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Organochlorine Pesticide Residues in Human Milk of Mothers Living in Northern Tunisia

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Abstract The levels of 13 organochlorine pesticides (OCPs) were measured in breast milk from 87 Tunisian mothers throughout their lactation periods. Breast milk levels of OCP residues were determined using a validated methodology that included capillary column gas chromatography with electron-capture detection. All samples contained detectable residues of p,p′-DDE (2.421 mg/kg milk fat) and HCB (0.260 mg/kg milk fat). The other OCP residues were detected in the following order of frequency: Dieldrin 38%, β-HCH 22.9% and γ-HCH 6.8%. It was made an attempt to point out the relationship between some factors and the transfer of OCP residues into breast milk. In this study we found that concentrations of OCPs increased with mothers' age, these levels decreased with the number of children.

Keywords Organochlorine pesticides · Breast milk · Breast-feeding · Tunisia

Organochlorine pesticides (OCPs) have been commonly used as insecticides worldwide since the 1940s. Due, primarily, to environmental concern, their use has been restricted in many countries since the 1970s. In Tunisia, most of OCPs have been prohibited in the late 1980s. As these compounds are highly lipophilic and persistent, human chronic exposure via food chain has led to the accumulation of both parent compounds and their metabolites in lipid rich tissues such as adipose tissues and human breast milk. OCPs have been shown to have a wide variety of toxic actions, although not all have been shown to have significance for human health. Recent reports suggested an association between exposure to pesticides and different types of human cancer (Safi 2002). Many of the OCPs are known as endocrine disrupters; they cause immune suppression and inhibit various enzymes (Iscan et al. 2002). Recently, hormone-like activities of some OCPs such as DDTs and HCHs have been suggested (Vos et al. 2000). In the United States, DDT has been reported to affect neurobehavioral functions and to be associated with premature births (Longnecher et al. 2001).

At our knowledge, previously only one study was carried out on levels of OCPs in human milk in Tunis area (Sabbah et al. 1987). However, our investigation is the first to study the exposure to the residues of OCPs in northern Tunisia (Beja and Bizerte).

The main objective of the present study was to identify and quantify the levels of OCP residues in mothers' milk and to determine factors that influence the levels of contamination.

Materials and Methods

Human milk samples were obtained from the maternities at Bizerte (n = 49) and Beja (n = 38) states hospitals in Tunisia, between September 2002 and February 2003, from 87 lactating mothers who were living these areas during at least the preceding 3 years. The volunteers signed their consent to participate in the study and completed a detailed survey regarding their age, number of deliveries, weight,

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occupation, smoking habit and place of residence. Samples (2–10 mL) were collected by manual expression 1–4 weeks post-partum into chemically clean glass bottles with Teflon caps. The milk was transported to the laboratory and stored at –20°C until analysis was done.

The solvents used in this study (*n*-hexane, ethanol, acetonitrile and dichloromethane) were pesticide quality and were obtained from Fluka (Buchs, Switzerland). Florisil (60–100 mech) was obtained from Merck (Darmstard, Germany), activated at 650°C and retreated at 130°C for 5 h. OCP standards were obtained from Polyscience Corporation Analytical Standards (Niles, IL, USA). Purities of the individual standards ranged from 97% to 99%. The studied OCPs are: HCB, β -HCH, γ-HCH, Heptachlor, Aldrine, Heptachlor epoxide, Dieldrine, o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD, o,p′-DDT and p,p′-DDT. A stock solution of the standard mixture containing 1,000 µg/L of each pesticide was prepared in hexane except β-HCH which is dissolved in acetone. The solutions were further diluted to obtain fortifying and GC calibration standard solutions for all pesticides.

