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Pesticide-Residue Relationship and Its Adverse Effects on Occupational Workers

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1. 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 (WHO, 2005) 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 (Pimental et al. 1992). There are several definitions of pesticide; the Food and Agriculture Organization (FAO) defines pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pest during the production, processing, storage or marketing of food in all agricultural commodities for controlling the pests (FAO 1986). Pesticides are playing a pivotal role in meeting the food, cotton fibre and tobacco demand of escalating population and control of vector-borne diseases. However, most of the applied pesticides get dispersed in the environment and affects the health of unprotected agricultural and industrial workers. Pesticides are used extensively throughout the world. The three major routes of entry for pesticides include contamination of the skin, mouth and the nose. Although pesticides furnish some benefits for crop, they entail a number of risks and problems. The public health issue of pesticide exposure is further complicated by the presence of impurities in so-called, inert-ingredients such as solvents, wetting agents and emulsifiers (Hashmi&Dilshad 2011).

The main similarity between pesticide exposure of farm workers' children and lead exposure of children living in poverty is that the substances are present in the home, are difficult for the family to control, and are inequitably distributed across ethnic and socioeconomic groups. Unlike lead, the potential developmental effects of childhood exposure to many

types of pesticides are greatly understudied. Some pesticides have been shown to cause behavioural effects in rodents such as hyperactivity, learning and memory problems, and altered habituation (Icenogle et al. 2004). In spite of the paucity of research on the effects of pesticides on human neurobehavioral development, there are reasons to be concerned about children's exposure. First, two widely used classes of insecticides, organophosphates and carbamates, inhibit cholinesterase. Cholinesterase inhibition leads to excess acetylcholine at the synapse, which in turn causes over activation of cholinergic neural pathways. There is evidence that organophosphate and carbamate pesticides can negatively affect early life rodent brain development by interfering with gene signalling by cholinesterases as well as by inducing faulty wiring of the brain via other mechanisms (Slotkin 1999; Aldridge et al. 2005; Sallam et al. 2006, 2009a,b).

These chemicals are suspected of producing adverse health effects based on their structural similarity to proven toxicants. Exposure to pesticides is one of the most important occupational risks among farmers in developing countries (Wesseling et al. 2001; Konradsen et al. 2003; Coronado et al. 2004; Shalaby et al. 2012). Occupational exposure to pesticides is of great interest in order to identify the hazards of pesticide use and the establishment of safe methods of pesticide handling. This is because pesticide misuse in various sectors of the agriculture often has been associated with health problems and environmental contamination worldwide (Soares et al. 2003; Mancini et al. 2005; Remor et al. 2009). Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides, is one of the major reasons for the high incidence of pesticide poisoning in developing countries (Konradsen et al. 2003; Hurtig et al. 2003; Atreya 2008). In general, knowledge of the main determinants of pesticide exposure in developing countries is often poor and also exposure situations may differ among countries (Hashmi&Dilshad 2011; Shalaby et al. 2012).

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 (Brown et al.1989; Karalliede&Senanayake 1999; Azmi et al. 2006). A major factor of pesticide contamination or poisoning in developing countries is the unsafe use or misuse of pesticides. Elements of unsafe use of pesticides that have been identified by past research include erroneous beliefs of farmers about pesticide toxicity, lack of attention to safety precautions, environmental hazards, and information about first aid and antidotes given by the label, the use of faulty spraying equipment or lack of proper maintenance of spraying equipment, and lack of the use of protective gear and appropriate clothing during handling of pesticides (Hurtig et al. 2003; Damalas et al. 2006a, b; Ajayi&Akinnifesi 2008; Chalermphol&Shivakoti 2009; Plianbangchang et al. 2009; Sosan&Akingbohunge 2009; Hashmi&Dilshad 2011).

In view of the adverse health effects from the unsafe pesticide use, the latency of the adverse effects, the reported lack of awareness of the adverse health effects of pesticides by some farmers, and the erroneous belief of invincibility by others, it becomes imperative that the potential hazards of unsafe pesticide use should be clearly communicated to the farmers. Research has often emphasized the need to increase the awareness of farmers about the consequences of unsafe pesticide use and the importance of communication and education programs aiming to reduction of risk (Ibitayo 2006; Hashemi et al. 2008; Oluwole&Cheke 2009; Sosan&Akingbohunge, 2009; Damalas&Hashemi 2010)

