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Persistent organochlorine in human breast milk from Al-Sharkia Governorate, Egypt

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ABSTRACT

In the present study, 23 human breast milk samples were collected in January 2009 from Fakous city, Al-Sharkia Governorate, Egypt. The samples were analysed for organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and its metabolites, α, β, and γ-hexachlorocyclohexane (HCH) isomers. The average concentrations of HCHs and DDTs were 225 and 1315 ng/g lipid respectively. There was no significant difference between the levels of OCP and mother age, while there was a significant difference and correlation between the levels of OCP and the number of times the mother had breast fed (primiparous and multiparous) ($p < 0.05$). The results suggested that DDT is still entering the environment depending on the observed ratio of DDE/DDT. The levels of OCP in human milk recommended that we need to do more regular pollutant monitoring programs.

Key word: Persistent organochlorine, Pesticide, Human milk, Egypt

INTRODUCTION

The worldwide production and use of organochlorine compounds (OCPs) since the 1950s has resulted in their widespread occurrence in the environment. Their chemical properties such as lipophilicity and persistency lead to bioaccumulation and biomagnifications in the fatty tissues of biological specimens, and within the food chain bring on a high degree of contamination in the organisms at the top of the food chain (Tanabe *et al*., 1984; Kucklic and 1998; Hoekstra *et al*., 2003). Bioaccumulation of these compounds has been related to serious toxic threats. For example, DDE has been reported to be responsible for several abnormalities in wildlife such as eggshell thinning in fish-eating birds (Lundholm, 1997), reproductive failure of seals and fish-eating birds (Reijnders, 1986).The bioaccumulative nature and endocrine disrupting potential of OCPs on humans and wildlife has been a matter of great concern.

OCPs have serious environmental and health hazards. Based on the reports of their toxicity and adverse harmful effects to wildlife and humans, many organochlorine pesticides were banned or restricted from use or trade by the Ministry of Agriculture. Since 1980 DDT and lindane have been officially prohibited from agricultural use in Egypt, and in 1996 a Ministerial Decree prohibited the import and use of 80 pesticides including aldrin, dieldrin, endrin, chlordane, heptachlor, DDT, toxaphene, mirex, lindane, endosulfan, pentachlorophenol, and heptachlor epoxide. Due to the great concern in protecting the human health and environment from POPs, Egypt signed the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2002 and ratified it in 2003. Nonetheless, many of the pesticides banned or withdrawn from developed markets are still produced and sold in developing country markets (Wood MacKenzie Consultants Ltd., 1994).

Organochlorine pesticides concentrations in human milk have been used to assess trends in OCP pollution since the early 1970s and in particular to evaluate the success of the ban of OCPs in numerous countries (Smith, 1999; Jaraczewska *et al.*, 2006; Szyrwinska and Lulek, 2007). Most studies consistently show peak concentrations of OCPs in the 1970s or early 1980s with a subsequent decrease in the concentrations (Newsome *et al.*, 1995; Schade and Heinzow, 1998; Noren and Meironyte, 2000; Konishi *et al.*, 2001; Bates *et al.*, 2002). Few studies have reported that daily intake for organochlorine pesticides for most infant and people were above the acceptable daily intakes established by FAO/WHO. As a result it remains unclear whether the decrease in OCP concentrations in human milk samples observed after the ban in the 1980s has continued or is approaching a steady state. This fact emphasizes the need for more detailed study on the accumulation of OCPs in human milk.

In the present study, human breast milk samples were collected from one City, Fakous in Al-Sharkia Governorate, Egypt. The main objective of this study was to determine the concentrations of persistent OCPs, such as DDTs and HCHs, and to evaluate the status of contamination in Egypt in comparison with other countries.

MATERIAL AND METHODS

Human milk collection

Human breast milk samples were collected in January 2009 from 23 mothers in Fakous City, Al-Sharkia Governorate Egypt. Among the donors, (9) were primiparous and (14) were multiparous with average ages of (20 and 32) years. Fakous is one of the largest cities in Al-Sharkia with its main economy based on agriculture.

