Original Article

Effects of Exposure to Pesticides on Blood Serum Components and Butyrylcholinesterase (BChE) in Pesticide Vendors of Punjab Province, Pakistan

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Abstract

Objectives: The present study is to see the effects of human exposure to selected pesticides on their liver and kidney function as well as variations in butyrylcholinesterase (BChE) were studied.

Patients and Methods: The study group consisted of 88 participants selected through purposive sampling technique and participated voluntarily. Sixty one pesticides vendors (male; mean age: 33.82 ± 11.5 years) and a control group consisting of 27 healthy male (mean age: 31.04 ± 11.96 years).

Results: Mean levels of total proteins (TP, g/dl), albumin (g/dl) and BChE (u/l) were significantly lower in pesticide exposed individuals than those of control group [6.96 ± 1.45 vs. 8.75 ± 1.05 (total protein); 3.47 ± 0.76 vs. 4.37 ± 0.23 (albumin); 4213.20 ± 1128.96 vs. 5955.90 ± 623.16 (BChE, u/l), $P < 0.001$]. Among the pesticide vendors, urea ($P < 0.01$) and ALT (alanine amino transferase) ($P < 0.05$) concentrations were increased and BChE ($P = 0.001$) level decreased significantly in smokers than in non-smokers. Pesticide vendors having longer exposure time to pesticides had significantly higher levels of urea ($P < 0.05$) and ALT ($P < 0.01$) but had lower levels oftotal proteins ($P < 0.01$) and BChE ($P = 0.001$). Vendors who were not using proper protective measures showed significant increase in ALT ($P < 0.01$) level and decrease in total proteins ($P < 0.05$) and BChE ($P = 0.001$).

Conclusion: The results of this study suggest that human exposure to pesticides has adverse effects on total proteins, albumin, urea, ALT, and BChE; these biomarkers were useful in studying adverse effects of pesticides in humans.

Key words: Pesticides, environmental toxicology, public health, buytrylcholinesterase activity, LFT, RFT.

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Introduction

In agriculture based world economies, such as Pakistan, where this sector contributes 21% of the total GDP, use of toxic pesticides is on the rise. For exa-

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mple, in the past 20 years, use of pesticides in Pakistan has increased by 11.9% and number of sprays per crop amounts to more than 10%.[1] This situation is alarming for the human population exposed to these chemicals during agricultural practices; the use of pesticides has increased from 665 tons in the 1980 to 45,680 tons in 1999.[2] Currently, a variety of pesticides, such as insecticides (108 types), fungicides (30 types), weedecides (39 types), agricides (5 types), and rodenticides (6 types) are being used in Pakistan.[3] Although the use of pesticides in the developing countries is only 20% compared to 80% in the developed countries, the death rate due to pesticide usage is 13% more in developing countries and the reason behind this differential may be the indiscriminate and/or improper use of pesticides in the developing countries.[4]

Human exposure to pesticides not only affects the farm workers but also affects the vendors. It was predicted that pesticides might affect the esterase enzymes[5]. However, some recent studies suggest a strong correlation between pesticide exposure and rise in the values of various liver and kidney related enzymes and other factors, such as alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphate (ALP), total protein(TP), urea, creatinine, blilirubin total proteins, etc.[6] Different ill-effects of pesticides have been reported, such as changes in concentrations of glutamic-oxaloacetic acid transaminase (GOT), glutamic-pyruvic acid transaminase (GPT), and ALP.[7] A significant decrease in buytrylcholinesterase (BChE) has been previously observed in humans exposed to pesticides compared to those not exposed.[8] The pesticides tend to cause modifications in biochemical biomarkers, such as inhibition in BChE, etc.[9] Variations in AST, ALT, urea levels due to pesticide exposure[10] and variations in BChE have already been reported.[11] The marked increase in the serum urea levels and creatinine suggests impairment of kidney functions.[12] These parameters may be used as biomarkers to evaluate the adverse effects of pesticides in retailers/vendors as well as farmers. Unfortunately, scarce data are presently available concerning the study of biochemical markers in occupationally exposed individuals to pesticides, such as retailers/vendors. Additionally, there are no guidelines available to the vendors and farmers concerning the handling of these toxic pesticides.[13] This necessitates initiation of such studies, particularly in the developing countries, such as Pakistan. Therefore, current study was undertaken to evaluate hazards to health due to exposure to pesticides by monitoring the changes in biochemical parameters involved in the liver, kidney functions as well as BChE levels in retailers/vendors. This study is the first report of its kind in Pakistan.