Exposure to pesticides at any point in the life cycle has the potential for causing a range of short-term or long-term health problems. Documented health effects include a wide variety of illnesses and diseases, from eye irritation, skin rashes and respiratory problems to neurological damage, birth defects, cancer and death. The risk for and severity of adverse health effects from pesticide exposure varies significantly depending on many factors, including individual characteristics such as age and health status, the specific pesticide, and exposure circumstances. Exposure to pesticides at certain developmental stages of life can result in irreversible damage to organ structure and function. Of particular concern is the effect of exposure at during the reproductive cycle, from pre-conception to breast feeding, because of the possibility of poor birth outcomes, congenital anomalies, developmental deficits, and possibly childhood cancer (Barthel 1981; Karabay et al. 2004; Hernandez et al. 2004; Sanborn et al. 2004; Strong et al. 2004; Hernandez et al. 2006; Hayes et al. 2006; El-Wakeil et al. 2009).

Farm worker families often live near or on the farms on which they work, and thus spend much of their time in close proximity to areas where pesticides are applied on a regular basis. Twenty-one percent of farm workers are women (Carroll et al. 2005), who may be directed to or inadvertently enter recently treated fields while pregnant. Women in farm worker households who do not work in the fields may still be exposed to pesticide residues brought home by farm worker household members on their shoes, clothes and skin; from nearby applications that drift or are directly sprayed on outdoor play areas; and from chemicals used to control pests in and around the home, especially in poor quality housing.

Agricultural extension is a major channel of communication between farmers and research experts which can improve crop production from many points of view as it provides a good link between farmers and research institutes where several agricultural technologies, including pesticides and the relative technology, are developed, tested, and modified accordingly. Training programs can play a crucial role in pest control decisions, providing farmers with the technical knowledge that is necessary for the selection of appropriate pest management methods and also for safe and effective pesticide use. Despite the appearance of homogeneity, often small farmers have different production practices, needs, and constraints (Carr 1989). A successful agricultural extension program, therefore, should not consider all individuals in a target group based on several variables such as age, gender, income, and types of crops (Sallam 2008; Shalaby et al. 2012).

2. Exposure pathways

Pesticides are used in 85% of homes in the US (Whitmore et al. 1992), but they or their residues can be found even on surfaces that have never been directly or peripherally treated. POPs introduced into the environment years ago are still around today, transported by human activity and through the food chain. Despite being banned in the US (and many other countries) some 30 years ago, traces of these insecticides are still found in the homes and bodies of individuals in the US who were not even alive when these products were used (Weiss et al. 2004; Wolff et al. 2007). Chlorpyrifos (a non persistent OP) has also been found to accumulate on newly-introduced surfaces, such as pillows, carpet and soft toys, when brought into a treated area up to two weeks after application, even if applied according to manufacturer's instructions (Gurunathan et al. 1998).

In agricultural settings, work-to-home exposure, or a "take-home pathway," has been identified as a key source of pesticide residues (primarily to OPs) in children's environment ((Fenske et al. 2000; Curl et al. 2002; Thompson et al. 2003; Rao et al. 2006; Coronado et al. 2006). Workers who are exposed on the job on a daily basis, whether as applicators or re-entry workers, are likely to carry home pesticides on their shoes, clothes, skin, and vehicles. Most workers are not provided with adequate washing or changing facilities to remove residues and put on clean clothes before leaving the work-site. If these workers do not take basic precautions (e.g., removing work shoes outside the dwelling, showering before picking up a child); they may transfer residues to the indoor environment or directly to other household members.

The primary routes by which pesticides enter the body are ingestion in food, soil, or water; inhalation, through the skin, and through the eyes (Arcury et al. 2000). OCs are absorbed through the lungs, stomach and skin, and excreted only slowly, sometimes over a period of years (e.g., DDT) (Pohl & Tylenda 2000; Cohn et al. 2007). Dietary ingestion is a significant source of exposure, especially for infants and children (Garry 2004). The residue monitoring program conducted by the FDA in 2003 found measurable levels of pesticides in baby foods, including DDT (6% of samples), captan + THPI (a possible carcinogen) (9%), carbaryl (carbamate) (6%), endosulfan (9%), dimethoate (4%), malathion (3%), and chlorpyrifos (all OPs) (2%) (FDA 2005; Sallam et al. 2006).

