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PESTICIDE-RESIDUE RELATIONSHIP AND ITS ADVERSE EFFECTS ON OCCUPATIONAL WORKERS IN DAKAHLYIA, EGYPT

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ABSTRACT

This study was designed to determine pesticide residues in blood, evaluate its effect on biochemical parameters and assess adverse health effects in farmers, pesticide market-workers and pesticide spray-workers. The level of awareness or knowledge about precautionary measures for pesticides safety was also assessed. The field survey indicated that 40.0, 6.7 and 12.0% of farmers, market-workers and spray-workers, respectively, did not wear protective clothing. However, most of them (83.3, 93.3 and 88.0%) had knowledge about the safety precautions to be taken during pesticide formulation and application. Significant decrease in hemoglobin level and platelet count was detected in spray-workers, but significant increase in white blood cell count was noticed in pesticide-market workers and spray-workers. No significant changes were observed in liver function, but significant inhibition in acetylcholine esterase activity and significant increase in prothrombin time were noticed in all pesticide-exposed subjects. A significant rise in urea concentration was observed in pesticide-spray workers, but no significant differences in creatinine level were seen. The residue analysis revealed that 76.7, 92.5 and 100.0% of farmers and market- and spray-workers, respectively, had insecticide residues in their blood. Also, most of the study subjects had multiple residues above the acceptable daily intake. Therefore, creating the awareness among pesticide users to improve and encourage safe use and handling of pesticides through education, proper guidance and fore-warning about the risks involved in misuse of poisonous materials are required.

Keywords: Biochemical markers, Egypt, occupational workers, pesticide exposure, pesticide residues, precautions, safety

INTRODUCTION

Pesticides use in agriculture is the most economical approach to control various pests, though they are considered major contaminant of our environment. The World Health Organization and United Nations Environmental Program have estimated one to five million cases of pesticide poisoning among agricultural workers each year with about 20,000 fatalities, mostly reported from developing countries (WHO, 1990; Pimental *et al.*, 1992). The spray-workers are directly exposed to pesticides during mixing, handling, spray, and through contaminated soil, air, drinking water, eating food and smoking at work places. The farm workers are, therefore, occupationally exposed to pesticides and may absorb them by inhalation, ingestion and dermal contact (Vega, 1994; Mathur *et al.*, 2005). The residue concentrations of these compounds in affected workers may lead to a variety of metabolic and systemic dysfunctions and, in some cases, outright disease states. Therefore, the excessive and repeated pesticide use has promoted toxicological problems in spraying community (Karalliede and Senanayake, 1999; Brown *et al.*, 1989; Azmi *et al.*, 2006). In Egypt the information on the impact of pesticides on health aspects of farm-workers and pesticide-dealers is lacking and base-line information needs to be generated so that risk exposure of farming community may be minimized. Hence, this study was designed to determine pesticide residues in blood and their correlation with biochemical markers for assessment of adverse health effects on farmers, market-workers and spray-workers as well as to assess the level of knowledge on precautions of pesticides safety.

MATERIALS AND METHODS

The study was conducted from July 2009 to June 2010 in seven villages (El-Mahmodia, Met Tarif, El-Yosifia, Deiarb, El-Daraksa, Hamada and Ali Hendi) located in Dekrnes, Meniate El-Nasr and Baniebad provinces, Dakahlyia Governorate, Egypt.

Basic design and sample size

Seventy healthy male individuals in age group of 30-55 year comprising of 30 farmers, 25 spray workers and 15 market workers were selected for the present study. The individuals selected had history of exposure to different classes of pesticides for 5 to 15 years. They were compared with 25 control individual residents of same area who had no history of pesticide exposure, either as farm worker or as pesticide dealer.

Field survey

All the individuals were provided a questionnaire seeking information on the kinds of pesticides they mostly used, protective equipment or cloths during preparation and application of pesticides, concentrations recommended for pesticides use. In addition, the questionnaire elicited information about the re-entry period (the minimum amount of time that must pass between the time of application of pesticide and the time the farmers could go into the field without wearing personal protective equipment). The individuals selected included those who worked in both field crops and vegetables on the same ground but in different seasons.

