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Pesticides in the Blood Samples of Spray-workers at Agriculture Environment: *The Toxicological Evaluation*

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Abstract

Pesticides are major contaminating chemicals in agriculture environment and a hazard to exposed population. These compounds are absorbed by inhalation, ingestion, and dermal contact. Their bioavailability in the individuals can lead to a variety of metabolic and systemic dysfunctions, and even outright disease states. Therefore, the tremendous usage of pesticides has promoted toxicological studies in spraying community. This study comprising pesticide spray-workers (n=140) and controls (n=110) indicated highly significant effects by analytical means on serum cholinesterase level ($p<0.001$) through ANOVA. Duncan Multiple Range Test ranking for monocrotophos and carbaryl were similar though different than endosulfan and cypermethrin, which conceptualized the effects on cholinesterase level. The GC-MS detection of residue concentrations in blood serum samples of spray-workers; calculated for endosulfan, monocrotophos, carbaryl and cypermethrin were 0.009, 0.005, 0.05 and 0.08 mg/kg body weight respectively. It was further seen in light of reported NOAEL that indicated the effect and extent of exposure among the study population.

Keywords: *Pesticides, Residues, Acetylcholinesterase*

Introduction

Pesticides widespread in agriculture sector are most economical approach to control the insects and pests, though are major contaminants of our environment and highly toxic to non-target organisms. The spray-workers during sprays on crops are directly exposed to pesticides while mixing, handling, spray, and through contaminated soil, air, drinking water, eating food and smoking at work places. Ultimately these are absorbed by inhalation, ingestion, and dermal contact [1]. The residue concentrations of these compounds in the exposed spray-workers can lead to a variety of metabolic and systemic dysfunctions, and in some cases outright disease states. Therefore, the tremendous usage of pesticides has promoted toxicological studies in spraying community. Common mode of action of the major pesticide products is to disrupt neurological function [2-3]. In addition to being neurotoxic, these compounds are profoundly injurious to the immune and endocrine systems as well [4-6]. Such ill health effects are not limited only to those systems, but can cause a variety of dermatological, gastrointestinal, genitourinary, respiratory, musculoskeletal, and cardiological problems [7-8].

Globally various products of different pesticide groups have been investigated by environmental toxicologists for their toxic end points; as reports indicated the accumulation of DDT, BHC and endosulfan which has been implicated in the pathogenesis of cardiovascular disorders, hypertension and other health related problems [9]. The residue concentrations of some organochlorine and organophosphorus pesticides were also detected in blood samples of school children [10] which prompt the adult studies in the directly exposed spray workers. Monocrotophos, a product of organophosphates group was massively used in agriculture sector and reported for environmental persistence [11] with half-life around seven days in soil exposed to natural sunlight [12-14]. Being cholinesterase inhibitor it was recorded as a potent neurotoxic agent [15-17].

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Similar in action to organophosphates, the carbamate pesticides also depress levels of acetyl cholinesterase (AChE) which can lead to neurological impairment. Analytically, AChE depression of 15% compared with baseline indicates overexposure [18], 40–50% depression is associated with mild neurotoxic effects and serious effects can occur at 80% depression [19]. Therefore inhibition of acetylcholinesterase enzyme (AChE) is broadly used for rapid detection to predict early warning of pesticide toxicity even in cases of organochlorines [20]. Hence, this important suggestion is accepted by toxicologists' to estimate and compare the level of acetyl cholinesterase among spray-workers and controls.

Many products of organochlorines and pyrethroids are hydrophobic molecules, which bind extensively to biological membranes, especially to the phospholipid bilayers [21], which indicate the bioavailability in body tissues. The evaluation of pyrethroid residues in the blood and body fluids gives an indication about the extent of exposure [22] and can be one reason for toxic symptoms in the exposed population. Thus few authors carried out studies on cypermethrin of pyrethroid group and found to affect the peripheral nervous system and an attack on the central nervous system [23-25].

In agricultural environment the spray-workers and their families can absorb a measurable quantity of pesticides; therefore most research concerns have focused on the acute or life threatening effects. In this regard little work was reported in Sindh and other provinces of Pakistan as reviewed by M.I. Tariq et al., 2007 [26] which recommended the need for further studies to monitor the exposure of highly hazardous pesticides by means of pesticide residues detection and their effects on cholinesterase enzyme among the spray-workers. This study was designed to evaluate the extent and effect of exposure of above mentioned pesticides by cholinesterase levels and residue concentrations in the blood samples of spray-workers. However, lack of national research and toxicological data on spray workers has hindered efforts to improve the agriculture environment for reducing probable risk exposures. Hence, the objective of this study was to explore the blood contamination and aimed to investigate the toxicity reason by means of residue analysis and acetyl cholinesterase activity in the exposed spray-workers.

