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Studies on Pesticide Residues in Human Blood

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Organochlorine, organophosphorus and synthetic pyrethroid pesticides, commonly used for cotton crop protection were examined in human blood samples of pickers and farmers of cotton growing areas of Multan division. A total of 75 samples, collected during 1992 and 1993 crop seasons were screened for the presence of the pesticides by analytical procedures involving extraction, cleanup and gas chromatographic determination. 85% samples were found to contain residues of studied pesticides. However, their quantities were quite low and not harmfull for health except in very few cases.

Key words: Pesticide residues, Human blood, Cotton pickers.

Introduction

Agro-chemicals are biologically active compounds. Its use for crop protection is essential to feed the fast growing population their misuse poses a serious threat to the environment and the ultimate victim is the man himself.

Pesticides pose several kinds of hazards to human health. The effect of the pesticides is essential that their absorption in the human body and examined with a standard method. Number of methods are available (Ribeiro and Minelli 1991; Zhang 1991; Hu-Yongshi and Yang-Xianging 1992; Drevenkar *et al* 1994; Krechniak and Laboda 1991; Rosell *et al* 1993; Dua *et al* 1996; Guardino 1996) for evaluating of chemicals by analyzing blood or urine from exposed industrial and agricultural workers. Many pesticides are widely used in agriculture and possible to detect the pesticides or its metabolites in blood urine at concentrations of several ng ml^{-1} (Nair *et al* 1992; Mes-Jos 1992; Gill *et al* 1996; Tayyab *et al* 1997; Namera *et al* 1997).

The total procurement of pesticides, nearly 80% is consumed for the protection of cotton crop and huge consumption, chances of misuse are relatively higher. Farmers working in cotton fields of punjab and sindh are affected during spray operation of pesticides and picking of cotton. There are also reports of fatalities occuring in cotton growing areas due to pesticide poisoning. It was, therefore, decided to examine pesticides in the cotton eco-system, i.e. cotton seed oil, human blood and milk. The studies were carried out in the cotton growing areas of Multan during 1992-93 crop seasons. The present paper describes the studies in detail in case of farmers' blood.

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Materials and Methods

Pesticide standards. Reference grade pesticides were furnished by the manufacturers.

Chemicals. All chemicals used in the study were AR grade and all solvents were redistilled before use.

Fortification. Atleast on pesticide, representing each group of studied compounds (OC, organochlorine; OP, organphasphorus; and SP, snthetic pyrethroid) was taken for fortification. Recoveries of gamma-BHC, p,p-DDT, monocrotophos and cyhalothrin were determined in triplicate at fortification levels of 0.005, 0.01, 0.10 and 0.01 $mgkg^{-1}$ and made directly on to 15.0 ml of control sample of human blood. The pesticides divided into three parts prior to addition of the extraction solvent. This was done to check reproducibility of results. Fortified samples were then processed according to the following procedures.

Extraction and cleanup. The influence of different solvents system for extraction of pesticide residues from human blood was examined. Chloroform was found to be the most suitable extraction solvent as it furnished highest residue recoveries and minimal amount of co-extractives. 5ml of control and fortified whole blood were placed in three 50ml screw cap glass tubes separately and 25ml chloroform was added to each tube. The tubes were capped and shaken thoroughly for 15 min on an electrical shaker. The mixture in each tube was then transferred separately to a 100 ml centrifuge tube used for centrifugation at 2000 rpm for 5 min and the upper chloroform layer was transferred to a 100 ml conical flask with a pipette. The extraction was repeated with two additional 25 ml portions of chloroform which were then combined in a

conical flask and filtered through anhydrous sodium sulfate on Whatman no.542 filter paper into a 250 ml round bottom flask to avoid loss of pesticide residues. The portions were combined, its volume noted and evaporated to dryness on a rotary vacuum evaporator at 40 °C and the residue then was taken up in 5ml petroleum ether for gas chromatography.

Gas chromatographic determination. For identification and quantification of studied compounds, a Varian AG Gas Chromatograph (Model 3600), equipped with Ni^{63} electron capture (ECD) and thermionic specific (TSD) detectors was employed. The equipment was used in conjunction with data system DS-650 series (Model DS-651) and printer (Hewlet-Packard, USA). When an unknown sample is experimentally identified on a particular GC column material, the findings have to be confirmed on another column packed with a different liquid stationary phase. Two different column materials were, therefore, used in the present investigations.

a) Operating parameters of Ni^{63} ECD: Two glass columns have 2 meters longx2 mm internal diameter and packed separately with a mixture of 1.5% OV-17+1.95% OV-210 on 80/100 mesh chromosorb W-HP and 3% OV-17 supported on 80/100 mesh chromosorb W-HP were used.

b) Other parameters. Temperatures: Column oven, 230°C; Injector, 250°C; Detector, 280°C; Attenuation, 32; Range, 10; Gas flow, nitrogen (carrier) 30 ml min^{-1}.

