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Organochlorine Pesticide Contaminants in Human Milk Samples Collected in Tebriz (Iran)

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Due to the accumulation capacity in human tissues and their toxicity, organochlorine pesticides (OCPs) have played an important role in the last few decades in our environment and daily life. OCPs are strong lipophilic compounds and can accumulate in man's body that is the last step of the food chain. Several studies have shown that milk secretion is the most important means of excretion of OCP in persons. Mother's milk is the primary source of infant and the infants being breast fed are exposed to these residues present in their mother's milk

Monitoring of the concentration of the OCP residues in human milk has been the subject of many studies (Matuo et al. 1992; Ejobi et al. 1996; Saleh et al. 1996; Waliszewski et al. 1996; Cok et al. 1997) however, very few reports did appear from Iran (Hashemy-Tonkaboy & Fateminassab 1977) To our knowledge, there are no sufficient data concerning the contamination of human milk by OCP in Iran since first study that had been realized among 1974 and 1976. Also only a few reports pertaining to the levels of OCP contaminants in adipose tissue are available from Iran (Hashemy-Tonkabony & Soleimani-Amiri 1978; Burgaz et al. 1995). The aim of this study is to estimate the levels of OCP contaminants in human milk samples collected in Tebriz (Iran) during the year 1991 and to investigate whether alterations in levels of these contaminants have occurred since 1974 in Iran.

MATERIALS AND METHODS

Human milk samples were obtained from the maternity unit at the Tebriz State Hospital in Tebriz City between April 1991 and June 1991 from 40 lactating mothers who were living in Tebriz area for at least 5 years. 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 3rd and 31th day of postpartum. Milk samples were kept frozen -20 °C until analysis. The age of mothers ranged from 19 to to 40 (mean age 28.2±6.74). Each donor completed a questionnaire to

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provide personal information such as weight, occupation, smoking, previous nursing time, dietary habits, and place of residence. All the donors were nonsmokers.

Gas chromatographic analyses were carried out on 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 25mx0.25 mm fused silica capillary column HP-5 from Hewlett-Packard. The operating conditions were as follows: 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 mobile phase was helium. Peak areas were used as the basis for quantification. Residue levels are expressed as mg/kg extracted fat (ppm). Standards of HCB, α–BHC, ß-BHC, γ–BHX, p.p'.DDE, p.p'.DDT, heptachlor epoxide, and aldrin were obtained from US. Environmental Protection Agency (EPA).

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

The organochlorine pesticides in human milk were extracted to the method by Krauthacher et al. (1986). Before extraction, the stored human milk samples were left to defrost, homogenized and kept 10 min. in 30°C water bath. After adding aldrin as an internal standard, 2.5 g of milk was mixed with 17 ml of chloroform/methanol 1:1(v/v); 7 ml redistilled water was added and 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 centrifuged, the organic phase concentrated to 2.0 ml and 0.5 µl injected into GC.

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, ß-BHC, γ–BHC, HCB, heptachlor epoxide, p.p'.DDE, p.p'.DDT were 1, 1, 1, 1, 1, 2, 3 ppb 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

Forty milk samples were analyzed by GC and the results are shown in Table 1. Residues of ß-BHC, HCB, p.p'.DDT, and p.p'.DDE were found to be the major contaminants in milk samples of Tebriz residents. Frequency of OCPs, mean of obtained values and ranges are listed in Table 1 as well. Our results show that the Tebriz population has detectable OCP levels.

Table 1. Organochlorine pesticide residues (mg/kg fat basis) in human milk samples in Tebriz residents.

Pesticide	Mean (±S.D)	Range	Frequency (%)
HCB	0.061±0.057	N.D - 0.273	87.5
α-BHC	0.022±0.051	N.D - 0.211	30
β-BHC	0.399±0.328	N.D - 1.274	92.5
γ-BHC	0.182±0.500	N.D - 3.062	42.5
Σ-BHC[a]	0.603±0.584	0.093- 3.430	
HEPTACHLOR EPOXIDE	0.054±0.074	N.D - 0.318	60
p.p'.DDE	1.701±0.721	0.523- 2.993	100
p.p'.DDT	0.302±0.212	0.066- 0.827	100
Σ-DDT[b]	2.199±0.915	0.680- 3.950	

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

Except for three the subjects are housewives, therefore, the relationship between occupational exposure and OCP was not investigated in this study. On the other hand, none of the subjects smoke or use tobacco, so no evaluation was made regarding OCP levels and use of tobacco.

Mothers were classified arbitrarily according to their age into three groups: 16-26 (n:16), 27-35 (n:l8), and 36-40 (n:6) year. There was no significant increase in three groups in terms of HCB, ß-BHC levels ($p>0.05$) though there was a trend towards higher HCB and ß-BHC residues increasing with age. In terms of p.p'.DDE levels significant increase ($pc<0.001$) between 16-26 age group with 36-40 age group and significant increase between 27-35 and 36-40 age groups were found ($p<0.05$).

In the study subjects were grouped into 3 as giving 1 birth, 2-3 births and 4+ births and no significant relationship between number of childbirths and analyzed pesticides was found ($p>0.05$). Similar results have been observed in some other studies (Burgaz et al. 1995; Krauthacher 1991).

