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Determination of Organochlorine Pesticides Residues in Human Adipose Tissue, Data from Cukurova, Turkey

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Abstract We determined and compared the levels of BHC and DDT isomers and metabolites and HCB in the adipose tissue of 82 cases of autopsies performed at the Morgue Department of Adana Branch of the Council of Forensic Medicine. The relationships between the age, gender, and body mass indexes of cases, and the accumulation of OCs residues were also investigated. Detectable concentrations of p,p'-DDE were found in 100% of adipose tissue samples. Concentrations of OCs in female adipose tissues were significantly higher than male adipose tissues ($p < 0.05$). The presented work is highly significant, being the first study pointing out the chronic exposure to organochlorine pesticides in Cukurova region.

Keywords Organochlorine pesticide · Subcutaneous adipose tissue · Gas chromatography · Cukurova region

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Organochlorine pesticides (OCs) have been used intensively in agriculture for a relatively long period of time. In Turkey, the use of some organochlorine pesticides was restricted in the mid-1980s. After 1985, the use of OCs has been banned except for endosulfan and toxaphene (Vural 1996). Due to their resistance to be metabolized and their lipophilic characteristics, OCs can spread out extensively. They tend to be accumulated in biological samples which has lipid serum, adipose tissue and breast milk (Waliszewski et al. 2001, 2002). The amount of fat in the subcutaneous fatty tissue is 80%, in serum 1% and 2%–4% in milk. Lipophilic contamination level in the subcutaneous fatty tissue is five times higher than milk and 100–350 times more than blood (Frias et al. 2004). The analysis of the levels of OCs in adipose tissue of human population remain reliable markers in determining the extent of chronic exposure and in the evaluating the hazards. Chronic exposure of humans to lipophilic OCs occur through different pathways; consumption of contaminated foods, specifically with high fat content (fish, meat, chicken etc.), local use and environmental contamination (Schildkraut et al. 1999). Although prohibition of the use of OCs by law has caused decrease in the amount used, OCs are still significant environmental pollutant in our country due to bioconcentration.

There have been many studies dealing with the biological follow up of exposure of pesticides and their metabolites in general population and occupational exposure (Waliszewski et al. 1995; Burgaz et al. 1994; Barkatina et al. 2002; Çok et al. 1998; Li et al. 2006). However, effects on human health after exposure to OCs are not adequately understood. Some of these have been implicated in the etiology of various diseases and endocrine-related disorders, such as non-Hodgkin's lymphoma, leukemia, brain and uterus cancer, soft tissue sarcoma and low sperm concentration (Quintana et al. 2004; Stellman et al. 2000). Pesticide consumption increased 45% between 1979 and 2002 in Turkey. Thirty two percent of Turkey's annual pesticide use takes place in the Cukurova region, which is one of the most important agricultural regions of Turkey (Akcan et al. 2009). In order to display chronic exposure of OCs, the present study determines the levels of pesticide residues in the human adipose tissue samples taken from 82 cadavers that were autopsied at the Morgue Department of Adana Branch of the Council of the Forensic Medicine. The relationships between the age, gender, and body mass indexes (BMI) of cases, and the accumulation of OCs residues were also investigated.

Materials and Methods

Eighty two samples of human adipose tissue were taken from adult cadavers, submitted to autopsy at the Morgue Department of Adana Branch of the Council of Forensic Medicine. Of all cases, 14 were female, and 68 were male and the age of cases ranged from 18 to 78 (mean ± SD; 40.51 ± 16.26 years).

The adipose samples were preserved at −20°C until analyses. The tissue samples were extracted according to previously described methods with modifications (Rivero-Rodriguez et al. 1997; Rivas et al. 2001). Concentrated sulfuric acid was used in the clean-up step of adipose tissue extracts in order to degrade the phthalate esters that interfere in the gas chromatographic identification of organochlorine pesticides.

Gas Chromatographic (GC) analysis was performed using a Perkin–Elmer Model Autosystem XL Gas Chromatograph (United Kingdom) equipped with a ^{63}Ni-EC detector. Chromatographic determination of OCs was carried out using a 30 m × 0.32 mm D.I. fused silica capillary column HP-5 from Hewlett-Packard (Agilent, U.S). The operating conditions were: injector temperature 250°C; detector 300°C; column 70–280°C (2 min at 70°C; 25°C/min., from 70 to 150°C; 3°C/min., from 150 to 200°C; 8°C/min., from 200 to 280°C; 10 min at 280°C. The carrier gas was helium. Peak areas were used as the basis for quantification. Residue levels are expressed relative to extracted lipid (ng/g).

