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Influence of exposure to pesticides on serum components and enzyme activities of cytotoxicity among intensive agriculture farmers

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Abstract

Although the effects of acute pesticide poisoning are well known for the pesticides most currently used, hardly any data exist on health effects after long-term low-dose exposures. Major unresolved issues include the effect of moderate exposure in the absence of poisoning. The increased utilization of pesticides other than organophosphates makes it even more difficult to find associations. In this study a cohort of 106 intensive agriculture workers were assessed twice during the course of a spraying season for changes in serum biochemistry, namely enzymes reflecting cytotoxicity (AST, ALT, LDH, CK, and amino-oxidase) and other biochemical parameters, such as markers of nephrotoxicity (urea, creatinine) and lipid profile (cholesterol and triglycerides). Several criteria for estimating pesticide exposure were used, the most important one being serum cholinesterase depression greater than 25% from baseline to peak exposure. Our results revealed an association of pesticide exposure with changes in AST (increased activity), LDH, and amino-oxidase (decreased activity) as well as with changes in serum creatinine and phosphorus (lower and higher levels, respectively). These results provide support for a very slight impairment of the liver function, but overall these findings are consistent with no clinically significant hepatotoxicity. Intriguingly, paraoxonase-1 R allele was found to be an independent predictor of higher rates of AST and lower rates of amino-oxidase, so that it may play a supporting role as an individual marker of susceptibility on pesticide-induced health effects. In conclusion, different biomarkers might be used to detect early biochemical effects of pesticides before adverse clinical health effects occur.

Keywords: Pesticides; Biomarkers; Cytotoxicity; Serum enzymes; Paraoxonase; Occupational health

1. Introduction

The extensive international use of pesticides (mainly organophosphorus compounds, OPs) results in numerous acute intoxications each year. A total number of 400 acute pesticide intoxications were reported to the Epidemiological Surveillance Program of Almeria (southeast Spain, the most important area of intensive agriculture in Spain) in 3 years (2000, 2001, and 2002). Eighty-four percent of them were of occupational origin. They resulted in an annual incidence of 26 cases of acute pesticide-related illness for every 100,000 inhabitants, 67 cases for every 100,000 workers, and 392 cases for every 100,000 agricultural workers. In contrast, in the United States, occupational exposure is known to result in an annual incidence of 18 cases of pesticide-related illness for every 100,000 workers (Calvert et al., 2004).

The effects of acute pesticide poisoning are well known for those most currently used (Wesseling et al., 1997). Evidence continues to accumulate that chronic pesticide exposure is associated with impaired health, including carcinogenesis, neurotoxicity, reproductive and development effects, and immunological effects. However, given the generally low incidence of toxic effects, direct evidence of toxic mechanisms or, at least, a plausible explanation

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based on mechanistic toxicology will be necessary, as the mechanisms by which these chemicals can contribute to these chronic events are largely unknown (Hodgson and Levi, 1996; Maroni and Fait, 1993).

Individuals are frequently exposed to many different pesticides or mixtures of pesticides, either simultaneously or serially, making it difficult to identify effects of particular agents. The relationship of pesticide-related cytotoxicity to overt clinical organ disease is still unresolved. In this regard, biomarkers may be used to detect the effects of pesticides before adverse clinical health effects occur.

"In vitro" studies have found that glyphosate and paraquat are able to inhibit certain enzyme activities: alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and acetyl cholinesterase (AChE) (El-Demerdash et al., 2001). Experimental studies in rats have reported significant changes in all these enzyme activities after suchronic administration of mancozeb in a dose-dependent manner (Kackar et al., 1999). Also, chronic exposure of rats and mice to OPs led to increased levels of serum ALT and AST (Gomes et al., 1999).

