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Levels and Trends of Chlorinated Pesticides in Human Breast Milk from Ankara Residents: Comparison of Concentrations in 1984 and 2002

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The detection of organochlorine compounds in breast milk and adipose tissue samples from the general population indicates widespread exposure to organochlorine pollutants from environmental sources. Therefore, several studies have investigated the residue levels of organochlorine pesticides (OCPs) in human adipose tissue and human milk collected from different countries in the world (Schoula et al., 1996; Cok et al., 1997; Harris et al., 1999; Konishi et al., 2001; Smeds & Saukko 2001; Waliszewski et al., 2002).

Persistent organochlorine pesticides are one of the most important groups of Persistent Organic Polutants (POPs). OCPs were produced in large quantities in the 1940-1950s and global production increased year by year. Most of the OCPs, excluding Endosulfan, were banned in Turkey in 1983. Despite the restrictions or bans of these pesticides, they still circulate in various food chains and eventually concentrate in man. Mother's milk is the primary nutritional source for the infant and the infants being breast-fed are exposed to these residues present in their mother's milk.

In Turkey, OCP residues have been monitored in breast milk and adipose tissue of the Turkish populations by carrying out regional surveys at given time intervals since 1976 (Kayaalp et al., 1979; Karakaya et al.,1987; Burgaz et al., 1994; Cok et al., 1997; Cok et al., 1998). However, there is no information concerning the contamination of human milk by OCPs in Ankara since the first study that had been performed in 1984 and 1985 (Karakaya et al., 1987).

The aim of this study is to present the actual concentrations of Hexachlorobenzene (BHC), p, p'DDT, p, p'-DDE, isomers of Hexachlorohexane (alpha, beta, gamma), and Heptachlor epoxide in human milk samples from Ankara.

MATERIALS AND METHODS

Between April 2002 and December 2002, 101 human milk samples were taken at the Ankara University, School of Medicine, Department of Obstetrics and Gynaecology, from different donors, which had been living in Ankara area for at least 5 years. The recruitment criteria were same as the 1984 study (Karakaya et

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al., 1987). All mothers participated in the study voluntarily. Milk samples (15 -30 ml) were taken from one of the breasts by manual expression at the end of the feeding, and between the 3rd and 45th days of postpartum. Milk samples were kept frozen at -20 °C until analysis. The age of mothers ranged from 17 to 39 (mean age 26.4±4.9). Each donor completed a questionnaire to provide personal information such as weight, occupation, smoking, previous nursing time, dietary habits, and place of residence. Most of the donors except seventeen were non-smokers. Of the mothers, 67 had given one birth, 29 had given two births and 5 were multiparous (more than 3). All subjects were mixed food consumers.

OCPs in human milk were extracted using the method by Krauthacher et al. (1986). Before extraction, milk samples were homogenized and kept in a 30° C water bath for 10 minutes. 2.5 g of milk was mixed with 17 ml of chloroform/methanol 1:1 (v/v); 7 ml re-distilled 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 determined gravimetrically. The fat was re-dissolved in 4 ml hexane and 5 ml -concentrated H_2SO_4 was added for purification. After centrifuging, aldrin was added as an internal standard and the organic phase was concentrated to 2 ml. 1µl of hexane phase was injected into the GC and seven organochlorine pesticides; HCB, α-BHC, β-BHC, γ-BHC, p.p'. DDE, p.p'. DDT, heptachlor epoxide, were quantified.

Standards of OCPs were obtained from the US. Environmental Protection Agency (EPA). All samples were run in duplicates. Recoveries from a fortified sample at 0.2 ppm each level were in the range of 86-94 %, 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 mg/kg respectively.

Gas chromatographic (GC) analysis was 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 OCPs was carried out using a 50mx0.2mmx0.33µm fused silica capillary column Ultra-2 (5% phenyl- and 95% dimethyl-polysiloxane) from Hewlett-Packard. The operating conditions were as follows: injector temperature 260 °C; detector 320 °C; column80 °C initial with 1 min. hold; 10 °C/min to 280 °C. The mobile phase was helium. Peak areas were used as the basis for quantification. Residue levels are expressed as mg/kg extracted fat.

