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Organochlorine Pesticide Residues in Human Breast Milk from Agricultural Regions of Turkey, 1995-1996

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Organochlorine pesticides (OCP) were produced in large amounts in the 1940-1950s and the global production increased year by year. They were banned or severely restricted during the early 1970s in most of the developed countries after understanding of their long term toxic effects. In Turkey , OCPs have been started to be used against pests in 1945, large quantities of these chemicals were used during 1960s and 1970s and since 1983 usage of these chemicals have been severely restricted. Breast milk is at the top of the food chain and one of the good markers for the determination of environmental pollution which is created by OCP (Jensen 1983). Since 1950 a large number of studies on the levels of OCPs and their metabolites in human milk in different countries have been published (Weisenberg et al. 1985; Dommorco et al. 1987; Dewailly et al. 1989; Bordet et al. 1993; Hernandez et al. 1993). In these studies DDT and its metabolites, BHC isomers, HCB, and heptachlor epoxide, are the most commonly reported.

The aim of this study is to present the last data of biological monitoring studies concerning the determination of OCP residue levels in the environment which began in 1986 and has been studied during certain time intervals (Karakaya et al. 1987; Burgaz et al. 1994). Results obtained will form part of an up-to-date report on OCP pollution in Turkey.

MATERIALS AND METHODS

104 human milk samples were collected between May 1995 and December 1996 from healthy donors living in two different regions of Turkey for at least 5 years. The age of mothers ranged from 17 to 44 (mean age 26±5.85 years). Milk samples (15-30 ml) were taken from one of the breasts by manual expression at the end of the feeding, and between 5th and 47th days of postpartum. Samples were kept frozen at -20 °C until analysis. Each mother completed a questionnaire to provide personal information such as number of births, smoking, occupation, dietary habits and place of residence. Nearly all the donors

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except six were nonsmokers.

The regions were selected on basis of similarities and differences in their environmental and socioeconomic characteristics: Manisa is an industrial and agricultural area, located in the west of Turkey; Van is an agricultural and stockbreeding region, located in the east of Turkey.

Before extraction, samples were homogenized and kept 10 min. in 30°C water bath. After adding aldrin as an internal standard, into the 2.5 g of milk it was mixed with 17 ml of chloroform/methanol 1:1 (v/v); 7 ml redistilled water was added and the mixture was centrifuged until separation. The lower phase containing fat was transferred into a weighed tube and the upper phase was re-extracted twice with an additional 5 ml of the solvent mixture. The pooled extracts were evaporated to dryness under a stream of nitrogen and the fat content was weighed. The fat was redissolved in 4 ml hexane and 5 ml conc. H_2SO_4 was added for purification. After centrifugation, the organic phase concentrated to 2.0 ml (Krauthacker et al. 1986) and 0.5 µl was injected into gas chromatograph.

Gas chromatographic analysis has been performed using a Hewlett-Packard Model 5890 gas chromatograph equipped with a ^{63}Ni electron capture detector and a HP 3396 integrator. Chromatographic determination of OCP was carried out using a 25mx0.25 mm fused silica capillary column HP-5 from Hewlett-Packard. The operating conditions were: injector temperature 260 °C ; detector 320 °C ; column 80 °C initial with 1 min. hold 10 °C/min to 280 °C ; 1/10 split ratio. The carrier gas was helium. Peak areas were used as the basis for quantification. Residue levels are expressed as mg/kg extracted fat (ppm).

All solvents used pesticide analytical grade reagents free of interfering residues as tested by gas chromatography.

Standards of HCB, α-BHC, β-BHC, γ-BHC, p.p'.DDE, p.p'.DDT, heptachlor epoxide, and aldrin were obtained from US.Environmental Protection Agency (EPA).

Recoveries from a fortified sample at 0.2 ppm for each level were in the range of 81-94 % for this method, including internal standard. Results were not corrected for the percentage recovery.