Regarding data on humans, Friedman et al. (2003) reported elevations in creatine kinase (CK) in patients more than 10 yr after acute exposure to anticholinesterases. An increased risk of liver dysfunction was observed in Air Force veterans responsible for the aerial spraying of herbicides in Vietnam, the effect being due primarily to increased AST, ALT, or LDH (Michalek et al., 2001). An increase in triglycerides, γ-glutamyl transpeptidase (GGT), and inorganic phosphorus was reported in 17%, 8%, and 7%, respectively, of a cohort of pesticide sprayers (Parrón et al., 1996). Kossmann et al. (1997) observed an elevation of AST and ALT in workers engaged in the production of chlorphenvinfos, an OP compound. Likewise, a positive association between occupational exposure to pesticides and an increased lipid profile was found with OPs (Nakagawa et al., 1982) and DDT (Kreiss et al., 1981). Plasma amine oxidase (PAO) is one of a group of enzymes involved in the biological oxidation of naturally occurring and xenobiotic primary amines (Ebong and Farkas, 1993). The liver enzyme has broad substrate specificity, including a variety of pesticide oxidations (Hodgson, 1982). It has been reported to be significantly lower in humans occupationally exposed to pesticides as compared to control individuals, while platelet membrane-bound monoamine oxidase was significantly higher (Zeinalov and Gorkin, 1990).

However, to our best knowledge, no study has been done on any of these parameters by taking into account two periods with different pesticide exposure during the course of a season or after adjusting for percent depression in cholinesterase activity (BChE) during in-season testing (used as a quantitative estimation of exposure). Taking these premises into consideration, the aim of this study was to ascertain if continuous exposure to mixtures of pesticides including OPs in farmers engaged to intensive agriculture leads to signs of cytotoxicity resulting in early biochemical changes, such as in serum enzymes and other standard serum parameters. The biochemical dysfunction could reflect either hepatic or renal cytotoxicity or changes in serum lipid profile.

2. Materials and methods

The study has a hybrid design that combines cross-sectional and follow-up design features.

2.1. Study subject

Subjects between the ages of 20 and 60 yr were recruited to participate in the study based on their potential for exposure to pesticides. They were from a small village located within a large intensive agricultural area of plastic greenhouses on the coastline of Granada (southeast Spain). All persons were initially contacted by phone via local telephone listing to afford a forum to explain the purpose of our study. As pesticides have been linked with various chronic diseases, individuals presenting diabetes, neurological disorders, liver dysfunction, or any other chronic condition were excluded from the population studied in order to avoid any interference with the biochemical parameters measured. Recruited individuals were requested to come to the general practitioner's clinic on a scheduled day to participate in the study. All individuals were offered health examinations consisting of a medical history, a physical examination, and a laboratory test. Given the small size of the village, the closeness between people and the general practitioner, and the strong awareness of pesticide toxicity in such a setting, most individuals who were eligible were willing to participate in the study, which allowed a high response rate. The overall response rate for greenhouse workers was higher than 90%.

The initial exposed group consisted of 135 workers engaged in intensive agricultural tasks for the whole year. The final study population consisted of 106 subjects, who provided two blood samples during the course of a spraying season (61 men, 45 women; mean age 37.4 yr). Of the subjects, 87 (82.1%) indicated that the nature of their work with pesticides was as applicators. The remaining 19 workers (17.9%) were engaged in regular greenhouse works, but they failed to spray pesticides. The five groups of pesticides most commonly used were carbamates (used by 16.2% of the individuals studied), dithiocarbamates (11.6%), organophosphates (11.3%, including parathion, malathion, and chlorpyrifos), pyrethroids (10.7%), and neonicotinoids (10.4%). Information on demographic characteristics (sex of the worker, age, height, and weight), smoking habits, alcohol consumption, lifetime exposure to pesticides, and use of personal protective equipment (PPE) was collected by means of a questionnaire administered by medical personnel at the general practitioner consultation.

2.2. Sample collection

After an overnight fasting period, two samples of venous blood were collected in tubes with clot activator and citrate-treated tubes, respectively, and preserved cooled less than 2 h until they reached the laboratory. Serum was separated by low-speed centrifugation (800g for 20 min) and analyzed immediately upon arrival at the Central Laboratory of Motril Hospital. Plasma was separated in a similar way and stored at $-20\,^{\circ}C$ until analyzed (within the first month) at the laboratory for toxicological analysis of the Granada School of Medicine. Blood samples were collected at two different periods during the course of a spraying season: when the spraying process peaked (higher exposure) and at the end of the season, when pesticide use was lowest (lower exposure period), considered as baseline. The two periods were identified by personal interviews with agricultural workers and their foremen. The time between exposure periods was 5 months. The spaying season was consistent for all participants. Written consent was obtained from the subjects who agreed

to participate, and they were allowed to drop out whenever they wanted. The proposal was approved by the Ethics Review Committee of Granada University.

