COMPARATIVE HISTOPATHOGENIC AND TOXICOLOGICAL EVALUATION OF IN VIVO EXPOSURE TO CHLORPYRIFOS AND ALDICARB IN MALE WISTAR RATS

Abd-Allah, Salwa M.
Mammalian Toxicology Dept., Central Agric. Pesticides Laboratory, ARC, Alexandria

ABSTRACT

The toxicological endpoints after chlorpyrifos and aldicarb exposure coupled with histological and ultrastructural examinations of multiorgan damage were assessed in the current study. The tested pesticides were applied orally in acute and subchronic manner to male Wistar rats at concentrations represent 1/3 and 1/10 LD_{50}; respectively. Animals under subchronic intoxication were treated daily for 28 day. Signs of toxicity morbidity and mortality patterns were observed in a dose-dependent manner. Hepatic-injury marked by significant elevation in serum indicator enzymes ($p<0.001$) after acute and subchronic exposures was shown in a type, dose and time-dependent manner. The effect exerted by aldicarb was more pronounced than chlorpyrifos either after acute or subchronic exposure. Kidneys showed signs of renal damage and nephropathy after exposure to both pesticides and a significant association ($p<0.01$) was observed between the length of exposure and level of damage. However, liver appears to be more sensitive to the effect of the tested pesticides where histological signs of liver hypertrophy were apparent and lesions were correlated with biochemical endpoints. The study provides evidence of both functional and ultrastructural damage elicited by acute and subchronic exposure to aldicarb and chlorpyrifos.

Keywords: Oral toxicity, chlorpyrifos, aldicarb, biochemistry, histopathology.

INTRODUCTION

Occupational exposure to organophosphorus and carbamate-type pesticides; the most used in agriculture in the region of Middle East for control a variety of indoor and outdoor pests, mites and nematodes; significantly inhibits acetylcholinesterase (AChE) activity and causes morbidity (Gomes *et al.*, 1999). Number of people exposed is substantial as are the exposure routes (contaminated food, water, indoor air and soil) (US EPA, 2000). The toddler, being the most sensitive receptor, is expected to have a mean daily dietary exposure to chlorpyrifos of 2.55% of the ADI (ANZFA, 2001). Also, poisoning has resulted from ingestion of produce such as melons and cucumbers containing low levels of aldicarb and its metabolites (Hirsch, *et al.*, 1987; Green, *et al.*, 1987). Developmental effects of chlorpyrifos and aldicarb involve mechanisms over and above cholinesterase inhibition, notably events in cell signaling cascades that are vital to cardiac and hepatic homeostasis (Qiao *et al.*, 2002).

The *in vivo* toxic symptomatology of aldicarb was related to the peak serum concentration of sulfoxide, suggesting that this metabolite is principally responsible for the aldicarb toxicity (Montesissa, *et al.*, 1994). While, the 3,5,6-trichloro-2-pyridinol (TCP) is the principal metabolite detected of chlorpyrifos (Nolan *et al.*, 1984).

Recently, it was documented that polymorphic cytochrome P450-2D6 is involved in the activation of chlorpyrifos but does not influence aldicarb toxicity (Costa *et al.*, 2003). Furthermore, cytochrome P-1A1, 2B1 and 3A1/2 are differentially involved in metabolism of chlorpyrifos to its oxon and the extent of plasma AChE inhibition was significantly greater in female than male rats (Dalvi *et al.*, 2004).

Low and increasing levels of chlorpyrifos can differentially modify endogenous antioxidants which may lead to development of oxidative stress and organ damage in some tissues (Bebe and Panemangalore, 2003). Organ damage was also noted after acute oral carbamate toxicity, where the respiratory system followed by CNS and liver were the mostly affected by carbamate toxicity (Winnik *et al.*, 1997). Additionally, chlorpyrifos has anti-androgenic activity compared with aldicarb by inhibition of increase in weight of accessory sex organs, but it does not show estrogenic and anti-estrogenic activity in immature female rats (Kang *et al.*, 2004).