Methodology

Study Areas /Population

This study was carried out on the agriculture farmers those were professionally spray-workers inhabitant to 14 districts; Badin, Dadu, Ghotki, Hyderabad, Jacobabad, Khairpur, Larkana, Mirpurkhas, Nawabshah, Nosheroferoz, Sanghar, Shikarpur, Sukkur and Thatta in Sindh province of Pakistan. Areas were selected according to the repeated sprays of pesticides; carbaryl, cypermethrin, endosulfan and monopcrotophos on crops of cotton, tomato, chili, okra vegetable and mango archers', during the year 2005 harvest season. Blood samples were taken from 250 farmers including 140 workers involved in sprays i.e. subjects (average age 45.6 years) and 110 controls (average age 44.8 years) who belonged to farmers' community but never spray pesticides on crops or any where else.

Experimental

Acetylcholinesterase: Serum samples of spray-workers (subjects) and control farmers were obtained after clotting and centrifugation at 2500 rpm. Samples of both the groups were initially analyzed for acetylcholinesterase level by manual method of Biggs et al., [27]. Data obtained were statistically analyzed through ANOVA and raking by Duncan Multiple Range Test DMRT, with help of SPSS version 11.

Pesticide Residue Analysis: It was difficult to analyze multiple pesticides and their metabolites in the blood samples of study population, therefore this work was limited to the areas and spray workers involved in pesticides. Residues analyses were carried out to determine the concentrations of above mentioned pesticides in the blood samples of the exposed subjects and similar exercise was carried out on the blood samples of the control group as well.

Chemicals and Reagents: Standards of the four pesticides i.e. endosulfan, monocrotophos, carbaryl and cypermethrin were obtained from Aldrich Sigma (Germany), always with purity higher than 99%. Stock standard solutions, 200 μgmL^{-1} were precisely weighed and dissolved in *n*-hexane (working solution 10 μgmL^{-1}). Working standard solutions were prepared by appropriate dilutions and stored in a refrigerator (4°C). Pesticide quality solvents *n*-hexane, methanol, diethyl ether and sulfuric acid were supplied by Merck (Germany).

Pesticide Extraction from Blood serum: Aliquots of 2.0 ml of serum samples of each the subjects and control separately were spiked by adding appropriate volumes of working standard solutions and equilibrated for 3.0 hours at room temperature in a test tube. Volumes of 1.0 ml of methanol were sequentially added to 2.0 ml of the samples, by mixing in a rotary mixer for 1.0 min, then 2.5 ml of n-hexane: diethyl ether (1:1 v/v) was added, agitating on a rotary mixer for 1.0 min and centrifuging at 2500 rpm for 5.0 minutes. The organic

phase was collected, and the aqueous phase was extracted twice more with 2.5 ml of n-hexane: diethyl ether (1:1 v/v). The combined organic phases were evaporated and concentrated to 1.0ml in a graduated test-tube under a gentle stream of nitrogen.

Clean-up Methods: A florisil (magnesium silicate) column of 20cm x 12mm topped with anhydrous sodium sulfate Na_2SO_4 was prepared and eluated with n-hexane. The extracts of each sample were passed twice through it. Eluate containing pesticides was evaporated and dried completely under a gentle stream of nitrogen. The samples were dissolved in 1.0ml of *n*-hexane and then injected into the GC/MS system. The reported procedure of extraction and clean up [28-29] was adapted as shown in Figure 1.