Breast milk samples were collected in clean 50 ml polypropylene centrifuge tubes with PTFE caps, kept in ice immediately after collection, and transferred to the Central Agricultural Pesticides Lab., Pesticide Residues and Environmental Pollution Department.

Informed consents were obtained from all donors. Questionnaires on dietary aspects and lifestyle were completed. The questions, concerned lifestyle factors, occupation, number of children, length of the lactation period, exposure to pesticides in the field and the use of the pesticides in home.

The pesticide standards for organochlorine pesticides were obtained from Dr. Ehrenstorfer Laboratories (Germany). Petroleum ether, diethyl ether, n-hexane, acetonitrile, anhydrous sodium sulfate, and methylene chloride were purchased from Merck (Germany). Florisil (PR Grade, 60–100 mesh) was purchased from BDH (England). All solvents were of pesticide residue grade. Florisil was activated at 130 °C overnight and cooled to room temperature in a desiccator.

Sample preparation

Procedures for extraction of pollutants and lipids from milk described by (Johansen *et al.*, 1994) were used with minor modifications, while the clean-up procedure was that described by (Covaci *et al.*, 2001). All samples were thawed and homogenized by shaking the milk for at least 5 min. An accurate amount of milk 10 g, was weighed and control samples were spiked with OCP standards (5ng α-HCH, 5ng of β-HCH, 5ng of γ-HCH, 2ng of p,p-DDT, 2ng of o,p-DDT, 2ng of p,p-DDE and 2ng of o,p-DDE) for recovery. After that 1 ml of formic acid was added to all samples. The extraction performed with 2×20 ml n-hexane/dichloromethane (5:1, v/v) by vortex shaking for 1 min. The organic layer was then subjected to clean up

onto a cartridge containing 1 g florisil. Complete elution of OCPs was realised with 10 ml n-hexane followed by 10 ml dichloromethane. The final eluate was concentrated first with a rotary evaporator and further under nitrogen to near dryness. The extract dissolved by 5ml n-Hexane when preparing for injection. Lipid determination was carried out on a separate aliquot of milk, by extracting 5 g milk with 2×5 ml n-hexane/ diethyl ether (1:1, v/v) for 2 min. The organic layer was transferred to a Petri-dish and evaporated. Lipids were gravimetrically measured after keeping the dish at 105 °C for 1 h.

Quantification

A Hewlett Packard (USA) 7890 gas chromatograph (GC) with a micro-electron capture detector (μECD) was equipped with a HB-5 capillary column (30m × 0.25mm i.d., 0.25μm film thickness). Nitrogen was used as carrier gas at a constant flow of 2.5 ml/min and Nitrogen as make-up gas (60.0 ml/min). One micro-liter was injected in the splitless mode. The temperature program applied was 120 °C held for 1 min, further by 20 °C/min to 180°C held for 2 min, further by 5 C/min to 220 °C, held for 5 min, and finally further by 3 °C/min to 245 °C, held for 30 min. injection port at 270°C. The detector temperature was 290°C. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the method reported by (JAN MOCAK *et al.*, 1997). The LOD ranged from 0.001 – 0.005 μg/g lipid, LOQ was 0.01 μg/g for p,p-DDE, p,p-DDT, 0.03 μg/g for α -HCH and γ-HCH and 0.04 μg/g for β-HCH, and the recoveries of OCPs from spiked samples were ranged from 87.5 % to 102 % across three spiking concentrations.

Questionnaires

Based on a self-administered dietary questionnaire, (Sasaki *et al.*, 1998; Sasaki *et al.*, 2000), which was modified for dietary habits, questions were asked about lifestyle factors, height and weight, occupation, past and current exposure to pesticides, smoking habits, status of breastfeeding, residence history, food consumption frequencies, per week or per monthly and the approximate amount of certain food items in one meal such as marine or freshwater fish, meat, eggs, cow's milk and so on.

