Evaluation of Some Organochlorine Pesticides in Human Breast Milk and Infants' Dietary Intake in Middle and Upper Egypt

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Abstract:

A total of fifty-four human breast milk samples were collected from Pediatric Department of Assiut University Hospital from lactating mothers during January-August, 2001. The samples were analyzed for organochlorine pesticide residues by using gas chromatography-electron capture detector (GC-ECD). The results revealed that all detected organochlorine residue levels were markedly lower in comparison with the previously documented levels although they still had the same frequency percentages as recorded previously. The study indicated also that p,p'-DDE and β-HCH were found to be the main contaminants and were detected in all analyzed samples (100%) followed by HCB (88.9%), p,p'-DDT (83.3%) and oxychlordane (33.3%). The mean total DDTs level in breast milk samples was 16.166 ± 14.82 µg/l formed mainly of p,p'-DDE (95%). The levels ranged from 3.353 to 67.159 µg/l, with levels exceeding the Extraneous Residue Limits (ERLs) issued by FAO/WHO, in 1994 &1996 (50 µg/l) in only 3 samples (5.6%). The total HCH isomers average was 1.599 ± 1.595 µg/l milk formed mainly of β-HCH. HCB and oxychlordane were detected at lower average levels of 0.389 ± 0.156 and 0.285 ± 0.175 µg/l milk, respectively. Within the detection limits no sample was found to contain aldrin, dieldrin, heptachlor, heptachlor epoxide, endrin, γ- and α chlordane residues. The estimated daily intakes (EDIs) for total DDTs, γ-HCH and total HCH residues never exceeded the recommended FAO/WHO acceptable daily intake (ADIs). The possible health impact of these pollutants was reviewed.

Introduction:

Intensive application of organochlorine pesticides in agriculture and medical parasitology with the high stability of these pesticides, results in their wide spreading. The concern about the environmental contamination by these persistent pesticides has led many countries to investigate the magnitude of their own environmental pollution. Residues of these compounds have been found at every level in the food chains.[1]

Human beings are placed at the top of the most food chains and it is not surprising that high levels of these compounds have been found in adipose tissue and milk fat.[2-6] Investigations on possible health and environmental hazards have led many industrial countries to restrict or ban the use of these chemicals and enforce the tolerance levels for the residues in food and feeds. Following the banning of these organochlorine pesticides e.g. DDT and HCH isomers in many countries, the levels in human milk have declined [7-9] and this trend is expected to continue.

Human milk is the most important and indispensable food for the newborn and young infant. During lactation, fat mobilization could take place from adipose tissue and therefore organochlorine compounds are mobilized and excreted together with milk. Thus, milk secretion is the most important route of excretion of those types of compounds in women.[10] Contamination of human milk by organochlorine and other related compounds has been reported throughout the world.[2] Limited studies were carried out by Dogheim et al.,[11,12] in 1991 and 1996, El-Hamid,[13] in 1993, Saleh et al.[14] in 1996 and Salem and El-Saied,[15] in 1997, in Egypt. These surveys indicated the existence of high levels of DDTs and total HCH isomers in samples collected from some Egyptian Governorates.

The present study was therefore initiated to investigate the levels of organochlorine pesticide residues in human breast milk and compare these levels with previously documented values. The study also contributes to the assessment of the environmental contamination in Middle and Upper Egypt and evaluate the toxicological hazard to the breast fed infants.

Subjects and Methods:

A total of fifty-four human breast milk samples were randomly collected over a period of 8 months from January to August 2001 from Pediatric Department of Assiut University Hospital. Mothers were admitted with their sick babies in the Pediatric Department,

Assiut University Hospital, and were coming from different urban and rural cities of Middle and Upper Egypt. Consent was taken from the lactating mothers to share in the research and to express their milk after explanation to them that there is no hazard from this research. The milk samples (15-20ml) were collected by manual expression into glass containers cleaned and rinsed in acetone and n-hexane. Milk samples were immediately taken to the laboratory, homogenized and 10 ml was taken into clean glass containers and frozen at -20°C till pesticide residue analysis was made in the laboratory of the Federal Institute of Veterinary Research, Moedling,Austria.