Patients and Methods

The universe of the present study included vegetable-grown (cabbage, brinjal, pea, potatoes, etc.) regions of central Punjab, including Lahore, Kasur, Sheikhupura, and Nankana Sahib, Pakistan (Figure 1). These regions were selected because of the high sales of pesticides, so leading to the rational that vendors of pesticides have more exposure to pesticides as compared to any other region, with low sales of pesticides.

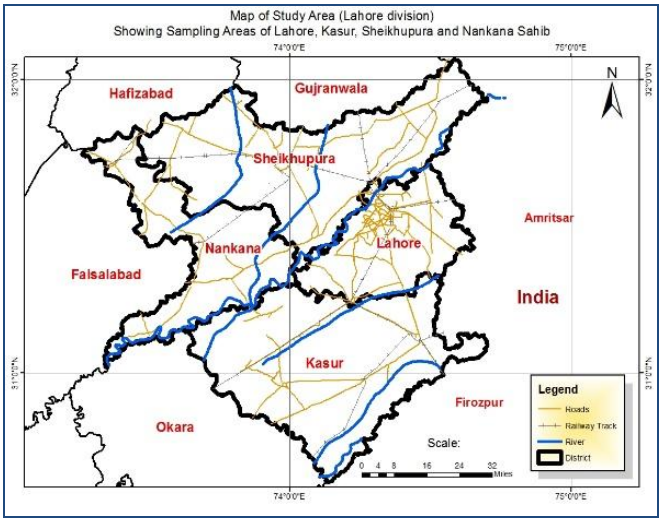

Fig. 1: *Map of Study.*

Only male vendors were included from the geographical areas of central Punjab including Lahore, Kasur, Sheikhupura, and Nankana Sahib. The age range of the subjects (vendors) was 18 to 60 years. In experimental group only those individual were included who worked in pesticide sales points, pesticide sales agencies and in pesticides shops. In control group only those individual were included who were not involved in any kind on pesticides related tasks and or handling. The participation of the subjects in both study and control group was voluntary and they have opportunity to withdrew from the research at any time during the research. This is to maintain the ethical standards of the research.

Individuals with ages less than 18 years and above 60 were excluded. It was ensured that all subjects with

apparent disease were excluded from research.

The non-probability purposive sampling technique was followed to draw 88 subjects. This including sixty one subjects (vendors, n = 61) from the pesticide retail shops selling chlorpyrifos (an organophosphate insecticide) and/or imidacloprid (a neonicotinoid insecticide). Moreover twenty seven subjects for control group (n = 27) were selected from the same geographical area using purposive sampling technique and following inclusion criteria. The study span stretched from October 2013 to September 2014. Both experimental and control groups belonged to similar socio-economic status and were generally in good health with no apparent serious ailment(s).

A total of eighty eight (N = 88) subjects were participated in this study. The participants were asked to fill in a structured questionnaire pertaining to information about different parameters, such as quality of life (physical), work experience as a pesticide vendor, health-related symptoms, and information concerning any personal protective/safety measures used during their routine work of working/ handling pesticides, etc.

Sample of blood was drawn from each participant in the study through vein punctures; the blood was collected into Gel and clot tubes properly labelled with an identification number. Sampling date, time, area and demographics of the sampled individual were subsequently noted and recorded against each of the identification number (sampled individual).

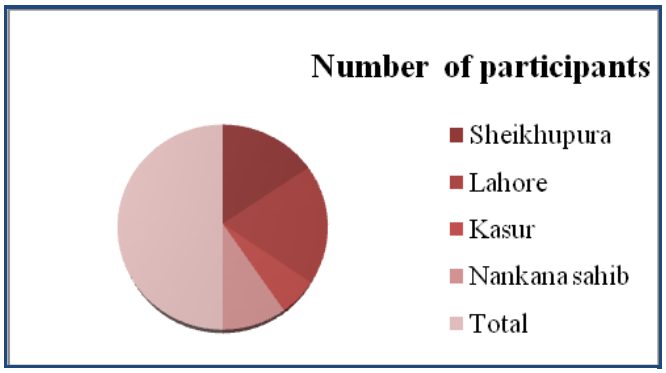

Fig. 2: *Pie chart showing proportion of study participants from different districts of Punjab, Pakistan.*

Blood serum was collected from the samples tubes that did not contain any anticoagulant. The blood samples were allowed to stand for a few hours. The samples were then centrifuged at 4000 rpm for 5 – 7 minutes and serum was isolated. The serum was pipette out and stored at -20°C for subsequent analyses.