2.3. Exposure measures

The variable used to describe pesticide exposure was change in BChE activity (expressed as % decrement of activity). It was calculated for each greenhouse worker by dividing enzyme activity at the high-exposure period by that at the baseline (low-exposure period). The resulting values were subtracted from unity and multiplied by 100 to give the percentage depressions of BChE. The study subjects were further divided into two groups according to whether their BChE was depressed by more than 25%, the cutoff level established in Spain for biological surveillance of subjects occupationally exposed to pesticides.

An additional estimate of exposure was performed by calculating the cumulative pesticide exposure for each farmer. It was estimated by multiplying the average number of days working with pesticides on a weekly basis by the average number of weeks per year and by the average number of years of pesticide use. Other indirect measures of exposure included utilization of protective gear during mixing/loading or during application of pesticides. Farmers can be expected to accurately recall details of their use of pesticides because it is a significant part of their farming operations.

2.4. Biochemical assays

Serum enzymes and parameters were measured using a Hitachi 717 biochemical autoanalyzer at the Central Laboratory of Motril Hospital (Granada) following standard procedures for clinical biochemistry purposes. The biological markers of cytotoxicity measured were ALT, AST, LDH, and CK. Other serum components also measured were urea, creatinine, cholesterol, triglycerides, and inorganic phosphorus.

2.4.1. BChE and paraoxonase-1 (PON1) phenotyping

Plasma BChE variants were determined by measuring the inhibition of benzoylcholine hydrolysis with dibucaine and fluoride (0.01 and 0.05 mM, respectively) at 240 nm (Whittaker, 1984). PON1 activity was measured in plasma as described previously (Hernández et al., 2004) following the rate of formation of *p*-nitrophenol at 405 nm in the presence and absence of 1 M NaCl (salt-stimulated and basal paraoxonase, respectively). PON1 R allele (i.e., the sum of individual phenotypes RR and QR) was phenotypically established as percentage of saline stimulation >60%, a cutoff derived from the corresponding histogram plotted after making the calculation [(salt stimulated PON1−basal PON1)/(basal stimulated PON1)] × 100, as reported by Eckerson et al. (1983).

2.4.2. Benzylamine oxidation

This spectrophotometric assay measures the plasma amino-oxidase (PAO)-catalyzed conversion of benzylamine to benzaldehyde. The assay conditions followed that of McEwen (1965) with minor modifications. Briefly, 0.5 mL of plasma was incubated with 8 mM benzylamine in 0.2 M phosphate buffer (pH 7.2) in open test tubes for 3 h at 37 °C with gentle shaking. The final volume was 1.4 mL. The reaction was stopped with 0.15 mL of 60% perchloric acid. The benzaldehyde was extracted into 2.0 mL of cyclohexane and the mixture centrifuged at 3000 rpm to separate the phases. The absorbance of the cyclohexane layer was then determined with a Perkin–Elmer Lambda 2 spectrophotometer at 242 nm against cyclohexane. Each sample was run in duplicate. To one tube, which was used for a zero time control, perchloric acid was added immediately after addition of the plasma.

2.5. Statistical methods

Log-transformed data were used for the statistical analysis after ascertaining that the biochemical parameters were approximately log-normally distributed. The Student *t*-test was used to compare group means of these parameters. Categorical variables were compared by the χ^2-test. The Pearson correlation coefficient was used to evaluate correlations among blood parameters. One set of multiple linear regression analysis was carried out to evaluate the contribution of pesticide exposure to log-transformed CK, AST, ALT, LDH, and PAO activities and on urea, creatinine, cholesterol, triglyceride, and phosphorus levels. A second set of models was used to compare the percentage change in serum enzymes between the two periods studied. Covariates considered were age, gender, body mass index (BMI), alcohol consumption, PON1 R allele, cumulative exposure to pesticides, BChE depression greater than 25%, and protective gear use during the mixing/loading and during the application of pesticides. All statistical analyses were performed with the SPSS statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA).

