Original Article

Determination of Pesticide Residues Hexachlorocyclohexane, Cyclodiene, Diphenylaliphate and their Metabolites in the Breast Milk of Women from Karachi- Pakistan

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ABSTRACT

Purpose: The aim of this study was to determine the presence of pesticide residues hexachloro-cyclohexane, cyclodiene, diphenylaliphate and their metabolites in the milk of women from different regions of Karachi, Sindh-Pakistan.

Study Design: An experimental study.

Place and Duration of Study: This study was conducted in the department of Zoology, University of Karachi from April 2009 to April 2011.

Materials and Methods: A total of 30 human breast milk samples were collected from the Gynae wards of Karachi hospitals. A breast pump was used to collect 5 ml milk sample from each women. The sample was taken in the sterilized and labeled vials. The collected samples were then stored at -20 ^{o} C for analysis. All the samples were analyzed for the presence of pesticide residues. Samples of milk were prepared accordingly and the purified samples injected into the Shimadzu GC-ECD apparatus. The peaks of the samples were compared by the retention time of the standard peaks. The chromatogram obtained indicated the quantity of pesticide residues.

Results: The standard chromatogram of organochlorine pesticides and their isomers were prepared and analyzed on GC-ECD. The standard chromatograms were then matched with the chromatogram of milk samples. The isomers like α, β, γ and δ were detected as major residues of HCH. The cyclodiene compounds such as aldrin and α-endosulfan were detected as common compounds. DDT was found in 43% of milk samples. The percentage of cyclodiene residues was higher than HCH and diphenylaliphate.

Conclusion: A significant bioconcentration of organochlorine residues was found in the breast milk. Total DDT concentrations were found higher than total HCH levels. It is thus concluded that this bioconcentration of pesticide residues clearly indicates that the mother contains a heavy amounts of such toxic chemicals.

Key Words: Hexachlorocyclohexane, Cyclodiene, Diphenylaliphate, Metabolites, Women breast milk.

INTRODUCTION

Pesticides or insecticides are among the most extensively used chemicals in the world today and they are also among the most hazardous compounds to the human being as well. As some pesticides can be beneficial in decreasing the populations of harmful or destructive insects while others can be damaging to the environment and can cause serious disturbances[1]. Organochlorine pesticides are organic compounds that are characterized by their lipophilicity persistence and semi-volatility has been used as pesticides including both insecticides such as DDT, lindane, aldrin, dieldrin and methoxychlor as well as herbicides. The chlorinated hydrocarbon unsecticides were introduced in 1940 and are widely used in agriculture sector to control insect pest species[2]. There is evidence of organochlorine pesticide residues in sediments, water, crops, meat and human fluids including human milk[3-8]. Restriction and bans on the preparation of organochlorine pesticides minimized their usage, as they persist in the environment for long period because of their accumulation in the soil[9]. Also in the industrialized sectors these chemicals are banned and restricted for their use on large scale but in the developing countries DDT is still being used[10]. As OC pesticides are hydrophilic and lipophilic compounds, they enter in the environment and human body via food chain and can cause various ailments such as reproductive, neurological and endocrine dysfunctions[11] as well as disturb the enzyme systems[12].

It has also been reported that mothers are exposed to OC's through food chain contamination and environmental pollution. These chemicals enter in the body through oral, dermal and inhalation and are then absorbed and distributed to different organs[13]. The fetus may receive these chemicals through the placenta of mothers[14] and also transferred from mother to newborn babies through breast milk like organochlronines[15-17]. As breast milk is an important carrier that provides all the necessary nutrients, growth factors and immunological components to the infants[18], several risk factors are involved for the accumulation of OC's in human breast milk e.g., parity, maternal age, timing of

sampling and the nature of diet that are consumed by the mother[19-22].

Very little work has been done to measure the body burden of organochlorines in the women of Karachi-Pakistan especially on human breast milk. Therefore, present study has been carried out to assess the residual levels of organochlorine pesticides and their metabolites in the breast milk of women particularly belonging to the different regions of Karachi, Pakistan.