Table 1: *Profile of the individuals under study.*

Parameters	Vendors
	n **(%)**
Age (Years)	
18 – 20	20 (22.7)
21 – 30	22 (25.0)
31 – 40	26 (29.5)
41 – 50	12 (13.6)
51 – 60	8 (9.1)
Literacy Rate	
Illiterate	10 (11.4)
Primary	61 (69.3)
Elementary	0
Secondary	14 (15.9)
Higher secondary	2 (2.3)
Master Level	1 (1.1)
Gender	
Male	61 (100)
Female	0 (0)
Family Constitution	
Joint Family	7 (8.0)
Separated Family	81 (92.0)
Marital Status	
Unmarried	13 (21.3)
Married	48 (78.7)
Duration of Pesticide Exposure/ Experience	
15 years or more	5 (8.2)
10years	8 (13.1)
5 years	31 (50.8)
1 years or 5 years	17 (27.9)
Smoking Habits	
Smokers	42 (68.9)
Non smoker	19 (31.1)
Total *n* = 61	

Table 2: *Comparison of age, height and body mass of pesticides-exposed (Vendors) and unexposed (Control) individuals.*

Parameter	Vendors Mean ± SD	Control Mean ± SD
Age (yrs)	33.82 ± 11.5	31.04 ± 11.96
Height (F)	61.459 ± 9.26	57.85 ± 7.72
Body weight (kg)	5.6 ± 0.34	5.6 ± 0.28

Prior to initiating the study**,** a consent/willingness-signed document was obtained from each participant in the study indicating that he/she was participating in the study program voluntarily and had waived his/her right to proceed for any legal action against the researchers.

Blood serum was analysed to determine different biochemical parameters (Urea; alanine transaminase 'ALT', Alkaline Phosphate 'ALP', Serum Creatinine, Bilirubin Total, aspartate transaminase 'AST' Serum Albumin, and Total Protein 'TP' by following instructions of diagnostic kits of fully automatic chemistry analyser (AU 480; Beckman Coulter) at the Certified Pathological Laboratory of the Jinnah Hospital, Lahore, Pakistan.

BChE was determined by colorimetric method following the kit instructions of Randox (Cat No. CE190) for the semiautomatic analyser (Metro Lab 1600 DR) in a certified pathological laboratory.

Collected data were statistically analysed using SPSS (SPSS version 18). Specifically, frequencies, descriptive analysis, independent sample *t*-test, and analysis of variance (ANOVA) were performed. Significance confidence levels for all the statistics was 95% ($p \leq 0.05$).

Results

Among the pesticides-exposed vendors showed significantly decreased levels of various parameters as compared to the control subjects were obtained, such as mean value of total proteins was 6.96 ± 1.45 g/dl versus 8.75 ± 1.05 g/dl ($p = 0.001$) and that of serum albumin was 3.47 ± 0.76 g/dl versus 4.37 ± 0.23 g/dl ($p = 0.001$) (Table 3).

BChE (an already established marker for pesticide exposure) levels decreased significantly ($p = 0.001$) in vendors as compared to their respective controls (Table 3).