Hematological effects

a) Sample collection: Fresh blood samples were collected from the arm vein (10 ml). Each blood sample was divided into three tubes, the 1st tube contained heparin for hematological assays. In 2nd tube the blood sample was left for a short time to allow blood coagulation for biochemical analysis (AST, ALT, AChE, urea, creatinine and prothrombin time) and the 3rd tube contained blood sample for the determination of pesticide residues.

b) Hematological analysis: The blood in heparinized ampoules was analyzed for white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) and platelets (PLT) counts as per the method of Schalm (1986).

c) Biochemical analysis: Plasma was separated by centrifugation at 1500 rpm for 15 min. Serum enzymes and biochemical analysis were carried out in Medical Biochemistry Lab (Faculty of Medicine, Mansoura University, Mansoura, Egypt). Plasma alanine aminotransferase (ALT) and aspartate aminotransfersae (AST) activities were determined according to IFCC method of Bergmeyer *et al.* (1998a,b); while plasma acetyl cholinesterase (AChE) activity was measured as per Ellman's colorimetric method (Elmann *et al.*, 1961). Urea and creatinine concentrations were determined according to the methods of Sampson and Baird (1979) and Spencer (1986), respectively. Prothrombin time (PT) was measured according to method described by Dacie and Lewis (1984).

Pesticide residue analysis

a) Extraction from blood serum: Extraction of pesticide residues in the serum was as per the method of Rivas *et al.* (2001). Aliquots of 2.0 ml serum samples of each individual and control were spiked separately by adding appropriate volumes of working standard solutions equilibrated for 3.0 h at room temperature in a test tube. Methanol (1 ml) was sequentially added to 2.0 ml sample by mixing in a rotary mixer for 1.0 min. and then 2.5 ml *n*-hexane: diethyl ether (1:1 v /v) was added. The solution was agitated and collected, and the aqueous phase extracted twice with 2.5 ml *n*-hexane: diethyl ether (1:1 v/v). The combined organic phases were evaporated and concentrated to 1.0 ml in a graduated test-tube under a gentle stream of nitrogen.

b) Clean-up: Clean-up of the extract of pesticide residues in serum was performed as per the method of Mercedes *et al.* (2004). A florisil column of 200 x 12 mm topped with anhydrous sodium sulphate was prepared and eluted with n-hexane. The extracts of each sample were passed twice through it. Elute containing pesticides was evaporated and dried completely under a gentle stream of nitrogen. The samples were dissolved in 1.0 ml *n*-hexane and then injected into GLC and HPLC systems.

c) Quantitative determination: The whole cleaned up extracts of organphosphorus and pyrothroid residues were performed by GLC (Hewlett Packard 6890 series) equipped with electron capture detector (ECD) under the following conditions: column: HP-17 (30 m x 0.32 mm x 0.25 µm film thickness), temperatures: column 240ºC; detector 350ºC and injection 320ºC. The quantitative analysis of carbamate pesticide residues was performed by HPLC (Agilent 1100 Series with workstation). UV Diod-array detector set at 220 nm and the analytical column Nucleosil-C18, 5 µm (4 x 250 mm) was used. The mobile phase was acetonitrile-water at flow rate 1 ml min^{-1}. All solvents and chemicals used were of analytical grade, free of interfering residues, as tested by Gas chromatograph. The statistical significance of data was assessed by Duncan and Tukey test at $p<0.05$ and $p<0.01$ (Snedecor and Cochran, 1980)**.**

RESULTS

Field survey

The most frequently used pesticides by the subjects in this study are shown in Table 1. Organophosphates, carbamates and pyrothrids represented 20.7, 20.7 and 10.3%, respectively, of the used pesticides in Egypt. Zinc phosphide was the most frequently used insecticide (97.14%) followed by chlorpyrifos and malathion (94.3%). The study revealed that the majority of study subjects were not taking necessary precautions to prevent hazards associated with their use (Table 2 and 3). The results of survey revealed that 60.0, 6.7 and 12.0% of farmers, market-workers and spray-workers did not wear protective apparels (such as overall, boots, gloves, etc.). Only 16.7, 60.0 and 80.0% of farmers, market-workers and spray-workers, respectively, wear overall, while 10.0, 20.0 and 84.0%, respectively, wear special boots and 6.7, 66.7 and 24.0%, respectively, wear gloves. The farmers did not use mask while 26.7 and 24.0 % of market- and spray-workers used masks. About 20.0 and 52.0% of farmers and spray-workers used hats, but pesticide-market workers did not use them.