3. Results

There were no significant differences between the two exposed groups in regard to age, BMI, gender, smoking habits, alcohol consumption, or serum BChE phenotypes. In contrast, significant differences were found in PON1 R allele and sprayer status after adjusting for BChE depression above or below 25% (Table 1).

Out of 106 greenhouse workers, 64 (60.4%) presented risky or unsafe levels of BChE depression greater than 25%, an indicator of exposure to OPs and carbamate pesticides. Of these, 66.7% were sprayers and 31.6% were

Table 1
Selected characteristics of the study population

Characteristic	Depression BChE <25%	Depression BChE >25%	*P*
N	42	64	
Mean age (yr)	37.3±7.6	37.4±8.3	0.936
BMI (kg/m^2)	27.6±4.5	27.4±4.9	0.795
Gender (%):			
Male	41	59	0.739
Female	38	62	
Tobacco consumption (%):			
Yes	45	55	0.329
No	36	64	
Alcohol users (%):			
Yes	38	62	0.892
No	40	60	
BChE variants (%):[a]			
Usual variants	40	60	0.646
Unusual variants	20	80	
PON1 R allele (%):			
Carriers	29	71	0.016
Noncarriers	52	48	
Sprayer status (%):			
Active sprayers	33	67	0.005
Nonsprayers[b]	68	32	
Past poisoning by pesticides (%):			
Yes	38	62	0.963
No (%)	40	60	

[a]Only five cases with unusual variants were found among the total population studied.

[b]Nonsprayers are greenhouse workers who failed to spray pesticides.

greenhouse workers who did not spray pesticides (Table 1), the differences being statistically significant ($P = 0.004$; OR: 4.33 C.I. 95%: 1.49–12.57). This means that exposure rates were higher among those who actually applied pesticides than among those who did not, suggesting exposure by direct contact associated with application. Nevertheless, 33% of the active sprayers had a BChE depression lower than 25% and 32% of the nonsprayers had a BChE depression higher than 25%. On the other hand, most of the individuals carrying the PON1 R allele showed a BChE depression greater than 25% ($P = 0.016$). Only five individuals were carriers of unusual BChE variants, and although four of them showed a decrease in enzyme activity by more than 25%, no statistically significant differences were observed (Table 1). Given this low number of cases, the variable was not used in further analyses. Last, 12.4% of subjects (13 out of 106) reported past poisoning by pesticides; most of them (61.5%) showed a BChE depression greater than 25%. Nevertheless, differences failed to be significant.

The levels of serum enzymes, reflecting cytotoxicity, and standard biochemical parameters measured at two periods with different pesticide exposure are presented in Table 2. Data have been adjusted for depression in BChE above 25%, used as a surrogate of exposure. Although mean values fell within the normal range, statistically significant differences were observed for LDH and phosphorus. PAO activity was near the significance level. Lower rates of these enzymes and higher levels of phosphorus were observed in the group of individuals for whom BChE was decreased by more than 25% (Table 2).

The results of the univariate analyses were further explored in a multivariate regression model (Table 3). Depression in BChE by more than 25% predicted lower AST activity and higher phosphorus level at the period with low pesticide exposure. The utilization of PPE during mixing/loading of pesticides predicted higher rates of LDH and PAO in the periods of high and low exposure to pesticides, respectively. In addition, the cumulative exposure to these chemicals predicted lower creatinine levels in the low-exposure period. A noticeable finding was that PON1 R allele was an independent predictor of higher AST and lower CK and PAO activities (Table 3).

Given that two analytical determinations of serum enzymes were performed during the course of the same spraying season, it raised the possibility of considering these parameters under a dynamical view. That is, we calculated the ratio of each enzyme activity between the two periods with different pesticide exposure and the resulting values were multiplied by 100 to give the percentage change between the two periods. The new built variables (% increased or decreased activity) were then used for further analyses.

The correlation between percentages of change in serum enzymes between the two periods of pesticide exposure is shown in Table 4. It can be observed that changes in BChE showed a direct correlation with changes in AST and LDH, and that changes in AST positively correlated with changes in all enzymes but PAO. Besides, changes in LDH also correlated with changes in all enzymes but ALT. Changes in PAO, in turn, only showed an inverse correlation with changes in LDH, and changes in ALT only correlated positively with AST. These results were further explored in another multivariate regression model (Table 5), where the most outstanding finding was that the percentage change in BChE predicted higher percentage change in AST and that PON1 R allele predicted higher percentage of change in PAO.

