in human milk and in the liver and adipose tissue of mice in group "100" were similar. In the remaining groups they were below the sensitivity threshold of the method. These results are presented in Table 2, 3 and 4.

Table 2. Residues of organic chlorine pesticides and PCBs in human milk (mg/kg of fat)

Compound	Arithmetical mean	Range
p,p' -DDE	4.95 ± 1.85	1.39 - 11.20
p,p' -DDT	0.46 ± 0.33	0.10 - 1.65
p,p' -DDD	0.03 ± 0.02	0.05 - 0.10
o,p -DDT	0.05 ± 0.02	0.01 - 0.10
S -DDT	5.50 ± 2.16	1.50 - 12.84
alpha -HCH	0.02 ± 0.01	0.00 - 0.04
gamma -HCH	0.05 ± 0.02	0.01 - 0.09
beta -HCH	0.13 ± 0.08	0.05 - 0.48
HCB	0.21 ± 0.14	0.04 - 0.67
PCBs	0.38 ± 0.20	0.05 - 0.98

Table 3. Residues of organic chlorine pesticides and PCBs in the liver of mice (mg/kg of fat)

Compound	Groups			
	"0"	"1"	"10"	"100"
DDE	0.053 ±0.012	0.210 ±0.107	0.857 ^{2a,2b} ±0.146	9.458 ^{3a,3b} ±2.373
DDT	0	0.013 ±0.019	0.100 ^{3a,2b} ±0.016	0.860 ^{3a,3b} ±0.169
alpha-HCH	0	0	0	0.095 ^{1a,1b} ±0.044
beta-HCH	0	0.177 ^{2a} ±0.031	0.293 ^{3a,2b} ±0.017	1.085 ^{2a,2b} ±0.336
gamma-HCH	0	0.043 ^{3a} ±0.005	0.123 ^{2a,1b} ±0.029	0.327 ^{2a,2b} ±0.118
HCB	0.017 ±0.012	0.137 ^{1a} ±0.054	0.353 ^{2a,1b} ±0.054	1.087 ^{3a,3b} ±0.178
PCBs	0	0	0	0.380 ^{2a,2b} ±0.137

1 - $p < 0.05$; 2 - $p < 0.01$; 3 - $p < 0.001$
a - in relation to group "0"; b - in relation to group "1".

Table 4. Residues of organic chlorine pesticides and PCBs in the mesenteric adipose tissue in mice (mg/kg of fat)

Compound	Groups			
	"0"	"1"	"10"	"100"
DDE	0.063 ±0.031	0.209 [a] ±0.071	0.844 [a,b] ±0.137	5.765 [a,b] ±2.068
DDT	0	0.026 [a] ±0.018	0.069 [a,b] ±0.012	0.672 [a,b] ±0.145
alpha-HCH	0	0	0	0.063 [a,b] ±0.011
beta-HCH	0	0.094 [a] ±0.035	0.291 [a,b] ±0.079	0.836 [a,b] ±0.318
gamma-HCH	0	0.033 [a] ±0.009	0.079 [a,b] ±0.025	0.252 [a,b] ±0.109
HCB	0.020 ±0.023	0.070 [a] ±0.014	0.230 [a,b] ±0.068	0.813 [a,b] ±0.173
PCBs	0	0	0	0.260 [a,b] ±0.102

a - in relation to group "0", b - in relation to group "1"
$p < 0.001$

Comparing the degree of accumulation of various compounds in the liver and mesenteric fatty tissue in mice in group "100" significantly higher concentrations of DDE, DDT and HCB were found in the liver.

The relationship between the concentration of the tested compounds in daily doses, and their accumulation in the liver and adipose tissue are presented in Table 5.

The statistical analysis demonstrated a very highly significant correlation between the daily dose and the concentrations of these compounds in the liver and mesenteric adipose tissue.

The problem of metabolism and distribution of these substances in the organism have been the subject of many studies and experimental works in late 1960s and early 1970s, when the risk of poisoning with high doses of preparations used for crop protection was high (Kutz et al. 1977, Niessen et al. 1984).

Presently, despite of ban of some organic chlorine compounds in the agriculture, they are still present in food of animal and plant

origin (Falandysz and Falandysz 1985, Nikonorow 1982) as well as in the other parts of the environment, which is partly due to their use in many branches of the industry. These persistent compounds can reach the food chain and ultimatively accumulate in vitally important human tissues and organs creating toxicological problems of great complexicity.

Table 5. Relationships between the concentration of organic chlorine compounds in daily dose and their accumulation in the liver and mesenteric adipose tissue

Compound	Correlation coefficient	Significance level	Regression coefficient
		Liver	
DDE	+ 0.947	0.001	0.094
DDT	+ 0.967	0.001	0.009
beta-HCH	+ 0.856	0.001	0.003
gamma-HCH	+ 0.898	0.001	0.010
HCB	+ 0.955	0.001	0.010
		Mesenteric adipose tissue	
DDE	+ 0.909	0.001	0.056
DDT	+ 0.964	0.001	0.007
beta-HCH	+ 0.844	0.001	0.002
gamma-HCH	+ 0.865	0.001	0.007
HCB	+ 0.948	0.001	0.007

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