Among the pesticides vendors who had poor protective habits (who used protective measures either

Table 3: *Comparisons of biochemical parameters of pesticide exposed subjects (Vendors) with pesticide non-exposed subjects (Independent t-test).*

	Vendor Versus Control				
Biochemical Parameter's	**NW**	**M**	**SD**	**t**	**p**
Urea (MG/DL)	Vendor	28.81	8.16	.245	.807
	Control	28.38	6.12		
ALT (SGPT; U/L)	Vendor	28.88	21.90	1.425	.124
	Control	21.72	14.47		
ALK. Phosphate (U/L)	Vendor	87.08	26.97	-1.425	.158
	Control	96.56	32.61		
S/ Creatinine (MG/DL)	Vendor	0.78	0.25	-1.425	.158
	Control	0.80	0.41		
Bilirubin T (MG/ DL)	Vendor	0.51	0.40	-.623	.535
	Control	0.56	0.25		

AST (SGOT; U/ L)	Vendor	43.68	24.67	.701	.485
	Control	39.34	31.05		
ALB (G/DL)	Vendor	3.47	0.76	-5.973	**.001**
	Control	4.37	0.23		
TP (G/DL)	Vendor	6.96	1.45	-5.778	**.001**
	Control	8.75	1.05		
BChE (U/L)	Vendor	4213.20	1128.96	-7.52	**.001**
	Control	5955.90	623.16		
n =88: vendors; 61, control, 27; *df* +86					

Significance confidence, $p \leq 0.05$
NW= nature of work, ALT (SGPT) = alanine transaminase, AST (SGOT) = aspartate transaminase, ALB = Serum Albumin, Bilirubin T = Bilirubin Total, S/ Creatinine = Serum Creatinine, TP = Total Protein, BChE = Butyrylcholinesterase

never, occasional, or on frequent basis) during pesticide handling had significant increase in the levels of ALT in comparison to the workers who used the protective measures on regular basis [mean: 52.40 ± 37.69 u/l (never), 42.34 ± 35.91 u/l (occasional), 26.01 ± 15.51 u/l (frequent);and 21.84 ± 10.79 u/l (always), p = 0.007]; however, vendors who had never used protective measures had significant decline in BChE (p = 0.001) and total proteins[mean: 5.55 ± 1.57 g/dl (never); 6.11 ± 1.61 g/dl (occasional); 7.09 ± 1.29 g/dl (frequent); and 7.43 ± 1.32 (always); p = 0.007)] in comparison to those who used the protective measures (Table 4).

Table 4: *Comparison of changes in selected biochemical parameters in relation to protective measures use by randomly selected pesticide Vendors in Punjab, Pakistan (one way ANOVA).*

Biochemical Parameters	Protective Measures Use	M ± SD	*F*	p
Alanine Transaminase 'ALT' (u/l)	Never	52.40 ± 37.69	4.47	0.007
	Occasional	42.34 ± 35.91		
	Frequent	26.01 ± 15.51		
	Always	21.84 ± 10.79		
Total Protein (g/dl)	Never	5.55 ± 1.57	3.75	0.01
	Occasional	6.11 ± 1.61		
	Frequent	7.09 ± 1.29		
	Always	7.43 ± 1.32		
Butryl Cholinesterase 'BChE'(u/l)	Never	2148.00 ± 171.76	210.10	0.001
	Occasional	2496.50 ± 141.72		
	Frequent	4252.40 ± 80.34		
	Always	5308.40 ± 543.86		

n = 61, Never = 5, Occasional = 8, Frequent= 27, Always = 21, Significance confidence, $p \leq 0.05$

Table 5: *Study of biochemical parameters in Vendors in relation to exposure duration to pesticides (One way ANOVA).*

		Vendors		
Biochemical Parameters	**Exposure Duration**	**M ± SD**	**F**	**p**
Urea (MG/DL)	15 years or more****	37.4 ± 5.03	3.132	0.032
	10 years or more***	29.63 ± 8.95		
	5 years or more**	29.05 ± 9.03		
	1 years or < 5 years *	25.48 ± 4.46		
ALT (SGPT; U/ L)	15 years or more****	52.4 ± 37.69	4.761	0.005
	10 years or more***	42.34 ± 35.91		
	5 years or more**	26.55 ± 14.72		
	1 years or < 5 years *	19.86 ± 10.51		
Total Protein (G/DL)	15 years or more****	5.55 ± 1.57	3.822	0.015
	10 years or more***	6.11 ± 1.61		
	5 years or more**	7.1 ± 1.24		
	1 years or < 5 years*	7.41 ± 1.4		
BChE (U/L)	15 years or more****	2148.00 ± 171.76	3004.00	0.001
	10 years or more***	2496.50 ± 141.72		
	5 years or more**	4248.10 ± 77.38		
	1 years or < 5 years*	5564.70 ± 51.43		