The extraction and clean-up of milk samples were carried out according to the method described by Sabbah and Bouguerre (1997). The stored human milk samples were left to defrost at ambient temperature and homogenized. Two to ten milliliters of milk were extracted three times with a solvent mixture (20 mL of *n*-hexane, 5 mL of acetonitrile and 1 mL of ethanol). The hexane layers were filtered on anhydrous sodium sulfate and evaporated to 5 mL by a rotating evaporator. Exactly 1 mL was pipetted into a pre-weighed bottle and evaporated to dryness. The flask was weighed until a constant weight was obtained. The difference between this weight and the original weight of the flask was used to calculate the fat content of the sample. The 4 mL extract was purified in a chromatographic mini-column 40 × 0.5 cm ID. The column was packed with 2 g of activated florisil and topped with 1 g of anhydrous sodium sulfate. The extract was eluted with 30 mL of elution mixture (dichloromethane and n-hexane 1:9; v/v). The eluate was evaporated in a Kuderna-Danish to 0.5 mL and was ready for GC analysis.

The analysis was done on an Agilent Model 6890 Gas Chromatograph equipped with a ^{63}Ni electron capture detector (GC-ECD). Two µL of extract was injected in splitless mode into a PTE-5 30 m × 0.32 mm ID, 0.32 µm film thickness capillary column, using nitrogen carrier gas with a 1.5 mL/min flow rate and the following oven temperature program: 50°C initial (2 min) to 160°C at 5°C/min and to 260°C at 2°C/min, hold 10 min. The temperature of the detector and injector were 300 and 250°C, respectively. The PTE-5 column was used as the primary analytical column. The data presented in this paper were obtained using this column. A second HP1 fused silica column 30 m × 0.32 mm ID, 0.25 µm film thickness was used as a confirmatory column. Quantitative and qualitative analysis were done by comparison with external standard. The pesticide residue levels of breast milk samples are reported on fat basis as it has been considered the most appropriate manner to express contamination with these residues due to the effects of variation in lipid levels during lactation (Norén 1983). The minimum determination limit expressed on fat basis for all OCPs studied was estimated to 1 ng/g. To determine the quality of the method, the recovery study was performed on ten over spiked replicates of blank cow milk samples, which revealed contamination levels below the detection limit. The percent recovery for each analyte was corrected for background concentration measured in unfortified sample. The recovery study, done at 10 ng/mL levels, showed mean values ranged from 80% to 93% of recovery. The relative standard deviations were below 10%, indicating acceptable repeatability of the method.

Table 1 Mean and relative standard deviation (RSD), median, range (mg kg^{-1} fat weight) and frequency of OCPs detected in human milk samples

	Mean[a] ± % RSD	Median	Range	Frequency[b] (%)
HCB	0.260 ± 1.71	0.114	0.003–3.127	100
β-HCH	0.050 ± 2.21	0.027	ND–0.470	22.9
γ-HCH	0.008 ± 4.38	0.006	0.019–0.225	6.8
Σ-HCH	0.067 ± 2.09		ND–1.375	
Dieldrin	0.059 ± 1.92	0.036	ND–0.529	38
o,p′-DDE	0.097 ± 3.80	0.060	0.006–2.541	31
p,p′-DDE	2.421 ± 1.37	1.389	0.001–24.975	100
p,p′-DDD	0.279 ± 1.51	0.119	ND–2.354	70
o,p′-DDT	0.029 ± 4.56	0.016	ND–0.932	12.6
p,p′-DDT	1.015 ± 1.57	0.484	0.029–8.442	88.5
ΣDDT	3.863 ± 1.20		0.058–31.325	

[a] The concentration below detection limit was treated as zero for calculation of arithmetic mean

[b] n = 87, *ND* not detected

Results and Discussion

Table 1 shows the levels of OCPs in analyzed breast milk samples. OCPs were detected in all samples with levels ranged between the determination limit and 24.975 mg kg^{-1} fat weight. This result suggests that the general population in these studied areas has been widely exposed to these toxic contaminants. The profile of concentration medians followed the order of p,p′-DDE > p,p′-DDT > p,p′-DDD > HCB>o,p′-DDE > Dieldrin > β-HCH > γ-HCH. Our results showed that almost all screened lactating mothers contained p,p′-DDE and HCB residues in their

Table 2 Comparison of OCP levels in human milk between Tunisia and other countries (mg kg^{-1})