Statistical analysis procedures:

The data was subjected to statistical analysis by a two-way ANOVA test using SPSS software for Windows version 10.0. Statistically significant differences between organochlorine levels and mothers' age, and number of children were tested by Duncan's multiple range (L.S.RD.) $p \leq 0.05$.

RESULTS

Characteristics of the participating women

The characteristics of the women who participated in the study (n = 23) are summarized in **Table 1.** The mean age was 24 years, ranging from 20 to 32 years. Only 39.1% of the mothers were nursing their first child, while 60.9% were multiparous.

Table 1: Characteristics of the women participating in the study

Characteristic	N	Mean	Median	Range
Maternal age	23	24	24	20 - 32
No. of Children	23	1.9	2	1- 4
Milk fat (% Lipids)	23	1.5	1.9	1.2 – 4.2

All women subjects were from rural areas. The results of the questionnaire revealed similar diets with low consumption of fish and relatively equilibrated amounts of animal meat and dairy products. Breast milk samples (5 g) were used for lipid measurements for all 23 subjects (Table 1). The mean fat content of the human milk samples ranged between 1.2 – 4.2%.

Breast milk is an ideal medium for assessing exposure to OCPs (Wong *et al.*, 2005), where they sequester in adipose tissue, serum and breast milk and equilibrate at similar levels on a fat basis (Hooper, 1999), so we analysed the chemical contamination status mainly on a lipid basis because the liposolubility rate is thought to be a major factor influenced by rates of accumulation and elimination from tissues and organs (Parham *et al.*, 1997), and because the existing differences depend principally on lipid content of the tissues (Henriksen *et al.*, 1998).

The present study is one of few reports on OCPs contamination in breast milk from Egypt. This study found organochlorine pesticides in the studied breast milk samples at various concentrations. The results of this study showed that p,p-DDE, p,p-DDT and β-HCH were the major OCPs found in human milk samples from Fakous, Sharkia, Egypt. The contaminants at the highest levels of were p,p-DDE (2980 ng/g Lipid), p,p-DDT (1962 ng/g Lipid). While the HCH were observed in moderate concentrations ranging (nd-23 ng/g Lipid), (nd-17 ng/g Lipid) and (0.4-760 ng/g Lipid) for α-HCH, γ-HCH and β-HCH respectively.

Distribution pattern of OCPs in human breast milk

The distribution pattern of the OCPs in human milk found was similar to the pattern previously observed in other studies which was reported by (How-Ran Chao *et al.*, 2006; Sudaryanto *et al.*, 2006; Mueller *et al.*, 2008) to follow the order of DDTs › HCHs. While the distribution pattern in the studies by Subramanian *et al.*, 2007; Tsydenova *et al.*, 2007; Behrooz *et al.*, 2009 followed the order of HCHs › DDTs.

We found no significant differences between the levels of OCPs and age among all age groups. While there is significant difference between levels of OCPs and number of breast-feedings, high levels were also observed among a group of women who had one-child primiparous. It is noteworthy that participants with the highest levels of DDE also had the highest levels of p,p-DDT and β-HCH. This association between DDE and β-HCH in the same subjects suggests that participant mothers in this study were exposed to high levels of environmental contamination and accumulation of these pesticides in fat bodies.

The DDE: DDT ratio can be used to indicate whether DDT is still entering the environment. A ratio of DDE:DDT is indicative of DDT entering the environment (Harris *et al.*, 1999), however, the ratio is not accompanied by high residue levels. The ratio of DDE:DDT, which presented in this study and residue levels suggested that the DDT is still being used in agriculture in Sharkia Governorate, where the samples were collected. Generally DDTs were detected at higher concentrations which ranged between (nd-2980 ng/g Lipid), followed by HCHs (nd-212.15 ng/g Lipid).

