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EFFECTS OF ORGANOCHLORINE PESTICIDE RESIDUES ON HEMATOLOGICAL PARAMETERS IN LIBYAN AGRICULTURAL WORKERS

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ABSTRACT

Objectives: Agricultural workers are at risk of exposure to occupational hazardous such as pesticides. Bio monitoring of effects in agricultural workers is necessary to assess the individual risk of handling pesticides. **Settings:** The study was conducted in the hematological unit at the Analytical Medical Laboratory at El-Beida Hospital, Libya. **Subjects and Methods:** This study was designed to study the hematological parameters effects of the pesticide pollution among Libyan agricultural workers at Aljebal Alakhtar, Libya. A total of 45 blood samples were collected from male agriculture workers who have been exposed to pesticides in crop fields for a long period time on Aljebal Alakhtar, while 25 blood samples have been taken from the group of people in meanwhile who were not dealing with pesticides (control). The hematocrit value (HCT), hemoglobin content, red blood cells count (RBCs) and mean corpuscular volume (MCV) concentration with increased white blood cells counts (WBCs), mean erythrocyte hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), lymphocytes and neutrophils levels were performed on Selectra E fully automatic hematological analyzer. The samples were analyzed for organochlorine pesticide residues by using gas chromatography-electron capture detector (GC-ECD). All the data from Libyan agricultural workers and age-matched controls from different experiments were analyzed and compared using Student's t-test. The results were expressed as mean ± SD. The significant test was applied at p value < 0.05. **Results:** The study indicated also detected four (p,p`-DDT, o,p`-DDT, o,p`-DDE and p,p`-DDD) of the 16 organochlorine compounds tested for in the whole blood samples were detected in all analyzed samples. The mean total p,p`-DDT, o,p-DDT, o,p`-DDE and p,p`-DDD levels in blood samples were 0.169 ± 0.001, 0.129 ± 0.003, 0.328 ± 0.007 and 0.510 ± 0.01 ppm, respectively. Assessment of hematological parameters revealed that the RBCs, hemoglobin, HCT, MCV. MCH, MCHC and neutrophils levels were significantly lower in workers than in controls, while WBCs and lymphocytes levels were significantly higher in workers. **Conclusions:** These biomarkers seem to be indicative of adverse effects of pesticides in agricultural workers, confirming their use for routine monitoring of effects.

KEY WORDS: Libyan agricultural workers, organochlorine pesticide, hematological parameters.

Abbreviations: HCT: hematocrit; RBCs: red blood cells counts; MCV: mean corpuscular volume; WBCs: white blood cells counts; MCHC: mean erythrocyte hemoglobin; MCH: mean corpuscular hemoglobin.

INTRODUCTION

The extent and complexity of health hazards inherent to workers who live with injuries caused by some types of work activities have highlighted the need to develop a model study of the future of occupational health.[1-2] Pesticides are chemicals used in agriculture to control weeds and pests and to fight plant diseases. Most pesticides are a mixture of several chemical components, characterizing them as a complex mixture.[3] Globally, the use of pesticides has increased in recent years. Among the groups involved in the preparation and the final distribution of pesticide mixtures, farmers and agricultural workers are commonly the most exposed individuals.[4] Pesticides are associated with the increase of various types of cancer at specific sites, such as lip, skin, prostate and brain tumors, non-Hodgkin lymphoma, Hodgkin's disease[5], leukemia[6] and lipid discords.[7] Agricultural workers chronically exposed to pesticides are at an increased risk of chronic toxicity. They are frequently exposed to many different categories of pesticides, either simultaneously or sequentially, making it difficult to identify the effects of particular agents.[8] Organochlorinated pesticides have been used globally for many years as solvents, fumigants, and insecticides. Organochlorine pesticides have very low water solubilities but are highly soluble in lipids and bioaccumulate in tissues.[9] These properties also contribute to bioaccumulation within the human organism by the ready absorption into the body and subsequent deposition into adipose tissue.[10] The agricultural sector is a very important part of the Libyan Gross Domestic Product (GDP). The agriculture sector was the main source of revenue for the Libyans making about 30% of the GDP. Agriculture accounts for about 20% domestic products and for about 17% of total employment.[11] In Libya use of pesticides is not well controlled as compare to the other developed countries due to ineffective legislation, lack of awareness and in appropriate pesticide management. They are completely unaware to the approach of integrated pest management (IPM). Additionally, the use of incorrect or high dosage of pesticides leads to the contamination of pesticides in their agricultural products, which may be health risk to the consumer. Due to these concerns, the monitoring of pesticide residues in food products become more essential requirement for consumers, producers and institutions concerned with standards and quality control management.[12-13] Several Libyan studies investigating the residual levels of pesticides in foods have reported hazardous concentrations in milk samples, fish, fruits and vegetables.[13-14] Therefore, the present study was undertaken to provide data on organochlorine pesticides contamination of agricultural workers from Aljebal Alakhtar, Libya. It is part of a more comprehensive evaluation of the exposure of the Libya population to persistent organochlorine pesticides. Due to the limited research in Libyan related to human monitoring of pesticide exposure, this study was carried out to evaluate the effects of pesticides on hematological parameters in agricultural workers chronically exposed to these compounds.