	Year	N	HCB	p,p′-DDE	p,p′-DDT	References
Mexico	1997–1998	60	0.030	3.997	0.651	Waliszewski et al. (1999)
Thailand	1998	25	ND	8.210	2.600	Stuetz et al. (2001)
Guangzhou	2000	54	ND	2.850	0.700	Wong et al. (2002)
Sweden	1997	40	0.012	0.129	0.014	Norén and Meironyté (2000)
Japan	1998	49	0.014	0.270	0.018	Konishi et al. (2001)
United Kingdom	2001–2003	54	ND	0.150	0.006	Kalantzi et al. (2004)
Tunisia	2002–2003	87	0.260	2.421	1.015	Present study

ND not determined, *N* number of analyzed samples

Table 3 Mean levels of organochlorine pesticides (mg kg^{-1} fat milk) in human milk samples according to number of parities

	Number of parities			
Pesticide	One (n = 29) (range)	Two (n = 28) (range)	Three (n = 16) (range)	Four and + (n = 14) (range)
HCB	0.166	0.289	0.085	0.595
	1.067–0.006	3.127–0.004	0.253–0.001	1.726–0.009
β-HCH	0.041	0.037	0.032	0.029
	0.282–0.042	0.241–0.009	0.378–0.050	0.221–0.193
γ-HCH	0.068	0.059	0.033	0.033
	0.470–0.024	0.461–0.021	0.411–0.019	0.277–0.057
Σ-HCH	0.109	0.096	0.066	0.063
Dieldrin	0.062	0.045	0.043	0.043
	0.529–0.002	0.218–0.034	0.344–0.020	0.295–0.009
p,p′-DDE	3.163	2.660	1.435	1.427
	20.822–0.321	24.975–0.076	9.128–0.024	5.531–0.001
p,p′-DDD	0.501	0.295	0.200	0.191
	0.860–0.012	1.394–0.075	0.784–0.004	0.659–0.001
p,p′-DDT	1.011	1.011	0.836	0.831
	6.475–0.092	5.498–0.065	8.442–0.029	2.648–0.043
Σ-DDT	4.489	4.065	3.125	2.569

breast milk. This result is in accordance with other researches investigated in Tunisia concerning contamination with OCPs of breast milk (Sabbah et al. 1987) and other biological matrixes: falcon eggs and honey (Driss and Bouguerra 1987; Driss et al. 1994) and human blood (Sabbah et al. 1987). This finding indicates that pollution sources by DDE and HCB are still present in Tunisia.

The predominant OCP detected in breast milk was p,p′-DDE with a mean concentration of 2.421 mg kg^{-1} milk fat. On the other hand, 70% and 88.5% of samples contained p,p′-DDD and p,p′-DDT, respectively. Apart from dietary sources, there is no obvious explanation for this finding taking into consideration that the use of DDT for agriculture purpose was banned by the Government since the late 1980s. The mean level of p,p′-DDE detected in the studied samples was generally lower than those reported from some developing countries in the tropics, such as Mexico (Waliszewski et al. 1999), Thailand (Stuetz et al. 2001) and Guangzhou in China (Wong et al. 2002) because of continuous human exposure to DDT used for malaria control until the end of 1990s. In contrast, this level was higher than those reported from some developed countries such as Sweden (Norén and Meironyté 2000), Japan (Konishi et al. 2001) and UK (Kalantzi et al. 2004) (Table 2). DDT' elimination from the body can take some time; its half-life in humans has been estimated at four years. DDE' half-life is estimated at approximately 6 years (Norén and Meironyté 2000). When the use of DDT reduces, human exposure to this compound decreases; however, exposure to its persistent metabolite DDE is still occurs because of continued exposure mainly via diet of animal origin and also to metabolic conversion of DDT in the body.

Among the other studied OCPs, HCB residue was found with a mean level of 0.260 mg kg^{-1} milk fat. To

understand the magnitude of contamination in human breast milk, HCB level was compared with those reported recently in some industrialized countries (tableau 2). This comparison shows that our value was higher than those measured in Sweden (12 mg kg^{-1}) (Norén and Meironyté 2000) and Japan (14 mg kg^{-1}) (Konishi et al. 2001). Although HCB is no longer used as a fungicide, it is still released in the environment by the discharge of plastic (Gómez-Catalán et al. 1987) and it has also been found in fly ash from fires or incinerators (DeVoto et al. 1998); therefore HCB continues to enter in the food chain.