The survey revealed that the most of subjects were washing themselves after pesticides operation (Table 3). Also 83.3 and 86.7% of farmers and market-workers smoke, drink and eat food during mixing or applying pesticides, while about 40.0% of pesticide-spray workers practice these habits. About 16.7% farmers and 20.0% pesticide-spray workers do have knowledge on re-entry periods. Majority of farmers and pesticide-spray workers do not bother to read the pesticide labels and contrarily 80% pesticide-market workers read labels. Interestingly 20.0, 13.3 and 40.0% of farmers, market-workers and spray-workers, respectively, reported that they re-used the pesticide containers, while the majority of farmers and spray workers (80.0 and 60.0%, respectively) leave it in the field after use.

Hematological effects

No significant differences were observed between RBC counts in pesticides-exposed subjects and control group (Table 4). However, a significant decrease in hemoglobin [Hb] level (-12.1%) and platelet count (-46.6% below control level) was observed in pesticide-spray worker group. On contrary, a significant increase in WBC counts was noticed in pesticide market- and spray-worker groups (34.6 and 73.9% above the control level, respectively) as compared with control.

Table 1: Pesticides frequently used by the subjects in this study

Pesticide	Group	*WHO category	Type of use	%**
Zinc phosphide	Inorganic compound	IB	Rodenticide	97.1
Chlorpyrifos	Organophosphorus	II	Insecticide	94.3
Malathion	Organophosphorus	III	Insecticide	94.3
Carbofuran	Carbamate	II	Nematicide	90.0
Abamectin	Avermectin	IB	Acricide	80.0
Lufenuron	Benzoylurea	U	IGR	80.0
Copper hydroxide	Inorganic compound	III	Fungicide	78.6
Lambdacyhalothrin	Pyrothrid	II	Insecticide	71.4
Profenofos	Organophosphorus	II	Insecticide	71.4
Mancozeb	Thiocarbamate	U	Fungicide	67.1
Brominal	Hydroxybenzonitrile	II	Herbicide	64.3
Pirimicarb	Carbamate	II	Insecticide	62.9
Fenpyroximate	Fenpyroximate	II	Acricide	61.4
Clodinafop - propargyl	Aryloxyphenoxy-propionate	III	Herbicide	61.4
Maneb	Thiocarbamate	U	Fungicide	58.6
Chlorpyrifos-methyl	Organophosphorus	II	Insecticide	57.1
Thiobencarb	Thiocarbamate	II	Herbicide	57.1
Diflubenzuron (IGR)	Benzoylurea	U	IGR	54.3
Dicofol	Organochlorine	III	Acricide	54.3
Dimethoate	Organophosphorus Juvenile	II	Insecticide	50.0
Pyriproxyfen	hormone mimic	U	IGR	44.3
Methomyl	Carbamate	IB	Insecticide	42.9
Fluazifop-ρ-butyl	Aryloxyphenoxypropionate	U	Herbicide	42.9
Glyphosate	Glyphosate-diammonium	U	Herbicide	35.7
Fenvalerate	Pyrothrid	II	Insecticide	32.9
Thiamethoxam	Neonicotinoid	III	Insecticide	28.6
Acetamiprid	Neonicotinoid	II	Insecticide	25.7
Triazophos	Organophosphorus	IB	Insecticide	25.7
Alpha-cypermethrin	Pyrothrid	II	Insecticide	24.3

*WHO (2005) classification: IB = Highly hazardous; II = Moderately hazardous; III = Slightly hazardous; U = Unlikely to pose acute hazard in normal use. **% = Percent of pesticide most frequently used by the subjects

Table 2: The response of farmers, pesticide market-workers and spray-workers regarding wearing of protective equipment

Protective equipment	Farmers (%)	Pesticide-market workers (%)	Pesticide-spray workers (%)	Mean (%)
Wear overall	16.7	60.0	80.0	48.57
Special boots	10.0	20.0	84.0	25.70
Gloves	6.7	66.7	24.0	25.70
Mask	0.0	26.7	24.0	14.29
Wide hat	20.0	0.0	52.0	27.14