All solvents used were pesticide analytical grade reagents free of interfering residues as tested by gas chromatography. All glassware used for the analysis was rinsed with n-hexane before use.

The different sets of data were examined for statistical differences ($p<0.05$) by the Mann-Whitney U test. Spearman rank correlation was calculated to

measure the association between residues. In order to test the variations between subgroups, Kruskal-Wallis nonparametric ANOVA test was applied.

RESULTS AND DISCUSSION

Table 1 presents the mean concentrations of OCPs in human milk samples from 101 women. Residues of β-BHC, HCB, p.p'. DDT, and p.p'. DDE were found to be the major contaminants in milk samples of Ankara residents. Frequency of OCPs, mean of obtained values and ranges are also listed in Table 1. Our results show that the Ankara population still has detectable OCP levels.

Table 1. OCP residues (mg/kg fat basis) in human milk samples of Ankara residents.

Pesticide	Mean (±S.D)	Range	Frequency (%)
HCB	0,15±0,24	N.D - 1.024	50.49
α-BHC	0.05±0.15	N.D - 0.881	14.85
β-BHC	0.49±0.65	N.D - 2.975	62.37
γ-BHC	0.01±0.05	N.D - 0.342	11.88
Σ-BHC[a]	0,55±0,69	N.D - 2.975	
HEPTACHLOR EPOXIDE	0,06±0,19	N.D - 1.200	17.82
p.p'.DDE	2,28±2,86	N.D - 12.220	95.05
p.p'.DDT	0,13±0,30	N.D - 2.734	82.17
Σ-DDT[b]	2,66±3,40	N.D - 14.090	

N.D: under the limit of detection; [a]Σ-BHC= α-BHC+β-BHC+γ-BHC
[b]Σ-DDT= 1.115xp.p'.DDE+p.p'.DDT

Subjects were classified arbitrarily according to their age into three groups: 17-23 (n: 41), 24-31 (n: 43), and 32-39 (n: 17). No correlations were found between OCP concentrations and age, except for β-BHC levels. The β-BHC levels are significantly higher for the 32-39 age group ($p<0.05$) compared to the 17-23 age

Table 2. The residue levels of OCPs in human milk by age (mg/kg fat basis).

OCP	Age Groups (Years)		
	17-23 (n:41)	24-31 (n:43)	32-39 (n:17)
α-BHC	0,04±0,15	0,07±0,18	0,02±0,04
β-BHC	0,35±0,15	0,48±0,63	0,74±0,90
γ-BHC	0,01±0,02	0,01±0,04	0,03±0,09
HCB	0,12±0,23	0,18±0,21	0,19±0,34
Heptachlor epoxide	0,05±0,16	0,08±0,25	0,05±0,19
p.p'.DDE	1,98±2,47	2,16±2,93	3,30±3,50
p.p'.DDT	0,11±0,19	0,09±0,14	0,27±0,65
Σ-BHC	0,44±0,60	0,56±0,66	0,79±0,90
Σ-DDT	2,33±2,92	2,51±3,35	3,82±4,46

group. Although there are differences in HCB, p.p'. DDE, p.p'. DDT, Σ-BHC and Σ-DDT amounts for all 3 age groups, they were not found statistically significant. The residue levels of OCPs in human milk by age are shown in Table 2.

In this study, subjects were grouped into two as giving 1 and 2+ births and no significant relationship between the number of childbirths and the levels of analysed pesticides was found ($p>0.05$) (Table 3). Similar results have been obtained in other studies (Burgaz et al., 1994; Krauthacher, 1991).

Table 3. Concentrations of OCPs found in primiparous and multiparous women.