4. Discussion

The present study revealed that certain serum enzymes (AST, LDH, and PAO), as well as serum components (creatinine and phosphorus), are to some extent influenced by pesticide exposure as determined using different criteria. A subtle biochemical dysfunction resulting in cytotoxicity is thus supported. The study therefore highlights the need to properly evaluate and control the potential health effects due to exposure to toxic substances among subjects employed in intensive agriculture.

Table 2
Biochemical parameters in serum (log transformed) of the study subjects adjusted for BChE depression above or below 25% (used as a surrogate of pesticide exposure) at two different periods during the same spraying season

Enzyme	High exposure period			Low exposure period		
	Depression BChE <25%	Depression BChE >25%	*P*	Depression BChE <25%	Depression BChE >25%	*P*
CK	4.66±0.49	4.74±0.57	0.456	4.63±0.71	4.61±0.61	0.878
AST	3.14±0.35	3.14±0.31	0.893	3.02±0.35	2.93±0.31	0.158
ALT	3.16±0.69	3.15±0.60	0.939	2.98±0.63	3.00±0.50	0.871
LDH	5.84±0.18	5.79±0.19	0.182	5.76±0.14	5.69±0.18	0.032
PAO	3.27±0.34	3.26±0.30	0.898	3.14±0.30	3.02±0.39	0.085
Urea	3.58±0.29	3.54±0.22	0.445	3.59±0.30	3.59±0.23	0.927
Creatinine	−0.02±0.13	−0.02±0.17	0.778	−0.05±0.15	−0.01±0.16	0.170
Cholesterol	5.36±0.19	5.35±0.16	0.781	5.34±0.20	5.34±0.26	0.970
Triglycerides	4.69±0.59	4.56±0.54	0.260	4.78±0.59	4.74±0.69	0.765
Phosphorus	4.01±0.47	4.20±0.49	0.059	3.58±0.48	3.85±0.51	0.008

Table 3
Forward stepwise multiple linear regression of biochemical parameters in serum (log transformed) of the study subjects at two periods with different pesticide exposure

Enzyme	High exposure period				Low exposure period			
	adj R^2	Predictor	B	P	adj R^2	Predictor	B	P
CK	0.441	Gender	−0.586	<0.001	0.418	Gender	−0.702	<0.001
		Age	−0.022	<0.001		Age	−0.018	0.009
		PON1 R allele	−0.198	0.023		PON1 R allele	−0.222	0.030
AST	0.385	Gender	−0.377	<0.001	0.401	Gender	−0.362	<0.001
		Alcohol	−0.168	0.006		PON1 R allele	0.215	0.002
						Depression BChE>25%	−0.220	0.003
ALT	0.410	Gender	−0.894	<0.001	0.368	Gender	−0.751	<0.001
LDH	0.041	PPE mixing/loading	0.105	0.043	0.310	Gender	−0.161	<0.001
						BMI	0.011	0.012
PAO	N.S.				0.231	Gender	0.255	<0.001
						PON1 R allele	−0.194	0.003
						PPE mixing/loading	0.166	0.025
BUN	0.092	Gender	−0.159	0.005	N.S.			
Creatinine	0.572	Gender	−0.243	<0.001	0.528	Gender	−0.227	<0.001
						Cumulative exposure	−0.223	0.024
Cholesterol	0.272	Gender	−0.177	<0.001	0.181	Gender	−0.171	<0.001
		Age	0.005	0.019				
Triglycerides	0.291	Gender	−0.541	<0.001	0.301	Gender	−0.706	<0.001
		BMI	0.033	0.021				
Phosphorus	0.050	Age	−0.016	0.029	0.220	Depression BChE >25%	0.395	0.001
						Age	−0.017	0.020
						Alcohol	0.265	0.016

Note: Predictors: age, gender (0: male; 1: female), BMI, alcohol consumption (1: yes, 0: no), PON1 R allele (1: RR or QR, 0: QQ), cumulative exposure to pesticides (log-transformed), depression of BChE >25% from baseline to peak exposure (1: yes; 0: no), utilization of personal protective equipment (PPE) during mixing–loading (1: yes, 0: no) or during application of the pesticide (1: yes, 0: no).
adj R^2: adjusted R^2, N.S.: not significant.