All solvents used were pesticide analytical grade reagents (Merck, Germany) free of interfering residues as tested by Gas chromatography. Pesticides' standards used in analysis were: α-BHC, β-BHC, δ-BHC, HCB, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT (Dr. Ehrenstorfer, Augsburg). For the investigation of pesticide retention times with gas chromatography, standard pesticide solutions were prepared with hexane, using aldrin as an internal standard. Excellent linearity was obtained in the concentration range studied, with correlation coefficients between 0.9970 and 0.9999. The mean relative deviation of OCs determination did not exceed 15%. To determine the quality of the method, a recovery study was performed on overspiked replicates of blank cow fat samples. The method validation studies done at 25–100 ng/g for fat samples, depending on the pesticide type, which showed ranging from 74 to 107% of recovery. The limit of detection was 0.48 ng/g of tissue for α-BHC, β-BHC and δ-BHC, 0.24 ng/g for HCB, and *p,p′*-DDE, 0.97 ng/g for *o,p′*-DDE, *o,p′*-DDT and *p,p′*-DDT. Control experiments with reagents had been carried out before the analysis.

All analysis results were given in units of ng/g. Statistical analyses were performed with SPSS 16.0 software pack. Categorical measurements (gender, age group etc.) were demonstrated as number or percentile, while progressive measurements (age, BMI etc.) were demonstrated as mean and standard deviation (median were demonstrated as min. and max. as required). For the comparison of residual values in fat tissue samples of different genders, age groups and BMI groups, Mann Whitney *U* test or Kruskal Wallis test was employed. Sperman Correlation coefficient was employed to investigate the correlation between pesticides. Linear Regression was employed to model Σ-BHC and Σ-DDT values. A $p < 0.05$ value was regarded as statistically significant.

Results and Discussion

The mean values of the organochlorine concentrations detected in adipose tissue are shown in Table 1. All samples studied were positive for at least one or more chemicals. The amount of OCs from highest concentration to lowest were as follows: *p,p′*-DDE, α-BHC, HCB, *p,p′*-DDT, *o,p′*-DDT, β-BHC, *o,p′*-DDE and δ-BHC. Detectable concentrations of *p,p′*-DDE were found in 100% of the adipose tissue samples. Among the remaining pesticides, DDT, *p,p′*-DDT was the most frequently (84.1%) available pesticide. However, HCB was detected in 62.2%, α-BHC was detected in 43.9%, β-BHC was detected 48.8%, *o,p′*-DDT was detected in 3.7% and *o,p′*-DDE was detected in 3.7% of samples. The DDE/DDT ratio was calculated as 8.87.

Subject cases were divided into three age groups: the first age group was 18–25 (n: 16) years, second group was 26–45 (n:38) ages and third group was ≥46 (n:28) age. Even though the levels of *p,p′*-DDE and Σ-DDT increased by age, statistically significant results were observed only

Table 1 Residue levels of studied pesticides in human adipose tissue (n = 82) (ng/g)

Compound	$X \pm SD$[a]	Min.	Max.	Percentage
HCB	5.34 ± 5.59	0.26	37.60	62.2
α-BHC	8.04 ± 7.17	0.72	35.80	43.9
β-BHC	1.77 ± 2.67	0.49	16.90	48.8
δ-BHC	ND	ND	ND	ND
Σ-BHC	6.05 ± 7.25	0.48	35.80	72.0
o,p′-DDE	1.41 ± 0.59	0.97	2.08	3.7
p,p′-DDE	52.27 ± 56.12	1.27	319.40	100
o,p′-DDT	1.92 ± 0.73	1.09	2.44	3.7
p,p′-DDT	4.13 ± 5.41	0.73	39.18	84.1
Σ-DDT	55.87 ± 59.03	1.27	327	100

ND under the limit of detection

[a] Arithmetic mean ± standard deviation, Σ-BHC = α-BHC + β-BHC + δ-BHC, Σ-DDT = *o,p′*-DDE + *p,p′*-DDE + *o,p′*-DDT + *p,p′*-DDT

between 18–25 to ≥46 and 26–45 to ≥46 ($p < 0.001$). Although the average pesticide levels were higher in 26–45 age group compared to 18–25, this difference was not statistically significant. In this study, pesticide levels were affected by gender, meaningfully. The levels of β-BHC, Σ-BHC, *p,p′*-DDE, *p,p′*-DDT and Σ-DDT were higher in female cases. However, no significant correlation were observed HCB and gender ($p > 0.05$).

There were no statistically significant relationship between BMI changes and the adipose tissue levels of HCB, Σ-BHC and Σ-DDT ($p > 0.05$). Since the authors were not able to obtain data regarding diets of the cases, a comparison was not performed using diet information.

