Risk Assessment of Organophosphate Pesticide Exposure on Greenhouse Workers in Menderes Region, Develi Village (Turkey)¶

MENDERES BÖLGESİ, DEVELİ KÖY'ÜNDEKİ SERA İŞÇİLERİ ÜZERİNE ORGANOFOSFORLU PESTİSİT MARUZİYETİ İLE İLGİLİ RİSK DEĞERLENDİRMESİ

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Abstract

Objective: Plasma cholinesterase (PChE) activities are widely used to determine the rate of exposure to organophosphate (OP) pesticides. The aim of our study was to determine the existence of any symptomatic and biochemical effects of OP pesticide exposure during pesticide application in workers.

Material and Methods: Blood samples were collected from 53 male greenhouse workers exposed to pesticides for 2-30 years in Develi Village. PChE levels were determined spectrophotometrically and chromatographic analysis were carried out on (gas chromatography/nitrogen phosphorus detector) (GC/NPD) for residue analysis.

Results: A decrease in PChE levels was observed (mean ± SD; 878 ± 90.2 nmol/min/mL plasma) when compared to control (mean±SD; 1706 ± 102.4 nmol/min/mL plasma). Symptoms were nausea, eye irritation and ophthalmia, headache, fatigue and anorexia, skin disease, coughing, gasping for breath and asthma, dizziness and coma. No organophosphate pesticide residue was detected in blood specimens of the workers.

Conclusion: Significant inhibition of PChE activity and clinical symptoms were observed in the workers, indicating a potential toxicological hazard from OP pesticide exposure.

Key Words: Organophosphate insecticide, worker, plasma cholinesterase activity, residue analysis

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Özet

Amaç: Plazma kolinesteraz aktivitesi, organofosforlu pestisitlere maruz kalma oranının belirlenmesinde sıklıkla kullanılmaktadır. Çalışmamızın amacı, işçilerde pestisit uygulaması esnasında oluşan organofosforlu pestisit maruziyetinin bazı semptomatik ve biyokimyasal etkilerinin varlığını belirlemektir.

Gereç ve Yöntemler: Develi Köy'de 2-30 yıl boyunca pestisitlere maruz kalan 53 erkek sera işçisinden kan örnekleri toplanmıştır. Plazma kolinesteraz seviyesi spektrofotometrik olarak belirlenmiş ve kalıntı analizi için kromatografik analizlerde HP GC/NPD (Hewlett Packard, Gaz Kromatografisi/Nitrojen Fosfor Detektör) kullanılmıştır.

Bulgular: Plazma kolinesteraz seviyelerinde (ort±SD; 878±90.2 nmol/dak/mL plazma) kontrole göre (ort±SD; 1706±102.4 nmol/dak/mL plazma) bir azalma gözlenmiştir. Semptomlar, mide bulantısı, göz irritasyonu ve oftalmi, baş ağrısı, bitkinlik ve anoreksi, cilt hastalıkları, öksürük, güçlükle soluk alma ve astım, başdönmesi ve komadır. İşçilerin kan örneklerinde hiç bir organofosforlu pestisit kalıntısı tespit edilememiştir.

Sonuç: Organofosforlu pestisit maruziyetinin potansiyel toksikolojik zararının göstergesi olarak işçilerde, plazma kolinesteraz aktivitesinin inhibisyonu ve klinik semptomlarda artış gözlenmiştir.

Anahtar Kelimeler: Organofosforlu insektisit, işçi, plazma kolinesteraz aktivitesi, kalıntı analizi

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Organophosphate (OP) insecticides exert their toxicity by irreversible inhibition of Acetylcholinesterase (AchE), (EC 3.1.1.7) in the peripheral and central cholinergic nervous system. These pesticides bind to cholinesterase and block the hydrolysis of the acetylcholine to choline and acetic acid at the post-synaptic junctions without junctioning acetylcholinesterase, acetylcholine accumulates.[1,2] The extent to which AChE is inhibited in workers is dependent upon the rate the OP pesticide is activated, metabolized to nontoxic products by tissue enzymes, its affinity for AChE concentrations in tissues.[3]