Table 4
Coefficient of correlation (Pearson) of percent change in serum enzymes between the two periods studied with different pesticide exposure

	BChE	AST	ALT	LDH	CK
AST	0.202*				
ALT	NS	0.418**			
LDH	0.208*	0.469**	NS		
CK	NS	0.436**	NS	0.297**	
PAO	NS	NS	NS	−0.198*	NS

*$P<0.05$.
** $P<0.01$.

Application of pesticides can result in exposure by either the dermal or respiratory route, and can produce illness even with low-grade depressions in cholinesterases (Gordon and Richter, 1991). It has been reported that AChE is better than BChE for the assessment of chronic exposure to OPs, since a cumulative inhibition is observed due to its lower recovery rate compared to that of BChE (Kamel and Hoppin, 2004). OPs also inhibit plasma BChE, but the effect lasts at most a few weeks and is therefore not useful for evaluating chronic exposure (Kamel and Hoppin, 2004). As subjects from our study were intensive agriculture workers continuously exposed to pesticides on a weekly basis, BChE was precluded from full recovery. This allowed us to monitor exposure by means of this biomarker. On the other hand, serum BChE activity has been reported to be a slightly more sensitive indicator of mixed exposure than red blood cell AChE activity (Richter et al., 1992). Experimental studies have also shown that subacute exposure to OP pesticides caused an 85% inhibition of BChE (Sachana et al., 2001).

A key limitation of using depressions in BChE for the assessment of exposure is high interindividual (and even intraindividual) variations in enzyme activity. This implies the need to set a rather high cutoff point to overcome the effect of these variations, so that depressions in BChE can only be associated with pesticide exposure. The WHO biological exposure index for individuals occupationally exposed to OPs pesticides has been set to 30% of BChE depression. In Spain, the Surveillance Health Programme established a slightly lower limit, 25%. Accordingly, in the present study a BChE depression above 25% was selected to quantitatively estimate exposure to pesticides. Thus, any factor associated with a BChE depression greater than

Table 5
Forward stepwise multiple linear regression of % change in serum enzymes of the subjects studied between the two periods with different pesticide exposure

Parameter	adj R^2	Predictor	*B*	*P*
% change in CK	0.295	% change AST	2.070	<0.001
		BMI	−6.073	0.001
		Age	2.637	0.014
% change in AST	0.416	% change in LDH	0.399	0.002
		% change in ALT	0.160	<0.001
		% change in CK	0.064	<0.001
		% change in BChE	0.172	0.044
% change in ALT	0.205	% change in AST	0.873	<0.001
		Gender	−21.77	0.004
% change in LDH	0.276	% change in AST	0.316	<0.001
		% change in PAO	−0.090	0.023
		BMI	−0.539	0.029
% change in PAO	0.143	Gender	−20.38	<0.001
		PON 1 R allele	11.84	0.036

Note: predictors: age, gender (0: male; 1: female), BMI, alcohol consumption (1: yes, 0: no), PON1 R allele (1: RR or QR, 0: QQ), and the percentage change in BChE, CK, AST, ALT, LDH, and PAO between the two periods with different pesticide exposure (depression in the case of BChE and stimulation of enzyme activity in the remaining cases).

25% could be strongly attributed to pesticide exposure. In spite of that, approximately one-third of active sprayers of our study had a depression in BChE below 25% and one-third of nonsprayers showed a depression above 25%, which has to be taken into account as another limitation in the use of this biomarker.

This study has found that AST is modified by the effect of pesticides, since workers presenting depressions of BChE above 25% had lower levels of AST after adjusting for several confounders and occupational covariates (Table 3). Also, percentage of depression in BChE predicted higher percentage variation in AST (Table 5), supporting those data. These data might reflect a subtle or subclinic hepatotoxic effect, although it warrants further investigation.