Post-natally, infants can be exposed to pesticides via breast feeding. The POPs, despite having mostly been banned, are still found in breast milk because they are stored in body fat (Weiss et al. 2004; Jurewicz et al. 2006). Postpartum weight loss increases the likelihood of the release of OCs into the breast milk (Jurewicz et al. 2006). There is some evidence that the maternal body burden is actually transferred to her children via breast feeding, as the pesticide concentrations decrease with the more times a mother has breastfed (Nickerson 2006). Fortunately, the benefits of breast feeding still far outweigh the possibility of harm from pesticide transfer in breast milk, and should be encouraged for all mothers regardless of exposure history (Nickerson 2006; Eskenazi et al. 2006). Pesticides exposure occurs in different ways: dermal, oral, respiratory and conjunctival routes.

2.1. Dermal exposure

It occurs by not washing hands after handling pesticides or their containers. Splashing or spilling of pesticide on skin by wearing pesticide-contaminated clothing and applying pesticides in the windy weather. Touching treated plants or soil also leads to dermal exposure. Exposures occur by rubbing eyes or forehead with pesticides contaminated gloves or hands, splashing pesticides in eyes, application in windy weather, drift exposure and mixing/loading of dry formulations without wearing goggles.

2.2. Oral exposure

Hands not washed before eating, smoking or chewing, pesticide splashed into mouth. Accidental application of pesticides to food, storing pesticides in drinking containers and drift on lip or in mouth also leads to oral exposure.

2.3. Inhalational exposure

Exposed to drift during or after spraying, mixing/loading, dusts, powders or other dry formulations; use of inadequate or poorly fitted respirators.

2.4. Exposure on respiratory system

The crops, activities, and exposure agents that can lead to respiratory disease are extraordinarily diverse and vary significantly by seasons, geography and type of agriculture. The number of substances affecting respiratory health to which a worker is exposed while working in an agricultural setting is enormous: pesticides, including insecticides, herbicides, and fumigants; other agricultural chemicals, including fertilizers and plant growth regulators; the crops and related allergens, such as pollens, pests, and microorganisms; and the land itself, including organic and inorganic dusts, to name just a few (Schenker et al 1998; Schenker 2005). Further complicating the issue, the likelihood that an individual worker has been exposed to but a single identifiable agent is small. Measuring exposure is also challenging, which makes dose-response relationships difficult to assess, and exposure limits have not been set for most relevant agents. Agricultural respiratory disease often goes untreated and unreported, especially by small operations not regulated by the Occupational Safety and Health Administration (OSHA), making it nearly impossible to determine the true extent of the problem (Kirkhorn & Garry 2000; Ross et al. 2001).

All children are at risk for contact with environmental toxins, but the burden of toxic exposures is disproportionately allocated to poor ethnic minorities (Schell 1997; Moore 2003; Dilworth-Bart & Moore 2006). "Economic factors not only constrain choices but also inequitably distribute human made stressors." and the psychosocial stress and environmental pollutants associated with poverty do not occur independently of one another. Rather, the effects may accumulate through risk focusing, a process by which exposures to toxic or infectious environmental materials are differentially allocated to a specific group partly because of previous exposure to those materials (Schell 1997; Yassin et al. 2002).

3. Pesticides poisonings

Agro-chemical industry has offered thousands of compounds. The climatic condition of Pakistan favors pest build up that destroys about 20 percent of potential agricultural crop. The health of the pesticides handlers and farmers in particular are at high risks due to irrational use of pesticides. Pesticides cause the acute and chronic health effects; organophosphate and carbamate groups are more important. These insecticides inhibit cholinesterase, an enzyme critical for normal functioning of the nervous system (Travisi&Nijkamp 1998; Gelman& Hill 2007; Soares& Porto 2012).

3.1. Prevalence of pesticides poisoning

In USA, more than 18,000 products are licensed for use and each year more than 2 billion pounds of pesticides are applied to crops, gardens, in homes etc. (U.S EPA 2002). The major economic and environmental losses due to the application of pesticides in public health were 1.1 billion dollars per year in USA (Pimentel 2005). Such wide spread use results in pervasive human exposure. Evidence continues to accumulate that pesticide exposure is associated with impaired health. Occupational exposure is known to result in an annual incidence of 18 cases of pesticides related illness for every 100,000 workers in U.S (Calvert et al. 2004). Pesticide poisoning is a major public health problem in many developing countries (Xue et al. 1987; Jeyaratnam 1990). In developing world, pesticide poisoning causes more deaths than infectious diseases. Pesticide poisoning among farmers and occupational workers in developing countries is alarming (McCauley et al. 2006). WHO estimated approximately 20,000 workers die from exposure every year, the majority in developing countries (Pimentel et al. 1992; Kishi et al. 1995). The number of intoxications with organophosphates is estimated at some 3000,000 per year and the number of deaths and casualties some 300,000 per year (Peter 2003). Ahmed and co workers have reported 64 percent of fatal cases of acute pesticides poisoning in Multan, Pakistan occurred due to Ops pesticide spraying (Ahmad et al. 2002; Ahmed et al. 2006) However another study revealed 21 percent of occupational pesticides poisoning in hospitalized patient (Afzal et al. 2006).