SUBJECTS AND METHODS

Sample collection and experimental design

A total of 70 individuals were selected as a sample size from different areas of El-Jabil Al Kadar area, in which 45 blood samples were collected from those male agriculture workers who have been routinely exposed to pesticides for a considerably long period of time but they did not had the previous history for any infectious diseases or other environmental exposures. The individuals for the study were enrolled during February 2015 and mean age was 61.24 ± 5.12 who have been working in the crop fields for an average of last 25 years. Similarly, 25 healthy subjects were collected within the same area but they did not possess any current/previous history for infectious diseases and exposer to pesticides or other environmental exposures (as a control). The age distribution of the control subjects was approximately similar to that of the patients (59.14 ± 3.21 years).

Data collection

A meeting interview was used for filling in the questionnaire, which designated for matching the study need. All interviews were conducted face to face by the researcher himself. During the study the interviewer explained to the participants any of the confused questions that will not clear to them. Most questions were the yes/no questions, which offer a dichotomous choice. After explaining the aim of study to the subjects and obtaining informed consent, a questionnaire seeking information on the age, smoking, sex and duration of the disease was administered to the subjects. The questionnaire was also used to gather information about medical history, like the presence of some signs and symptoms of kidney disease, cardiovascular disease, liver disease and recurrent infection.

Sampling

All subjects were anonymised by numbering them and numbering the blood samples taken from them. Blood specimens (7 mL) were taken from each individual and blood was collected in anticoagulant sterile vacutainer tubes. The blood specimens were immediately kept in an ice-cold chamber, transported and delivered to the laboratory for processing within 2 h of blood donation.

Determination of hematological parameters

Hematocrit value (HCT), whole blood hemoglobin concentration, white blood cell counts (WBCs), mean erythrocyte hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red blood counts (RBCs), mean corpuscular volume (MCV), lymphocytes, neutrophils and platelets count (PLT) analyses of blood samples collected into test tubes with anticoagulant were performed on Selectra E fully automatic hematological analyzer from Hungary was calibrated by standardized commercially available calibrated kit. Blood samples for hematological analyses were delivered to the laboratory within 2 h of collection and promptly assayed.

Extraction of pesticide residues from the whole blood

Extraction

Extraction was based on the method followed by Agarwal et al,[15] Blood (5 ml) was diluted with 25 ml distilled water and a 2 ml of saturated brine solution was added, then it was transferred to a 125 ml capacity separatory funnel. It was extracted with hexane: acetone (1:1) (20 ml) (thrice), by shaking the separatory funnel vigorously for 2-3 min, thereby releasing the pressure intermittently. As a result, the layers were allowed to separate. The three combined extracts were passed through anhydrous sodium sulfate and concentrated to about 1-2 ml using a rotary vacuum evaporator. Consequently, the whole blood was used.

Sample analysis

The samples were analyzed for organochlorines by using gas chromatography in Faculty of Science, Cairo University. The standard solutions of organochlorine pesticides were prepared in n-hexane.[16]

Statistical analysis

All the data from agriculture workers and healthy controls from different experiments were analyzed and compared using Student's t-test. The results were expressed as mean ± SD. The significant test was applied at p-value < 0.05.

