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Determination of insecticide residues and their adverse effects on blood profile of occupationally exposed individuals

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ABSTRACT

Insecticides, essential for crop protection measures, leave behind several toxic residues that can result in a series of human health disorders. Therefore, this study was planned for the determination of residues and adverse effects of insecticides in blood samples of sprayers, pesticide-industry workers and controls by using blood parameters of these individuals as biomarkers. Optimized analytical methods using GC-MS and HPLC for the simultaneous detection of 22 currently used insecticides were adopted. Eight of twenty-seven (22.22%) sprayers' blood samples were found positive for five different insecticides. Eleven of twenty-seven (40.74%) pesticide-industry workers were found positive for eight different insecticides. The blood samples of both the exposed groups, sprayers and industry workers had significantly ($P < 0.001$; Mann-Whitney *U*-tests) low hemoglobin-Hb concentrations (12.17 ± 2.13 and 12.22 ± 2.37 g/dl respectively) than the average value of the control group with 14.23 ± 2.37 g/dl. The erythrocyte sedimentation rates (ESRs) in sprayers and insecticide industry workers (28.78 ± 20.72 and 28.17 ± 25.14 mm/1st h respectively) were greater significantly ($P < 0.001$; Mann-Whitney *U* test) than the control blood samples (9.53 ± 3.34 mm/1st h). These results indicate that the exposed individuals have experienced significant hemotoxic effects during insecticide exposure. The study also predicts the risk to exposed individuals in developing countries like Pakistan and demands realization of safety measures to prevent such dangerous effects of pesticide exposures.

1. Introduction

The term pesticide is generally used for any substance or a mixture of substances found naturally or synthesized by man, which can kill, deter or repel any pest (EPA, 2009). Internationally two million tons of noxious pesticides are being incorporated into the environment annually (De et al., 2014). This is especially true in the United States; where approximately 12,000 various active ingredients of pesticide are approved and sprayed on crops in 18,000 combinations (Frazier et al., 2011). Out of them, approximately 500 pesticides, like organochlorinated, organophosphates, pyrethroids and neonicotinoids with mass applications, contain lead, mercury and arsenic which are highly toxic to living organisms. Only one percent of sprayed chemicals are utilized against the pests, while almost 99% of applied chemicals are released into the environment which, ultimately are absorbed by human through food chain (Zhang et al., 2011). Among all pesticides the insecticides and rodenticides are most toxic to humans (Mathur et al., 2005). In Pakistan, population of insect pests is favored by the climatic conditions (MINFAL, 2004). The use of pesticides on vegetables and fruits is a routine practice in Pakistan for previous two decades. Pesticide import has increased to 23,033 t in 2013–14 from 15,692 t in the same period of the previous fiscal year 2012–2013. The insecticides volume has increased by 1368 t (61.35%) to 3598 t in 2014 from 2230 t in 2013 (Pakistan Bureau of Statistics, 2013-14).

About one third of the plant products is obtained using these pesticides. The loss of fruit, vegetables and grains caused by pests can reach up to 78%, 54% and 32% respectively without these pesticides (Liu et al., 2002; Cai, 2008). Yield losses decrease by 35–42% by the use of pesticides (Pimentel, 1997; Liu and Liu, 1999). Out of which 80% are used to control pests of cotton during the cotton growing season, from July to October (Alam, 2006).

Excessive use of pesticides is fraught with unwanted side effects due

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to contamination of the food chain. Many insecticides cause acute and chronic health and environmental problems (World Health Organization (WHO), 1990; Conway and Pretty, 1991). These chemicals interfere with the normal physiology of lymphocytes and erythrocytes (Banerjee et al., 1999). Undesirable health tribulations comprise a number of detrimental problems such as cancer (Alavanja et al., 2004; Settimi et al., 2003), immune and neurotoxic effects (Kamel and Hoppin, 2004; Galloway and Handy, 2003; Colborn, 2006), reproductive problems (Yucra et al., 2006; Garcia et al., 1999) and endocrine diseases (Barlow, 2005). In man, little conclusive information has so far been produced by epidemiological observations mainly due to drawbacks in exposure assessment. Therefore, information on the type and levels of exposure is fundamental in order to better understand and characterize risk to human health. Exposure assessment of these pesticides in man is carried out by the analysis of integral chemicals or their metabolites in the plasma, peripheral blood, or urine (Aprea et al., 2002).

Most methods of detecting insecticides and their key metabolites in biological

samples involve gas chromatograph (GC) attached with the electron capture detector (ECD) (Cruz et al., 2003), gas chromatograph coupled with mass spectrometer (GC-MS) (Hayat et al., 2010; Weiyue et al., 2010; Vasilic et al., 1999), liquid chromatograph with ion mass spectrometer (LC-IMS) (Kawasaki et al., 1992; Futagami et al., 1997). High performance liquid chromatograph (HPLC) in combination with a UV detector (Azmi et al., 2006; Itoh et al., 1996) or the liquid chromatograph in combination with the tandem mass spectrometer (LC/MS/MS) (Araoud et al., 2010). Clean up is required for analysis of insecticide in organic tissues to remove turbulence and to lower the detection limits of the chemicals. There are several methods to prepare samples for pesticide-residual analysis of biological substances. Reproducible analysis results, cleaner extracts and better selectivity can be obtained by solid phase extraction (SPE) than liquid to liquid extraction (LLE) (Aprea et al., 2002).

