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Levels of organochlorine pesticides in human breast milk in Marivan, west of Iran

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ABSTRACT

The present study aimed to assess the levels of organochloride pesticides (OCPs) in the human breast milk obtained from the nursing women admitted for delivery to a hospital in Marivan county, located in Kurdistan, Iran. Milk samples were collected from 30 women. Gas chromatography analysis was performed using a Dani 1000 (Italy) gas chromatograph equipped with the Ni electron capture detector (ECD). The findings showed the high concentrations of OCPs in the samples. On the other hand, DDTs were ranked as the most abundant OCPs, followed by HCHs and HCBs, with the concentrations estimated at 2,345, 2,617, and 570 ng/g lipid/wt, respectively. Therefore, it could be concluded that the tested women might have been exposed to OCPs. However, further investigation is required on larger sample sizes to take proper measures.

Keywords: Biomonitoring, Breast milk, Marivan, Nursing women, Organochlorine pesticides

Introduction

Breast milk is the most fundamental nutritious support to the infant, which contains optimal amounts of proteins, carbohydrates, and fats. With its remarkable properties, breast milk is considered an irreplaceable source of food humans in infancy. Recently, there have been growing concerns regarding the presence of chemical contaminations. Several studies have confirmed the presence of lipophilic xenobiotic, such as polyhalogenated chemicals and organochlorine pesticides (OCPs) in human breast milk.[1, 2]

Pesticides have caused a revolution in the history of agriculture, increasing food products and supplies for humans. Despite these benefits, their toxicity for human health and their high persistence in the environment have restricted the use of OCPs, which have particularly been known to be highly toxic and even lethal. However, their use has continued in developing countries, and their long-term persistence in the environment is another contributing factor in this regard, which make it vulnerable to be analyzed in human breast milk.[3]

Lipophilicity is a prominent feature of OCPs, which leads to the sustainability of these compounds in living organisms, enabling them to persist and be transferred through food chains and accumulate in the fatty tissues of animals and humans.[4]

OCPs such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyl, and hexachlorobenzene (HCB) are a class of persistent organic pollutants that are distributed into surface and ground water, soil, plants, animals, and humans, causing disturbances in the endocrine, reproductive, neurologic, and immunologic systems and may even be oncogenic.[5, 6]

Hexachlorocyclohexane (HCH) is a synthetic OCP with eight isomers (e.g., α, β, ϒ, θ, and γ-HCH [lindane], which is still used in the treatment of ectoparasites. HCHs and DDTs have been detected at higher concentrations than the tolerance limits recommended by the Food and Drug Organization (FAO) and World Health Organization (WHO) in fish and dairy

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products in Asian and Oceanian countries.[2, 7] Several decades ago, organic and inorganic chemical pollutants were traced in human breast milk. Since the 1960s, human milk monitoring programs for environmental pollutants have initiated mainly in industrialized countries. The first reports on the level of OCPs have indicated that DDTs and its metabolites, HCB, and heptachlor epoxide are the most frequent agents.[1, 2, 8]

In Stockholm (Sweden), studies on breast milk have initiated since 1967, and sampling in different periods have shown that PCB levels decreased by 30% during 1972-1997. In addition, DDT levels in 1997 were reported to be 1% of the levels stated in 1967. In 1997, the concentration of p,p-dichlorodiphenyldichloroethane (DDE) also decreased to 5% of the concentration 1972. Moreover, in 1997, HCB declined to 5% of the levels reported in 1974. In Sweden, the mentioned data are representative of applying restrictions and the efforts of health systems in the monitoring and lowering of these toxic agents. However, many developing countries and even some industrialized countries, such as the United States and Australia, are far from these achievements. In a developing country such as Uganda, DDTs and DDEs have been detected in all the sampled cases with the mean concentration of 3240 ng/g of milk fat, while in an industrialized country such as Switzerland, 75.5% of the tested samples contained OCPs in significantly higher concentrations than the permitted limits of the FAO and WHO.[6, 9]

According to a review by Pirsaheb *et al.*, OCPs are still used in some countries, including Iran.[10] However, no reports are available in the western regions of Iran regarding the levels of these compounds in human milk.