HCH residues were detected only in a few number of analyzed samples. We have detected β-HCH at a mean level of 0.050 mg kg^{-1} milk fat and γ-HCH at 0.008 mg kg^{-1} milk fat (Table 3). A previous study of HCH isomers contamination in Tunisian mothers' milk had already shown that the β-HCH isomer is the most abundant one (Sabbah et al. 1987). This finding confirms two facts: β-HCH is the most persistent HCH isomer and the widespread use of this pesticide in the past.

Dieldrin, which is an oxygenated metabolite of aldrin, is more persistent in the environment. This pesticide was found in 38% of analyzed samples at a mean level of 0.059 mg kg^{-1} fat milk. This result is comparable to that observed in samples from other countries such as in Uganda: 0.07 mg kg^{-1} fat milk (Ejobi et al. 1996).

The levels of excreted OCPs are influenced by several factors such as the donor's age, number of children and duration of breastfeeding (Czaja et al. 2001). Table 2 presents the mean values of pesticide residues according to the parity.

In this study, subjects were grouped into four as giving 1 birth (n = 29), 2 birth (n = 28), 3 birth (n = 16) and 4 + birth (n = 14). In the primagravidae group the p,p′-DDE metabolite level reached 3.163 mg kg^{-1} fat milk and diminished with a subsequent child to 2.660 mg kg^{-1} fat milk and up to 1.427 mg kg^{-1} fat milk in mothers with a third or four + breastfed child. No significant relationship between number of childbirths and concentrations of HCB, HCH, dieldrin and p,p'-DDT was found ($p > 0.05$). Similar results have been observed in some other studies (Stuetz et al. 2001; Čajka and Hajšlová 2003). These results support the theory that the total body load of organochlorines via the breast milk is shed with each successive pregnancy and lactation.

Mothers were classified according to their age into four groups: ≤25 (n = 21), 26–30 (n = 36), 31–40 (n = 22) and >40 (n = 8). There was no significant increase in four groups in terms of HCB, ∑-HCH, dieldrin and p,p′-DDE levels ($p > 0.05$) though there was a trend towards higher HCB, dieldrin, HCH and p,p′-DDE residues increasing with age (Fig. 1). In terms of p,p′-DDT levels, significant increase ($p < 0.05$) was detected between 26–30 and 31–40

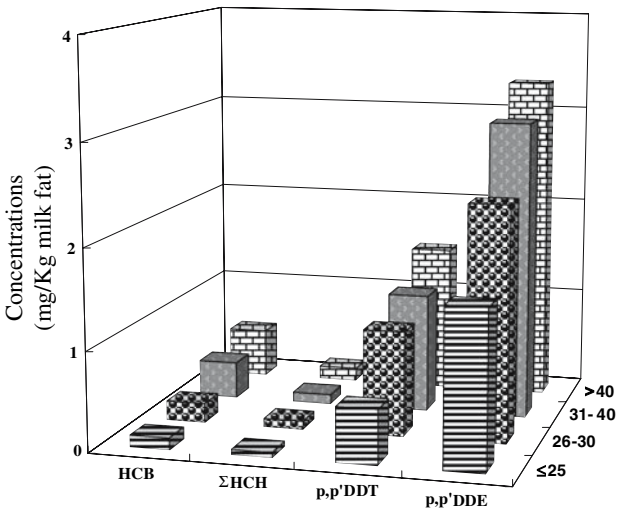

Fig. 1 Comparison of HCB, ∑-HCH, p,p′-DDT and p,p′-DDE content in human milk by the age of mothers

age groups. An increase in organochlorine compound concentration along with the age of donors was described by Czaja et al. (1997), and Çok et al. (1999).

Our findings suggest a need to investigate the state of organochlorine compounds in other Tunisian areas and their effects on wide life and human health. In addition, further detailed studies on breast milk regarding health of infant should be performed. Despite the presence of toxic compounds in human milk, breast-feeding is a highly desirable practice. The beneficial effects generally relate to improvements in host defence, digestion and absorption of nutrients, neurodevelopment, gastrointestinal function as well as psychological effects on the mother (Schanler et al. 1999).

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