Pesticide residue analysis:

Samples were extracted according to Suzuki et al.[16] Pesticide residues were eluted from extracted fat by using florisil column according to the method established in the Federal Veterinary Research Institute in Moedling, Austria and used by Salem,[17] in 1993 and Salem et al.,[18] in 1995.

Gas chromatographic analyses were carried out on a Perkin-Elmer Autosystem XL Gas chromatograph equipped with an electron capture detector (PE-GC) with split-splitless injector. Chromatographic determination of organochlorine residues was carried out using a 50 m X 0.2 mm X 0.11 um capillary column HP Ultra 2 from Hewlett-Packard.

The operating conditions were as follows: injector temperature 250°C; 1/50 split ratio; 4 mm i.d. wide bore liner; detector 350°C. Oven program: initial temp: 80°C; initial hold: 1 min; Ramp 1: 45.0°C/min to 160°C, hold for 1 min; Ramp 2: 2.0°C/min to 220°C, hold for 1 min; Ramp 3: 45.0°C/min to 280°C, hold for 5 min. Carrier gas: helium (1 ml/min) and Make Up-gas: nitrogen (35 ml/min). Peak areas were used as the basis for quantification. Residue levels are expressed as µg/l milk and µg/kg extracted fat (ppb).

All solvents and chemicals used were pesticide residue analytical grade reagents free of interfering residues as tested by Gas chromatography. All glasswares used for the analysis were rinsed with distilled water and then with acetone and petroleum ether before use to prevent contamination. Pesticide reference standards were obtained from SUPELCO SA (Switzerland).

Recoveries from fortified samples at 200 µg/l each level were in the range of 94-116% on this method, including internal standard. Recovery percentages, detection and quantitation limits are presented in table I. All residue levels were corrected according to their recoveries.

Table I. Recovery percentages (%), detection and quantitation limits (µg/l) of studied organochlorine pesticide residues in breast milk.

Pesticide residues	Recovery percent	Detection limit	Quantitation limit
pp-DDE	105	0.01	0.02
pp-DDD	96	0.02	0.03
op-DDT	102	0.01	0.03
pp-DDT	99	0.01	0.02
α-HCH	97	0.02	0.05
β-HCH	96	0.06	0.13
γ-HCH	106	0.04	0.07
δ-HCH	98	0.02	0.03
HCB	100	0.03	0.05
Oxychlordane	108	0.01	0.01
γ –Chlordane	94	0.01	0.01
α –Chlordane	99	0.01	0.01
Aldrin	100	0.01	0.02
Dieldrin	108	0.01	0.02
Heptachlor	95	0.03	0.06
H. epoxide	112	0.01	0.01
Endrin	116	0.02	0.03
Nonachlor	95	0.01	0.01

Results:

The average fat content in milk samples was 2.7g/dl, individual level ranged from 1.41 to 5.19 g/dl. Estimated daily intakes of different pesticides were calculated based on assumed quantity of breast milk intake and concentration of pesticides in milk and are shown in table III. Frequency of positive samples, mean values ± standard deviation and ranges of organochlorine residues are listed in table II. The main contaminants were p,p'-DDE and were detected in 100% of the samples followed by HCB in 88.9%, p,p'-DDT in 83.3% and oxychlordane in 33.3%. The mean value of total DDTs was 16.166 ± 14.82 µg/l milk being mostly formed of p,p'-DDE (95%), that of total HCH was 1.599 ± 1.595 µg/l milk being formed mainly of β-HCH(96%). The values of total DDTs ranged from 3.353 to 67.159 µg/l milk; 3 samples (5.6%) were above the Extraneous Residue limits (ERLs) issued by FAO/WHO in a whole milk basis for the total DDTs (50 µg/l). The HCB and oxychlordane mean values were 0.389 ± 0.156 and 0.285 ± 0.175 µg/l milk, respectively. Aldrin, dieldrin, heptachlor, heptachlor epoxide, endrin, γ- and α chlordane residues could not be detected. The estimated daily intakes (EDIs) for total DDTs, γ-HCH and total HCH residues never exceeded the recommended FAO/WHO acceptable daily intake (ADIs).