Among HCH isomers, β-HCH was the predominant isomer contributing 93.9% of the total HCHs (Fig.1). In addition, the ratio between different HCH isomers changes from lower tropic level in food chains to

human milk. However, in some individuals of the present study, α-HCH exceeded over 50% of the total HCHs, indicating that technical HCH is still being used recently. The average values of (α-HCH& γ-HCH) /β-HCH ratios in the present study are (1/15).

In general, this study found that OCPs levels in human breast milk had no significant association with maternal age among all age groups ($p>0.05$), while there is significant association with the number of breast feeding ($p < 0.05$), (primiparous and multiparous) mother and milk lipid content. (Fig.1).

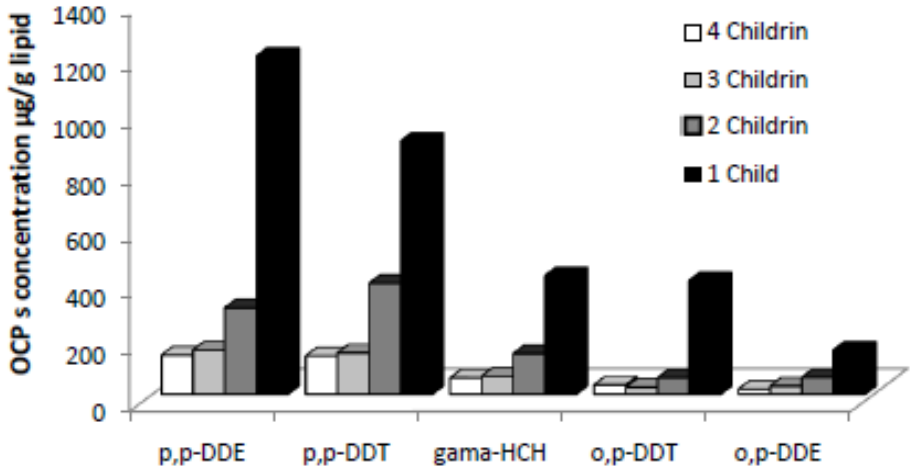

Fig. 1
The relation between number of children (primiparae and multiparous mother) and kevel of organochlorine pesticides in human breast milk

Comparison of samples from Egypt with other countries

Comparison of OCP levels between different countries is presented in Table 2. For Egypt mean concentration of sum-HCHs in the total sample set was 225 ng/g lipid (Table 2). This was comparable to levels found in breast milk samples from India (Mumbai), Malaysia, Russia (Murmansk and Barents region) and higher than the results from Jakarta, Purwakarta, Bogor, Lampung, Norway by 16, 7.5, 20, 32 and 16 times respectively. While the levels of the sum-HCH concentration in human milk from China (Hong Kong, Dalian and Shenyang), Iran (Nour, Countryside of Nour and Noushahr), Turkey, Russia (Irkutsk region, Kola peninsula and Murmansk), Buryatia were higher than what we are found in human milk samples by 4, 6, 25, 13, 11, 25, 2, 10, 3.5, 4 and 3.6 times, respectively. The data in Table 2 showed that the mean concentration of sum-DDTs in total samples was 1315 ng/g lipid in Egypt. This finding was comparable with the levels found in breast milk samples from India (New Delhi and Chennai), Purwakarta, Bogor, Malaysia, Russia (Murmansk and Barents region). The concentration of DDTs found in human milk samples from China (Hong Kong and Dalian), Iran (Noushahr), Turkey, Russia (Irkutsk region) were higher than the concentrations we found by 2, 1.5, 2, 3, 2 and 1.5 times, respectively. While the level of DDTs we found in human breast milk samples was higher than the concentration levels found in breast milk samples from India (Mumbai), China (Shenyang), Jakarta, Philippines, Norway, Belgium, Russia (Kola peninsula, Murmansk and Buryatia) by 3, 3, 1.5, 2, 8, 12, 8, 1.5, 1.5 and 2 times, respectively.