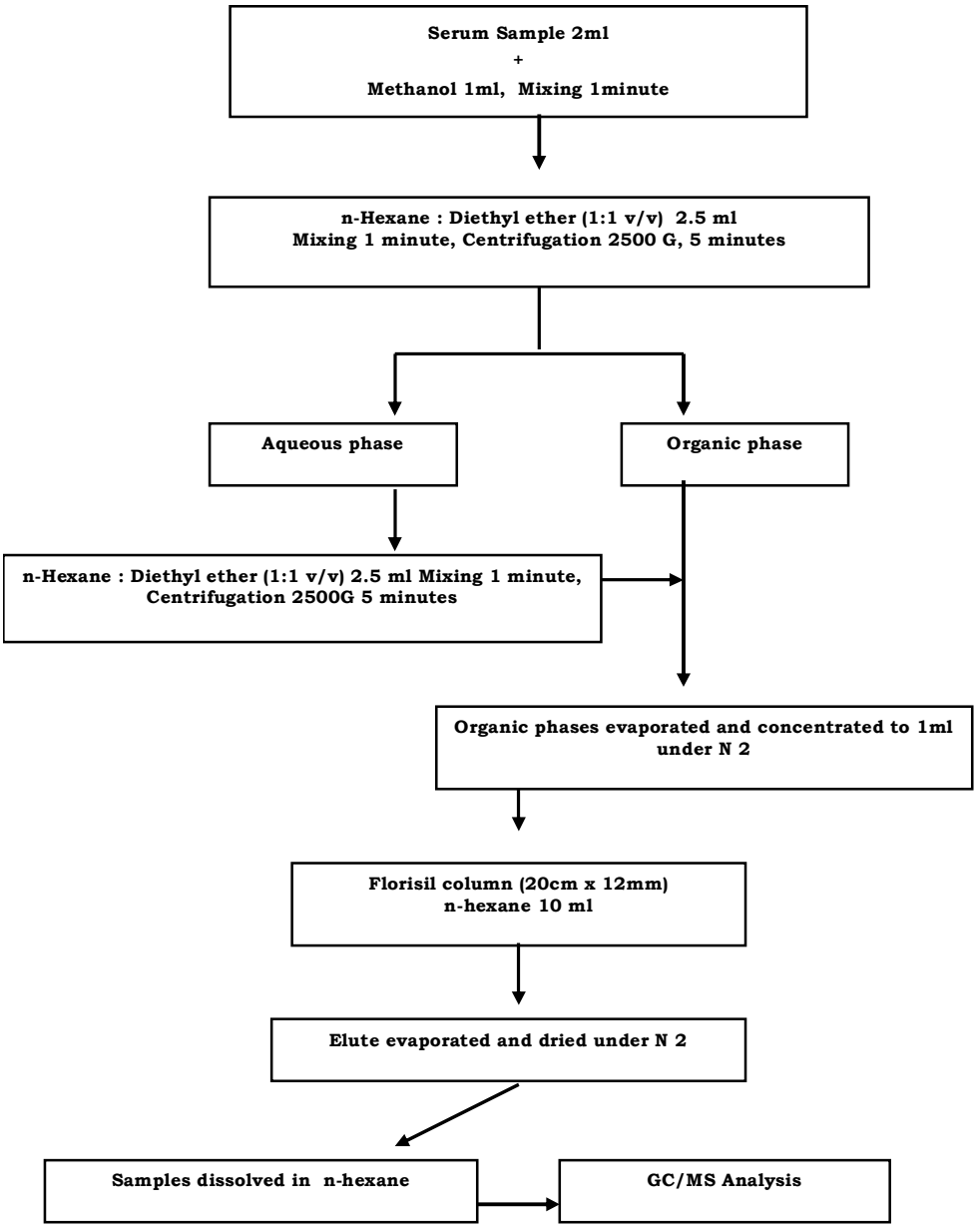

Figure 1. **Extraction and Clean-up scheme for Serum Samples**

GC-MS: Analysis of prepared samples was carried out on Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA). A capillary column HP- 5MS (5% phenyl methylsiloxane) with dimension of 30 m x 0.25 mm i.d x 0.25 µm film thickness (Agilent Technologies, Palo Alto, CA, USA) was used. Volumes of 2.0 µl were injected by the autosampler at a flow rate of 1.0 µl/s. The GC employed a septum equipped temperature-programmable injector that was initially held at 90°C for 0.1 min before being ramped to 280°C at a rate of 200°C/min. The GC oven was initially held at 80°C for 2.5 min and then ramped at 50°C/min to 140°C, and finally from 140°C, the temperature was increased at 5°C/min to 260°C and held for 3 min. The GC-MS conditions were; solvent delay 11 min; 70 eV of electron impact energy; scan rate 0.6 scans/s; scanned-mass range 85–450 *m*/*z*. The transfer line was kept at 260°C and the ion trap manifold at 200°C. The automatic gain control was switched on with a target fixed at 5000 counts. Helium (99.99%) at a flow rate of 1.0 ml/min was used as the carrier and collision gas.

Results

Serum Cholinesterase: ANOVA for serum cholinesterase activity result the highly significant differences ($p<0.001$) in the detected values of both the groups as shown in Table 1. F-statistics value was 283.31 whereas according to ranking groups; values having same letters are not significantly different at 0.01 levels of confidence intervals which, was determined by Duncan Multiple Range Test (DMRT).