RESULTS AND DISCUSSION

The current study was conducted to evaluate the effects of pesticides on farmer's health of Aljebal Alakhtar, North-East of Libya by determining their levels of the hematological parameters. For this purpose, the people lacking any history of diseases were sampled after conducing preliminary investigations.

Socio-demographic characteristics of male agriculture workers

Table 1 illustrates general characteristics of the study population. Mean age of the control and spraying peoples were 59.14 ± 3.21 and 61.24 ± 5.12 years, respectively, which were not significantly different. The mean time of pesticide exposures in all farm workers were 21 ± 3.7 years. All studied populations were not use personal protective equipment (PPE). Pesticide sprayers of the present study are generally exposed to different classes of pesticides (insecticides, fungicide and herbicides) during their work. In the exposed group, all agricultural workers were regularly exposed (2-3 times per week) to complex mixtures of pesticides. The use of a wide range of pesticides mostly of "moderately hazardous" to "slightly hazardous" category among our study farmers. Food and Agriculture Organization (FAO) recommends that World Health Organization (WHO) (Highly hazardous) pesticides should not be used in developing countries.[17] It also suggests that class II (Moderately hazardous) pesticides be avoided. Individuals are frequently exposed to many different pesticides or mixtures of pesticides, either simultaneously or serially, making it difficult to identify effects of particular agents. The relationship of pesticide-related cytotoxicity to overt clinical organ disease is still unresolved. In this regard, biomarkers may be used to detect the effects of pesticides before adverse clinical health effects occur.[18]

Residues of organochlorine pesticides

In recent years, man has become increasingly conscious and critical of the environmental pollution by chemicals. Organochlorines have been a major cause of concern to ecologists because they are relatively resistant to bio-degradation. Organochlorine deposition in biolipids is due to their low solubility in water because of their strongly lipophilic character. These properties explain why organochlorine tends to accumulate in the adipose tissue of the human body to levels that are considered as significant organochlorine residue burdens.[19] Although organochlorine are very persistent, some biodegradation can occur yielding the metabolites shown in Figure 1.

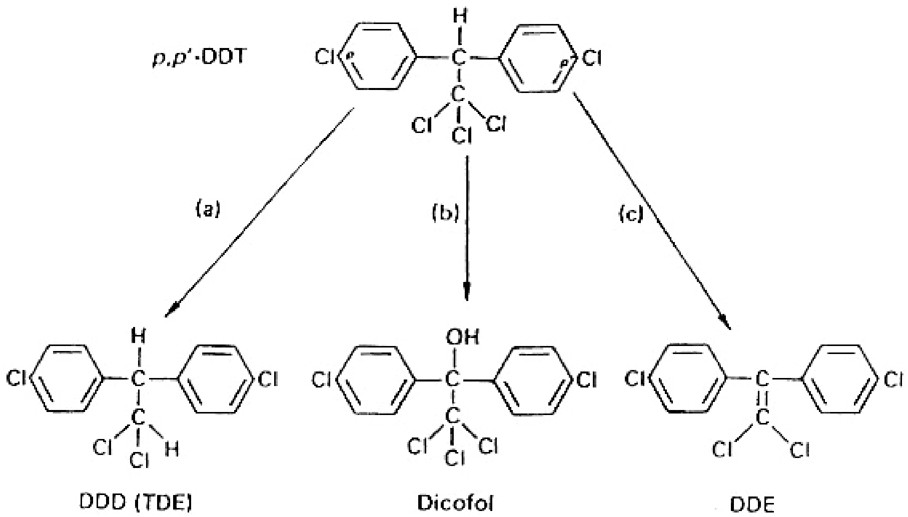

Figure 1: Metabolites formed from DDT, (a) reductive dechlorination, (b) oxidation, (c) dehydrochlorination (Kresis et al., 1981).[19]