As related to carcinogenicity, chlorpyrifos is not considered as carcinogen (CCINFO, 1991). Comparatively, aldicarb is suspected to be a mutagen; although very little concerning studying chronic effects has been done; where one of the possible conversions of aldicarb is to that of a N-nitroso derivative, which is known to induce tumors in rats (Wagner, 1983). Aldicarb was also found to have a clastogenic effect in rats in accumulative manner since it induces a significant increase in sister chromatid exchange in cultured human lymphocytes, both with and without metabolic activation (Kevekordes *et al.*, 1996).

In the current study, biochemical indices and ultrastructural examinations of hepatocellular injury and renal affection are investigated as markers for evaluation of acute and subchronic exposure to such pesticides, as well as an attempt to determine and compare the relationship between the progression of histological changes and the biochemical alterations detected in the sera of treated rats.

MATERIALS AND METHODS

Chemicals

A technical grade of chlorpyrifos (94% purity), o,o-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate and aldicarb (96% purity), 2-methyl-2(methylthio) propionaldehyde 0-(methylcarbamoyl)oxime were obtained from Dow Elanco, Indianapolis, IN, USA. All other chemicals used in this study were obtained from Sigma and were the highest grade available.

Animals

Young adult male Wistar rats, weighing 50-70 g were used and allowed to acclimate to the environment for a week prior to initiation of study. LD_{50} was tested for each compound. The doses administered represent 1/3 and 1/10 LD_{50} of each pesticide given orally via a stomach gavage needle containing the insecticide of choice suspended in corn-oil at concentrations providing a 0.5 ml maximum dosing volume.

Experimental protocol

Rats were divided randomly into 6 groups, each included 10 rats. Two groups (1,2) were treated once with 1/3 LD_{50} of either chorpyrifos or aldicarb. Two other groups (3,4) were treated daily with 1/10 LD_{50} of each pesticide for 4 weeks. The last group serving as a control, was divided into 2 subgroups (5,6) and both received an equal volume of the vehicle for the same experimental period as for the tested insecticide. All animals were weighed periodically before each treatment to ensure the maximum dose effect.

Bleeding regimen

Rats grouped as 1,2,5 were bled after 24 hr of dose administration while groups (3,4,6) were bled weekly. Blood was withdrawn from the retro-orbital plexus **(Schalm, 1986)** of the anesthetized rats using heparinized micro-capillary tubes.

Biochemical examinations

Sera were separated from coagulated blood after spinning at 3000 rpm for 10 min and stored at $-20^{0}C$ until use. Hepatic injury was assessed by measuring levels of transaminases (AST, ALT), phosphatases (ACP, ALP),gamma glutamyl transferase (γ-GT), bilirubin, total protein, albumin in addition to kidney function tests (urea and creatinine) using commercial diagnostic kits, Stanbio Co., Spain). The activity of both AChE and lipase were measured according to Ellman *et al.* (1961) and Tietz (1966); respectively.

Histopathological examinations

At the end of exposure period, rats were anesthetized and both liver and kidney were quickly removed, cleaned of extraneous tissues, cut into small pieces and put into 10% Bouin solution as a fixative. The extracted tissues were then dehydrated in 70-100% ethanol series, cleared in terpineol and embedded in paraffin wax. Serial sections of 6 um thickness, were cut and stained with haematoxylin and eosin according to (Carleton *et al,* 1967). Slides were examined and the cells were photographed from prints projected from the negatives.

Statistical analysis

Data was represented as mean± standard error. The significance was analyzed using student two-paired t-test (Snedecor and Cochran, 1989). A p values of <0.05, <0.01 and <0.001 were considered significant.

RESULTS AND DISCUSSION

Due to their high fat-soluble properties, these compounds easily penetrate through cell membranes and are quickly distributed throughout the body and may penetrate through the blood-brain barrier (Marrs and Dewhurst, 2000). The toxicity of these compounds is dependent on the vehicle and nature of administration, possibly owing to reduced bioavailability of the compound or to the bolus effect of certain forms of administration (e.g., gavage).

In the course of general toxicological evaluation, signs of acute toxicity of chlorpyrifos and aldicarb are consistent with AChE inhibition and the resulting cholinergic over stimulation, including excessive salivation, rapid breathing, body tremors, lacrimation, convulsions, respiratory failure and death. Recovery in surviving animals was evident within one week post-dosing.