Biochemical effects

A slight insignificant increase was observed in AST and ALT activities in all the subjects (Table 5). Higher level of both these enzymes was observed in pesticide spray-workers (8.41 and 34.41% above the control level, respectively). A significant decrease in AChE activity was observed in pesticide-exposed groups in comparison to control. High inhibition rate was observed in spray-workers followed by market-workers (-48.7 and -41.5%, respectively). On the contrary, a significant rise in urea concentration was noticed in spray workers (+50.0%), but no significant differences were observed in creatinine concentration. This study revealed a positive correlation between the pesticides exposed with prothrombin time (PT). PT was significantly raised among the farmers, market workers and spray workers (12.0, 23.5 and 44.7% above the normal level, respectively).

Table 3: The response of farmers, pesticide market-workers and spray-workers regarding practices on safety measures during pesticide operations

Precautions	Farmers (%)	Market-workers (%)	Spray-workers (%)	Mean (%)
Wash after pesticide operation	66.7	93.3	88.0	80.00
Smoke, drink and eating food	83.3	86.7	40.0	60.57
Re-entry period	16.7	0.0	20.0	14.29
Read pesticide labels	36.7	80.0	24.0	41.43
Re-use pesticide containers	20.0	13.3	40.0	25.70
Didn't follow precautions	16.7	6.7	12.0	14.29

Table 4: Effect of pesticide residues on hematological parameters of farmers, pesticide market-workers and spray-workers

Treatments	RBC (10^6)	Hb (mg)	Platelets (10^3)	WBC (10^3)
Control	5.02[a]	13.74[a]	299.0[a]	4.68[c]
Farmers	4.83[a] (-3.78)*	14.04[a] (+2.18)	240.2[ab] (-19.67)	4.66[c] (-0.43)
Market workers	4.70[a] (-6.37)	13.42[a] (-2.33)	263.4[ab] (-11.91)	6.30[b] (+34.60)
Spray workers	4.68[a] (-6.77)	12.08[b] (-12.10)	159.6[b] (-46.60)	8.14[a] (+73.93)
$LSD_{0.05}$	0.85	1.30	103.9	1.12

The figures superscripted with same alphabets in the same column do not significantly differ from each other as per Duncan test. *The values in parenthesis are the percent content in comparison to the respective control.

Table 5: Effect of pesticide residues on biochemical parameters of farmers, pesticide market-workers and spray-workers

Treatments	AST (u ml^{-1})	ALT (u ml^{-1})	AChE (u ml^{-1})	Urea (mg dl^{-1})	Creatinine (mg dl^{-1})	Prothrombin time (seconds)
Control	21.4[a]	18.6[a]	1874.6[a]	28.8[bc]	1.25[a]	10.96[d]
Farmers	21.8[a] (+1.8)*	22.4[a] (+20.43)	1573.4[b] (-16.1)	23.4[c] (-18.75)	1.12[a] (-10.4)	12.28[c] (+12.00)
Market-workers	23.2[a] (+8.4)	22.6[a] (+21.51)	1096.2[c] (-41.5)	36.8[ab] (+27.8)	1.21[a] (-3.2)	13.54[b] (+23.54)
Spray-workers	23.2[a] (+8.4)	25.6[a] (+34.4)	962.0[c] (-48.7)	43.2[a] (+50.0)	1.33[a] (+6.4)	15.86[a] (+44.70)
$LSD_{0.05}$	11.2	14.8	165.11	12.72	0.298	0.99

The figures superscripted with same alphabets in the same column do not significantly differ from each other as per Duncan test. *The values in parenthesis are the percent content in comparison to the respective control.

Pesticide residues

The detection limits of pesticides ranged between 0.001 to 0.0025 µg ml^{-1}. Percent recoveries in reference samples were 82.0 to 93.0%. Accordingly, the sample analysis data was corrected for these recoveries. About 76.7, 92.5 and 100% of farmers, market-workers and spray-workers had varied levels of insecticide residues in their blood (Table 6). About 60.0 and 23.3% of farmers had chlorpyrifos and lambada-cyhalothrine (0.022 and 0.014 mg kg^{-1}) residues above the acceptable daily intake (ADI) in their blood. In addition, most of the pesticide market-workers were observed to have multiple pesticide residues above ADI. About 80.0% of them had carbofuran residues and 73.3% had chlorpyrifos (0.217 and 0.137 mg kg^{-1}). All pesticide spray-workers had high amount of residues in their blood; most of them had chlorpyrifos (84.0%), profenofos (72.0%), lambda-cyhalothrine (64.0%), pirimicarb (52.0%), carbofuran (28.0%) and triaziphos (24.0%) residues above the recommended ADI levels because of their extensive use. Further, about 23.3 and 7.5% of farmers and market-workers, respectively, had no insecticide residues in their blood.

