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MONITORING OF PESTICIDE RESIDUES IN HUMAN MILK

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After establishing proper analytical methodology for multiple pesticide residues, cotton-growing areas of Multan Division of Pakistan were surveyed and 40 samples of human milk from cotton pickers were collected during two crop seasons. Screening of these samples showed 72.5% contamination with 19 different pesticides/metabolites. The most frequently occurring pesticides were DDT and its metabolites, dimethoate, cyhalothrin, monocrotophos, profenofos and quinalphos.

Keywords: Pesticide residues, Human milk, Pakistan.

Introduction

Organochlorine pesticides were used extensively some time back. However, their use was officially banned in 1980s. These compounds are persistent, have high absorbance on sediments and soil and are highly lipophilic in fatty animal tissues. Organophosphorus and synthetic pyrethroid compounds have low persistence and are readily decomposed (Morifusa 1977) These are extensively used for pest control of cotton crop in Multan division, Pakistan.

Screening procedures for organochlorine and other compounds in human milk were developed by Schenck and Wagner (1995), Petreas *et al* (1996), Hooper *et al* (1997) and Schenck and Casanova (1999).

Monitoring studies on the presence of organochlorine and organophosphorus pesticides in human milk have been reported by several workers, eg., Dogheim *et al* (1996), Waliszewski *et al* (1998), Schoula *et al* (1996), Czaja *et al* (1997), Chikuni *et al* (1997), Cok *et al* (1997), Kredl *et al* (1997), Barkantina *et al.* (1998), Nasir *et al* (1998) and Okonkwo *et al* (1999).

There were reports of adverse health effects on cotton pickers particularly the lady pickers during spray operations/picking of cotton in the cotton growing areas (Khan *et al.* 2000). Since sufficient data on pesticide residue levels in the agro-ecosystem of Pakistan is not available, this work forms part of our investigations in that direction. The paper, therefore, presents results of our pesticide-monitoring programme in samples of human milk drawn from lactating lady cotton pickers of Multan division.

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Materials and Methods

Control milk sample was taken from a housewife from a Maternity Hospital. Milk (10g) was taken in triplicate for each experiment in a 250-ml beaker. Calculated amounts of standards of γ-BHC, endosulfan, dieldrin and p,p'-DDT (organochlorine compounds), chlorpyrifos, dimethoate, methamidophos, monocrotophos, profenofos and quinalphos (organophosphorus compounds), α–methrin, cyhalothrin, cyfluthrin, cypermethrin and fenpropathrin (synthetic pyrethroids) were separately added to milk and shaken for 5 minutes. Fortification levels for each of these pesticides were 0.001, 0.01, 0.1, 0.2, 0.5 and 1 ppm. Each fortified sample was allowed to stand for half an hour at room temperature and then processed for extraction, cleanup and determination.

Analytical methodology A rapid and convenient procedure for milk was developed which eliminated a fat isolation step, provided an extract acceptable for GC determination after it was defatted with Florisil (BDH, 100-12- mesh activated at 450°C for 3 h) and at the same time enabled us to analyze several samples in a short period of time. All chemicals/solvents used in this study were of analytical reagent grade.

Extraction and cleanup Milk sample (10 g) was taken in a beaker to which an equal quantity (10 g) of activated Florisil (100-120 mesh) was added. It was then mixed thoroughly and left for 15 minutes at room temperature. The mixture of milk and Florisil was transferred to a glass column (350 mm long x 10 mm i.d.) and pesticides were eluted with a mixture of diethyl etherin-hexane (1:1). 150-ml of eluate was collected. Flow rate through the column was 0.5 ml min^{-1}. It was then

Table 1
Recovery of studied pesticides from fortified human milk

Pesticides	Fortification (ppm)	Recovery (%)	Mean±SE
Synthetic Pyrethroids:			
Alpha-methrin	0.10	78.27, 81.05, 80.92	80.08±0.92
Cyhalothrin	0.10	93.19, 91.73, 90.82	91.91±0.70
Cyfluthrin	0.10	83.81, 85.01, 83.67	84.16±0.43
Cypermethrin	0.10	95.23, 94.83, 94.37	94.81±0.25
Fenpropathrin	0.10	96.08, 95.87, 97.23	96.39±0.43
Organophosphorus:			
Chlorpyrifos	0.10	86.73, 87.05, 86.57	86.78±0.14
Dimethoate	0.50	92.25, 93.38, 92.91	92.84±0.33
Methamidophos	0.50	79.57, 78.97, 79.02	79.19±0.19
Monocrotophos	0.20	86.28, 85.92, 86.20	86.13±0.11
Profenofos	0.10	94.17, 93.75, 94.03	93.98±0.14
Quinalphos	0.20	87.48, 86.92, 87.23	87.21±0.09
Organochlorine:			
Gamma-BHC	0.001	98.72, 98.15, 99.07	98.65±0.27
Endosulfan	0.01	93.28, 92.63, 93.06	92.99±0.19
p,p'-DDT	0.01	97.83, 98.16, 98.09	98.03±0.10
Dieldrin	0.005	97.57, 96.98, 97.36	97.30±0.17

evaporated to dryness in a rotary vacuum evaporator and taken-up in petroleum ether for GC determination.