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The toxic effects of OPs are largely associated with short-term exposures to high concentrations of pesticides, during the manufacture, formulation, mixing and application of them. Inhalation, ingestion and dermal contact are potential routes of absorption. Under agricultural working conditions OP pesticides primarily are absorbed through the skin. Occupational exposure limits for OPs usually have skin notations to warn of the potential for dermal absorption to contribute to systemic toxicity. Consequently, biological monitoring is an essential component of any comprehensive assessment of exposure. Biological effect monitoring by measurement of reduction of blood ChE activity involved the measurement of (PchE) (EC 3.1.1.8).[4] Inhibition of PChE in plasma serves as a diagnostic tool for the risk assessment of exposure to toxic OPs.[5] PChE activities are indicators of exposure to OP pesticide and widely used to determine the rate of exposure to OP pesticide. Cholinesterase activity levels and symptom interviews are useful for monitoring workers at risk. Whole blood and plasma cholinesterase activity were slightly more sensitive indicators of pestiside exposure than red blood cell cholinesterase activity.[6] OPs can affect ChE activity in both RBCs and in blood plasma, and can act directly, or in combination with other enzymes, on ChE in the body.[7]

The typical human exposure assessment approach is to measure metabolic biomarkers in blood. No study was reported in Izmir (Turkey) on exposed workers previously, therefore this study was conducted with the aim of evaluating impact on health produced by use of OP pesticides in greenhouses.

Material and Methods

Subject Selection and Blood Sample Collection: The protocol was approved by Ethical Committee of Ege University, Faculty of Medicine (00-7/5M-285, 07.03.2000) and informed consent was obtained. Greenhouses were identified in Menderes region, Develi Village. The blood samples were collected from 53 male greenhouse workers (aged 18-57 years, mean ±SD; 32±10 years), exposed to OP pesticides for 2-30 years and 30 male control subjects (aged 20-50 years, mean ±SD; 29 ±7 years), (workers not occupationally exposed to pesticides at their work site). The blood samples (2x10 mL) were collected into tubes (Vacuette® heparin tubes, Kremsmuenster, Austria) by a registered nurse.

Plasma Cholinesterase Activity: Serum was separated from blood by centrifugation at 10000 g for 15 min at ambient temperature. Samples were stored at –70 °C until assay of ChE activity. The PChE activities were measured according to the procedure of Ellman et al. based on the colorimetric estimation of unreacted acetylcholine.[8] The assay mixture contained 0.259 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) in 67 mM phosphate buffer, pH 7.4, 0.154 mM butyrilthiocholin iodide and 20 µL of an appropriate dilution of enzyme source in a total volume of 3.02 mL. Reaction was followed at 410 nm (Shimadzu UV/VIS-1601 PC Spectrophotometer) for 10 minutes intervals at 37°C against blank containing butyrilthiocholin iodide and phosphate buffer. The detection limit is approximately 10 U/L and the sensitivity is equivalent to $\Delta A/\Delta t$ 0.002 per 5 min. The extinction coefficient of the product of the chemical reaction, 5-thio-2-nitrobenzoate is $\varepsilon = 3.61\ mM^{-1}\ cm^{-1}$. The reference range for human plasma ChE activity is 1.8-5.0 kU/L plasma for women and 2.0-5.2 kU/L plasma for men at 25 °C.[9] Serum total protein levels were determined by Lowry method.[10]

Residue Analysis: Plasma (0.5 mL) was prepared for extraction by addition of 100 µL (1 ppm) of dichlorvos as internal standard and homogenized. Benzene (6 mL) was added and agitated for 2 min. Samples were centrifuged (6000 g for 5 min) and the organic layer was transferred to a tube. After three times recentrifugation, organic layers were accumulated to the tube. A glass column contains 5 g anhydrous sodium sulfate, 0.5 g active charcoal (activated 170 ºC' de for 2 h) and 2 g neutral aluminum oxide was conditioned with 6 mL benzene. The samples were eluted (5 mL/ min) from the column under vacuum. The fractions were evaporated (N gases, 40º C) to about 1 mL. Gas chromatographic analyses were carried out HP 6890 gas chromatograph directly connected nitrogen phosphorus detector. The samples were analyzed using a method described by Jing et al.[11]

Statistical analysis

Enzyme activities and serum total protein levels were expressed as mean ± standard deviation of mean (SD). Differences between group means were detected by analysis of variance, Student's t-test and the non-parametric Mann-Whitney U-test. Data were analyzed with the SPSS 11.0 statistical package. P<0.05 was considered significant.