Table 6: The percent of farm workers, pesticide-market workers and pesticide-spray workers having different pesticide residues and mean concentration of residues (mg kg^{-1} b.w.) in their blood

Pesticides	ADI* (mg kg^{-1} b.w**)	Farmers			Pesticide-market workers			Pesticide-spray workers		
		%	Pesticide residue (mg kg^{-1})		%	Pesticide residue (mg kg^{-1})		%	Pesticide residue (mg kg^{-1})	
			Range	Average		Range	Average		Range	Average
Chloropyrifos	0.01	60.0	0.07-0.34	0.022	73.3	0.1-2.08	0.137	84.0	0.31-3.76	0.247
Malathion	0.30	33.3	0.009-0.26	0.016	53.3	0.09-1.78	0.097	72.0	0.16-2.44	0.183
Profenofos	0.01	36.7	0.03-0.178	0.008	53.3	0.3-1.67	0.088	72.0	0.26-2.67	0.208
Pirimicarb	0.02	40.0	0.007-0.09	0.004	33.3	0.05-0.95	0.034	52.0	0.47-1.8	0.117
Carbofuran	0.02	26.7	0.006-0.04	0.009	80.0	0.14-2.6	0.217	28.0	0.07-0.49	0.026
Lambdacyhalothrin	0.005	23.3	0.05-0.17	0.014	40.0	0.07-0.53	0.046	64.0	1.08-2.11	0.136
Methomyl	0.03	0.0	nd†	nd	26.7	0.04-0.09	0.007	32.0	0.36-0.97	0.067
Triazophos	0.001	3.3	0.001	0.001	0.0	nd	nd	24.0	0.0005-0.004	0.002
Dimethoate	0.002	0.0	nd	nd	6.67	0.001	0.001	12.0	0.0007-0.0017	0.001
Fenvalerate	0.02	0.0	nd	nd	0.0	nd	nd	12.0	0.004-0.01	0.008
Persons having no residues		23.3			7.5			0.0		

*Acceptable daily intake; **b.w. = Body weight; †nd = Not detected

DISCUSSION

The present study was carried out in various villages located in Dakahlyia Governorate, Egypt, where infestation level of pests is very high. Ever growing demand for enhancing crop production to meet the requirements of increasing population and the need to enhance farm income tends farmers to use pesticides excessively and irresponsibly.

The major pesticides used (58.62%) by the farmers, pesticide market-workers and spray-workers in the study area include zinc phosphide, chlorpyrifos, malathion, carbofuran, abamectin, lufenuron and copper hydroxide. As per WHO (2005) most of the pesticides used in these villages are classifies either highly or moderately hazardous.

Interviews showed that the majority of farmers and pesticide spray-workers do not take the necessary precautionary measures to prevent hazards associated with their use. Also, the low education level of study groups coupled with lack of good training of pesticides resulted in high exposure hazards (Tijani, 2006; Damals *et al.*, 2006). Similar results were obtained by Tchounwou *et al.* (2002) who reported that in Menia El-Kamh in Sharkia Governorate (Egypt) more than 95% of farm workers do not practice safety precautions during pesticide formulation and application.

Our studies revealed significant changes in Hb level and platelets counts in pesticide spray workers and WBC counts in pesticide market-workers and spray-workers. The results are in agreement with Amr (1999), Abu Mourad (2005) and Al-Sarar *et al*. (2009) who reported that occupational exposure to pesticides resulted in alterations in hematological parameters.