OCP	Primiparous women (n:67)		Multiparous women (n:34)		P
	(Mean ± S.D.)	(Max-Min)	(Mean ± S. D.)	(Max-Min)	
α -BHC	0,05±0,14	(N.D- 0,71)	0,05±0,16	(N.D- 0,88)	>0,05
β -BHC	0,44±0,57	(N.D- 2,02)	0,58±0,79	(N.D- 2,97)	>0,05
γ -BHC	0,01±0,04	(N.D- 0,18)	0,02±0,07	(N.D- 0,34)	>0,05
H.E*	0,07±0,23	(N.D- 1,20)	0,02±0,06	(N.D- 0,34)	>0,05
HCB	0,15±0,24	(N.D- 0,81)	0,16±0,26	(N.D- 1,24)	>0,05
p-p -DDE	1,93±2,26	(N.D- 10,8)	2,95±3,69	(N.D-12,22)	>0,05
p-p -DDT	0,11±0,16	(N.D- 0,89)	0,17±0,47	(N.D- 2,73)	>0,05
Σ-DDT	2,27±2,68	(N.D-12,71)	3,39±4,42	(N.D-14,09)	>0,05
Σ-BHC	0,49±0,62	(N.D- 2,39)	0,65±0,80	(N.D- 2,97)	>0,05

N.D: under the limit of detection; * Heptachlor epoxide

Smoker subjects did not have more OCPs in their milk samples compared to nonsmokers ($p>0.05$). As previously reported, tobacco is of minor importance as a source of OCPs (Abbott et al., 1985).

Table 4 compares earlier results of Karakaya et al. (1987) to the results obtained in the present study. As seen in Table 4, OCP levels in human milk samples from subjects who live in Ankara have decreased by time.

Table 5 shows the levels and trends of the main residues of OCPs in adipose tissue samples over the 15 years that surveys were carried out in Ankara.

Heptachlor epoxide was clearly detected in some of the samples (17.82%). The presence of heptachlor epoxide in different regions of Turkey included in Ankara have been reported earlier (Burgaz at al., 1994; Çok et al., 1998). The level of heptachlor epoxide in human adipose tissue samples of Ankara residents has been reported as 0.021 ppm by Burgaz et al. (1994). In this study, the amount of heptachlor epoxide was found as 0.06 ppm which is significantly different than the previous one ($p<0.05$). The heptachlor epoxide levels in human body might have originated from heptachlor as an oxidation product, given that it was used as a pesticide in Turkey until 1985.

HCB is a widespread contaminant that has entered the environment through its past manufacture and use as a pesticide and its formation as a by-product during

the production of a variety of chlorinated compounds. Average HCB residue levels in adipose tissue of Ankara residents have been reported as 0.164 ppm (Burgaz at al., 1994). In this study the level of HCB was found as 0.152 ppm. Indeed, HCB is the most striking one among the OCPs that have been analysed. The values tend to be consistent over the years, even though expected to decrease. Concentration of HCB was found to be lower in other parts of Turkey (Çok et al., 1998; Çok et al., 1999). The presence of HCB in human milk can be attributed to various activities and other pesticides (PCNB, PCP, DCPA etc) (Tobin, 1986). Although the agricultural usage of HCB was prohibited in Turkey in 1959, the usage of pesticides and industrial activities such as waste by product in specific manufacturing processes have created considerable amounts of HCB impurities. The need for more comprehensive studies on the causes and sources of HCB exposure, is indicated by this research.