Some of these methods consist of tiresome extraction procedures that take a long time and are not valid in case of acute intoxication due to production of late results. Therefore, it is essential to selectively develop and validate fast and reliable methods for detecting multi-residues that may be useful in identifying and quantifying as many insecticides as possible in human blood. Many insecticide products are hydrophobic and lipophilic molecules that can bind to biological membranes; in particular the double layers of phospholipids, which indicate their deposition in body tissues (Lee et al., 1991). The interacting mechanisms of pesticides, with diverse chemical assemblage, with lipid bilayers depend on the degree of lipophilicity, the molecular polarity and certain other characteristics of the pesticide compound. Generally, this interaction occurs through assimilation of the pesticide molecules in the hydrocarbon region within the bilayer or adsorption of pesticide molecules in the area of polar membrane phospholipids, or incorporation of amphiphilic pesticide molecules in both the non-polar and polar regions of the membrane. The formation of pesticide complex with lipids in liposomal or cellular membrane can occur with covalent bonding upon the elimination of water and HCl (Golubev, 2007). The covalent chemical link between the pesticide and proteins or DNA is termed as biomolecular adduct. After exposure to an insecticide, a part of it might be absorbed by blood, distributed into body tissues, metabolized or excreted through urine. The process of absorption, distribution, metabolism and excretion make together the toxicokinetic process after a pesticide entry into the body (Klaassen, 2001; Barr et al., 2006). Usually, blood measurements are more specific for a pesticide as parent chemical is usually measured opposite to its metabolites in urine. For instance, after exposure to chlorpyrifos, it can be measured in the blood easily as compared to its metabolite, 3,5,6-trichloro-2-pyridinol (TCPY) which offer more chemical specificity than urinary TCPY measurement. Additionally, the whole blood volume remains constant so the pesticide level measured for specific period after pesticide contacts also remains constant until the absorbed pesticide quantity is constant (Wessels et al., 2003).

Globally numerous cytogenetic bio-monitoring investigations have been reported on insecticide workers (Kocan et al., 1994). Other studies conducted on animals have reported that the pesticides alter animal hematology. Decreased white and red blood cell counts, hemoglobin-Hb and packed cell volume (PCV) values were recorded after exposure to two concentrations (0.15 and 0.30 µl/L) of cypermethrin. Mean corpuscular volume (MCV) value was reduced in response to lower concentration and increased in response to higher concentration whereas mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were increased at low concentration and decreased at high concentration of cypermethrin as compared to control fish. Oppositely, WBCs and MCHC values were increased at higher concentration of diazinon exposure while RBCs, Hb, PCV, MCV and MCH values were increased at both concentrations of diazinon as compared to control (Khatun et al., 2014). Even some studies, in this regard, indicate the adverse effects of insecticides exposure on circulating blood hormones such as thyroid stimulating hormone (TSH) and triododirone (T3) in addition to affecting the blood parameters of agricultural workers in Swat, Pakistan. In the population of people exposed to pesticides, a decrease in TSH and an increase in T3 hormone were observed as compared to control (Quraishi et al., 2015).

In certain studies, already conducted in Pakistan, hematological parameters such as hemoglobin-Hb measurement, hematocrit (HCT) or packed cell volume (PCV), red blood cell (RBC) counts and platelets were significantly differed in pesticide sprayers as compared to controls (Bhalli et al., 2006; Fareed et al., 2013; Quraishi et al., 2015). Different cell indices, mean cell hemoglobin (MCH) which refers to the mean mass of Hb in single RBC, mean cell volume (MCV) which indicate the size of RBC in term of volume occupied by only one RBC and MCH concentration (MCHC), which is the average concentration of Hb in packed volume of RBCs, have also been shown to be affected by pesticide exposure (Bhalli et al., 2006; Fareed et al., 2013; Quraishi et al., 2015). Longer exposure to insecticides may lead to DNA damage as well as variations in blood cell profile (Bhalli et al., 2006, 2009; Khan et al., 2013).

The presence of pesticide remains amongst Pakistani farmers has been reported in some studies (Latif et al., 2012). The effect of pesticides on enzymes was reported through blood analysis of Pakistani farmers (Bhalli et al., 2006). Soomro et al. (2008) investigated the sprayers' blood for insecticide residues in 14 districts of Pakistan's Sindh Province. GC-MS detection method was used and blood samples of spray workers were found positive for five insecticides viz. endosulfan, monocrotophos, carbaryl and cypermethrin (0.009, 0.005, 0.05 and 0.08 mg/kg body weight respectively). Analytically, these concentrations showed significant effects on blood serum cholinesterase level. Khan et al. (2008) determined six pesticides in blood samples of tobacco farmers using HPLC and GC-NPD analytical techniques. The farmers exposed to pesticides had significant changes in enzyme activity as compared to controls. The tobacco farmers had multiple pesticides residues above acceptable daily intake (ADI) in their blood consisting of methomyl, thiodicarb, cypermethrin, imidacloprid, methamidophos and endosulfan (0.74, 0.51, 0.01, 031, 0.30, and 0.39 mg/Kg body weight). Butyryl cholinesterase (BChE) activity was significantly decreased in the pesticides exposed farmers as compared to controls. Plasma biochemical markers including, alanine aminotransferase (ALT), the aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) and phosphate were significantly raised in the pesticides exposed farmers as compared to control group. Bhalli et al. (2009) detected four insecticides in blood samples of exposed workers using HPLC technique and significantly correlated to DNA damage. Latif et al. (2012) also conducted quantitative analysis for 30 pesticides residues in human blood samples of 188 individuals in two district of Sindh Pakistan using gas chromatograph (GC) connected with micro electron capture detector (µECD) and detected chlorpyrifos,