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Measurement of Organochlorine Pesticides Level in Milk Agricultural Women Workers (Mazandaran-Iran)

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Abstract: Main purpose of this study levels of some organochlorine pesticides were determined in milk samples of mothers living in two agricultural cities of Iran (Sari and Babol in Mazandaran province) in order to assess the trends of woman workers exposure to persistent pollutants. The milk samples gathered from breastfeeding women routine determination of OCPs was performed with GC/ECD. The resulte showed the highest amounts were found for p,p'-DDE, which was 0.056 $\mu g\ g^{-1}$ in fat in Sari and 0.045 $\mu g\ g^{-1}$ fat in Babol , followed by β-HCH, which was 0.037 $\mu g\ g^{-1}$ fat and 0.024 $\mu g\ g^{-1}$ fat, respectively. The low level of OCPs in human milk as found in the present study support the Iranian policy of encouraging breast-feeding. The fact that the mother breast-feeds her child and that she originally comes from a region where DDT is still in use as a vector control agent, as well as the former use of organochlorine pesticides OCPs in agriculture, seems to be the main factors for high DDT and other OCP residues in the mothers' milk.

Key words: Organochlorine pesticides, milk, women workers, Iran

INTRODUCTION

Organochlorine pesticides are used mostly as insecticides and composed primarily of carbon, hydrogen and chlorine. Specific uses take a wide range of forms, from pellet application in field crops to sprays for seed coating and grain storage[1,2]. In Mazandaran province some organochlorines are applied to surfaces to kill insects that land there.

Most of them break down slowly and can remain in the environment long after application and in organisms long after exposure. Several commonly known organochlorines have been banned for use in the US including DDT, aldrin, dieldrin, toxaphene, chlordane and heptachlor .

Organochlorines contribute too many acute and chronic illnesses. Symptoms of acute poisoning (exposure to a large dose) can include tremors, headache, dermal irritation, respiratory problems, dizziness, nausea and seizures. Exposure to low doses of organochlorines-more representatives of exposures due to pesticides accumulated in the body-are associated with many chronic diseases. Studies have found a correlation between organochlorine exposure and various types of cancer, neurological damage (several organochlorines are known neurotoxins), Parkinson's disease, birth defects, respiratory illness and abnormal immune system function. Many organochlorines are known or suspected hormone disruptors and recent studies show that extremely low levels of exposure in the womb can cause irreversible damage to the reproductive and immune systems of the developing fetus[3,4].

Breastfeeding has been intensively encouraged, especially in developing countries, due to its beneficial properties, i.e., increase infant immune factors and resistance to chronic diseases such as asthma, diabetes or allergies. In addition, human exposure to environmental pollution has led the scientific community to study the pathways of these contaminants and the possible risks they pose to human health[3,4]. Exposure to organochlorine pesticides (OCs), in special, has been the subject of great interest during recent years given their potential toxicity, resistance to degradation and bioaccumulation through the food chain[5,6]. The major source of OC has been agriculture and public health campaigns to vector control. General population exposure occur mainly through the diet and human milk can be an indicator of exposure since OCs are lipophilic xenobiotics that accumulate in adipose tissue and breastfeeding is the main pathway of elimination through the fatty fraction of milk[7,8]. In this study milk samples of mothers living in two agricultural cities of Iran (Sari and Babol in Mazandaran province) were evaluated in order to assess the woman workers exposure to persistent pollutants.

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MATERIALS AND METHODS

Milk sampling: Milk samples collected in the city of Sari and the other in the city of Babol. Both were part of a total of 10 samples collected in order to exposure study on Levels of OCPs. All samples were obtained in July 2004 and donors were selected according to the protocol established by WHO[9]. All mothers were informed about the objectives of the study and gave their consent. At each area, 10 samples of 100 mL each were collected from breastfeeding mothers. Milk was expressed manually directly in a jar and kept frozen at -20°C. Before, each jar was cleaned with organic solvents and three tablets of potassium dichromate were added to avoid degradation during shipping. After finishing the collection of the 10 sub-samples, they were sent frozen to the central laboratory in Sari city, where they were defrosted at room temperature and pooled to give a total volume of 1000 mL. One part of 500 mL was sent to the reference laboratory in Shaheed Beheshti University of Tehran and the other aliquot was kept frozen for safety purposes (e.g., damage during transportation).

Analytical procedure: The method was followed baded on Methods in Yu[10]. For determination of organochlorine pesticides, human milk samples were centrifuged and from the cream the compounds of interest extracted together with fat by use of sodium sulfate and light petroleum. After evaporation of the solvent the fat content was determined by weighing out the mass of the remainder. Up to 0.5 g of the fat extract was redissolved in cyclohexane ethyl acetate and the internal standards were added. For separation of fat, gel permeation chromatography was performed on Bio-Beads S-X3 with cyclohexane/ethyl acetate as eluting solvent. after concentration of the eluate almost to dryness and redissolution in iso-octane chromatography on a small column of partially deactivated silica gel was used as final clean up step using toluene as eluent.routine determination was performed with GC/ECD using generally two different GCs (Fisons Mega 2) with two columns of different polarity (fused silica no. 1: 30 m PS-088 [97.5% Dimethyl- 2.5% diphenyl siloxane copolymer], 0.32 mm i.d., 0.32 μm film thickness, fused silica No. 2: 30 m OV-1701-OH, 0.32 mm i.d., 0.25 μm film thickness, both columns custom-made). Results were confirmed routinely by GC-LRMS[11].