Experimental and epidemiological studies show that DDE/DDT ratio increases with timeafter exposure or after the limitation or restriction of DDT usage. In this study, DDE/DDT ratio was found as 17.67 which is quite higher than that obtained in the 1987 study by Karakaya et al which was 4.50 ($p<0.001$). On the other hand, in the studies that have been performed on adipose tissue samples, this value was calculated as 3.19 in 1976 (Kayaalp et al., 1979), 9.4 in 1984 (Karakaya & Özalp 1987) and 13.53 in 1991 (Burgaz et al., 1994). When we consider DDE/DDT ratios, it is seen that the limitation and legislation for OCPs in Turkey has been effective and the exposure to these compounds tends to decrease in time. Obtained DDE/DDT ratios in human milk were higher than the values reported for Slovak Republic (3.15) (Veningerova et al., 2001), Ukraine (3.36) (Gladen et al., 1999), Mexico (5.60) (Waliszewski et al., 2002), Iran (5.67) (Çok et al., 1999), Egypt (7.30) (Saleh et al., 1996), Japan (15.16) (Konishi et al., 2001) and in England (11.32) (Harris et al., 1999), but were lower than those in Spain (46.75) (Hernandez et al., 1993), Finland (48.00) (Smeds & Saukko 2001) and Brazil (21.08) (Beretta & Dick 1994).

We calculated the daily intakes of γ-BHC, HCB, heptachlor epoxide, Σ- DDT by breast-fed children assuming that a child consumes 130 g milk per day per kilogram of body weight (WHO, 1986). In these calculations, the fat content is regarded as 4.09% (w/w), but the individual values ranged widely from 0.49 to 13.81 %. On this basis, the mean and maximum daily intakes are shown in Table 6 together with Acceptable Daily Intakes (ADIs) established for adults by FAO/WHO Expert Groups.

As can be seen from Table 6, the daily intake of Σ- DDT and γ-BHC by infants were generally low, while the maximum intakes of HCB and heptachlor epoxide were greater than the ADI.

In Turkey, most of the research which aimed to determine the level of OCPs and other organochlorine compounds have been conducted in Ankara (Çok et al., 2003). Further studies should be done in part of the country where agriculture as well as industry is prevalent in terms of highlighting general exposure to these

Table 4. Mean levels of OCP residues in human milk from residents of Ankara over the period 1984-2002.

Year	n	α-BHC	β-BHC	γ-BHC	HCB	Heptachlor epoxide	p.p'DDE	p.p'DDT	ΣDDT	ΣBHC	DDE/DDT
1984-1985*	61	0.14	1.30	0.02	--	--	3.28	0.73	4.15	4.39	4.50
2001-2002**	101	0.05	0.49	0.01	0.15	0.06	2.28	0.13	2.66	0.55	17.67
P***		<0.001	<0.001	<0.001	-	-	<0.001	<0.001	<0.001	<0.001	<0.001

(The mean levels experessed as mg/kg excracted fat basis),*Karakaya et al (1987) ;** Present study; ***ANOVA

Table 5. OCP residue levels in adipose tissue in Ankara residents.

Year	n	α-BHC	β-BHC	γ-BHC	HCB	H.E	p.p'DDE	p.p'DDT	ΣDDT	DDE/DDT
1976-1977*	41	--	--	--	--	--	10.2	3.20	14.60	3.19
1984-1985**	48	0.19	1.52	--	--	--	5.83	0.62	7.12	9.40
1991-1992***	60	0.16	1.52	0.017	0.16	0.062	3.72	0.27	4.42	13.53

(The mean levels experessed as mg/kg excracted fat basis), * Kayaalp et al., 1979 ** Karakaya and Özalp 1987; *** Burgaz et al., 1994

Table 6. Calculated daily intake of OCPs (μg/kg b.w/day) by breast fed infants.

Residue	n	Mean.± S.D	Median	Range	ADI*
γ-BHC	12	0.56±0.53	0.47	0.064-1.81	8
HCB	51	1.63±1.56	1.14	0.047-8.16	0.6**
H.Epoksit	18	1.68±1.95	0.59	0.01 - 6.36	0.1
Σ DDT	98	14.31±18.22	7.12	0.06-74.66	20

* Acceptable Daily Intake;**Conditional

compounds in Turkey. In addition, studies should focus on the toxicological implications (for instance, the effects on fertility) on humans and wild life, which could be made possible by measuring exposure levels in these different sources.

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