Table 2 shows the trend for the mean levels of the main residues of OCPs in human milk over the 17 years that these surveys have been carried out

in Iran

Table 2. Mean Levels of OCP residues in human milk from Iranian population over the period 1974-l991.

Year	p.p.'DDE	p.p.'DDT	Σ- DDT	DDE/DDT	Reference
1974-76	1.13	1.01	2.88	1.12	1
1991-92	1.70	0.30	2.20	5.67	*

(*Present Study; Reference: 1) Hashemy-Tonkabony &Fateminassab 1977)

Hashemy-Tonkabony and Fateminassab (1977), did not report heptachlor epoxide levels in 131 milk samples that they collected between years 1974-76. Also in the studies on adipose tissue samples from Iran (Hashemy-Tonkabony & Soleimani-Amiri 1978; Burgaz et al. 1995) there was no record for heptachlor epoxide. But in present study heptachlor epoxide was clearly detected in 60% of the samples analyzed. Confirmation of heptachlor epoxide was made by only spiking during GC analysis.

γ– BHC amounts has been found higher than most of those found in other countries such as India (Banerjee et al. 1997), Egypt (Saleh et al. 1996), Turkey (Cok et al. 1997) United Kingdom (Dwarka et al, 1995). The main reason of this could be the use of technical Lindane (25% γ-BHC) instead of DDT in this country.

When p.p.'DDE and p.p.'DDT values were compared with those of study by Hashemy-Tonkabony & Soleimani-Amiri (1977), it is found that as p.p'.DDE increased, p.p'.DDT decreased. In addition to that DDE/DDT ratio was found 5.67 in this study which is quite higher than those obtained in 1977 study which was 1.12 . Obtained DDE/DDT ratios has been found higher than those studies which was found in human milk in Brazil (Matuo et al. 1992), Uganda (Ejobi et al. 1996) and in Mexico (Waliszewski et al. 1996) but has been found lower than those in Spain (Hernandez et al. 1993) Canada (Dewailly et al. 1989) France (Bordet et al. 1993), United Kingdom (Dwarka et al. 1995) Egypt (Saleh et al. 1996) and Turkey (Cok et al. 1997). When the use of p.p'.DDT ceases, human exposure to this compound decreases initially fairly rapidly. However, exposure to its persistent metabolite p.p'.DDE is still occurs due to continued exposure mainly via foodstuffs of animal origin and also to metabolic conversion of p.p'.DDT to p.p'.DDE in the body. Thus the ratio of DDE and DDT in the human body will increase when the use of DDT ceases (Slorach & Vaz 1983). When we consider DDE/DDT ratios, it is seen that limitation and

legislation for OCPs in Iran has been effective and the exposure to these compounds tends to decrease in time.

In Iran, beginning from 1979, restrictions are imposed on the usage of DDT in agriculture, and since 1983 its use has been prohibited, however DDT has only been used for malaria control. HCB has not been used directly in agriculture in Iran. Technical Lindane (25% γ-BHC) is widely used instead of DDT in this country. The reason of HCB residues are produced as by product, presence in other pesticides as an impurity and the biotransformation of BHC to HCB biologically (Tobin 1986; Menzei 1986). Both usage of technical Lindane and industrial activities could help to explain the source of HCB in human milk samples of Tebriz residences. Burgaz et. al (1995) has determined HCB residues 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 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 Iran.

Table 3. OCP residue levels in human milk and adipose tissue in Tebriz residences.

	Year	∑- BHC	p.p'.DDE	p.p'.DDT	∑-DDT	DDE/DDT	Ref.
Adipose Tissue	1991-92	0.77	2.45	0.19	2.921	12.89	1
Human Milk	1991	0.60	1.70	0.3	2.199	5.67	*

(* Present Study; Reference:1) Burgaz et al. 1995)

Table 3 shows previous results of Burgaz et al. (1995) based on the findings of adipose tissue samples from subjects who live in Tebriz in the same years and the present study's finding of milk samples which was obtained from Tebriz. The values seem to be parallel when we consider results of the study.

We calculated the daily intakes of γ–BHC, HCB, heptachlor epoxide, Σ–DDT by breast-fed children assuming that a child consumers 130 g milk per day per kilogram of body weight. In these calculations the fat content of is regarded as 3.44% (w/w) but individual values ranged widely from 0.82 to 9.82 %. On this basis the mean and maximum daily intakes are shown in Table 4 together with Acceptable Daily Intakes (ADIs) established for adults by FAO/WHO Expert Groups.

As can be seen from Table 4 the daily intake of Σ - DDT by infats were in

general low, maximum intake of γ-BHC and all intakes of HCB and heptachlor epoxide were greater than the ADI.

Table 4. Calculated daily intake of OCPs (µg/kg b.w/day) by breast fed infants.

Residue	n	Median	Maximum	ADI
γ-BHC	37	-	10.53	8
HCB	35	0.13	0.94	0.6**
HE*	24	0.10	1.09	0.1
∑-DDT	40	6.56	13.59	20

*Heptachlor epoxide
**Conditional

With the results of this study, it could be suggested that limitation of OCP usage in Iran has caused the decrease of OCP exposure and exposure amounts has been decreased in human body. It is not the case for ß-BHC as Lindane is still used, it showed high amount of exposure of this compound than the previous studies.

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