There are no studies on blood levels of organochlorine in the Libyan population. Organochlorines and their isomers residual levels in the whole blood of agriculturalist and non-agriculturalist were quantified. An analytical method GC-electron-capture detection using a capillary column was implemented to determine DDT and its metabolites (p,p-DDD and p,p-DDE), as well as other organochlorine pesticides in whole blood samples from 45 farmers and 25 non-occupationally exposed workers. As shown in Table 4,we detected four (o,p-DDE, o,p-DDD, o,p-DDT and p,p-DDT) of the 16 organochlorine compounds tested for in the whole blood samples (Table 2). The DDE concentration in whole blood samples for Libyan farm workers was 0.328 ppm is hampered by the fact that different analytical methods were used. In our study capillary column separation and GC/MS were used whereas other authors used packed columns.[20-21] Having the above mentioned restrictions in mind one can nevertheless conclude those blood concentrations of Libyan farm workers were very high by comparison with values determined for workers in other countries.[19, 23-24] It is proposed that this difference is due to different methods of pesticides application in the fields. In the Libya, especially on Aljebal Alakhtar area, pesticides are often applied manually without any protective devices. DDT is a potent nonsystemic insecticide. A major metabolite of DDT is 2,2-bis (p-chlorophenyl)-1,1-dichloroethylene (p,p`-DDE) was detected at mean levels of 0.328 ppm in whole blood samples analysed from Libyan farm workers. DDE is more persistent than DDT. p,p`-DDD another metabolite of DDT was detected at mean levels 0.510 ppm in whole blood samples. DDT was detected in blood samples perhaps due to its persistent nature. Since DDT is known to undergo metabolic conversion and dehydrochlorination, presence of metabolites of DDT i.e. DDD and DDE encountered in this study might be due to such metabolic processes. Among DDT, p,p`-DDE was the most predominant compound in all Egyptian and international published data like those of Dogheim et al in 1996, Saleh et al. in 1996, Salem and El-Saied in 1997, Barkatina et al. in 1998, Pardio et al. in 1998, Cok et al. in 1997 and 1999 and Waliszewski et al. in 2001.[25-31] This might be due to the high solubility and tendency of DDT and its metabolites to accumulate and store in fatty tissues.[32] Therefore, since 1970s DDT was banned in most of the Western countries (Biscoe, 2004). Although DDT has long been banned in Libya, its residues can still be detected in the environment after a decade. This is due to the high persistent nature. Evaluation of this concentration is valuable in detecting the source of contamination and may be due to the persistence and long-range transport nature of DDT and its metabolite DDE[33] and also their ability to bioaccumulate and biomagnify in the food chain.[34]

Effects of pesticides exposure on hematological parameters

There are three main types of blood cells, the red blood cells (RBCs) or erythrocytes, the white blood cells (WBCs) or leukocytes and platelets (PLT) or thrombocytes.[35] Hematological parameters can be used easily and rapidly for prediction and diagnosis of pesticide toxicity. Alterations in these parameters reflect toxic stress in the treated animals.[36] Hematological analysis is very much important in many fields of research such as toxicology and monitoring of environmental pollution. Several studies have reported on the effects of pesticides on hematology of various fish species in different parts of the world.[37] The data of analysis of full blood counts in serum-included measurements of RBCs and its related indices of healthy controls and exposed group are shown in Table (3). In exposed group HCT, whole blood hemoglobin concentration, RBCs, MCHC, MCH and MCV values were found to be lower (36.09 ± 10.14 %, 10.39 ± 3.08 g/dl, 3.13 ± 0.52 (x106/μl), 24.52 ± 10.15 g/dl, 28.30 ± 5.13 pg and 70.13 ± 29.82 fL, respectively) compared to control (47.14 ± 5.02 %, 12.86 ± 1.51 g/dl, 4.76 ± 0.10 (x106/μl), 34.54 ± 4.37 g/dl, 37.03 ± 3.15 pg and 89.33 ± 13.21 fL, respectively). This decreased was statically significant ($p<0.05$). The primary reasons for assessing the RBCs and related indices are to check anemia and to evaluate normal erythropoiesis. Hemoglobin level indicates the amount of intracellular iron, while hematocrit, representing the volume of RBCs in 100 ml of blood helps to determine the degree of anemia or polycythaemia.[38] The blood is a unique mirror in which all the internal process taking place in an organism is reflected. A fall in the red blood cell count, hemoglobin concentration and haematocrit volume worsening of an organism state and developing anemia. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. The anemic condition in fish results from an unusually low number of red blood cells or too little hemoglobin in the red blood cells.[39] According to[40] the pesticide induced anemia in agricultural workers may be due to the inhibitory effect of the toxic substance in the enzyme system responsible for the synthesis of hemoglobin. These alterations were attributed to direct responses of structural damage to RBC membranes, resulting in hemolysis and impairment in hemoglobin synthesis.[39] Similarly, several studies have been conducted in animals, which report that pesticides alter the hematology of animals.[37, 41-42] The increased RBCs count and PCV in the present study are similar to the finding of Emam et al.[43] Similarly increased RBCs counts in human having high DDT concentration in the blood serum has been reported by Dunstan et al. (44). Contrary to this Patil et al.[45] found significantly decreased packed cell volume and RBCs counts in sprayers. The study revealed that occupational exposure of agriculture workers to pesticides significantly changes some of their hematological parameters. Thus, exposure to pesticides may pose a serious threat for human health. The careful application of pesticides in agriculture and other practices may be highly recommended.

