

Impact of pesticide residues (chlorpyrifos, cypermethrin, profenophos) on health and liver marker values alkaline phosphate (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) among workers of pesticide formulation & packing plants in Pakistan

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ABSTRACT

Objective: To evaluate the impact of pesticide residues (chlorpyrifos, cypermethrin, profenophos) on health and liver marker values alkaline phosphate (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT) among workers of pesticide formulation & packaging plants in Pakistan

Materials and Methods: A hundred workers of the age group between 20-50 years working, for at least a year, at various pesticide formulation & packaging units were included in the present study.

One hundred workers, apparently healthy, with no history of pesticide exposure - after matching age, sex and social status - were taken as control. Biochemical tests ALP, AST and ALT, were carried out using chemistry autoanalyzer, Dimension RXL (Dade Behring). Pesticide residues (chlorpyrifos, cypermethrin, profenophos) were carried out employing gas chromatograph electron capture detector GCECD 2010, Shimadzu Japan.

Results: In current study 100 workers of pesticide industry were taken and ALP, AST and ALT of 80 workers out of 100 were found to be within the normal limits. However, 20 workers had unusually raised levels of ALP ALT and AST. These twenty workers showed pesticide residues of chlorpyrifos, cypermethrin, profenophos in their blood.

Conclusions: the pesticide residual level of all the workers were very low (below the no observable adverse affect level NOAEL) and were not too significant to create health problems.

Key Words: Pesticide industrial workers; Residue; Serum; Enzymes

INTRODUCTION

It is a well established fact that pesticides are frequently used substances worldwide, despite the fact that the use of some of them is forbidden due to the recognized adverse effect they have on the health of those that consume the contaminated products. In addition, these pesticides display markedly high toxicological activities among the people who apply the pesticides. It is worth mentioning that undesirable off target effects of pesticides impinge on various tissues. These pesticides have considerable tissue toxicity. In Pakistan there is a tremendous use of pesticides without any proper monitoring. In accordance with this assumption, only a single research group evaluated side effects of improper use of pesticides on farmers in Gadap (rural area) Karachi. Results indicated that ALP, ALT and AST levels were considerably raised in the samples of the subjects as compared to the controls Azmi *et al.* (2005)¹. It is becoming increasingly apparent that there is a tight correlation between enzymes and pesticides as demonstrated in various researches Thakur M. *et al.* (2019)². In addition, remarkably high level of ALT and AST in the blood of occupational workers chronically exposed to organophosphate pesticides was observed Kamal *et al.* (1990)³; Mostafalou S *et al.* (2017)⁴; Karami-Mohajeri S, *et al.* (2017)⁵. It is intriguing to note that wide ranging pesticides severely disrupt the enzymatic levels in persons exposed to the pesticides Goel *et al.* (2000)⁶; Altuntas *et al.* (2002)⁷; Dahamana *et al.* (2004)⁸; Jayaraj R. *et al.*, (2016)⁹; Volodymyr I. Lushchak. *et al.* (2018)¹⁰.

Recently animal studies have indicated that synthetic pyrethroids-organophosphate insecticides induce oxidative stress and hepatotoxicity Lushchak VI *et al.* (2014)¹¹; Lushchak VI *et al.* (2016)¹². More detailed investigational study currently conducted highlighted the notion that Mexican farm workers exposed to pesticides, had substantial hepatotoxicity Payán-Rentería *et al.* (2012)¹³; Ji G *et al.* (2016)¹⁴. Increasing sophisticated information underlined the fact that there was substantial hepatotoxicity in pregnant women exposed to pesticides Cecchi *et al.* (2012)¹⁵; Addissie, Y.A *et al.* (2020)¹⁶

MATERIALS AND METHODS

100 workers (20-50 years) working in pesticide formulation unit for more than one year were included in this cross sectional study.

100 age and gender matched healthy persons with no history of exposure to pesticides were taken as controls.

Venous blood samples (6-8 ml) were drawn from all subjects to obtain serum from them. Levels of Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST) were estimated employing chemistry autoanalyzer, Dimension RXL (Dade Behring). Screening for Hepatitis B and C virus was done by immune chromatographic technique. The samples which showed any abnormal level of ALP, ALT, and AST were analyzed for pesticide residual levels (chlorpyrifos, cypermethrin, profenophos) by gas chromatography with electron capture detector.