RESULTS AND DISCUSSION

The age of donors in each sample was about 23 years old in average. The fat amount of fresh weight was 4.2% for Sari and 4.9% for Babol city. Concentrations, based on lipid weight, of organochlorine pesticides in human milk are given in Table 1. In both samples, the highest amounts were found for p,p'-DDE, which was 0.056 μg g^{-1} fat in Sari and 0.045 μg g^{-1} fat in Babol , followed by β-HCH, which was 0.037 μg g^{-1} fat and 0.024 μg g^{-1} fat, respectively. The concentrations of the other analyzed compounds were near or, in most cases, below detection limit (0.001 μg g^{-1} fat).

The high proportion of p, p'-DDE / p,p'-DDT was 5.6 for Sari and 11.25 for Babol, indicate that exposure happened through environmental contamination by former application of DDT since DDE/DDT ratio will increase when the use of DDT ceases[5]. The exposure to its persistent metabolite DDE still occurs mainly via foodstuffs and also through metabolic conversion of p,p'-DDT. The same assumption can also be made for the HCH group, as only the more stable beta isomer was present in both samples. In 1989, the use of DDT and HCH has been restricted by the Iranian Government to public health campaigns for vector control. Until 1998, both compounds were used as insecticides, which is concentrated in the North region (98% of cases), northern part of Iran. Both sampled Cities are located in the north part of Iran.

Table 1: Organochlorine pesticide concentrations in human milk from two agricultural city, Sari and Babol collected in July 2004 (μg g^{-1} fat)

Compound	Sari city $\bar{\chi} \pm SEM$	Babol city $\bar{\chi} \pm SEM$
HCB	0.006	0.003
o,p'-DDT, o,p'-DDE, o,p'-DDD	< 0.001	< 0.001
pp'-DDE	0.056	0.045
pp'-DDT	0.010	0.004
pp'-DDD	< 0.001	< 0.001
dieldrin	0.001	0.001
endrin, endrin ketone	< 0.001	< 0.001
cis-heptachlorepoxide	0.001	0.001
α-chlordane, *gamma*-chlordane	< 0.001	< 0.001
oxy-chlordane	0.003	0.001
trans-nonachlor	0.002	0.002
Ó Parlar (toxaphene)	< 0.001	< 0.001
α-HCH	< 0.001	< 0.001
β-HCH	0.037	0.024
γ-HCH	< 0.001	< 0.001
Ó Endosulfane	< 0.001	< 0.001

Table 2: Average concentrations of organochlorine pesticides from different countries (μg g^{-1} fat)

Compound	Brazil xi 1999 (n = 40)	Mexico xii 1999 (n = 40)	Indonesia xiii 2000 (n = 70)	Turkey xiv 2003 (n = 37)	Germany 2001/02
HCB	0.061	0.025	0.03	0.020	0.042
pp'-DDE	1.701	3.997	0.28	1.522	0.148
pp'-DDT	0.302	0.651	0.06	0.065	0.005
pp'-DDD	n.a.	0.002	n.a.	n.a.	< 0.005
Ó DDT	2.199	4.696	-	1.595	0.152
α-HCH	0.022	0.001	n.a.	< 0.001	< 0.005
β-HCH	0.399	0.061	0.09	0.149	0.021
γ-HCH	0.182	0.002	0.01	0.003	< 0.005

n.a. = not analyzed

Levels of HCB, DDT and HCH have been reported in human milk from different countries and some data are given in Table 2. Data from Germany are also from samples from the third round of WHO-coordinated exposure studies x and reflect the situation in central Europe. Comparison of the levels from the current study in Iran with those in other countries showed that HCB is at the Lower end of concentrations, whereas sum DDT and β-HCH are at the upper end[12,13].

CONCLUSIONS

Ocs concentrations in the environment have been decreasing gradually worldwide. Legally banning of OCs can lead to low concentrations in the environment and consequently to low human exposure which can be observed by considering human milk as good indicator of general population exposure[13,14]. This process is supported by the Stockholm Convention which is a global treaty signed now by 55 parties to take action against certain POPs, among them PCBs, PCDDs and PCDFs[9,15]. After ratification by France as the 50th Party, the Convention entered into force in May 2004. The effectiveness should be evaluated four years after the date of entry into force and periodically thereafter at intervals[10,16]. Therefore, a Global POPs Monitoring Programme was developed. United Nations Environment Programme (UNEP) organized a workshop to provide a scientific basis for this programme. One of the conclusions was to select the following matrices: air; bivalves; wildlife species (fish, bird's eggs, marine mammals) and human milk[11].Therefore, future analyses of these POPs in breast milk also from Iran are encouraged to show tendencies over time.

Levels of OCP residues in human milk from Mazandaran province were comparable to those from developed countries and significantly lower than those from developing countries. The low level of OCPs in human milk as found in the present study support the Iranian policy of encouraging breast-feeding.

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