Organochlorine pesticides are lipophilic agents that are persistent in the environment because of their chemical stability. Chronic exposure mainly occurs through the contaminated foods and poor environmental conditions in the general population. As these chemicals are lipophilic, resistance and metabolized slowly, they tend to concentrate in fatty tissue of organisms. In this respect, use of OCs is controlled and partly prohibited throughout the world. There have been several biological follow up studies investigating residue levels of these chemicals accumulated in the adipose tissue of humans and animals. The levels detected have been considered as an indicator measuring general population exposure of these compounds. Furthermore, such biological follow up reports might lead authorities to make regulations preventing chronic exposure of OCs and to decrease their environmental levels (Rivas et al. 2001). Interestingly, there is not a previously conducted study displaying residue levels of OCs in Cukurova region that is responsible for a great deal of pesticide use.

The experiments revealed statistically significant relationship between gender and pesticide levels. Higher amount of residual pesticides were detected in females compared to males. Various pesticide residue levels have been obtained from studies carried out in different countries. Some of these studies revealed higher residue levels in men (Çok et al. 1998), while others found higher levels in women (Barkatina et al. 2002; Burgaz et al. 1994). No significant correlation was reported between residue levels and gender (Molina et al. 2005). On the contrary, pesticide residue levels were changed by gender meaningfully. The levels of β-BHC, Σ-BHC, *p,p′*-DDE, *p,p′*-DDT and Σ-DDT were detected to be higher in females. However, no significant correlation were observed between HCB levels and gender ($p > 0.05$). While, a study conducted by Arrebola et al. (2009) found that levels of HCB in adipose tissue were three times higher in women than in men.

Due to chronic exposure, these chemicals continually accumulate in the body tissues during a person's life. Additionally their metabolisms and elimination occur at a slow pace (Çok et al. 1998). Thus, the increase of levels of OCs with increasing age is an expected finding (Waliszewski et al. 1995; Burgaz et al. 1994). Even though *p,p′*-DDE and Σ-DDT levels increase by age, the correlation of residual amounts and BMI was not statistically significant ($p > 0.05$).

DDT had widely been used in Turkey between the 1950s and the 1970s. Although its use in agriculture was banned in 1972 (Vural 1996), it is allowed in manufacturing of the pesticides currently produced and in use, in Turkey, such as

Table 2 Organochlorine pesticides' residue level in human adipose tissue in different regions of Turkey (mg/kg fat)

Year	Region	n	Σ-BHC	*p,p′*-DDE	*p,p′*-DDT	Σ-DDT	DDE/DDT	Ref
1976–1977	Ankara	41	4.20 ± 0.73	10.2 ± 0.64	3.20 ± 0.63	14.6 ± 1.38	3.19	1
1984–1985	Ankara	48	1.72 ± 0.83	5.83 ± 3.31	0.62 ± 0.50	7.12 ± 4.10	9.40	2
1991–1992	Ankara	60	1.54 ± 1.04	3.72 ± 3.59	0.27 ± 0.32	4.42 ± 4.16	13.7	3
1995–1996	Manisa	56	0.59 ± 0.39	1.83 ± 0.89	0.09 ± 0.21	2.13 ± 1.03	20.82	4
2007–2008	Adana*	82	0.006 ± 7.25	0.052 ± 56.12	0.0041 ± 5.41	0.056 ± 59.03	8.87	*

1 Kayaalp et al. (1979), *2* Karakaya and Özalp (1987), *3* Burgaz et al. (1994), *4* Çok et al. (1998)

* Present study

Table 3 OCs levels in human adipose tissue in different countries (mg/kg)

Country	Year	p,p′-DDE	o,p′-DDT	p,p′-DDT	Σ-DDT	α-HCH	β-HCH	γ-HCH	δ-HCH	Σ-HCH	HCB	Ref
Germany	1990	0.436	0.013	0.093	0.556	–	0.042	0.038	–	–	–	1
Poland	1990	15.00	0.009	0.67	15	–	0.02	0.004	–	–	–	1
Finland	1991	2.60	–	–	2.80	–	–	–	–	–	–	1
Japan	1991	2.40	–	–	–	–	0.84	–	–	–	–	1
USA	1991	0.679	0.014	0.294	0.987	–	0.163	–	–	–	–	1
Canada	1992	0.585	0.006	0.032	0.623	–	0.028	–	–	–	–	1
India	1994	5.79 ± 6.12	–	6.77 ± 7.23	13.86 ± 12.70	3.63 ± 3.39	5.98 ± 4.52	3.54 ± 3.22	4.77 ± 4.42	17.92 ± 12.4	–	2
Kenya	1992	3.26	0.15	2.49	5.91	–	0.034	–	–	–	–	1
Spain	1993	6.00	–	1.18	7.18	–	1.97	0.06	–			1
Mexico	1992	18.91	1.19	4.72	24.82				–			1
Mexico	1997	4.04	0.05	0.56	4.67	0.01	0.17	0.02	–	0.18	0.07	3
Turkey	1991–1992	3.722 ± 3.59	–	0.275 ± 0.32	4.42 ± 4.16	0.014 ± 0.06	1.552 ± 1.03	0.002 ± 0.01	–	1.539 ± 1.04	0.164 ± 0.22	4
Turkey	1995–1996	1.832 ± 0.889	–	0.088 ± 0.212	2.130 ± 1.026	0.102 ± 0.067	0.374 ± 0.311	0.043 ± 0.095	–	0.519 ± 0.3390	0.033 ± 0.036	5
Argentina	2006	0.918 ± 935.5	–	0.0079 ± 25.7	–	–	0.3674 ± 470.2	–	–	–	0.0683 ± 65.26	6
Belarus	2000	1.019 ± 1.448	–	0.010 ± 0.021	1.029 ± 1.430	–	0.319 ± 0.427	–	–	0.319 ± 0.427	0.061 ± 0.054	7
Mexico	2003	1.22 ± 0.612	0.074 ± 0.078	0.638 ± 0.428	1.949 ± 0.885	0.392 ± 0.254					0.055 ± 0.0418	8
Spain	2006	0.501 ± 496.63	0.014 ± 65.35	0.014 ± 32.92	0.660 ± 744.35							9
Turkey*	2007–2008	0.052 ± 56.12	0.0019 ± 0.73	0.0041 ± 5.41	0.056 ± 59.03	0.008 ± 7.17	0.0017 ± 2.67	–	–	0.006 ± 7.25	0.0053 ± 5.59	10