Table II. The mean, standard deviation, range values, the number and percentages of positive samples of organochlorine pesticide residues detected in breast milk collected from Pediatric department , Assiut University Hospital.

Pesticide Residues	Levels of Organochlorine Pesticide Residues						Positive samples	
	Whole milk basis (µg/l)			Fat basis (µg/kg)				
	Mean ± S.D.	range	Median	Mean ± S.D.	range	Median	Number	Percent
pp-DDE	15.493± 14.377	3.02-65.159	11.394	495.789 ± 460.055	96.5 – 2085.1	364.6	54	100
pp-DDD	0.319 ± 0.06	0.266 – 0.384	--	10.2 ± 1.931	8.5 – 12.3	--	3	5.6
op-DDT	0.180 ± 0.058	0.109 – 0.244	--	5.767 ± 1.87	3.5 – 7.8	--	9	16.7
pp-DDT	0.750 ± 0.484	0.278 – 2.003	0.628	24.007 ± 15.483	8.9 – 64.1	20.1	45	83.3
Total-DDTs	16.166± 14.820	3.353–67.159	12.222	517.317 ± 474.245	107.3 – 2149.1	391.1	54	100
A-HCH	0.217 ± 0.031	0.183 – 0.25	--	6.5 ± 0.917	5.50 – 7.30	--	3	5.6
B-HCH	1.545 ± 1.583	0.453 – 7.387	1.09	46.344 ± 47.49	13.6 – 221.6	32.7	54	100
γ-HCH	0.29 ± 0.139	0.110 - 0.42	--	8.7 ± 4.181	3.3 – 12.6	--	9	16.7
Δ-HCH	ND	ND	ND	ND	ND	ND	ND	ND
Total HCH	1.599 ± 1.595	0.453 – 7.387	1.203	47.976 ± 47.854	13.6 – 221.6	36.1	54	100
HCB	0.389 ± 0.156	0.2 – 0.867	0.396	9.268 ± 3.739	4.8 – 20.8	9.5	48	88.9
Oxychlordane	0.285 ± 0.175	0.1 – 0.568	0.258	7.133± 4.376	2.1 – 14.2	6.45	18	33.3
γ –Chlordane	ND	ND	ND	ND	ND	ND	ND	ND
A –Chlordane	ND	ND	ND	ND	ND	ND	ND	ND
T. Chlordane	0.285 ± 0.175	0.1 – 0.568	0.258	7.133± 4.376	2.1 – 14.2	6.45	18	33.3
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND
Aldrin & Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor	ND	ND	ND	ND	ND	ND	ND	ND
H. epoxide	ND	ND	ND	ND	ND	ND	ND	ND
T. heptachlors	ND	ND	ND	ND	ND	ND	ND	ND
Endrin	ND	ND	ND	ND	ND	ND	ND	ND

ND = *not detected*

Table III. Estimated daily intake (EDI) of some organochlorine pesticide residues detected in breast milk collected from Pediatric Department, Assiut University Hospital in relation to international guidelines and other published EDI from Egypt.

Pesticides	ERLs* (whole basis)	No. of samples exceeded limits	ADIs** FAO/WHO	EDIs This study	Salem and El-Saied (1997)	Dogheim et al. (1991)	El-Hamid (1993)	Dogheim et al. (1996)	
	(ug/L)		ug/kg bwt/day	Middle & upper Egypt	Assiut	Cairo	El-Mono-fiya	Cairo	Kafr El-Zayat
Total DDTs	50	3/54	20.0	1.94	5.2	8.2	0.76	11.56	17.19
Lindane (γ-HCH)	10	0/9	8	0.035	0.07	0.09	14.03	<0.12	0.31
∑HCH isomers	***	0/54	***	0.192	5.00	1.94	7.99	52.27	14.07
Hept. & epoxide	6	ND	0.1	--	0.168	0.11	--	--	--
Ald. & Dieldrin	6	ND	0.1	--	0.173	1.22	--	--	--
Endrin	0.8	ND	0.2	--	0.169	--	--	--	--
HCB	20	0/48	withdrawn****	0.047	0.036	1.40	--	--	--
T. chlordane	--	--	0.5	0.034	--	1.9	--	--	--