The present study aimed to determine the concentrations of OCPs in the breast milk of the mothers in Marivan county, Iran.

Materials and Methods

Sample collection and pre-treatment

In total, 50 milk samples were randomly collected from the women living in Marivan and the surrounding villages in 2017. The containers used for sample collection were washed with detergents and distilled water and placed in nitric acid solution (65%) for 12 hours. Following that, they were washed with distilled water again in order to become completely free of impurities and contamination.

Human breast milk samples (5-50 ml) were collected from the mothers admitted to the hospital in Marivan county for delivery. Marivan county is located in the west of Iran (35.5211°N, 46.1757°E). The samples were immediately stored in ice and transferred to the laboratory at the temperature of -20 °C until analysis.[15]

Written informed consent was obtained from all the donors with various durations of residence in Marivan (7-31 years). All the donors were housewives. The milk samples were warmed and homogenized for five minutes so as to determine the level of OC pesticides.

Analytical procedures

The levels of the determined OC pesticides, including α, β, and γ-HCH, HCB, o,ṕ -DDE, p,ṕ -DDE, p,ṕ-DDT, and pṕ-DDD in the deforested milk samples were measured using the methods proposed by Ogunfowokan *et al.*[11] Depending on the available amounts (1-6 g), specific amounts of milk were added to the internal standard (15 ng ε-HCH). After adding two milliliters of formic acid, the extraction was performed with the ratio of 5:1 v/v (12 ml of n-hexane/dichloromethane [DCM]) by vertexing the solution for two minutes.

At the next stage, a column (length and internal diameter: 15×1 cm) was filled with five grams of activated silica gel, which was prepared in a slurry form in n-hexane. Approximately 0.5 centimeter of anhydrous sodium sulfate was placed at the top of the column in order to absorb the water in the samples or the solvent. Afterwards, the column was pre-eluted with 15 milliliters of n-hexane without the air exposure of the sodium sulfate layer. The reduced extract was placed in the column and allowed to sink below the sodium sulfate layer. Elution was performed using the extracted solvent (DCM; 2×10 ml). Following that, the eluate was collected, dried with

anhydrous sodium sulfate, and evaporated to dryness under a stream of analytical grade nitrogen (99.999%).

Lipid determination was performed on a separate aliquot of milk by extracting one gram of milk using 2×3 milliliters of n-hexane/diethyl ether (1:1 v/v) for two minutes. Afterwards, the organic layer was transferred to an aluminum dish and evaporated. Moreover, the gravimetrical measurements were performed after preserving the dish at the temperature of 105 °C for one hour.[12]

Gas chromatography (GC) analysis was performed using a Dani 1000 (Italy) gas chromatograph equipped with ^{63}Ni electron capture detector (ECD). The GC system was equipped with a DB-5 capillary column (diameters: 60 m×0.25 mm, film thickness: 0.25 µm, Macherey-Nagel). Helium gas was used as the carrier at the low rate of 2 ml/min. The oven temperature was initially maintained at 100 °C for one minute, and the rate increased at 10 °C/min to the temperature of 240 °C and maintained for one minute. Afterwards, the oven temperature was set with the rate of 1 °C/min until reaching the temperature of 260 °C for one minute, increased at the rate of 20 °C/min to the temperature of 300 °C, and remained at the same temperature for 10 minutes.

The injection port was operated in the splitless injection mode, and the injection and detector temperatures were set at 250 °C and 300 °C, respectively. In addition, an injection sample volume (1 µl) was used for liquid analysis.