DISCUSSION

Organochlorine pesticides were widely used in agriculture and pest control until research and public concern regarding the hazards of their use led to government restriction and bans. In Egypt, the use of these compounds has been officially banned for about 30 years. Despite restrictions and bans on the use of many organochlorine pesticides, they continue to persist in the environment. In fact, in many ways, human have become reservoirs for these substances.

Public concern about the adverse environmental and human health impacts of organochlorine contaminants led to strict regulations on their use in developed nations more than two decades ago. Nonetheless, DDT and several other organochlorine pesticides are still being illegally used for agriculture in many developing countries and have led to the contamination of foodstuffs, especially those having a high fat content such as meat and meat products which

Table 2: Comparison of OCPs in human milk from various countries

Country	Year of collecting	N	ΣHCHs	ΣDDTs	ΣCHLs	References
Developing countries						
Egypt	2009	23	225	1315	-	This study
India	2000	8	720	430	5.5	Kunisu, *et al.*, 2002
New Delhi	2005-2006	21	340	1500	2.6	(Devanathan *et al.*, 2009)
Mumbai	2005-2006	26	220	450	3.4	(Devanathan *et al.*, 2009)
Chennai	2002-2003	12	4500	1200	7.3	(Subramanian *et al.*, 2007)
China						
Hong Kong	1999	132	950	2870	-	(Wong *et al.*, 2002)
Dalian	2002	-	1400	2100	16	(Kunisue *et al.*, 2004)
Shenyang	2002	-	550	870	6.7	(Kunisue *et al.*, 2004)
Indonesia						
Jakarta	2001	16	14	630	2	(Sudaryanto *et al.*, 2006)
Purwakarta	2002	19	30	1300	7.7	(Sudaryanto *et al.*, 2006)
Bogor	2003	15	11	1200	1.8	(Sudaryanto *et al.*, 2006)
Lampung	2003	6	7	1000	3.5	(Sudaryanto *et al.*, 2006)
Iran						
Nour	2006	-	3005	2680	-	(Behrooz *et al.*, 2009)
Countryside of Nour	2006	-	2588	1571	-	(Behrooz *et al.*, 2009)
Noushahr	2006	-	5742	3563	-	(Behrooz *et al.*, 2009)
Malaysia	2003	17	230	1600	23	(Sudaryanto *et al.*, 2005)
Philippines	2004	33	-	170	5.5	(Malarvannan *et al.*, 2009)
Developed countries						
Turkey	1995-1996	-	460	2400	-	(Cok *et al.*, 1997)
Norway	2000-2002	29	14	110	14	(Polder *et al.*, 2008)
Belgium	2006	197	12	156	7.8	(Colles *et al.*, 2008)
Russia						
Irkutsk region	1988-1989	-	2100	2000	18	(Schecter *et al.*, 2004)
Kola peninsula	1993	-	800	860	46	(Polder *et al.*, 1998)
Murmansk	1993	30	858	1474	-	(Polder *et al.*, 1998)
Murmansk	2005	14	235	900	22	(Polder *et al.*, 1998)
Barents region	1996-1997	-	320	1200	37	(Polder *et al.*, 2003)
Buryatia	2003-2004	-	810	660	19	(Tsydenova *et al.*, 2007)
Japan	1998	-	210	290	85	(Konishi *et al.*, 2001)
Japan	2001-2004	93	110	340	80	(Kunisue *et al.*, 2006)
Germany	1995-1997	246	40	240	-	(Schade and Heinzow, 1998)
England	2001-2003	-	40	220	-	(Kalantzi *et al.*, 2004)

contribute to the high dietary intakes of most of the organochlorines (Kannan *et al.*, 1994). As a consequence, humans in this region are exposed to dietary levels of organochlorines at least 5–100-fold greater than those in more developed nations (Kannan *et al.*, 1997).