: Extraneous Residue limits (ERLs) issued by FAO/WHO (1994),* *: ADI (Acceptable Daily Intake) issued by FAO/WHO (1994, 1996).*
****: Not applicable and compared with γ-HCH limit,* *****: Conditional ADI of HCB= 0.6 ug/kg bwt was withdrawn in 1978.*

Discussion:

Organochlorine pesticides such as DDT and HCH are major environmental pollutants. They are either known or suspected carcinogens.[19] The incidence of malignant tumors in terminally ill patients from the general population was significantly correlated with the concentration of DDE in the adipose tissue.[20]

The environmental exposure to organochlorine residues especially β-HCH may be an important etiologic factor in breast cancer[21,22] and increased amounts of organochlorines have been detected in certain cancerous tissues.[23] Newcomb et al.,[24] in 1994, reported a reduction in breast cancer risk with duration of lactation linking elimination of organochlorins through breast-milk secretion as a possible mechanism. These pesticide residues may have deleterious effects on the immune system[25-27] exposing the mothers and their babies to high incidence of infectious diseases. Furthermore, they carry neurological risk to the breast fed infants since a significant increase in the number of infants showing hyporeflexia, associated with an increase in the DDE concentration in breast milk has been reported.[28] Convulsions and/or EEG abnormalities, hepatic disorders and blood dyscrasias can occur in

cases of intoxication with chlorinated hydrocarbons.[29]

DDT metabolite -DDE was detected in all investigated milk samples (100%) followed by the parent compound p,p'-DDT (83.3%). Among DDTs, p,p'-DDE was the most predominant compound in all Egyptian and international published data like those of Dogheim et al.,[11,12] in 1991 and 1996, Saleh et al.,[14] in 1996, Salem and El-Saied,[15] in 1997, Barkatina et al.,[30] in 1998, Pardio et al.,[31] in 1998, Cok et al.,[32,33] in 1997 and 1999, Ntow,[5] in 2001 and Waliszewski et al.,[6] in 2001. This might be due to the high solubility and tendency of DDT and its metabolites to accumulate and store in fatty tissues.[34] Under natural conditions, in a living organism, DDT is usually transformed into its more stable metabolite DDE[1,30,35]

Total DDT values ranged from 3.353 to 67.159 µg/l, with a mean value of 16.166 µg/l (whole milk basis). p,p'-DDE (mean level 15.493±14.377) was the main contributor to total DDTs detected in the milk samples (95%), with only a small amount of the parent compound p,p'-DDT (0.75±0.484 µg/l, 4.6%), indicating the continuous degradation of DDT to a less toxic and more persistent derivative. The study indicated that DDTs and DDE detected values were lower than those previously recorded in some Egyptian governorates by Dogheim et al.[11,12] in 1991 and 1996, El- Hamid,[13] in 1993, Saleh et al.,[14] in 1996 and Salem and El-Saied,[15] in 1997.

Almost all studies throughout the world have shown DDT to be present in all lactating mothers. The obtained data showed that the concentrations of total DDTs in our samples were lower than those found in Iran by Cok et al.,[33] in1999, in Kenya by Kanja et al.,[4,36] in 1986 & 1992 and Kinyamu et al.,[37] in 1998, in Turkey by Cok et al.,[32] in 1997, in Spain by Hernandez et al.,[10] in 1993, in Ghana by Ntow,[5] in 2001, in Mexico by Pardio et al.,[31] in 1998, and by Waliszewski et al.,[6,38] in 1996 and 2001 and in the Republic of Belarus by Barkatina et al.,[30] in 1998, while it is higher than levels found in Japan by Nakagawa et al.,[39] in 1999. This indicates the role of the restriction measures taken in our country

The ratio of p,p'-DDT to p,p'-DDE was also evaluated. This ratio is usually higher in countries where DDT is still used. In countries like Norwey where use of DDT was banned in 1970,[7] the ratio of p,p'-DDT to P,p'-DDE decreased over the years from 0.32 in 1970 to 0.154 in 1979. Evaluation of this ratio is valuable in detecting the source of contamination and assessing recent or previous exposure and direct or indirect exposure through the food chain. The ratio in this study (0.048) was much lower than the ratio obtained in earlier studies in the same region and other regions in Egypt.[11,12,14,15].