Results and Discussion

Table 1 shows the concentrations of OCPs (ng/g lipid/wt) in the human breast milk samples collected in Marivan, located in the west of Iran. The concentration of lipids in the breast milk samples was 2.28%. In a study conducted by Subramanian *et al.*, which aimed to assess the levels of OCPs in the breast milk of the women in four regions in India, the concentration of lipids was reported to be 2.1%, 2.4%, 2.6%, and 1.8%, respectively, which is in line with the results of the present study. Therefore, it could be inferred that the concentration of lipids in human breast milk might not be affected by geographical region or lifestyle. In the current research, the mean concentration of α-HCH was 1,123 ng/g lipid/wt, while the concentration of α-HCH in the mentioned study was estimated at 4.6, 4.7, 9.1, and 7.9 ng/g lipid/wt.[13]

Table 1. Concentration (ng/g lipid wt) of organochlorine (OCs) pesticides in human breast milk from Marivan, West of Iran

Parameters	Mean	Median	Range
Lipids %	2.28	2.1	0.5–6.3
α-HCH	1123	740	45 - 6715
β-HCH	1520	854	15- 5870
γ-HCH	419	170	10 - 2740
∑HCHs	2617	1567	50 - 12850
HCB	570	337	30 - 4120
o,ṕ -DDE	530	10	20 - 2580
p, *ṕ* -DDE	2125	1430	80 - 7230
p, *ṕ* -DDT	530	310	n.d - 3510
p, *ṕ* -DDD	15	n.d	n.d - 230
∑DDTs	2345	1120	70 - 10550

nd: Not Detectable

In Norway, the concentration has been reported to be 2 ng/g lipid/wt[14] and 0.133 ng/g lipid/wt in the Taiwanese population.[5] The estimated concentration of α-HCH in the present study was significantly higher compared to the previous studies in this regard. In the current research, the mean concentration of β-HCH in the breast milk samples was 1,520 ng/g lipid/wt, while the concentration of β-HCH in Norway was reported to be 0.120 ng/g lipid/wt,[14] and 240, 210, 680, and 4,500 ng/g lipid/wt in the study by Subramanian *et al.*[13] In another study performed in northern Germany, this concentration was estimated at 26.8 ng/g lipid/wt,[2] which indicated the higher levels of β-HCH in Marivan compared to the previous studies in this regard, with the exception of some regions in India.

According to the results of the present study, the mean concentration of γ-HCH was 419 ng/g lipid/wt. In Norway, the mean concentration of γ-HCH was reported to be 0.0914 ng/g lipid/wt,[14] while it was estimated at 82, 1.1, 2, and 1.1 ng/g lipid/wt in the four

regions in India,[13] 3.6 ng/g lipid/wt in northern Thailand,[1] and 3.7 ng/g lipid/wt in northern Germany.[2] Therefore, the mean concentration of γ-HCH in the present study was significantly higher than the previous studies in this regard.

This spatial pattern in the current research could be due to the lower latitudinal position (350 m), vicinity of the studied area to the oil refinery area in Kirkuk city, Iraq (230 km distance in the west of Marivan), and typical tropical climate, which might facilitate the higher volatilization of HCHs. Another reason may be the location of Marivan county at a higher latitude toward the east and vicinity to Iraq, which is one of the largest producers/users of HCHs. The higher export of HCHs to these areas may lead to the higher concentrations of these compounds in the environment and ecosystem.

The global comparison of human breast milk in North Vietnam indicated intermediate levels of HCHs, which are lower compared to China, Hong Kong, India, and Japan, while higher than the concentrations reported in the other developing countries in Asia, such as Cambodia and the Philippines.[15]

In the present study, DDT concentration was estimated at 15 ng/g lipid/wt, which is relatively higher compared to the values reported in the regions in the world (e.g., 0.161 ng/g lipid/wt in Taiwan,[5] 8.1 ng/g lipid/wt in southern Spain,[16] 7.9 ng/g lipid/wt in Thailand,[1] and 260-5910 ng/g lipid/wt in the villages in South Africa).[17] However, the mean concentration of DDT in India has been reported to be 14.32 ng/g lipid/wt,[13] which is consistent with the findings of the current research.