Vendors; *n* = 61, **** = 5, *** = 8, ** =27, * = 21

Table 6: *Comparison of selected biochemical parameters of smoker and non-smoker pesticide vendor groups (one way ANOVA).*

Biochemical Parameters	**Smoking Habit**	**M ± SD**	***F***	***p***
Urea (mg/dl)	Smokers	30.38 ± 8.55	5.38	0.01
	No	25.34 ± 6.06		
ALT (u/l)	Smokers	32.23 ± 24.32	3.29	0.05
	No	21.46 ± 12.90		
BChE(u/l)	Smokers	3764.2 ± 1018.67	32.539	0.001
	No	5205.7 ± 612.09		

n =61; smokers = 42; non-smoker=19: Significant confidence, $p \leq 0.05$

Majority (50.8%) of the studied subjects had dealt with pesticides for at least 5 years. The pesticide vendors having rather prolonged exposure duration (15 years or more or 10, 5, and 5years) to pesticides

showed significant increases in urea (mean: 37.4 ± 5.03; 29.63 ± 8.95; 29.05 ± 9.03; and 25.48 ± 4.46 mg/dl, respectively, $p = 0.032$) and ALT (mean: 52.4 ± 37.69; 42.34 ± 35.91; 26.55 ± 14.72; 19.86 ± 10.51 u/l respectively, $p = 0.005$) concentrations while showing decreases in total protein (mean: 5.55 ± 1.57; 6.11 ± 1.61; 7.1 ± 1.24; and 7.41 ± 1.4g/dl, respectively, $p = 0.015$) and BChE (mean: 2148.00 ± 171.76, 2496.50 ± 141.72, 4248.10 ± 77.38, 5564.70 ± 51.43 u/l, respecttively, $p = 0.001$) levels (Table 5).

It was also noted that most of the pesticides vendors (68.9%) were smokers. Among the pesticides vendors group, smokers were more affected compared to non-smokers as indicated by their increased ALT (mean: 32.23 ± 24.32 u/l versus 21.46 ± 12.90 u/l, $p = 0.05$) and urea values (mean: 30.38 ± 8.55 mg/dl versus 25.34 ± 6.06 mg/dl, $p = 0.01$) and significantly decreased BChE level (3764.2 ± 1018.67 u/l versus 3764.2 ± 1018.67 u/l, $p = 0.001$) in smokers than in non-smokers, respectively (Table 6).

Discussion

Hazardous impacts on the health of the human population under investigation and exposed to pesticides, such as organophosphates, neonicotinoids, pyrethroids, carbamates and organochlorines etc. have been found. Various parameters have been discussed as follows.

Pesticide exposure in vendors seems to stimulate significant decline of albumin and total proteins in the pesticides-exposed population compared to non-exposed subjects (Table 1). These findings are in agreement with a number of previously reported similar studies.[14] A recent study, using mice as a model animal, revealed that deltamethrin (a synthetic pyrethroidpesticide) caused decrease in total proteins.[15] The decrease of serum proteins, especially albumin, might be due to alteration in protein metabolism of free amino acids and their production in liver under the influence of pesticides.[16] Another explanation of protein decline may be the result of protein loss either due to protein synthesis reduction/degradation or increase in the proteiolytic activity.[17]

Butyrylcholinesterase (BChE) is a non-specific enzyme that can cause hydrolyses of a lot of diverse choline esters. BChE is reliable biomarker and confirmed indicator of organophosphate exposure.[18] In humans, primarily it is present in the liver.[19] The decline in BChE level can cause the delay in the metabolism of some of clinically significant compounds, such as succinyl choline, procaine, mivacurium, cocaineand heroin, etc.[20]

Depressed level of butyrylcholinesterase (BChE) levels in vendors (pesticide handlers) in comparison to their respective controls in this study confirms the adverse impact of exposure of organophosphates to these populations. This theory is further strengthened by the findings significant variations in the values those using protective measures as compared to those not using any of these. Similar findings have already been reported in other related studies previously conducted on workers handling pesticides.[21,11] For instance, in a pesticide-exposed population in India, significant decrease in cholinesterase level was reported.[10] Several other studies have also suggested a decrease in BChE level in response to exposure to organophosphates.[22,23] Similarly, in other parts of the developing world, including Mexico, Kenya, and Bangladesh, similar findings have been reported.[10,24] In mammals, such as rats, a significant decrease in BChE levels was also observed in the groups exposed to an insecticide.[25]

Varying responses of the populations exposed to insecticides by varying degree indicate that the impact rendered on part of these insecticides depends on a number of factors, such as the duration of exposure, the use of protective measures, etc.