This study was conducted to assess organochlorine pesticides levels in human breast milk among Egyptian women. The majority of participants in this study had high levels of DDE and β-HCH, this was expected because of the high organochlorine levels in food in Egypt (El-Dib and 1985; Nabawi *et al.*, 1987; Dogheim *et al.*, 1988; Dogheim *et al.*, 1990; El-Gendy *et al.*, 1991; Amr *et al.*, 1995; Badawy *et al.*, 1995). The correlation between organochlorine pesticides levels and number of children and prolonged lactation, suggests that breast feeding may modulate excretion of organochlorine pesticides and prolonged lactation may have reduced the body burden of organochlorine levels through breast milk transfer (Rogan *et al.*, 1980; Rogan *et al*., 1986; Soliman *et al*., 2003).

The high human breast milk OCP levels in the majority of our study subjects, confirmed that the role of lactation prolongation in reducing OCP levels from human body (Rogan *et al.*, 1980; Rogan *et al.*, 1986). Our finding may also be supported by many studies from India, Croatia, United States and Egypt (Rogan *et al.*, 1986; Nair *et al.*, 1996; Laden *et al.*, 1999; Soliman *et al.*, 2003).

CONCLUSION

The study shows the presence of high levels of organochlorine pesticide in human milk in the Fakous, Sharkia Governorate. Total DDT concentrations were found to be higher than total HCH levels, which can be attributed to the illegal use of DDT in agriculture in Sharkia Governorate and/or earlier anti-malaria activities. The present data clearly indicates a significant bioconcentration of DDT and HCH residues in the breast milk, and the newborn is a recipient of this bio-concentrated form of pesticides. The result demonstrates that considerable amounts of DDT and HCH are transferred from the mother through breastfeeding. There were no significant differences between the levels of OCPs among the different age groups ($p > 0.05$). While there were significant differences between number of breast feeding (primiparous and multiparous) mothers ($p \leq 0.05$). So a positive correlation between age and OC body burden is expected. Breast milk can be considered as a suitable indicator for monitoring the burden of the persistent lipophilic chlorinated pesticides in the human body. Government and other relevant agencies should educate farmers in Good Agricultural Practices (GAP) in the use of pesticides in agriculture. Regular monitoring of OCP levels in the environment should be conducted.

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ARABIC SUMMARY

المركبات الكلورونية العضوية الثابتة في ألبان الامهات بمحافظة الشرقية، مصر

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قسم بحوث متبقيات المبيدات وتلوث البيئة- المعمل المركزي للمبيدات- مركز البحوث الزراعية، الدقي، مصر

في هذه الدراسة تم تجميع عدد ٢٣ عينة من ألبان الامهات في يناير ٢٠٠٩ من المركز الصحي، بمدينة فاقوس، محافظة الشرقية، مصر. وقد تم تقدير بعض المبيدات الكلورونية العضوية الثابتة ونواتج تمثيلها مثال مركب داي كلورداي فينيل تراي كلورايثان (ددت) و نواتج تمثيله، وكذلك مركبات الفا، بيتا و جاما هكساكلوروهكسان. وقد كان متوسط تركيز مركبات الهكساكلوروهكسان والددت هو ٢٢٥ و ١٣١٥ نانوجرام/جرام دهن علي الترتيب. وقد لوحظ عدم وجود فرق معنوي في تركيز المبيدات الكلورونية بإختلاف عمر الأم، بينما كان هناك فرق معنوي وارتباط بين تركيز المبيدات الكلورونية وعدد مرات الإرضاع (ولادة واحدة و عدة ولادات) للأم (معنوية $\leq$ ٠.٠٥). وتشير النتائج الي أنه هناك احتمال أن هذه المركبات لازالت تدخل الي البيئة في هذه المنطقة طبقا لـ نسبة دداي / ددت. ومن خلال النتائج المتحصل عليها ودراسة مستويات تركيز المبيدات الكلورونية العضوية توصي الدراسة بإجراء المزيد من برامج تقصي الملوثات في البيئة بشكل دوري.