Statistical Analysis: Results of ALP, ALT, and AST, were expressed as mean \pm SD. Data were analyzed according to the unpaired student's t test. ANOVA technique was applied for the comparison of three groups i.e., controls group and workers of pesticides industry having normal and abnormal ALP, ALT and AST Level. A p value of less than 0.05 was considered statistically significant.

RESULTS

In the present work blood samples of 200 workers were taken; 100 workers from other than pesticides industry called as control group and 100 pesticides industrial workers. ALP, ALT AST tests were performed on Auto Analyzer Dimension RXL, Dade Behring. Hepatitis B and C virus were screened by immunochromatographic technique to ascertain that all samples included in the present study were negative for both of these viruses.

The data of 100 pesticides industry workers was taken and ALP, ALT and AST titre of 80 out of 100 workers was within the normal range whereas 20 workers had abnormally high level of ALP, ALT and AST.

Pesticide Residue Analysis: Regarding pesticide residue analysis, data of known concentration of standards of three commonly used pesticides i.e., Cypermethrin, Chlorpyrifos and Profenophos are shown in (Table 1, Figure 1), (Table 3, Figure 3) and (Table 5, Figure 5) respectively. Data of samples of 20 pesticide industrial workers having abnormally raised levels of ALP, ALT & AST for the three above mentioned commonly used pesticides are shown in (Table 2, Figure 2), (Table 4, Figure 4) and (Table 6, Figure 6), respectively.

From the above data it is noted that 18 blood samples had positive residues of cypermethrin, ranged between 0.002 µg/ml to 0.093 µg/ml. Eighteen samples had positive residues of chlorpyrifos and ranged between 0.01 µg/ml to 0.099 µg/ml whereas, only 03 samples showed pesticide residue of profenophos ranging between 0.018 to 0.030 µg/ml.

Table 1: Concentration of Cypermethrin and peak area of chromatogram obtained from GCECD

Concentration of Cypermethrin (µg/ml)	0.1	0.5	1	2	5
Area (AU)	15340	19034	27644	34842	56087

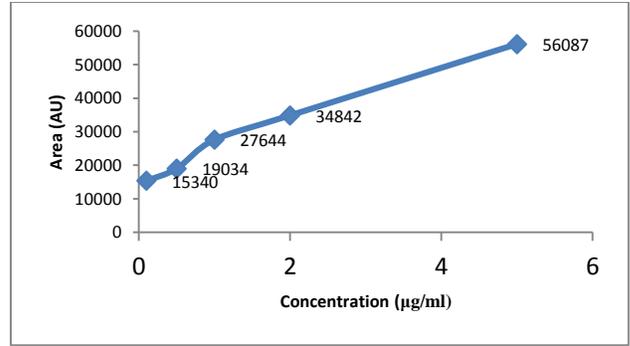


Figure 1: Standard line graph of Cypermethrin (of known Concentration 0.1, 0.5, 1, 2,5) which shows the peak area obtained through GCECD

Table 2: Concentration of residue of Cypermethrin and peak area of samples of Pesticides workers ("W" is meant for worker sample No)

Sr. No	1	2	3	4	5	6	7	8	9
Sample No.	W41	W75	W18	W92	W26	W58	W87	W12	W89
Concentration of Cypermethrin (µg/ml)	0.093	0.092	0.081	0.054	0.051	0.049	0.047	0.043	0.037
Area (AU)	14249	14188	12394	8337	7841	7590	7182	6632	5677

Sr. No	10	11	12	13	14	15	16	17	18
Sample No.	W14	W82	W72	W56	W64	W28	W44	W27	W71
Concentration of Cypermethrin (µg/ml)	0.028	0.026	0.023	0.012	0.012	0.01	0.009	0.009	0.002
Area (AU)	4233	4009	3508	1896	1786	1495	1412	1410	302

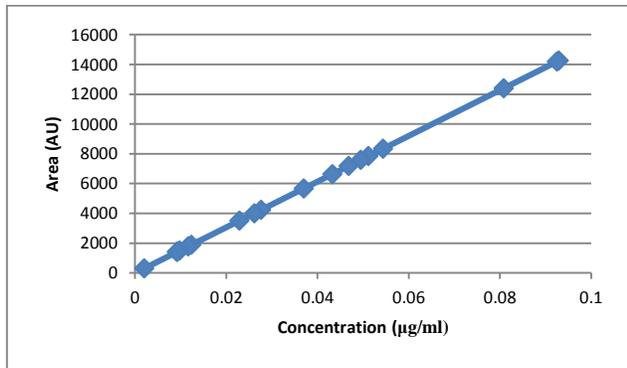


Figure 2: Line graph of Concentration of residue of Cypermethrin and peak area of samples of Pesticides workers