3.2 Acute toxicity

Organophosphorous compound exert acute systemic toxicity by inhibiting the enzymes AChE through a process of phosphorylation. Pesticides bind to cholinesterase and block the hydrolysis of the acetylcholine and acetic acid at the post synaptic junctions without junctioning acetyl cholinesterase; acetylcholine accumulates (Chan & Critchley 1998; Mason 2000). OPs induced neuronal symptoms are a consequence of axonal death. Following OPs exposures inhibition of neuronal enzymes, called neuropathy target esterase, occurs and many of them are irreversible.

4. Occupational health & safety (OHS) (clinical recommendations)

Health care providers are in an ideal position to identify and assess a patient's risk for exposure. The first step is to obtain an environmental history that covers residential and employment histories, types of work activities performed currently and in the relevant past, and possible sources of exposure to biological or chemical agents. For each exposure source identified, additional information needs to be collected, such as frequency, duration, and intensity. Women who are pregnant or planning a pregnancy, especially those currently performing farm work, should be informed of the implications of exposure before, during and after pregnancy, and assisted in making decisions that are appropriate for their individual work and home situations (McDiarmid&Gehle (2006). In addition, providers should encourage mothers to avoid exposure that might contaminate breast milk without unduly alarming them, perhaps by associating it with the importance of not smoking or drinking alcohol during pregnancy and nursing (Pohl &Tylenda 2000; Nickerson 2006). Of course, breast feeding should continue to be strongly encouraged since all evidence indicates that the known benefits far outweigh the potential risks (Eskenazi et al. 2006). Education about pesticide safety is an important measure for preventing exposure. The Migrant Clinicians Network has recently developed a 14-page full-color Spanish language comic book and Wake Forest University School of Medicine has produced patient education handouts and posters in English and Spanish. Women living in farm worker households should be offered additional education on ways they and the farm workers with which they live can reduce take-home exposure:

- remove work clothes and shoes before entering the home
- shower or bath upon returning home and before touching other people
- store and launder dirty work clothes separately from other clothing (Rao et al. 2006)

As the evidence continues to accumulate of the overall hazards that pesticides pose to human health, it is important that health care providers consider the possibility and consequences of occupational, dietary and residential exposure to pesticides for their female patients. Occupational exposure is almost certainly the primary source of exposure for farm workers and their families (McDiarmid&Gehle 2006; WHO 2006). Awareness of the ways in which pesticide exposure occurs and the danger it poses are a crucial component of comprehensive preconception and prenatal care for farm worker women.

Most of the units are seriously concerned with workers health issues. Generally, they have their own OHS plans and policies, which they endeavour to, implement and follow. Following OHS issues, which require more attention are identified because without following these practices a proper assessment of the workers exposure cannot be made:

- i. Most of the units are not carrying out the required monitoring of the working air quality, with respect to pesticides and solvents.
- ii. Records of accidents and disease are not being properly maintained.
- iii. Many of the antidotes are not available readily in the market; this situation is not satisfactory to cope with emergency.

5. Local study

In Egypt the information on the impact of pesticides on health aspects of farm workers and pesticides dealers is lacking and base-line information needs to be generated so that risk exposure of farming community may be minimized. Hence, this book chapter is planned to explain how pesticides are dangerous for humankind, animals and food products; as well 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.

5.1. Materials and methods

The study was conducted from July 2009 to June 2010 in seven villages (El-Mahmodia, Met Tarif, ElYosifia, Deiarb, El-Daraksa, Hamada and Ali Hendi) located in Dekrnes, Meniate EL-Nasr and Baniebad provinces, Dakahlyia governorate, north 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 types 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 times 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.

5.1.1. 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 the 2 tubes the blood sample was left for a short time to allow the blood to coagulate for biochemical analysis (aminotransferase (AST), Plasma alanine aminotransferase (ALT), acetyl cholinesterase (AChE), urea, creatinine and prothrombin time) and the 3rd tube contained blood sample for determination of pesticide residues.

b. Hematological analysis

The blood in heparinised 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).