Table (4) shows WBCs and differentials and platelets measured in serum of healthy controls and farm workers. There was a significantly higher ($p<0.05$) total white blood cell counts, (9.11 ± 1.68 million/mm3), lymphocytes (49.75 ± 7.16 %) and neutrophils (34.95 ± 9.61 %) in the farm workers compared to control subjects (5.89 ± 0.31 million/mm3), lymphocytes (31.18 ±1.21 %) and neutrophils (48.13 ± 3.10 %). There did not appear to be differences in platelet count levels between diabetic cases and the control group (Table 4). The present study demonstrated that the total and differential leukocyte counts were significantly altered in farm workers. Shahi and Singh[46] notions are also in agreement with the current findings who reported an increase in the WBCs count in response to the pesticides exposure. Similarly, an increase in the levels of lymphocytes, neutrophils and WBCs has also been reported by Dunstan et al. al.,[44] which again justifies our findings. Jamil et al.[47] have reported that WBCs count was increased in agriculture workers who were exposed to pesticide. Khan et al.[48] observations are also in consistency with the present finding who revealed a significant increase in the WBCs upon exposure of the individuals to pesticides. No significant differences were noted in the PLT count in response to pesticides exposure of the studied field workers. Leukocytes are involved in the regulation of immunological function and a protective response to stress in fish. The leucopoenia observed here, can be attributed to generalized stress response that cause increase pituitary internal activity[49], which also may be due to dysfunction in hematological tissues (spleen and kidney) or certain infectious diseases. Lymphopenia can be an indicator of immune system deficiency. In agreement with these results Banaee et al.,[50] The most common and important cause of neutrophilia is tissue damage, Poisonings and severe disease, like kidney failure all cause neutrophilia.[51] Ghosh and Banerjee[52] reported lymphopenia and increased both neutrophil and eosinophil after an effect of pesticides.

Table 1. Characteristics of the study population.

Characteristics	Control group	Exposed farm workers
No of subjects	25	45
Age group [mean ±SD]	59.14 ± 3.21	61.24 ± 5.12
Wt (kg)	76.36 ± 6.75	74.76 ± 7.34
Years of exposure [mean ±SD]	-	21 ± 3.7
Personal protective equipment Yes No	- -	- 100%

Table 2. The distribution concentrations (ppm) of organochlorine pesticide residues detected in whole blood samples collected from Libyan farm workers.

Pesticides detected in farmers group	Concentrations of pesticide residues (ppm)
Dichlorodiphenyltrichloroethane DDT/metabolites	
p,p`-DDT	0.169 ± 0.001
o,p`-DDT	0.129 ± 0.003
Dichlorodiphenyldichloroethylene DDE total	
o,p`-DDE	0.328 ± 0.007
Dichlorodiphenyldichloroethane DDD total	
p,p`-DDD	0.510 ± 0.01

Table 3. Effects of chronic exposure to a mixture of pesticides on red blood cells (RBCs) and related indices measured in serum of controls and exposed group. All results are expressed as mean ± SD, *p<0.05 for exposed group compared to control group.