C) Operating parameters for TSD. The columns as mentioned above were used for Thermoinic Specific Detector. Other conditions employed in this case were: *temperatures:* Column oven, 200°C; Injector, 225°C; Dectector, 250°C; Attenuation, 32; Range, 12; Bead current, 3.2 Amp.; Gas flows, nitrogen (carrier) 30 ml min^{-1}; Hydrogen, 5.4 ml min^{-1} and Air, 175 ml min^{-1}.

Table 1 shows fortification levels and % recovery of pesticides from fortified human blood samples.

Sampling. Cotton farmers were physically examined and interviewed during sampling period for adverse effects of pesticides on their health. Spraymen complained of abnormal facial sensations that developed mostly 2-3 h after the start of pesticide spraying and usually disappeared by next day after the exposure ceased. Some complained of dizziness, headache and

Table 1

Percent recovery of studied pesticides from fortified human blood

S.no.	Pesticide	Fortification	Recovery %	Mean±SE
Synthetic pyrethroids				
1	Alpha-methrin	0.10	88.37, 87.79, 88.03	88.06±0.17
2	Cyhalothrin	0.01	93.15, 94.09, 93.80	93.68±0.28
3	Cypermethrin	0.01	96.62, 95.82, 96.02	96.15±0.24
4	Deltamethrin	0.10	89.04, 88.61, 88.72	88.79±0.13
5	Fenpropathrin	0.01	98.34, 97.49, 98.04	97.96±0.25
6	Fenvalerate	0.10	78.43, 77.07, 77.68	77.73±0.40
Organophosphorus				
7	Chloropyriphos	0.01	87.03, 86.92, 86.51	86.82±0.16
8	Dimethoate	0.01	83.68, 84.21, 84.17	84.02±0.17
9	Methamidophos	0.10	92.73, 91.69, 92.07	92.16±0.31
10	Monocrotophos	0.10	98.27, 97.69, 98.03	97.99±0.10
11	Profenofos	0.01	97.03, 96.62, 96.24	96.63±0.23
12	Quinalphos	0.50	79.71, 81.09, 80.42	80.41±0.41
Organochlorine				
13	Alpha-BHC	0.001	98.43, 97.89, 98.23	98.18±0.16
14	Dieldrin	0.01	96.32, 99.03, 97.78	97.71±0.80
15	Endosulfan	0.01	89.92, 91.07, 91.44	90.81±0.47
16	p,p -DDT	0.01	95.89, 97.28, 97.15	96.77±0.45
17	Diafenthiuran	0.10	93.83, 94.21, 94.11	94.05±0.12

Table 2
Pesticide residues in human blood

S. no.	Pesticide detected	No. of samples contaminated	Quantity present (mg kg^{-1})
Synthetic pyrethroids			
1	Alpha-methrin	1	Traces
2	Cyhalothrin	4	0.286, 0.370, 0.730, 0.760
3	Cypermethrin	1	Traces
4	Deltamethrin	1	Traces
5	Fenpropathrin	9	0.035, 0.220, 0.240, 0.350, 0.410, 0.510, 0.520, 0.580, 0.590
Organophosphorus			
6	Chloropyriphos	2	Traces, Traces
7	Dimethoate	5	0.310, 0.316, 0.387, 0.500, 0.570, 2.100
8	Methamidophos	3	0.210, 0.428, 1.020
9	Monocrotophos	5	Traces, Traces, Traces, 0.420, 1.000
10	Profenofos	9	Traces, Traces, Traces, Traces, 0.060 0.080, 0.100, 0.100, 0.210,
11	Quinalphos	6	Traces, 0.135, 0.321, 0.632, 1.030, 1.590
Organochlorine			
12	DDT & its metabolites	16	Traces, Traces,Traces, Traces, 0.070, 0.080, 0.080, 0.110, 0.130, 0.150, 0.170, 0.230, 0.260, 0.640, 0.650, 1.440
13	Gamma-BHC	1	0.350
14	Endosulfan	2	Traces, Traces

Area samples drawn, cotton growing area of Multan; Period of sampling, December 1992; No. of blood samples drawn, 25; No. of contaminated samples, 22; Percentage contamination, 88.

nausea during picking. 25 and 50 random samples of whole blood (15 ml each) were drawn with the help of sterilized injection syringes during December 1992 and November 1993 respectively. Each sample was divided into three sub samples which were then transferred to sterilized glass tubes containing 20ml chloroform. The tubes were properly labelled, marked, sealed and kept in ice containers during sampling time. After sampling all samples were transferred to the laboratory as soon as possible. In the laboratory, the blood samples were placed in a deep freezer at -20°C until screened for residues.