MATERIALS AND METHODS

Chemicals and Standards Pesticides: The standard mixtures of organochlorine compounds were purchased from Supelco Company of USA. All the chemicals and solvents used were of pesticide scan grade. For the extraction of milk samples methanol, sodium oxalate, diethylether and hexane were used.

Table I. Pesticide residues (mg/kg) in the milk samples.

Name of Pesticides	Positive samples	Positive test (%)	Mean	SD	SE	Range min-mix
α-HCH	10	33	0.006	0.012	0.002	0.007—0.016
β-HCH	15	50	0.405	1.013	0.185	0.432—1.157
γ-HCH	13	43	0.019	0.033	0.006	0.027—0.050
δ-HCH	9	30	0.009	0.020	0.003	0.010—0.024
Σ HCH			0.439			
Heptachlor	4	13	0.002	0.006	0.001	0.001—0.006
Hepta-endo-epoxide	3	10	0.001	0.006	0.001	0.0006—0.005
Hepta-exo-epoxide	9	30	0.022	0.043	0.007	0.028—0.059
Aldrin	10	33	0.062	0.229	0.041	0.040—0.205
Endrin	9	30	0.087	0.443	0.081	0.012—0.329
α-Endosulfan	16	53	0.346	0.945	0.172	0.341—1.018
β-Endosulfan	5	16	0.086	0.466	0.085	0.001—0.335
Endosulfan Sulfate	00	-	-	-	-	-
Dieldrin	7	23	0.029	0.123	0.022	0.014—0.102
Σ Cyclodienes			0.635			
4,4-DDT	7	23	0.016	0.077	0.014	0.004—0.183
4,4 DDE	13	43	0.032	0.012	0.002	0.036—0.066
4-4,DDD	9	30	0.222	0.473	0.086	0.266—0.604
Methoxychlor	8	26	0.175	0.202	0.037	0.271—0.416
Σ Diphenylaliphatic			0.444	-	-	-
Hexachlorobenzene	1	3	0.003	0.016	0.003	0.000—0.013
Σ OCs			1.518			

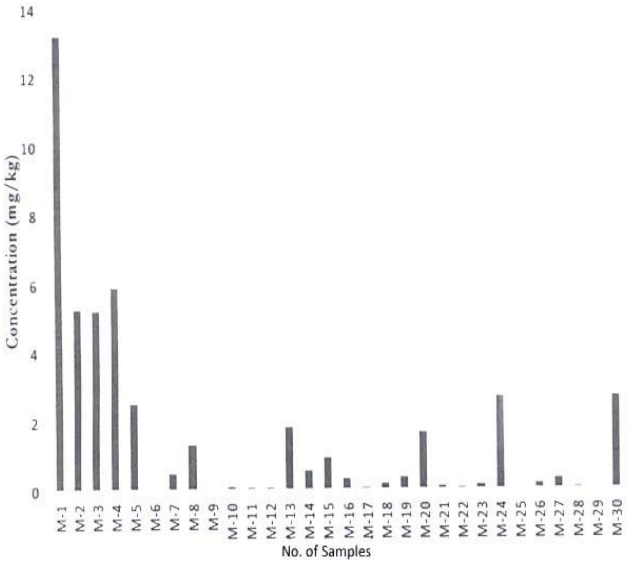

Figure I: Bar graph showing total OC concentration in 30 milk samples

Collection of milk samples: The milk samples were collected from the Gynae wards of Karachi hospitals. A total of 30 breast milk samples were collected for this study. Each milk samples comprises of 5 ml that were taken in the well cleaned and labeled vials. All the samples were kept and stored at -20 °C till analysis.

Preparation of Samples: Out of total 5 ml of milk samples, 4 ml of sample was mixed with equal amount of methanol and shaken for 5 min for proper mixing. Sodium oxalate 0.1 gm was then added and the solution was again shaken by vortex mixer. The mixture was extracted with 10 ml of diethyl ether and n-hexane (1:1 v/v) and was then centrifuged for 15 min at 3000 rpm. The organic phase was separated out after centrifugation. The pellet was again extracted with diethyl ether and n-hexane. This was repeated twice to obtain three organic phases from the mixture. The organic phases were then dried by nitrogen. The dried residues were re-dissolved in 1 ml of hexane for clean up procedure.