Pesticide Residue Analysis: The residues concentration of the selected pesticides in serum samples were detected in percentage of the spray-workers (n=140) at limits of detection (LOD) specified for each pesticide (Table 2), while residues in control samples were untraceable. The pesticides under study were identified by comparing their retention times on the recording integrator, with that of the pesticide standards. Quantitative determinations were done by comparing the peak areas of unknown samples with those of standards through calibration curves.

Discussion

Toxicological assessment of pesticides is carried out by several methods including acetyl cholinesterase inhibition, residues detection and No Observable Adverse Effect Level (NOAEL) in the exposed population. In present work initially serum cholinesterase was determined and ANOVA results indicated highly significant ($p< 0.001$) effects in the spray-workers (grouped according to pesticide products in their use) as compared to controls (Table 1), whereas, DMRT ranking of means indicated the toxic effect level for the pesticides under study. Mean ± S.D for both pesticides carbaryl and monocrotophos was significantly different than controls whereas, both were same at DMRT ranking. This can be related with the presence of detected residue quantities of carbaryl (0.05) and monocrotophos (0.005) mg/kg per kg body weight (Table 2), though the residue concentration of these pesticides were untraceable in the blood samples of the control group. Baron 1991, recorded 0.06 mg/kg NOAEL for carbaryl [30], the present work revealed nearest concentration of carbaryl in 13.57% of the spray-workers and seen affecting the cholinesterase activity at highly significant level ($p< 0.001$). Therefore this indicates harmful contamination of the blood samples of the farmers. While in a human volunteer study, monocrotophos oral intake of 0.0057 mg/kg body weight per day for 28 days produced inhibition of serum cholinesterase [31], which seemed consistent to this work.

As endosulfan, a product of organochlorine pesticides is lipophilic in nature, its presence in human tissues and fluids [32-33] is reported to cause neurotoixc effects [34]. Its bioavailability may produce toxic effects, as the highest percentage of spray-workers (44.28%) having endosulfan residues averagely 0.009 mg/kg body weight (Table 2), so this contamination of blood can be predicted for environmental effects on their health. Needham et. al., [35] in his NOAEL animal study reported mean concentrations of endosulfan residues in plasma (0.081) and whole blood (0.856) mg per kg body weight following repeated daily oral administration of 1.0 mg/kg body weight for up to 28 days. It is observed that endosulfan residues (Table 2) and inhibited serum cholinesterase level significantly, Mean ± S.D and statistical mean ranking as given in Table 1, designate the pesticide-environmental impact, though endosulfan does not directly affect its cholinesterase enzyme activity.

Pyrethroid products are said to be safer and effective as compared to other groups of pesticides; so preferred for crop protection, among these cypermethrin is reported for its maximum use. When its effect on cholinesterase level analyzed in the selected study population, it was noticed as highly significant (Table1). This effect may be due to cypermethrin residues (0.08 mg/kg body weight) averagely detected among 10% of the subjects, which is comparatively less proportion of blood contamination. U S. EPA [36] suggested NOAEL

of 1.0 mg/kg/day, a dog based study for cypermethrin, though previously cypermethrin was reported for its toxic effects on nervous system and immunosuppressive [37]. Whereas, DMRT ranking of means for cholinesterase was calculated statically similar to endosulfan, which point out the consistent toxic effects of cypermethrin in spray workers inhabitants in Sindh province of Pakistan.

Table 1. **Product wise Effect of pesticide on Serum Cholinesterase u/ml level in the Spray-workers compared to controls**

Groups	Mean ± S. D	DMRT Ranking
Controls	3.32 ± 0.66	c
Carbaryl	1.53 ± 0.28	a
Endosulfan	2.40 ± 0.33	b
Monocrotophos	1.55 ± 0.26	a
Cypermethrin	2.52 ± 0.27	b

$p<0.001$ at 0.01 level of confidence intervals

Table 2. **Detected Amount of Pesticides in Number and Percentage of Serum Samples of the Spray-workers**

Pesticide	Number and % age of Spray-workers	Average amount (mg/kg body weight)
Endosulfan	(62) 44.28	0.009
Monocrotophos	(45) 32.14	0.005
Carbaryl	(19) 13.57	0.05
Cypermethrin	(14) 10	0.08

Conclusion

Generally the residues in blood are likely to appear at very low concentrations because as soon as it enters into the body most of the chemical may get metabolized and the metabolites may accumulate to induce toxic effects. However, the ultra low quantities of the contaminants present in the body indicate toxicological impact on exposed population. Present results which revealed blood contamination and cholinesterase inhibition in the spray-workers in Sindh, Pakistan, noticed the effect and extent of exposure in the spray-working community. This implies the need for further toxicological studies particularly with reference to agrochemicals and their metabolites in the farmers' community at agriculture environment.

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