	Control (n = 25)		**Exposed farm workers (n = 45)**	
Parameters	Mean ± SD	Max-Min	Mean ± SD	Max-Min
RBCs (x106/µl)	4.76 ± 0.10	5.43-4.01	3.13 ± 0.52*	4.34-2.12
Haemoglobin (g/dl)	12.86 ± 1.51	14.34-11.01	10.39 ± 3.08*	8.01-11.83
HCT (%)	47.14 ± 5.02	49.11-45.32	36.09 ± 10.14*	29.04-38.67
MCV (fL)	89.33 ± 13.21	92.62-85.97	70.13 ± 29.82*	81.65-62.45
MCH (pg)	37.03 ± 3.15	41.32-35.71	28.30 ± 5.13*	23.61-32.73
MCHC (g/dl)	34.54 ± 4.37	39.76-31.88	24.52 ± 10.15*	30.61-19.12

Table 4. Effects of chronic exposure to a mixture of pesticides on white blood cells (WBCs) differentials and platelets measured in serum of control and exposed group. All results are expressed as mean ± SD, *p<0.05 for exposed group compared to control group.

	Control (n= 25)		**Exposed farm workers (n = 45)**	
Parameters	Mean ± SD	Max-Min	Mean ± SD	Max-Min
WBCs (million/mm3)	5.89 ± 0.31	6.63-4.71	9.11 ± 1.68*	11.74-8.22
Lymphocytes (%)	31.18 ±1.21	33.54-28.61	49.75 ± 7.16*	56.11-40.63
Neutrophils (%)	48.13 ± 3.10	51.01-44.12	34.95 ± 9.61*	37.14-22.37
Platelets (x103/µl)	218.95 ± 17.24	262.52-185.77	221.88 ± 39.12	271.45-192.05

CONCLUSION

Some organochlorine pesticides evaluated in this study some are lower and some are higher than previously documented values. The still high incidence and levels of some pesticides especially p, p`-DDT, o, p`-DDT, o, p`-DDE and p, p`-DDD enforce the responsible aspects to take more effective and continuous defenses. Frequent and continuous monitoring of these pesticides will help to take cautions in consuming food of animal origin, fishes, vegetables, fruits and grains from contaminated areas. The results obtained from this research work revealed that there are still residues of some organochlorine pesticides in the environment. Sixteen different organochlorine pesticides namely p, p`-DDT, o, p`-DDT, o, p`-DDE, p, p`-DDD, γ-HCH, β-HCH, heptachlor, aldrin, Endrin, endrin-aldehyde, endrin-ketone, γ-endosulphan, endosulphan-sulphate, γ-chlordane, dieldrin, and methoxychlor were detected. Four out these fourteen organochlorine pesticide residues; p, p`-DDT, o, p`-DDT, o, p`-DDE and p, p`-DDD. Total number of pesticides detected in blood samples from Aljebal Alakhtar was 4 out of 16 pesticides analysed which indicates that each person is exposed to and carries a body burden of multiple pesticides, which might be due to a combination of direct and indirect exposure to these pesticides. Major contribution to total pesticide concentration in blood samples from Aljebal Alakhtar is of organochlorine pesticides. Presence of organochlorine pesticides in blood means that they do persist n the body for good amount of time. It also indicates the presence in the body of the pesticide in its form as a primary compound. It can be concluded from this study that human pesticide residue is a biological index of pesticide exposure and studies on blood can be used for assessing the total body burden data of pesticides in the occupationally exposed and unexposed population. This study revealed that long-term exposure to various pesticides leads to signs of cytotoxicity resulting particularly in biochemical alterations. It can be concluded that chronic exposure to organochlorine pesticides can cause effects in hematological systems. So further researches on large scales are needed to confirm these findings and to obtain detailed information about mechanisms of toxicity. Also, official regulations and interventions to reduce farmer’s overexposure to pesticides are needed. These findings reinforce the need for heightened efforts to better protect from pesticide exposure among farm workers. As an adjunct to the regulation of pesticides, regular monitoring is necessary to ensure that control measures are satisfactory.

Recommendations

- Protective measures such as the use of facial masks, socks and gloves must be undertaken when dealing with pesticides.
- Government agencies must implement strict legislation over selling, purchase and on the use of pesticides.
- The public should be properly trained and educate about the use of pesticides and its safety measures.
- Building the capacities and capabilities of the institutions or provision of projects concerned with chemicals management.
- Establishment of sound chemical management system involving all concerned parties from government, agricultural workers, industry, research institutes, non-governmental organizations and academia through multi-stakeholder committee.
- Establishment of poison control centers with an adequate clinical and analytical capacities in addition to functions of treatment and prevention.

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