Detection and Quantification of Pesticide Residues: Quantitative estimation of pesticide residues in all the extracts was done by Schimadzu GC 17 – A, equipped with Ni 63 Electron Capture Detector (ECD) attached with CBM-102 chromatogram recorder system. Purified nitrogen gas was used as the carrier gas and a known volume 1µl of milk sample was injected in the column through specialized syringe. Different peaks of the samples were identified by comparing the retention times of the standard peaks and the quantification of residues was then obtained from each sample.

RESULTS

The standard chromatogram of 18 organochlorine agricultural pesticides and their isomers were prepared on GC-ECD. The standard chromatograms were then matched with chromatogram of 30 milk samples. Sample 1 has the highest OC concentration 13.2 mg /kg than the rest samples. Samples 6, 9, 25 and 29 had negligible quantities (Fig.1). The results obtained from all samples (n=30) was statistically interpreted (Table 1). The mean concentration of OC ($\sum OC$) was calculated as 1.518 mg /kg. The mean concentration of Hexachlorocyclohexane isomers α, β, γ and δ were calculated as 0.006 mg /kg, 0.405 mg /kg, 0.019 mg/kg and 0.009 mg /kg respectively. The concentration of total HCH isomers was then found to be 0.439 mg /kg.

Among cyclodiene group of compounds, heptachlor was detected in 4 samples out of total 30 samples and the mean value was calculated as 0.002 mg /kg. The isomers of heptachlor i.e., heptachlor endoepoxide and heptachlor exoepoxide were calculated as the mean value of 0.001 mg /kg and 0.022 mg /kg. Aldrin was detected in 10 samples and Dieldrin was found in 7 samples with their mean values of 0.062 mg /kg and 0.029 mg /kg respectively. Endosulfan and their isomers like α-endosulfan and β-endosulfan were detected with their mean values as 0.346 mg /kg and 0.086 mg /kg respectively.

Regarding diphenylaliphate compounds, the mean value of 4,4-DDT was calculated as 0.016 mg/kg and of DDE (major metabolite) was 0.032 mg /kg, where as for DDD it was found to be 0.222 mg/kg. The mean of total DDT (i.e., sum of DDT, DDE and DDD) was found to be 0.270 mg /kg.

DISCUSSION

This study was conducted in the different localities of Karachi to collect the breast milk samples of women from different hospitals for the detection and quantification of pesticide residues and their metabolites due to pesticide exposure.

The present finding of the milk samples showed the heavy concentration of HCH as compared to Diphenylaliphate insecticides. Among all metabolites, β-HCH was reported to be more stable in the environment than its isomers[23]. The present study however indicated that the β-isomer of HCH was in a higher concentration of 0.405 mg/kg than α, β, γ and δ isomers. A study reported that about sixty milk samples were found to be contaminated with HCH isomers[24]. The same finding was also noticed by the present study. Another study reported that the breast milk of Russian women[25] and Chinese women[26] indicated higher β-HCH than α and γ isomers. The present study also agreed with the findings of these reports. Similarly a study reported that the total HCH found in four villages of India[27] showed 0.123 ppm, 0.129 ppm, 0.131 ppm and 0.127 ppm and these values are found significantly in lower level as compared to the present study that showed mean concentration $\sum 0.439$ ppm of HCH.

A study from Victoria, Australia reported that the breast milk samples showed lower levels of aldrin, dieldrin, heptachlorepoxide and HCB[15], while the present investigation indicated higher values of these compounds in the milk samples. The pesticide residues dieldrin and HCB were found to be 60% higher values in the milk samples of Ghana women[28]. However the present study also indicated higher values of these compounds in the milk samples. Many researchers also reported the high concentration of DDT in the breast milk of Victorian's mother[15]. This is almost in line with the present study.

CONCLUSION

A significant bio-concentration of organochlorine residues was noticed in the breast milk of Karachi women by the present work. Total DDT concentrations were found to be higher than total HCH levels. As milk is the most initial natural food source of infants, so there are more chances of the bioaccumulation of the residues and their metabolites in infants and new born provided if their mothers contain or are heavily exposed by such toxic chemicals.

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