According to the results of the present study, the mean concentrations of *o,ṕ*-DDE and p, *ṕ*-DDE in human breast milk were 530 and 2,125 ng/g lipid/wt, respectively. In this regard, various values have been reported in different countries across the world. On the other hand, the literature review indicated the range of 0.148-14,580 ng/g lipid/wt.[18] In the current research, the mean concentration of *ṕ*-DDT in human breast milk was 530 ng/g lipid/wt. Table 2 shows various concentrations reported throughout the world. Accordingly, the concentration of DDT in the human breast milk in Indonesia was as high as 15,000 ng/g lipid/wt.[19] The values reported in the literature reveal the potential utilization of DDTs for other purposes, such as sanitation and control of some tropical diseases, rather than in agriculture.

Table 2. Different levels of OCs (ng/g lipid wt) reported from various parts of the world[15, 19]

		Year	n	DDTs	CHLs	HCHs	HCB
Developed countries	Ukraine	1993-1994	197	2700	38	730	168
	Turkey	1995-1996	104	2100	-	457	50
	Germany	1995-1997	246	240	-	40	80
	Czech Republic	1996	17	1050	-	70	-
	Russia	1996-1997	140	1040	37	280	91
	Sweden	1997	40	143	-	-	12
	UK	1997-1998	168	470	-	103	43
	Canada	1992	497	244	34	24	15
	Australia	1995	60	1200	-	350	-
	Japan	1998	49	290	85	210	14
Developing countries	Mexico	1998	60	4100	-	60	30
	India	2000	8	420	0.9	650	1
	China (Hong Kong)	1999	132	2870	-	950	-
	China (Guangzhou)	2000	54	3550	-	1110	-
	Cambodia	2000	49	1500	1.6	5.2	1.7
	Philippines	2000	10	190	15	4.7	-
	Vietnam (North)	2000-2001	48	2200	4.5	36	3.2
	Marivan (IRAN)	2018	30	2345	-	2617	570
	Data not available						

The high levels of residue in human breast milk in developing countries could be attributed to the widespread and increased DDT contamination in the environment and food chains. Fortunately, DDT concentrations in the human breast milk samples in Marivan were lower compared to the values reported in other countries. Due to the excretion of DDTs via milk, it seems that giving milk in multiparous women may play a key role in reducing the burden imposed by DDT contamination on nursing mothers.

Although the samples in the present study were preserved in the freezer, there was a long interval from sample collection to the analysis since meeting the required standards for analysis was lengthy. This could be another reason for the high concentration of OCPs in the human breast milk samples.

According to the current research, the mean concentration of HCB in the tested samples was 570 ng/g lipid/wt, which is relatively higher compared to the global levels, indicating the maximal exposure of the general population to this chemical agent.

A review study in this regard revealed a significant, positive correlations between maternal age, multiparty, and concentration of chlorinated pesticides in breast milk.[10] The sample population in the current research was selected from only one hospital, and the subjects were within the same age range; as a result, no statistical analysis could be carried out on the obtained data.

Limitations of the study

Due to financial limitation, the present study was conducted on a small sample size; therefore, the findings may not be generalized to the entire population in Iran. Moreover, considering the sensible dietary patterns in Kurdistan province, it essential to further investigate various contaminations throughout the area. Another limitation of the study was the poor cooperation of the nursing mothers due to their negative subculture.

Conclusion

The present study aimed to evaluate the current contamination status of OCPs in human breast milk in Marivan county, located in the west of Iran. Among the analyzed OCPs, ∑HCHs was predominant, followed by ∑DDTs and HCB. However, no association was observed between OCP concentrations and maternal age, which could be attributed to the narrow age range of the participating mothers. Therefore, further investigation is required to clarify the status of the entire population within the province.

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