Table 3: Concentration of Chlorpyrifos and peak area of chromatogram obtained from GCECD

Concentration of Chlorpyrifos (µg/ml)	0.1	0.5	1	2	5
Area(AU)	37025	51824	71727	82868	158072

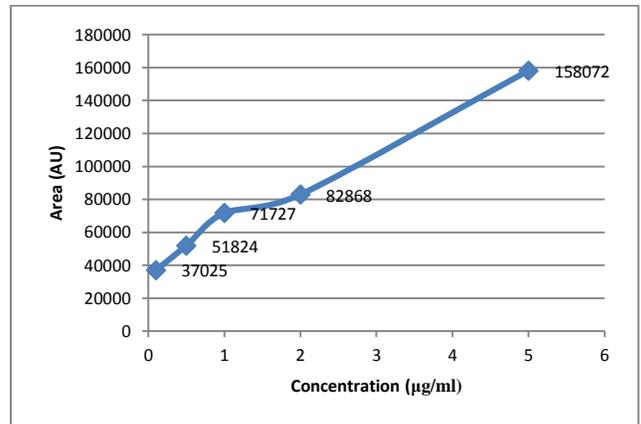


Figure 3: Line graph of Chlorpyrifos (of known Concentration 0.1, 0.5, 1, 2, 5) which shows the peak area obtained from GCECD

Table 4: Concentration of residue of Chlorpyrifos and peak area of samples of Pesticides Workers ("W" is Meant for worker sample No)

Sr. No	1	2	3	4	5	6	7	8	9
Sample No	W75	W71	W56	W72	W82	W87	W4	W44	W18
Concentration of Chlorpyrifos (µg/ml)	0.099	0.050	0.050	0.040	0.040	0.040	0.030	0.030	0.030
Area (AU)	36688	18586	17498	15033	14878	14694	12210	11994	11413

Sr. No	10	11	12	13	14	15	16	17	18
Sample No	W64	W89	W28	W10	W12	W27	W14	W58	W92
Concentration of Chlorpyrifos (µg/ml)	0.030	0.020	0.020	0.020	0.010	0.010	0.010	0.010	0.010
Area (AU)	9557	7935	7407	6940	5207	3442	2315	2208	1977

Table 5: Concentration of Profenophos and peak area of chromatogram obtained from GCECD

Concentration of Profenophos (µg/ml)	0.5	01	2	5
Area (AU)	32188	80409	106454	139547

Table 6: Concentration of residue of Profenophos and peak area of samples of Pesticides workers ("W" is Meant for worker sample No)

Sr. no	1	2	3
Sample No	W87	W58	W27
Concentration of Profenophos (µg/ml)	.0307	.0299	.0181
Area (AU)	1980	1928	1169

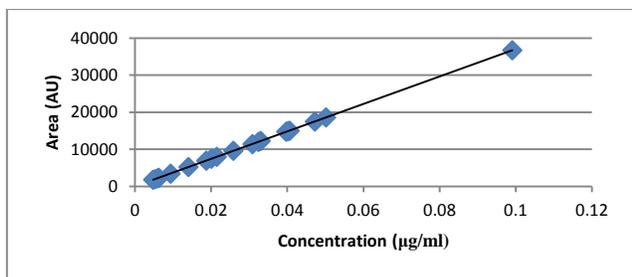


Figure 4: Line graph of Concentration of residue of Chlorpyrifos and peak area of samples of Pesticides workers

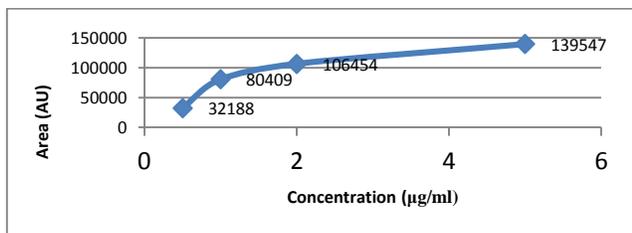


Figure 5: Line graph of Profenophos (of known Concentration 0.5, 1, 2, 5) which shows the peak area obtained from GCECD