Results

This paper describes the measurement of PChE enzyme activities in greenhouse workers exposed to the OP pesticides (pesticide exposure periods: 2-10 years for 44 workers (83 %) and >11 years for 9 workers (17 %)) and control subjects. There was correlation noted between the number of working years and PChE activity. The PChE activity of greenhouse workers declined with increasing OPs exposure. The means of ages of the exposed and control groups are 32 and 29 years, respectively. There were no statistically significant differences between our exposed and control groups regarding age. All members of both groups were in the age range where PChE activities do not change significantly with age.[12,13]

It was reported that 26.4, 35.0, 12.2, and 1.9 % of the workers used OP combined with fungicide; synthetic pyrethroid, carbamate, chlorinated hydrocarbons; fungicides, chlorinated hydrocarbons, fumigants; and carbamate, respectively, while 24.5 % of the workers used only OP. Active ingredients of these pesticides and their chemical class were determined as chlorpyriphos, methamidophos, diclorvos, diazinon and parathion-methyl (OPs); delthamethrine and lambda-cyhalothrine (synthetic pyrethroids); endosulphan (chlorinated hydrocarbones); furathiocarb (carbamates); captan, propyreb, chlorphluazurone and mancozeb (fungicides), and methyl bromyd (fumigant).

Total protein levels in workers exposed to pesticide were determined as mean ± SD; 5.8 ± 2.3 g/dL and total protein levels of 29 workers (53.5 %) were less than the control. The mean ± SD of PChE activities in workers was 878 ± 90.2 nmol/min/mL plasma (range from 372 to 3907) and 48 workers (90.7 %) were significantly lower than the control (mean ± SD; 1706 ± 102.4 nmol/min/mL plasma, range from 1603 to 5824). Table 1 summarizes the data as mean ± SD of PChE activity and total protein levels, and the number of subjects per group.

Table 1. Summarized data (mean, SD, *N*) for PChE activity and total protein levels of greenhouse workers exposed to organophosphate in Develi Village (Turkey)

		PChE (nmol/min/mL plasma)		Total protein (g/dL)	
	N	Mean	SD	Mean	SD
Controls	30	1706	102.4	7.5	3.1
Workers	53	878*	90.2	5.8	2.3

* Significantly different from control (p = 0.032)

Discussion

The PChE activity has a long history of use in monitoring both workers at risk of OP pesticide exposure and in investigating accidental exposures to OP pesticides. The decreasing ChE level in plasma arises from OP pesticides exposure has also been reported in several studies.[4,5,13,14] Similarly, in a study that regularly examined of the blood ChE activity, the number of hours engaged in termite-control work, general conditions, and various test values in workers at a termite-control office, the lowest ChE level observed during the full-scale termite-control season was less than 50 % of the mean value for each worker and was less than 10 % of the pre-season values.[15] It was reported in other studies that PChE activity was 50 % lower in OP-exposed group than in controls.[16,17]

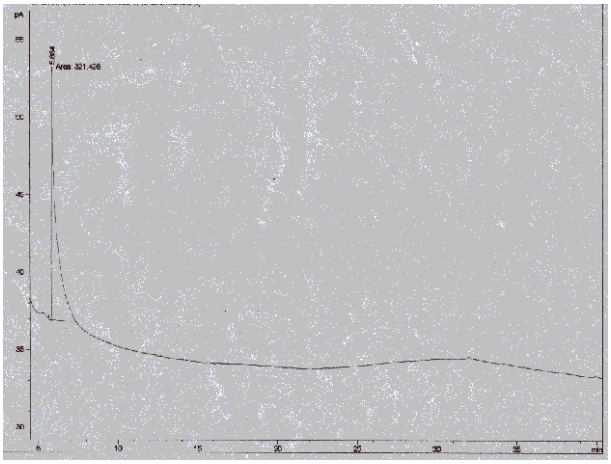
Twenty-one of the workers (approximately 40%) referred some sing and symptoms after use of pesticides. The symptoms were nausea, eye irritation and ophthalmia, headache, fatigue and anorexia, skin disease, cough, to gasp for breath and asthma, dizziness and coma. The symptoms and the correlating PChE activities were stated below in Table 2. Frequency of serious symptoms in workers was increased with decreasing in ChE activity (for example, coma was observed in 428 nmol/min/mL plasma, which is the lowest ChE activity). OP-related inhibitions of AChE in the brain, neuromuscular junction, and peripheral nerves are character-