5.1.2. Biochemical analysis

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

5.1.3. 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, 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 according to the method of Mercedes et al. (2004). A florisil column of 200 x 12 mm topped with anhydrous sodium sulfate was prepared and eluted with *n*-hexane. The extracts of each sample were passed twice through it. Eluate 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 organophosphorus and pyrethroid 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 μ m film thickness), temperatures: column 240°C; detector 350°C and injection 320°C. The quantitative analysis of carbamate pesticide residues was performed by HPLC (Agilent 1100 Series with workstation). UV Diode-array detector set at 220 nm and the analytical column Nucleosil-C18, 5 μ m (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 tests at $p < 0.05$ and $p < 0.01$ (Snedecor & Cochran 1980).

5.2. Results

Field survey

The most frequently used pesticides by the subjects in this study are shown in Table 1. Inorganic compound, organophosphates, carbamates and pyrethroids are the most pesticides used in Egypt. Zinc phosphide was the most often used insecticide (97.14 %) followed by chlorpyrifos and malathion (94.3%). The study revealed that the majority of study subjects were not taking the necessary precautions to prevent hazards associated with their use (Tables 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.). While 16.7, 60.0 and 80.0% of farmers, market workers and spray workers, respectively, wear overall only 10.0, 20.0 and 84.0% wear special boots and 6.7, 66.7 and 24.0% farmers, market workers and spray workers, respectively, wear gloves. The farmers did not use mask while 26.7 and 24.0 % of market and spray workers used masks. About 20 and 52 % of farmers and spray workers use hats, but pesticides marketing 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 or drink and eat food during mixing or applying pesticides, while about 40.0% of pesticide sprayers 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.

5.2.1. 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 (-6.6% below control level) was observed in pesticide-sprayer group. On contrary, a significant increase in WBC counts was noticed in pesticides market and spray workers groups (34.6 and 73.9% above the control level, respectively) as compared with control.

5.2.2. 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 sprayers (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 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).

Pesticide	Group	WHO category	Type of use	% *
Zinc phosphide	Inorganic compound	IB	Rodenticide	97.14
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	78.57
Lambdacyhalothrin	Pyrothrid	II	Insecticide	71.42
Profenofos	Organophosphorus	II	Insecticide	71.42
Mancozeb	Thiocarbamate	U	Fungicide	67.14
Brominal	Hydroxybenzotrile	II	Herbicide	64.3
Pirimicarb	Carbamate	II	Insecticide	62.86
Fenpyroximate	Fenpyroximate	II	Acricide	61.42
Clodinafop - propargyl	Aryloxyphenoxypropionate	III	Herbicide	61.4
Maneb	Thiocarbamate	U	Fungicide	58.57
Chlorpyrifos-methyl	Organophosphorus	II	Insecticide	57.14
Thiobencarb	Thiocarbamate	II	Herbicide	57.1
Diflubenzuron (IGR)	Benzoylurea	U	IGR	54.29
Dicofol	Organochlorine	III	Acricide	54.29
Dimethoate	Organophosphorus Juvenile	II	Insecticide	50.0
Pyriproxyfen	hormone mimic	U	IGR	44.29
Methomyl	Carbamate	IB	Insecticide	42.86
Fluazifop- <i>p</i> -butyl	Aryloxyphenoxypropionate	U	Herbicide	42.9
Glyphosate	Glyphosate-diammonium	U	Herbicide	35.7
Fenvalerate	Pyrothrid	II	Insecticide	32.86
Thiamethoxam	Neonicotinoid	III	Insecticide	28.57
Acetamiprid	Neonicotinoid	II	Insecticide	25.71
Triazophos	Organophosphorus	IB	Insecticide	25.71
Alpha-cypermethrin	Pyrothrid	II	Insecticide	24.29

*Source: WHO (2005) classification: Ib = Highly hazardous, II = Moderately hazardous, III = Slightly hazardous, U = Unlikely to pose acute hazard in normal use. *% = Percent of most frequently by subjects.

