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Organochlorine Pesticide Residue Levels in Human Adipose Tissue of Residents of Manisa (Turkey), 1995-1996

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Persistent organochlorine pesticides (OCPs) have been used intensively in agriculture for a relatively long period of time. The occurrence of OCPs in the environment and subsequently in parts of the food chain resulting in the intake of these compounds by man and animal already been noted since the early sixties (Kutz et al. 1991). To estimate the effect of certain government measures to ban the use of persistent toxic chemicals, most countries have conducted initial monitoring programs to determine organochlorine pesticides and polychlorinated biphenyls (PCBs) in human tissues (Jensen 1983; Kutz et al. 1991).

The measurement of the levels of OCP in adipose tissue of human populations are good markers in determining the extent of exposure and in the evaluating the hazards. The levels of OCP in human adipose tissues has been the subject of a number of studies reporting the last two decades. Most interest has centered on DDT and its metabolites and BHC isomers.

In Turkey OCP residues have been monitored in the Turkish population in human adipose tissue samples by carrying out regional surveys at given time intervals since 1976 (Kayaalp 1979; Karakaya & Özalp 1987; Burgaz et al. 1994). Although there are some studies reporting OCP levels in mother milk in different regions of Turkey (Karakaya et al. 1987; Çok et al. 1997), studies on OCPs in adipose tissue in Turkey are limited only in Ankara residence. This study is significant in regard to reporting OCPs in adipose tissue in another province rather than Ankara.

MATERIALS AND METHODS

Between April 1995 and December 1996, 56 human adipose tissue samples (35 female and 21 male) were taken during surgical operations in Manisa State Hospital from different donors living in Manisa province for at least 5 years. The age of subjects ranged from 18 to 70 (mean±SD; 41.2±14.2 years). Each subjects completed a questionnaire to provide personal information such as smoking, occupation, dietary habits, weight, height and number of births. 40 of the subjects were nonsmokers. Of the mothers 3 had one, 10 had two children and 22 were multiparous (more than 3). All the subjects were mixed food consumers.

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The adipose tissue samples were kept frozen (at -20 ° C) in glass containers until analysis. Manisa is an urban city and has low altituted and warm climate, main economic activities of Manisa region are agriculture (fruit trees, cotton, tobacco, olive), tourism, alimentary, electronics, textile and mine industries.

100 mg samples were ground with anhydrous sodium sulphate by using a tissue grinder. Aldrin was used as an internal standard and also added to the adipose tissue before extraction. Then tissue samples were extracted with 4x2x2 ml of n-hexane. The combined n-hexane extract was evaporated to dryness to determine extractable fat content (Smrek and Needham 1982) using 8 ml n-hexane as the solvent, the sample was cleaned up with 1-2 ml of conc. sulphuric acid (Murphy 1972; Wickström et al. 1983).

Gas Chromatographic (GC) analysis has been performed using a Hewlett-Packard Model 5890 Gas chromatograph equipped with a ^{63}Ni-EC detector and a HP 3396 integrator. Chromatographic determination of OCPs was carried out using a 25m x0.25 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/mm 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 relative to extracted lipid (mg/kg; ppm). Recoveries from a fortified sample at 0.2 ppm each level were in the range of 81-94% on 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 ppb respectively.

All solvents used pesticide analytical grade reagents free of interfering residues as tested by Gas chromatography. All glassware was washed with detergent and distilled water and rinsed with etanol and n-hexane. Standards of HCB, α-, b-, g-BHC, p.p'-DDE, p.p'-DDT, heptachlor epoxide and aldrin were obtained from US.Environmental Protection Agency (EPA).

The different sets of data were examined for statistical differences ($p<0.05$) by the Mann-Whitney 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

Fifty-six adipose tissue samples were analyzed by GC and the results of the subjects are shown in Table 1. Residues of α-BHC, β-BHC, HCB, heptachlor epoxide and p,p'-DDE were found to be major contaminants in the adipose tissue samples of Manisa residents. A detectable amount of α-BHC was found in 93%, β-BHC in 96%, γ-BHC in 36%, HCB in 84%, heptachlor epoxide in 100%, p,p'-DDE in 100%, p, p'-DDT in 25% of the samples. Our results indicated that the

donors' obesity index (weight/height^2) did not correlate with residue levels of OCP compounds in human adipose tissue.

Table 1. Chlorinated hydrocarbon residues in adipose tissue in Manisa Residents (The mean levels expressed as mg/kg extracted fat basis).

Compound	Mean (±S.D)	Range
HCB	0.033±0.036	N.D - 0.177
α-BHC	0.102±0.067	N.D - 0.339
β-BHC	0.374±0.311	N.D - 1.581
γ-BHC	0.043±0.095	N.D - 0.479
Σ-BHC	0.519±0.339	0.129 - 1.694
HEPTACHLOR EPOXIDE	0.121±0.063	0.030 - 0.316
p.p'-DDE	1.832±0.889	0.305 - 3.938
p.p'DDT	0.088±0.212	N.D - 1.039
Σ-DDT	2.130±1.026	0.305 - 4.325

N.D: under the limit of detection
Σ-BHC=α-BHC+β-BHC+γ-BHC
Σ-DDT=1.15xp.p'-DDE+p.p'-DDT

The increase of level of OCPs in increasing age is an expected finding. Because it is well known that these chemicals accumulate in the body during life course and their metabolisms and elimination take place at slow pace. Donors were classified arbitrarily according to their age into four groups: 18-30 (n: 13), 31-40 (n: 16), 41-49 (n: 14) and 50-70 (n: 13) years. Nevertheless, in this study, Σ-DDT levels were significantly different between age 31-40 and 41-49 ($p<0.01$) and DDE levels were also found significantly differences were determined between age groups 18-30 and 41-49 ($p<0.05$). For all 4 age groups although there were differences in HCB, α-BHC, β-BHC, Σ-BHC and especially p,p'-DDT amounts, these were not found statistically significant.