Our data also pinpoint a role for pesticides on LDH and PAO, because (a) lower enzyme levels were observed in subjects with a BChE depression greater than 25% (Table 2) and (b) those individuals wearing PPE while mixing/loading or spraying pesticides presented higher rates of these enzymes (Table 3). It must be taken into account that the utilization of PPE prevents pesticides from being absorbed into the body. A previous study showed that spray personnel without protective clothing presented a marginal reduction in their blood cholinesterase activity during the exposure period (More et al., 2003). Our findings are consistent with in vitro, experimental, and epidemiological studies reporting changes in LDH, PAO, and AST after exposure to different kinds of pesticides (Al-Qarawi and Adam, 2003; El-Demerdash et al., 2001; Gomes et al., 1999; Gupta et al., 1991; Kackar et al., 1999; Kossmann et al., 1997; Michalek et al., 2001; Zeinalov and Gorkin, 1990). Some pesticides, such as paraquat and glyphosate, have been reported to cause inhibition in the activity of serum AST and LDH, while other pesticides (OPs, organochlorines, and pyrethroids) are able to cause inhibition of LDH (El-Demerdash et al., 2001). On the other hand, an inhibition of PAO has been observed in the presence of polyphosphates, since the phosphate ion is able to bind free polyamines, so that it may behave as an apparent competitive inhibitor of the enzyme (Corazza et al., 1992; Di Paolo et al., 1995).

Intriguingly, PON1 R allele was an independent predictor of serum AST, PAO, and CK. This finding, reported by the first time, deserves further attention and should be investigated in depth, as it highlights the role of individual biomarkers of susceptibility on pesticide-induced biochemical changes in target organs. The individual susceptibility is caused by polymorphic key enzymes like esterases (PON1, BChE), transferases (GST), and CYP450 (Hernández et al., 2005; Thier et al., 2003). Human serum PON1 hydrolyzes OPs pesticides entering the blood circulation and tissue fluid, thus limiting toxicity. Several epidemiological studies have highlighted the involvement of PON1 in xenobiotic susceptibility in different scenarios: Gulf War veterans, sheep dippers, acute poisoning with OPs, and chronic exposure to pesticides in agricultural workers (Costa et al., 2005).

Regarding other serum parameters, individuals showing higher BChE depression presented increased levels of inorganic phosphorus (Tables 2 and 3). This finding suggests that subchronic exposure to pesticides leads to an elevation of phosphorus in serum. Previous epidemiological studies reported levels beyond the normal range in 7% of the pesticide applicators studied (Parrón et al., 1996). At first glance, it could be accounted for by a higher than normal absorption of OPs compounds; however, the hydrophility of their alkyl phosphate metabolites does not allow them to accumulate into the body, so that alternative explanations should be considered.

The last interesting finding of our study is the association of cumulative exposure to pesticides with serum creatinine (Table 3), although the fact that it is an inverse association makes it difficult to interpret. Previous studies have reported subtle nephrotoxic changes in workers occupationally exposed to pesticides (Al-Qarawi and Adam, 2003), because of their higher levels of serum creatinine and/or blood urea. Further studies using more specific and sensitive biomarkers of nephrotoxicity should be done to ascertain the potential selectivity of pesticides on the nephron. In contrast to other epidemiological studies (Al-Qarawi and Adam, 2003; Nakagawa et al., 1982), our data do not support changes in the serum lipid profile (Tables 2 and 3).

The observed significance of lower LDH activity at the low exposure period, as well as the fact that depression in BChE greater than 25% predicted lower AST activity and higher phosphorus levels also at the period of low pesticide

exposure, may indicate that the potential biochemical outcomes are not expected to occur immediately after exposure, but a long-term hazard may arise because of the persistence of the biological effects induced by low-dose exposure to pesticides (Gomes et al., 1999).

This study has some limitations. The criterion used for classifying workers into groups makes it difficult to reach significant differences, since they were all greenhouse workers with either direct or indirect exposure to pesticides. It would have been of most interest to have had a nonexposed group of individuals, but it was not feasible to get two set of blood samples during the course of the same spraying season as with workers. This might weaken our study by making associations more difficult to detect, although it does not undermine the validity of any observed associations (Kamel and Hoppin, 2004). On the other hand, the study population was exposed to mixtures of pesticides, as many classes of pesticides are commonly used on the same crop. As a result, interactions between them may occur, making it almost impossible to determine the cumulative risk posed to humans.

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