Detection limits for α-BHC, β-BHC, γ-BHC, HCB, heptachlor epoxide, p.p'.DDE, and p.p'.DDT were 1,1,1,1,1,2,3 ppm respectively.

The different sets of data were examined for statistical differences by the Mann-Whitney U test. Sperman rank correlation was calculated to measure association between residues . In order to test differences between subgroups Kruskal-Wallis nonparametric ANOVA test was applied.

RESULTS AND DISCUSSION

The average finding from these two provinces which have quite different conditions are thought to represent the general pattern of OCP levels in Turkey. For this reason, average values of OCP residues are considered in the results.

104 milk samples were analysed by GC and the results are shown in Table 1. Residues of α-BHC, β-BHC, HCB, Heptachlor epoxide, and p.p'.DDE were found to be the major contaminants in milk samples of Manisa and Van residences. A detectable amount of α-BHC was found in 93 % , β-BHC in 100% , γ-BHC in 45%, HCB in 96%, heptachlor epoxide in 96%, p.p'.DDE in 100%, p.p'DDT in 44% of the samples.

Table 1. Levels of OCP in human milk in Van and Manisa provinces (Mean±S.D.)

Compound	Van	Manisa	Average
HCB	0.058±0.029	0.044±0.027	0.050±0.030
α-BHC	0.050±0.020	0.067±0.037	0.060±0.032
β-BHC	0.417±0.140	0.355±0.137	0.380±0.141
γ-BHC	0.016±0.023	0.017±0.028	0.017±0.026
Σ-BHC[a]	0.483±0.152	0.441±0.220	0.457±0.167
HEPTACHLOR EPOXIDE	0.078±0.028	0.069±0.037	0.072±0.034
P.P'.DDE	2.263±1.188	1.851±0.700	2.013±0.939
P.P'.DDT	0.141±0.168	0.072±0.130	0.100±0.149
Σ-DDT[b]	2.670±1.470	2.153±0.906	2.357±1.182

[a]Σ-BHC= α-BHC+β-BHC+γ-BHC
[b]Σ-DDT= 1.115xp.p'.DDE+p.p'.DDT

As 91% of the subjects were housewifes, therefore, the relationship between occupational exposure and OCP was not investigated.

Mothers were classified arbitrarily according to their age into three groups: 17-25 (n:48), 26-32 (n:40), and 34-44 (n:16). Concentration of p.p' DDE , p.p'DDT increased significantly with selected age groups ($p<0.01$). However, concentration of HCB, β-BHC, heptachlor epoxide, Σ-BHC, and Σ-DDT increased strongly with selected age groups ($p<0.001$). This is a habitual finding usually explained by the longer exposure time and the long life of OCP residues (Kutz et al. 1991). In our study there was no clear decreasing influence of the number of childbirths on the Σ-DDT and Σ-BHC concentrations.

Heptachlor epoxide was clearly detected in most of the samples analyzed. The presence of heptachlor epoxide in Turkish human milk (Üstünbas et al. 1994) and adipose tissue (Burgaz et al. 1994) has been reported earlier. The heptachlor epoxide level in human body originated as an oxidation product from heptachlor as pesticide used in Turkey until 1985. Heptachlor epoxide levels found are lower than those reported from France, (Bordet et al. 1993) higher than those obtained in Spain (Hernandez et al. 1993).

Table 2 shows the trend for the mean levels of the main residues of OCPs over the 10 years that these surveys have been carried out in different cities of Turkey.

HCB is one of the most persistent and toxic organochlorine contaminants. This compound has not been used since 1959 in Turkey. In this study it was found that 96% of all the milk samples contained HCB residues. The reason of HCB residues are shown as in various industrial activities HCB residues are produced as by product , presence in other pesticides (Tobin 1986) as an impurity and the transformation of BHC to HCB biologically. In the latest study which is on the detection of OCP levels in adipose tissue, HCB level is found as 0.164 ppm fat basis (Burgaz et al. 1994). Present study which yields considerable level of HCB residue in human milk shows parallel results with previous research and also maintains the presence of HCB exposure in Turkey.