Monitoring studies. 75 samples drawn during two crop seasons were screened for the determination of multiple pesticide residues in human blood, in accordance with the above described procedures and results are presented in Table 2 and 3. 63 samples were found to contain residues of different pesticides.

Results and Discussion

In the present study, an evaluation of each of the analytical parameters involved has been undertaken with the goal of achieving good recoveries. Thus methods involving extraction, cleanup and gas chromatographic determination were developed for studying OC, OP and SP pesticides using fortified and control samples of human blood. Recoveries of the studied pesticides were between the range of 77.73-97.99% (Table-I). Cleaned up extracts were free from any interferring impurity and the methods were efficient, reliable and sensitive down to 0.005 ppm for OC and SP compounds & 0.1 ppm for OP compunds. It can be effectively used for monitoring of cotton pesticides in human blood. After developing appropriate analytical procedures as described above, 75 samples of human blood, drawn from cotton pickers of Multan during two crop seasons, were monitored for different pesticides. 85% samples were found contaminated with residues of OC, OP and SP pesticides (Table 2 and 3). Maximum residue limit (MRL) of pesticides in human blood are not available in the scientific literature but a galance at the tables show that the overall picture of pesticide residues in the screened samples is not alarming as the quantities of different pesticides found to be present are quite low except in very few cases.

Organophosphates contaminated 61 samples while synthetic pyrethroids were present in 80 samples. Two years monitoring study showed contamination of human blood with 18 different OC, OP and SP pesticides and their metabolites but the most frequently occurring pesticides were DDT and its metabolites, fenpropathrin, cyhalothrin, profenofos and monocrotophos present in 32, 30, 23, 15 and 15 samples respectively. The use of these compounds is relatively high in cotton growing areas of Multan division where greater pest activity prevails due to long summer and rainy season. Therefore, regular monitoring of the pesticides in cotton growing areas of Pakistan is essential from human health point of view.

Table 3
Pesticide residues in human blood

S. No.	Pesticide detected	No. of samples contaminated	Quantity present ($mgkg^{-1}$)
Synthetic pyrethroids			
1	Alpha-methrin	2	Traces 0.340
2	Bifenthrin	1	0.397
3	Cypermethrin	19	Traces 0.024, 0.026, 0.096, 0.234, 0.252, 0.278, 0.317, 0.366, 0.381, 0.416, 0.444, 0.660, 0.913, 1.202, 1.329, 1.571, 1.730, 2.471
Organophosphorus			
4	Cypermethrin	8	0.204, 0.232, 0.236, 0.327, 0.382, 0.389, 0.481, 1.021
5	Deltamethrin	5	0.174, 0.293, 0.350, 0.327, 0.780
6	Fenpropathrin	21	0.011, 0.012, 0.014, 0.015, 0.021, 0.032, 0.044, 0.068, 0.069, 0.071, 0.072, 0.240, 0.240, 0.300, 0.303, 0.482, 0.550, 0.570, 0.680, 0.870, 1.201
7.	Fenvalerate	8	Traces, Traces, Traces, 0.180, 0.192, 0.623, 0.7702, 0.821
Organochlorine			
8	Methamidophos	6	0.428, 0.430, 0.458, 0.609, 0.621, 1.007
9	Monocrotophos	10	0.509, 0.542 0.614, 0.628, 0.629, 0.714, 1.080, 1.312, 1.317
10	Profenofos	6	0.189, 0.292, 0.461, 0.463, 0.579, 0.618
Organochlorine			
11	Delta-BHC	2	0.210, 0.291
12	Gamma-BHC	3	0.029, 0.080, 0.640
13	DDT & its metabolites	16	0.014, 0.100, 0.100, 0.107, 0.162, 0.189, 0.217, 0.234, 0.240, 0.321, 0.329, 0.393, 0.432, 0.501, 0.521, 0.632
14	Endosulfan	7	Traces, Traces, 0.039, 0.051 0.130, 0.330, 1.032
15	Dieldrin	1	Traces
16	Diafenthiuran	7	0.448, 0.571, 0.627, 0.712 0.714, 1.017, 1.071

Area samples drawn, cotton growing area of Multan; Period of sampling, November 1993; No. of blood samples drawn, 50; No. of contaminated samples, 41; percentage contamination, 82.

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