Although both DDT and its metabolites and BHC and its isomers were expected to be lower in women than those of in the men due to the factors like giving birth and menstruation, in this study our gender difference was not determined except the Σ-BHC level difference ($p<0.01$). However, there were gender differences between β-BHC, γ-BHC and p.p'-DDT levels which were not statistically significant. For this point, different findings were obtained in different countries. While in of some these studies higher residue levels were being determined in man (Mussalo et al. 1984) some of the others found higher levels in women (Focardi et al. 1986). No gender differences were observed in some studies (David et al. 1984). It is striking to determine HCB levels of 84 % of the 56 analyzed samples. Because the agriculture use of HCB was prohibited in 1959 in Turkey. In various countries many studies on adipose tissue and mother milk, high levels of HCB residues was detected (Camps et al. 1989). These studies maintain that various industrial activities and other pesticides (PCNB, PCP, etc) as a source (Tobin, 1986).

In Turkey such industrial activities are known to be used present and also pesticides including HCB impurities are know to be used a considerable amount. In Turkey in a latest study on people living in Ankara by using adipose tissue was found HCB level as 0.164 ppm while in this study it is found as 0.033 ppm. This is relatively lower value than those found in other studies which are held on in Turkey (Burgaz et al. 1994), and in some countries such as Spain (Gomez-Catalan et al. 1995) and Poland (Ludwicki & Goralczyk, 1994).

The level of α- and γ- isomers of BHC in organisms and in ecosystem were found low because of transformation to β- isomer and because of the persistent of β-isomer (Kutz et al. 1991). The distribution of BHC isomers that was found in our study tend to support the results of this study. Heptachlor epoxide was detected in all adipose tissue samples (100%). The presence of heptachlor epoxide in Turkish human adipose tissue has been reported as 0.021 ppm by Burgaz et al. (1994). In this study the amount of the heptachlor epoxide is found as 0.121 ppm which is significantly different than the previous one ($p<0.01$). This result might be attributed to the fact that the collected samples from Manisa where agriculture is predominant and before prohibition of the use of heptachlor which is the source of heptachlor epoxide was common. Levels of OCPs in Turkey depending on the results of adipose tissue studies are shown in Table 2.

Table 2. OCP levels in adipose tissue in different regions of Turkey (mg/kg fat basis

Year	Region	n	Σ-BHC	p.p'-DDE	p.p'-DDT	Σ-DDT	DDE/DDT	Ref
76-77	ANKARA	41	4.20±0.73*	10.2±0.64	3.20±0.63	14.6±1.38	3.19	1
84-85	ANKARA	48	1.72±0.83	5.83±3.31	0.62±0.50	7.12±4.10	9.40	2
91-92	ANKARA	60	1.54±1.04	3.72±3.59	0.27±0.32	4.42±4.16	13.77	3
95-96	MANISA*	56	0.59±0.39	1.83±0.89	0.09±0.21	2.13±1.03	20.82	**

(1)Kayaalp et al.1979; (2)Karakaya&Ozalp 1987; (3)Burgaz et al.1994;* as γ-BHC, **Present study

In present study, the ratio of DDE/DDT is found as 20.82. This value is higher than the previous studies which held on different regions in Turkey (Kayaalp et al. 1979; Karakaya & Ozalp 1987; Burgaz et al. 1994). In Table 3, the levels of p.p'-DDE and p.p'-DDT in human adipose tissue found in the present study and DDE/DDT ratios are compared with those of other countries. Ratio of DDE/DDT is significantly higher than those found in other countries such as Germany and Poland, where DDT was banned in 1970s and in such countries as India and Kenya, where DDT is still used in sanitary actions . The amount of Σ-DDT was found to be decreased as compared to Karakaya et al. (1987) and Burgaz et al. (1994) studies ($p<0.001$) (Table 2).

Table 3. Mean levels (mg/kg exctracted fat basis) of p.p'-DDE and p.p'-DDT residue levels in human adipose tissues from various countries

Country	Year	p.p'-DDE	p.p'-DDT	DDE/DDT	References
Spain	1991	3.93	0.40	10.70	Gomez-Catalan et al. 1995
India	88-89	0.71	0.88	0.81	Nair et al. 1992
Kenya	1992	3.26	2.49	1.31	Kanja et al. 1992
Mexico	1991	10.00	4.02	2.49	Waliszewski et al. 1996
Germany	1990	0.44	0.09	4.69	Teufel et al.1990
Poland	89-92	5.75	0.54	10.70	Ludwicki & Goralczyk 1994
Iran	91-92	2.45	0.19	12.89	Burgaz et al. 1995
Turkey	95-96	1.83	0.09	20.82	Present study

Since the year 1976 the increase in DDE/DDT ratio and decrease in Σ-DDT and Σ-BHC levels in adipose tissue demonstrate that the influence of limitations and inhibitions for OCPs and the decrease of expence to these compounds in time.

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