The major metabolite of p,p'-DDT is the more persistent metabolite p,p'-DDE.[1] When the use of p,p'-DDT is banned or severely restricted, the levels of this substances in foods of plant origin fall rapidly, but exposure to its metabolite p,p'-DDE still occurs through the consumption of foods of animal origin that continue to accumulate this substance from the environment long after the use of DDT has ceased. Furthermore, when food-producing animals are exposed to p,p'-DDT, they metabolize this substance and retain the metabolite.[4] Contamination of human milk by DDT reflects relatively recent exposure of the mother to DDT from foodstuffs or through direct exposure. Contamination with DDE, on the other hand, reflects either earlier exposure of the mother to DDT, which has subsequently been metabolized to DDE and retained in her body fat, or exposure of the mother to DDE as such through consumption of foods of animal origin. In this respect, surveys carried out in Assiut Governorate showed a widespread contamination (100%) with DDE and DDT residues in animal tissues, milk and fresh water fish.[17,18] In Egypt, we still use the dicofol (*Kelthane*®) as acaricide which also replaced DDT as the primary source of environmental DDE and contains as much as 0.6 % p,p' and o,p'-DDT.[40] This indicates a continuous contamination of the environment by DDTs.

Hexachlorocyclohexane is one of the most widely used organochlorine insecticides for agriculture and public health programs. The large–scale application of HCH over a number of years, coupled with its extreme stability and slow metabolism, has led to environmental contamination and potential health hazards.[26]

HCH was detected as β-isomer in all samples (100%). Sometimes besides β-HCH, α-HCH was detected (5.6%) or χ-HCH (16.7%) while δ-HCH couldn't be detected. The level of β-HCH, the most persistent and lipophilic fraction, reached a mean value of 1.545 ± 1.583 µg/l while total HCH isomers mean value was 1.599 ± 1.595 µg/l. Total HCH levels were mainly due to the beta isomers. The commercial insecticide HCH is a mixture of different isomers mainly χ-HCH, α-HCH and β-HCH. χ-HCH (*Lindane*) is the most important isomer that is used as insecticide and is the most toxic. β-HCH, the most symmetric and stable isomer, is also the most persistent in nature, thus it is usually found in highest concentrations in human adipose tissues and milk. In addition, α-HCH and. χ-HCH isomers

may isomerise into β-isomer in living organisms.[37] This observation may explain the higher levels of β-HCH and the frequent occurrence of this isomer as compared to the other two isomers. Differences in persistence and bioaccumulation potential between isomers change the ratio of different HCH isomers from the start of food chain until the excretion in human milk; β-HCH with a five times slower rate of elimination in comparison to χ-HCH, has been considered as the predominant and persistent isomer in human milk.[41] Other HCH isomers presented considerably lower levels approximated to hundredth parts of μg/l.

We found low levels of HCH residues in the analyzed samples in comparison with the previously recorded data. Its mean values were 41.716 μg/l in Assiut,[15] 193.44 and 115.97 μg/l in Cairo and Kafr El-Zayat.[13]

Hexachlorobenzene (HCB) is one of the most persistent organochlorine contaminants known. It is used in the industrial production of pentachlorophenol and it is formed as a byproduct in many industrial processes. HCB may be formed during chlorination of wastewater.[42] It was translocated in foods and human tissues from non-agricultural sources such as industrial high-temperature processes involving chlorine and production of organic solvents.[43,44] HCB residues were present in 88.9% of the analyzed samples which indicates an increase in HCB frequency in the present study but the levels were low. It was detected with higher values in almost all samples in Mexico,[38] Turkey,[32] and Iran.[33] HCB values ranged from 0.2 – 0.867 μg/l with a mean value of 0.389 ± 0.156 μg/l. Its mean value was lower than those detected by Dogheim et al.,[11] in 1991 in Cairo (11.67 μg/l) and Salem and El-Saied,[15] in 1997 in Assiut (0.5 ± 0.18 μg/l).