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

Precautions 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

Table 2. The response of farmers, pesticide marketing and spray workers regarding wearing of protective equipment

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

Treatments	RBCs (10 ⁶)	HGB (mg)	Platelets (10 ³)	WBCs (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	4.7 a (-6.37)	13.42 a (-2.33)	263.4 ab (-11.91)	6.3 b (+34.6)
Spray workers	4.68 a (-6.77)	12.08 b (-12.08)	159.6 b (-46.62)	8.14 a (+73.93)
LSD 5 %	0.844	1.30	103.89	1.121

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 4. Effect of pesticide residues on hematological parameters of farmers, pesticide market 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 (second)
Control	21.4 a	18.6 a	1874.6a	28.8 bc	1.25 a	10.96 d
Farmers	21.8 a(+1.8) *	22.4 a(+20.43)	1573.4b(-16.1)	23.4 c(-18.75)	1.12 a(-10.4)	12.28 c(+12.0)
Market - workers	23.2 a(+8.41)	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)	15.86a(+44.7)
LSD _{0.005}	11.2	14.76	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.

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

5.2.3. Pesticide residues

The detection limits of pesticides ranged between 0.001 to 0.0025 µg ml⁻¹. Percent recoveries in reference samples were 82-93%. 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 lambda-cyhalothrine (0.022 and 0.014 mg kg⁻¹) residues above the acceptable daily intake (ADI) in their blood. In addition, most of the pesticides 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 pesticides spray workers had high amount of residues detected 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.

5.3. Discussion

The present study was carried out in some 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, 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 pesticides used are classified 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-level education 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.

Pesticides	ADI * (mg / kg b.w **)	Farmers			Pesticides market workers			Pesticide spray workers		
		%	Range mg / kg	Average mg / kg	%	Range mg / kg	Average mg / kg	%	Range mg / kg	Average mg / kg
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	0.3	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	N.D ***	N.D	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	N.D	N.D	24.0	0.0005 – 0.004	0.002
Dimethoate	0.002	0.0	N.D	N.D	6.67	0.001	0.001	12.0	0.0007 – 0.0017	0.001
Fenvalerate	0.02	0.0	N.D	N.D	0.0	N.D	N.D	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

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

Our studies revealed significant changes in HGB level and platelets counts in pesticide spray workers and WBC counts in market workers and spray workers. The results are in agreement with Amr et al. 1997; Amr (1999), Abu Mourad (2005) and Al-Sarar et al. (2009) who reported that occupational exposure to pesticides resulted in alterations of 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, 2009, 2010); and in sprayers of 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 pesticides induced toxicity in occupationally exposed workers (Dasgupta et al. 2007; Khan et al. 2008; Shalaby&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 market workers thereby indicating that the study subjects had pesticide residues in their blood especially organophosphorus and

carbamate compounds. Similar results were noticed among the cotton growers in India (Mancini et al. 2005); in Palestinian farm workers (Abu Mourad 2005) and the tobacco farmers in Pakistan (Khan et al. 2008). In orchard farmers of Kashmir about 31.9% patients (124 out of 389) were orchard-farm workers and the orchard residents and orchard playing children had higher serum cholinesterase (<6334 U/l) level (Bhat et al. 2010).

In present study there was significant increase in urea concentration in pesticide sprayers, 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 sprayers 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 & Clarke 1978; Leck & 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 pesticides marketing workers did not have 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, 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 the 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% imidacloprid; 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 lindane 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.

Generally, controlled studies have shown mixed results about chemical insecticides and chemical fertilizers. We are looking for a solution for the actual problem with intensive insecticide uses. Some support the conclusion that organic production methods lead to increases in nutrients. Other studies show no demonstrable differences. A recent analysis conducted by the London School of Hygiene & Tropical Medicine provides a comprehensive

review of the available literature (Dangour et al. 2009). The authors identified 46 studies with sufficient documentation and quality upon which they performed a systematic review. Eleven nutritional categories were evaluated. The nitrogen content of conventionally-grown plants was higher, and the phosphorus and titratable acidity levels were higher for organically-grown plants. These differences were considered biologically plausible due to differences in fertilizer use (nitrogen and phosphorus) and ripeness at harvest (titratable acidity). There was no difference for the remaining eight categories, including some key ones, including Vitamin C, phenolic compounds, magnesium, calcium, potassium, zinc, total soluble solids, and copper.

6. Conclusion

The present study revealed that most of persons studied did not take necessary precautionary measures to avoid the hazards associated with pesticides marketing, handling and application. Analysis showed that low level of education of users and lack of proper training about safe pesticide usages and handling resulted in high occupational hazards. Most of individuals studied had multiple pesticide residues above ADI in their blood which is injurious to health. 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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