Table 2. Average levels of OCP in milk from mothers in different regions of Turkey (mg/kg fat basis)

City	*Year*	*n*	*α-BHC*	*β-BHC*	*γ-BHC*	*HCB*	*HE***	*pp'DDE*	*pp'DDT*	*ΣDDT*	*DDE/DDT*	*Ref.*
Sivas	1983	18	0.26	0.94	0.3	0.08	---	---	---	13.97	---	1
Ankara	84-85	61	<0.01	0.92	<0.01	---	---	2.71*	0.42*	3.66	6.45	2
Adana	84-85	52	<0.01	1.43	<0.01	---	---	8.55*	1.17*	10.57	7.31	2
Kocaeli	84-85	50	<0.01	0.72	<0.01	---	---	2.56*	0.37*	3.30	6.92	2
Kayseri	1989	51	0.096	0.522	0.156	0.084	0.011	2.39	0.41	3.07	5.61	3
Van	95-96	41	0.05	0.417	0.016	0.058	0.078	2.26	0.141	2.67	14.74	***
Manisa	95-96	63	0.067	0.355	0.017	0.044	0.069	1.85	0.072	2.15	17.45	***

(*Median; **Heptachlor epoxide; ***Present study; References: 1)Cetinkaya et al. 1983; 2)Karakaya et al. 1987; 3)Üstüntas et al. 1994)

Experimental and epidemiologic studies show that due to the faster transformation kinetics of DDT, DDE/DDT ratio increases with time after exposure or after the limitation or restriction of DDT usage. In our study, DDT and DDE correlated significantly ($r=0.604$, $p<0.05$) and DDE/DDT ratio is increased from 6.78 to 16.04 in milk samples. This ratio of DDE/DDT in the present study is as high as those found in most developed and developing countries where DDT use has been prohibited since the 1970’s (Table 2).

As shown in Table 3 Σ-DDT ratio in milk tends to decrease gradually in our country. A significant difference has been found in Σ-DDT levels between the present study and the 1987 study (Karakaya et al. 1987) ($p<0.001$).

Table 3. Average OCP residues in human milk from various countries (µg/kg whole milk)

Country	Year	p.p'.DDE	p.p'.DDT	DDE/DDT	References
Israel	1985	79.0	8.46	9.33	Weisenberg et al.1985
Italy	1985	1.4	0.25	5.6	Dommorco et al. 1987
Canada	1987	29.22	2.45	11.92	Dewailly et al. 1989
France	1990-1991	21.83	0.79	7.3	Bordet et al. 1993
Spain	1991	18.7	0.4	46.75	Hernandez et al. 1993
Egypt	1993	21.37	2.93	7.3	Saleh et al. 1996
Turkey	1995-1996	20.13	1.0	16.04	Present study

We calculated the daily intakes of HCB, Heptachlor epoxide, Σ-DDT by breast-fed children assuming that a child consumes 130 g milk per day per kilogram of body weight. The daily intakes were calculated from median and maximal concentrations of the compounds. In these calculations the fat content is regarded as 3.21% (w/w) but individual values ranged widely from 0.4 to 8.8 %. The comparison of these intakes with the ADI set by WHO is shown in Table 4.

Table 4. Comparison of daily intake of OCPs (µg/kg b.w/day) from milk

	n	*Median*	*Maximum*	*ADI*
HCB	100	0.21	0.53	0.6**
HE*	100	0.31	0.69	0.1
Σ-DDT	104	9.32	28.5	20

*Heptachlor epoxide
**Conditional

Although the daily intakes of HCB and Σ-DDT by infants were in general low, all intakes of Heptachlor epoxide were greater than the ADI.

When the results of this study are considered, it could be argued that due to the prevention, OCP residues tend to decrease in Turkey in terms of exposure to HCB both in industry and in agriculture more strict preventions are needed.

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