Oxychlordane, γ-chlordane and α-chlordane were the major metabolites of the technical chlordane. Lower levels of oxychlordane were measured at a detection rate of 33.3%. Its mean value ranged from 0.1-0.568 with a mean value of 0.285 ± 0.175 18 μg/l. γ-chlordane and α-chlordane could not be measured in the analyzed samples. Oxychlordane was detected in 12.9% of the investigated breast milk in Cairo,[11] with a mean value of 1.8 ppb and a maximum of 2.6 ppb while it couldn't be found in a previous study in Assiut,[15] even with its presence in some foods of animal origin.[17] *Trans*-Nonachlor and *cis*-nonachlor are necessary ingredients of technical chlordane,[45] which was once used as a termite exterminating agent for houses in Japan. Nonachlor was used as internal stander in the analyzed samples for quality control of the used method. We noticed that the measured values of the internal stander in some samples were higher than the expected values which indicating that these samples had some residues of this pesticide. We didn't calculate nonachlor in the analyzed samples.

Table III shows that 3 breast milk samples out of 54 (5.6%) exceeded the Extraneous Residue limits (ERLs) for total DDTs (50 μg/liter whole milk) issued by FAO/WHO in 1994 & 1996; γ-HCH and HCB didn't exceed their limits (10 and 20 ug/l). Total HCH isomers couldn't be evaluated, as their limit is not applicable, but their values didn't exceed γ-HCH limit (10 ug/l).

Estimation of dietary intake of organochlorine pesticide residues due to contamination of breast milk could be obtained from human milk data. Studies from industrialized countries suggested that the average breast milk consumption of 2-3 months old infant is 700-800 ml/day as compared to 500-700 ml/day in developing countries. An infant consumes an average of 120 g/day per kg body weight during the first 3 months of life. After that, the volume consumed per unit weight decreases with age.[46] EDI was calculated and compared with international limits.

FAO/WHO[47,48] in1994 & 1996, have proposed as acceptable daily intake (ADI) 20 ug/kg body weight for total DDTs, 8 ug/kg bwt for γ-HCH, 0.5 ug/kg bwt for chlordane. HCB ADI was withdrawn. Table III shows the results of calculation of estimated daily intakes (EDIs) of organochlorine pesticides by breast-fed infants and the corresponding ADIs. EDIs were generally below established ADIs.

Table III shows also a comparison of this study results with the data from the same region conducted by Salem and El-Saied,[15] in 1997 and other Governorates by Dogheim et al.,[11,12] in 1991 & 1996, and El-Hamid,[13] in 1993. EDIs for different pesticide residues estimated in Middle and Upper Egypt were generally lower than those in other Governorates.

Finally, it could be concluded that among the various organochlorine pesticides, DDT derivatives and HCH isomers were the most frequent in human milk. The persistence of these pesticides and their metabolites in the environment means that much of these materials are used for control of insect-borne diseases and elimination of agricultural pests still contaminates soil and water. Dogheim et al.,[12] in 1996 detected high levels of DDTs, HCH isomers, dieldrin and heptachlor in groundwater, followed by Nile River water and tap water. They found also that soil samples contained the highest levels, especially for DDTs that could be an important source of

contamination. It was also reported that 96.3% of human exposure to organochlorine is due to ingested food. In addition, Nakagawa et al.,[39] in 1999 reported that the most lipophilic-pollutants are due to fish and meat, especially with egg, milk and dairy processed food.

Organochlorine pesticides should be continuously monitored in breast milk to see if a downward trend is maintained and appropriate measures would be taken to minimize its occurrence. Caution should be observed in consuming foods of animal origin, fish, vegetables, fruit or grains from contaminated areas.

Conclusion/Recommendation:

- Some organochlorine pesticides evaluated in this study are lower than previously documented values while others couldn't be detected indicating the efficiently political protective measures.
- The still high incidence and levels of some pesticides especially p,p'- DDE, p,p'-DDT and β-HCH enforce the responsible aspects to take more effective and continuous defenses.
- Frequent and continuous monitoring of these pesticides will help to take cautions in consuming food of animal origin, fishes, vegetables, fruits and grains from contaminated areas.

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