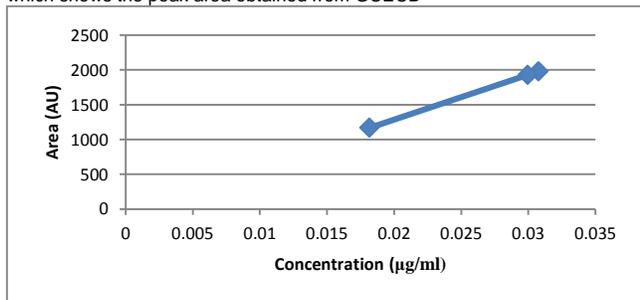


Figure 6: Line graph of Concentration of residue of Profenophos and peak area of samples of Pesticides workers

DISCUSSION

The present study was carried out to evaluate the impact of exposure to pesticides on ALP, ALT, and AST workers of pesticides industry. For this purpose blood samples of 100 pesticide industry workers having exposure to pesticides during formulation, filling and packing were selected after observing the inclusion and exclusion criteria. Similarly, 100 subjects from other than pesticide industry were also selected as control. It was observed that the entire control group had ALP, ALT, and AST not only within normal limits but also, on average, on the lower side of normal levels. Out of 100 pesticide industry workers, blood samples of 80 workers showed normal, albeit, slightly higher level of ALP, ALT and AST, as compared to control subjects. Other 20 pesticide industry workers had elevated levels of these enzymes which also show pesticide residues of chlorpyrifos, cypermethrin, profenophos.

High ALP level in serum has also been reported by previous researchers such as, ALTuntas *et al.* (2002)⁷ and Kaur and Dhanju (2004)¹⁷ Yaqub, S *et al.* (2015)¹⁸ Reyes, J *et al.* (2018)¹⁹ in the persons involved in spraying.

High level of ALT and AST has also been noted in the serum of the persons exposed to pesticides by different research scientists; Kossmann and Magner (1992)²⁰ Goel *et al.* (2000)⁶, Sahin *et al.* (2002)²¹ Kaur and Dhanju (2004)¹⁷, El-Nahhal Y *et al.* (2016)²², Jamal F., (2016)²³, Faustin Pascal Tsagué Manfo *et al.* (2020)²⁴ Ahmed A *et al.* (2021)²⁵.

In Pakistan, studies have been conducted by SNH Naqvi *et al.* (2007)²⁶ at Karachi and they reported that there was significant increase in the liver enzyme levels in the persons exposed to

pesticides (Cypermethrin, Chlorpyrifos, DDT) and they complained about liver and kidney dysfunction and respiratory tract infection. Also target persons of above research work were the males/females involved in spraying of pesticides in the field. However, the complaints reported by spray men / women were more as compared to the workers of current study probably due to high exposure to pesticides of the farmers, as pesticides are more volatile with addition of water and spray men / women were not following adequate safety measures.

Pesticides residue analysis of three commonly used pesticides (i.e., Cypermethrin, Chlorpyrifos and Profenophos) in serum of 20 pesticide industrial workers having abnormal levels of ALP, ALT and AST was carried out in current study. It was noted that in the blood samples of pesticide industry workers, the pesticide residues of cypermethrin ranged between 0.002 µg/ml and 0.93 µg/ml, chlorpyrifos ranged between 0.018 to 0.03 µg/ml and profenophos ranged between 0.018 to 0.03 µg/ml. Average body weight of these workers was more than 70 kg. The no observable adverse affect level (NOAEL) established by codex alimentarius commission, joint FAO /WHO²⁷ in 2008 for cypermethrin 0.2 mg/kg body weight, for chlorpyrifos 0.1mg/kg body weight and for profenophos is 0.3mg/kg body weight. The pesticide residual level of all the workers were very low (below the NOAEL) and were not too significant to create health problems. Presently, no worker was suffering from any severe acute or chronic health problem.

CONCLUSIONS AND RECOMMENDATIONS

Following conclusions were drawn from the present study.

- Pesticide industry workers were affected more as compared to control subjects due to continuous exposure of pesticides.
- Normal levels (but in high range) of ALP, ALT, AST were seen in serum of pesticide industrial workers.

On the basis of above conclusions following recommendations are made:

- The pesticide industrial workers should be educated about the hazardous or toxic effects of pesticides.
- General public sector regulatory authorities and the local pesticides industry should be sensitized to ensure periodical medical examination of the workers after appropriate time interval by a qualified Doctor.

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