One of the major causes of this scenario is that there are no “personal protection guidelines “implemented by the local government or any other relevant authority in Punjab for the insecticide handlers. These observations reveal that most of the farmers never use any protection; on the other hand, however, some of the workers use some of the protective measures at their own, such as wearing head covering gear (caps/hats/turban), handkerchiefs, gloves, boots, face mask and eye glasses, etc.

In cases of subject vendors, significantly elevated level of ALT and significantly depressed level of BChE and total proteins in population more exposed to insecticides (Table 2) indicate the altered health parameters in them. This implies that the risk of pesticide toxicity is directly proportional to the extent of exposure, No or inappropriate use of protective measure involved in his business lead to more influx of the insecticides in the body, either through inhalation, skin contact or even through exposed living surface, such as eyes, etc.; this might lead to altered physiological and biochemical processes.

Similar findings have been suggested by the previous studies concerning insecticide toxicity.[26] Similar trend was also reported by a study carried out in Egypt

where significant incidence of health related problems were reported in agricultural community.[27,28]

Another study also pointed out that the incidence of pesticide exposure increased among workers who had not followed instructions/guidelines for proper handling of pesticides and.[29]

Since this problem of using appropriate measure is directly linked to the behaviour and attitudes of the workers, it is predictable that there is involvement of a number of factors in this scenario, such as low literacy rate, lack of information, poor spraying practices/habits, lack of appropriate training, inability to read the instructions written on the insecticide packing, etc. These factors have already been reported in some other investigations carried out separately.[30]

Significantly altered biochemical responses in the populations under investigation with more number of years (< 10) and particularly that BChE levels support the idea that the toxicity of insecticide increases with the passage of time. These findings are in agreement with the previously undertaken study on agricultural workers in Kenya, where cholinesterase inhibition was recorded due to prolonged exposure of the workers to pesticides.[31] Similarly the elevated levels of ALT in more exposed population in our study endorse the previously reported similar findings.[32]

Elevated levels of vital biochemical indicators, such as urea, ALT, serum creatinine, and AST and the depressed values of serum albumin, total proteins, and BChE levels in smokers (comprising of major proportion of the studied population; 69% of vendors). These findings are confirm the already reported assumptions (Abbassy *et al*., 2014; Asif, 2013; Inal *et al*., 2014).[33-35] This implies that tobacco smoking stresses vital physiological mechanisms and puts the body the body on elevated risk of insecticide toxicity.

Various pesticides, such as chlorpyriphos and imidacloprid were found to exert hazardous health effects on human populations exposed to these insecticides. Blood analyses of pesticides-exposed (vendors) showed significantly elevated values of urea, createnine, aspartate transaminase (AST) and alanine transaminase (ALT), whereas significant decrease in levels of BChE, serum albumin and total proteins were observed in pesticides-exposed individuals compared to the level of these parameters in control subjects.

The above-mentioned rather alarmingly altered values of various health-related parameters in the exposed groups to pesticides indicate vital health risks to humans due to exposure to pesticides. These markedly elevated toxicity-related effects of pesticides can be attributed primarily to the lack of appropriate legislative framework concerning the handling of pesticide in place at present in the state of Punjab, Pakistan. This situation adversely affects the individuals involved in the pesticide business one way or the other. Most of the staff members handling pesticides at the retail establishments are poorly trained; these establishments often lack first aid kits and personal protecttive gear. Moreover, unhygienic practices while handling of pesticides probably contribute to increased risks in pesticides exposure of retailers as well as end users. Due to lack of pesticides safety, training and knowledge concerning handling of pesticides, it is expected that pesticides retailers may misguide the end users who could result in severe pesticide poisoning.

Conclusion

The results of this study suggest that human exposure to pesticides has adverse effects on total proteins, albumin, urea, ALT, and BChE; these biomarkers were useful in studying adverse effects of pesticides in humans.

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