Gas chromatographic determination For identification and quantification of studied pesticides, Varian GC-3600 Gas Chromatograph with the following operating parameters was employed:

GLC was equipped with ^{63}Ni electron capture (ECD) and flame ionization detection (FID) systems employing 2mx2mm i.d. glass columns packed with 1.5% OV-17+1.95% OV-210 and 3% OV-17 on 80/100 mesh chromosorb W-HP, respectively.

Prior to monitoring work, the efficiency of analytical methodology was evaluated in model experiments in which human milk was spiked with known quantities of each studied pesticide separately. It was then carried through the developed procedures and finally analyzed by gas chromatography. Recoveries of different pesticides ranged between 79.19% and 98.65%. Recovery data are presented in Table-I. The developed analytical methodology is reliable, rapid, easy to standardize and suitable for monitoring of multiple pesticides in human milk.

The minimum detectable residues of studied pesticides are mentioned in Table 1 on a whole milk basis.

Monitoring studies. Sampling. Forty samples of human milk were drawn from the lactating mothers during the picking period from the cotton growing areas of Multan during two crop seasons of 1992-93 and 1993-94. These samples were considered as random samples. 100 ml quantity was sufficient for sub-sampling. They were kept in clean and dry propylene bottles, sealed, properly labelled and brought to the laboratory. In the laboratory, all the samples were stored at –20°C for subsequent residue analyses.

Pesticide residue analysis. All human milk samples were subjected to extraction, cleanup, identification and determination of multiple pesticide residues in accordance with the above-described procedures.

Results and Discussion

Each human milk sample was sub-divided into three subsamples of equal size and analyzed by the developed methodology for triplicate analyses. Results of GC analysis are presented in Table 2. Twenty-nine samples were found to be contaminated with residues of different pesticides used on cotton crop during study period. Several scientists in different countries also studied the pesticide contamination in human milk. Czaja *et al* (1999) studied the persistence of organochlorine compounds in breast milk from two consecutive lactations of the same donors. Their results show the mean concentrations of all compounds. No significant decrease was found in the mother with the longer interval. In other study (Polder *et al* 1998) PCBs, toxaphenes and other chlorinated pesticides were determined in human milk from the Archangels district, Russia. In our study cyhalothrin, cypermethrin, chlorpyrifos, dimethoate, monocrotophos, profenofos, quinalphos, endosulfan and DDT were used by farmers throughout the two crop seasons and found to contaminate the human milk of cotton pickers. The presence of pesticides in human milk samples of lactating mothers obviously indicates that infants are exposed to hazards of these pesticides. A glance at Table 2 shows that those organochlorine pesticides i.e. BHC and DDT are still present in the cotton ecosystem.

Conclusion:

Internationally approved FAO/WHO maximum residue limits of pesticides detected in human milk are not yet available in the literature. However, Table 2 shows that several contaminated samples contained high amounts of detected pesticides. This can have various short and long term adverse health effects on mothers as well as their children. Organochlorine pesticides still contribute to the problem of human exposure. These pesticides are persistent and banned world-over including Pakistan. Being cheaper, these products some how find their way in the agricultural market thus creating a seri-

Table 2

A comparative picture of pesticide residues detected in cotton pickers milk (192-93 and 1993-94) from cotton growing area of Multan

Pesticide detected	Crop season 1992-1993		Crop season 1993-1994		Hazardous to human beings (FAO/WHO)
	No.of samples conta-minated	Quantity found (ppm)	No.of samples conta-minated	Quantity found (ppm)	
SyntheticPyrethroid:					
Alpha-methrin	-		2	0.325, 1.210	Moderately
Cyhalothrin	2	0.363.0629	3	0.241, 0.299, 0.371	
Cyfluthrin	-	-	1	Traces	
Cypermethrin	2	Traces, 0.327	1	Traces	
Fenvalerate	-		1	Traces	
Organophosphorus:					
Chlopyifos	1	0.417	3	0.012, 0.013, 0.381	"
Dimethoste	2	0.550, 0.621	5	0.510-2.964	"
Methamidophos	-		1	0.712	Highly
Monocrotophos	2	0.621, 2.050	3	0.302, 0.896, 1.374	"
Profenofos	3	Traces, Traces, 0.810	2	Traces, 0.173	Moderately
Quinalfos	3	0.715, 0.823, 1.003	2	0.558, 0.710	"
Organochlorine:					
Alpha-BHC			1	0.019	"
Garmma-BHC			1	0.410	"
Delta-BHC	-		1	0.114	"
Endosulfan	2	0.471, 0.717	3	0.134, 0.627, 0.732	"
P'p'-DDT	5	0.068-0.715	9	Traces-0.903	"
P'p-DDD	5	0.710-1.410	6	0.413-1.327	"
P'p' DDE	2	Traces, 0.379	4	Traces 1.210	

Total No. of samples analyzed= 40; Total No. of samples contaminated= 29; Samples contaminated= 72.5%

ous environmental problem. The data also emphasize the importance of the pesticide residue monitoring programme and highlights the adverse health effects of pesticides on children and infants. Hence there is the need to create awareness amongst the farming community to use pesticides prudently and in accordance with "Good Agricultural Practice" in order to minimize contamination of our agro-ecosystem and hazards to human lives.

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