The increase in ALT and AST activities are good indicators of hepatic toxicity (Hall, 2001). The present study showed that insignificant elevation in liver functions (AST and ALT levels) of all study subjects. Similar observations have been made by Al-Sarar *et al.* (2009). Nevertheless, significant increase in the levels of these enzymes was seen in occupationally exposed workers (Tomei *et al.*, 1998; Khan *et al.*, 2008); and in sprayer-workers in grape gardens in India (Patil *et al*., 2003). Plasma AChE activity has been used for several years as indicator to estimate the risks associated with pesticide-induced toxicity in occupationally exposed workers (Dasgupta *et al*., 2007; Khan *et al*., 2008; Shalaby and Abd El-Mageed, 2010). Present study revealed a negative correlation between pesticide residues with AChE activity. We found significant inhibition in its activity in exposed subjects than that of the control. High inhibition rate was observed in pesticide-spray workers followed by pesticide-market workers thereby indicating that the study subjects had pesticide residues in their blood especially organophosphorus and carbamate compounds. Similar results were noticed in cotton growers in India (Mancini *et al*., 2005); in farm workers in Palestine (Abu Mourad, 2005) and in tobacco farmers in Pakistan (Khan *et al*., 2008). Of the orchard farmers of Kashmir about 31.9% patients (124 out of 389) were orchard-farm workers; and the orchard residents and orchard playing children showed higher serum cholinesterase (<6334 U/l) level (Bhat *et al*., 2010).

In present study there was significant increase in urea concentration in pesticide spray-workers, with no significant changes in creatinine level in all cases. Some previous studies have shown subtle nephrotoxic changes in workers occupationally exposed to pesticides with higher levels of creatinine and urea (Attia, 2006; Shalaby, 2006; Khan *et al*., 2008). Nevertheless, Al-Sarar *et al.* (2009) observed insignificant elevated levels of serum urea and creatinine among pesticide spray-workers of Riyadh Municipality, Kingdom of Saudi Arabia. In addition, this study revealed significant increase in prothrombin time (PT) in all cases studied. In general, this noticeable effect of pesticide upon blood coagulation could be attributed to the great defect in blood coagulability and to the severe damage of capillaries (Clarke and Clarke, 1978; Leck and Park, 1981).

Pesticide 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 be metabolized and the metabolites may accumulate to induce toxic effect (Soomro *et al*., 2008). However, the ultra low quantities of contaminants present in body indicate toxicological impact on exposed population. Present results revealed that all pesticide spray-workers had insecticide residues in their blood; however, 23.3 and 7.5% farmers and pesticide-market workers did not show presence of residues. In addition, the amounts of these residues are high in spray-workers than its values in other groups, but these amounts are very low in farmers. The spray workers during spray on crops are directly exposed to pesticides while mixing, handling and spraying as well as through contaminated soil, air, drinking water, eating food and smoking at work places. Also, pesticides-market workers are directly exposed to pesticides while handling and opening of pesticide containers in pesticide stores, but farmers are working in the field after pesticides operation. In our study, most of subjects had multiple pesticide residues above ADI in their blood, which is injurious to health. Most of them had chlorpyrifos, profenofos, carbofuran and lambda-cyhalothrin residues. Similar results were obtained by Coye *et al*. (1986) and Khan *et al*. (2008) who reported that the tobacco farmers had multiple pesticide residues above ADI in their blood consisting of 63% methomyl; 56% thiodicarb; 62% cypermethrin; 49% imidaclprid; 32% methamidophos and 27% had endosulfan residues. Sosan *et al*. (2008) has reported that 42 out of 76 cacao farmers had residues of diazinon, endosulfan, propoxur and lindine in their blood. Similarly Bhat *et al.* (2010) has reported that 90.04% brain tumour patients (389 out of 432) in Kashmir were orchard farmers exposed to high levels of multiple types of neurotoxic and carcinogenic pesticides for more than 10-20 years.

Conclusion: The present study revealed that most of persons studied did not take necessary precautionary measures to avoid the hazards associated with pesticide marketing, handling and application. Analysis showed that low education level of users and lack of proper training about safe pesticide usage and handling resulted in high occupational hazards. Most of individuals studied had multiple pesticide residues above ADI in their blood. The study suggests promotion of awareness among pesticide-users with right practices for safe use and handling of pesticides. Pesticides are absorbed by inhalation, ingestion and/or dermal contact so pesticide users should wear protective clothing. Also, they are highly advised to read pesticide labels before use. The role of government regulations and agriculture experiment services as well as the cooperation of pesticide manufactures of is vital in reducing the risks and hazards of pesticides.

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