1 Waliszewski et al. (1995), *2* Dua et al. (1998), *3* Waliszewski et al. (1998), *4* Burgaz et al. (1994), *5* Çok et al. (1998), *6* Munoz-de-Toro et al. (2006), *7* Barkatina et al. (2002), *8* Waliszewski et al. (2003), *9* Cerrillo et al. (2006), *10* present study dicofol. In our country dicofol is used as an acaricide, of which concentration is allowed to contain a maximum 0.1% of DDT. However, a study previously conducted in our country revealed that the amount of DDT in commercially dicofol formulation was between 0.3% and 14.3% (Turgut et al. 2009). As a result, although DDT use has been banned, licensed DDT containing pesticides are still on the market, in Turkey. Residue levels of DDT and metabolites were detected in this study. In this respect, despite their prohibition, the presence of these compounds in the body reflects either a relatively recent exposure or cumulative past exposure.

Adipose tissue residue levels of OCs detected by previously conducted studies in Turkey are shown in Table 2. In the present study the Σ-DDT level was detected as 0.056 mg/kg, which indicates that Σ-DDT levels tend to decrease in the course of years, comparing to previous studies. The ratio of DDE/DDT was found as 8.87. Even though this value was not too high compared to other studies, it was significantly higher than the value (Waliszewski et al. 2001) observed by Karakaya and Özalp (1987) between 1976 and 1977. A statistically significant correlation was not observed between *p,p′*-DDT to *o,p′*-DDT ($p > 0.05$), in this study.

In our series, the percentage *o,p′*-DDT frequency for positivity was 3.7%. This finding was expected as DDT is commercially available as *p,p′*-isomer and *o, p′*-isomer was only 15% of commercial DDT. Therefore, residue levels of *o,p′*-isomer in human adipose tissue are either too low or below detectable limits. This study revealed a remarkable decrease in Σ-BHC levels, compared to other studies performed on adipose tissue in our country (Table 3). Likewise, investigation in terms of Σ-DDT and DDE/DDT ratio showed that the DDT levels have decreased gradually. These results are indicative of the influence of the restriction and prohibition of DDT and the decrease in exposure to these compounds over the past few decades.

According to a study by Çok et al. (1998), HCB pollution has decreased by sixfold between 1993 and 1996. Residue levels of HCB (5.34 ng/g), detected in our study was considerably lower than previous studies conducted in countries given on Table 3. HCB residue levels detected in adipose tissue lead to the necessity of careful examination of pollutant resources.

In all examined samples α-BHC was detected in 43.9%, and β-BHC was detected in 48.8% however, δ-BHC was not detected at all. Similarly, in related studies, detectability rates of β-BHC were higher than α and other isomers (Burgaz et al. 1994; Li et al. 2006), while detectability rates of these compounds were in approximate values. Compared to other studies conducted in our country Σ-BHC levels were considerably lower (Table 2). Likewise, residue levels of α-BHC and β-BHC were considerably low δ-isomer was not found at all, compared to other countries.

In Table 3 the residue levels of OCs in human adipose tissue detected in the present study are compared with those of other countries. Mean concentrations of residual OCs were significantly low when compared with other countries.

In summary, this is the first report regarding residue levels of OCs, from Cukurova Region. More significantly, *p,p′*-DDE was detected in all cases. We believe that further similar studies on this subject in other regions of Turkey would confirm our findings. Forensic toxicology has an important position in terms of legislating new laws to control the effects of environmental toxins on human health.

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