Table 2. The symptoms and the correlating ChE activities

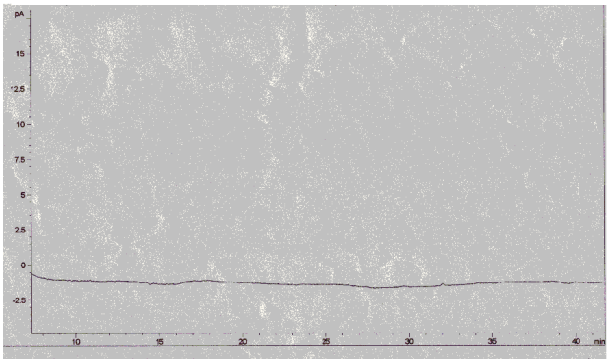
Symptoms	Number	%	ChE activities
No symptoms	32	60.4	2670
Nausea	5	9.5	1876
Eye irritation, ophtalmia	4	7.6	1443
Headache	1	1.8	733
Fatique, anorexia	2	3.8	669
Skin disease	4	7.6	630
Cough, to gasp for breath, asthma	3	5.7	600
Dizziness	1	1.8	558
Coma	1	1.8	428
Total workers	53	100.0	

ized by central nervous system (CNS) effects and muscarinic and nicotinic effects in the periphery. Generally it was known that, CNS effects include restlessness, tremor, slurred speech, ataxia, generalized weakness, seizures and delirium, and peripheral nicotinic effects include muscle cramps and weakness, paralysis and respiratory failure, while muscarinic effects include salivation, lacrimation, urination, diarrhea, gastrointestinal distress and emesis.[18] OPs cause numerious cases of acute and even fatal poisonings.[19,20] In the acute phase of OP poisoning, low serum ChE (> 50 % of minimum normal value) supports the diagnosis of OP poisoning but it does not show a significant relationship to the severity of poisoning. The serum ChE activity may be a useful parameter in following the acute prognosis of OP poisoning.[21] Exposure to OPs is also a potential cause of longer-term damage to the nervous system, with reports of poor mental health and deficits in memory and concentration.[3,22,23] Weissmann et al[24] also reported that one patient developed severe weakness, fasciculation, disorientation and sleepiness had low levels of plasma AchE.[24] As an overview of these symptoms, similar results were observed in our study too.

Since some pesticides have shown to be ubiquitous environmental pollutants due to their great chemical stability and lipid solubility, these are routinely detected in human adipose tissue, blood and breast milk. Many analytical methods for OPs have been reported using GC-MS (Gas Chromatography- Mass Spectroscopy).[25,26] Various common pesticides were found at the ng/mL and ng/g levels in some demonstration blood and dirty samples, respectively.[27] The blood and urine specimens from human were analyzed by GC-MS, and organochlorine pesticide residues were detected in urine but not in blood.[28,29] In current study, no OP insecticide residue was also observed in the blood specimens using the conditions of GC/ NPD chromatography. Figure 1b shows that no peak to indicate OP insecticide residue. Many OPs are unstable in aqueous solution and especially in blood because of the presence of esterases and complete degradation of the OPs during storage was frequently observed.[30-32] Our study may also indicate that OP compounds are degraded more rapidly by esterase activities than by chemical mechanisms, because we could not detect OP compound residues in blood specimens.



(a)



(b)

Figure 1. a. Peak of dichlorvos as an internal standard. **b.** Result of residue analysis.

Biological monitoring by determination of blood ChE activity has important role in assessing the exposure of workers to OP pesticides. The approach of blood ChE measurement has a role in reassuring most workers that their exposure is unlikely to result in any toxicity. It has well defined guidance values to help interpret results and is directly related to risk.[14] Anyone exposed to ChE-affected pesticides can develop lowered ChE levels. Regular checking of ChE levels provides to alert the exposed person to any change in the level of this essential enzyme before it can cause serious illness. Ideally, a pre-exposure baseline ChE value should be established for any individual before they come in regular contact with OPs.[23]

Clinical symptoms with statistically significant (p=0.032) inhibition of PChE activity were observed in the 48 workers (90.7 %) that indicating a potential toxicological hazard from OP exposure. In conclusion, occupational exposure to OP pesticides may be monitored by the measurement of the activity of ChE enzymes in order to predict the risk of pesticide poisoning. For preventing the toxicity, required safety precautions can be taken in advance.

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