Oral LD_{50} identified under the experimental conditions was 148 mg/kg for chlorpyrifos and 0.93 mg/kg for aldicarb. These values agree with others who stated that they lie within the known average determined value of LD_{50}. For chlorpyrifos, oral LD_{50} ranged from 118-270 mg/kg for male rats (McCollister *et al.*, 1974), WHO rat oral LD_{50} ranged between 135 and 163 (WHO, 1975) while NRA rat oral LD_{50} was about 96 mg/kg in rats (NRA, 2000). On the other hand, oral LD_{50} of aldicarb in rats ranged from 0.6 to 1.1 mg/kg (Gaines, 1969) and depends on the vehicle where it is most acutely toxic when administered in corn or peanut oil (Risher *et al.*, 1987).

The metabolic pathways of chlorpyrifos and aldicarb are different although both are bioactivated in the liver. Cytochrome P-450 dependent desulfuration or dearylation catalysis by microsomal enzymes are responsible for bioactivation and metabolism of the first while, mixed function oxidases are responsible for metabolism of the later (Casarett *et al.*, 1980 and Nolan *et al.*, 1984). Accordingly, the toxicological endpoints measured are based on the potential effect on liver marked by measurement of transferases, phosphatases, lipase, protein and bilirubin coupled with histopathology in addition to measurement of urea and creatinine strengthening with kidney histopathology for precise evaluation of the hepatic-renal nexus.

Acute exposure to both chlorpyrifos and aldicarb caused a significant increase in ACP, ALP, AST, lipase ($p<0.001$) as compared to the control group as shown in Fig. 1. Chlorpyrifos activated ALP in a trend higher than ACP and similarly AST more than ALT compared with control. Aldicarb-treated rats showed significantly higher serum AST and ALT levels when compared with those exposed to chlorpyrifos. Also, acute dosing of aldicarb resulted in a significant elevation of γ-GT and bilirubin levels ($p<0.001$) while no statistical difference was found between the level of these parameters in chlorpyrifos-treated group and the control one. Both compounds resulted in a significant decrease ($p<0.001$) in serum total protein accompanied with a significant decrease in serum albumin ($p<0.001$) and serum globulins in case of chlorpyrifos treatment while aldicarb did not have significant effect on serum globulins.

The release of enzymes into blood stream particularly ACP is an index of cellular degeneration and lysosomal activity linked to Xenobiotic elimination after exposure (Verplanke *et al.*, 2000). Such increase might also be due to the increase of synthesis of these enzymes as an adaptive mechanism of the toxicant stress. These data are coincide with others reported that both ACP, ALP elevation is considered biomarkers for tissue damage following intoxication stress particularly for Ops (Rahman *et al.*, 2000).

With regard to renal affection, acute exposure to the tested pesticides induced highly significant increase in serum urea and creatinine ($p<0.001$) as

compared to control group. Aldicarb exhibited the highest increase in serum creatinine (Table 1). Moreover, livers of rats exposed to acute treatment of both chlorpyrifos and aldicarb exhibited several histological variations (Fig. 3). Both of them caused loss of the normal architecture accompanied with enlargement of hepatocytes with absence of hepatic sinusoid in case of the first insecticide and disarrangement of hepatic sinusoid in case of the later. Vaculated and lysed hepatocytes, and presence of small dark nucleus with vaculated cytoplasm were observed in case of chlorpyrifos treatment.

Table 1: Effect of acute treatment of chlorpyriphos and aldicarb on kidney function tests (KFTs) in male Wistar rats (data are expressed as mean±SE).

Treatment	KFT	
	Urea (g/L)	Creatinine (µmol/L)
Control	11.30±0.03	0.25±0.02
Chlorpyriphos (49.0 mg/Kg)	21.88±0.4***	0.55±0.02***
Aldicarb (0.35 mg/Kg)	34.63±0.56***	0.93±0.03***

*, **, ***: Significance at $p < 0.05$, 0.01, and 0.001 respectively.

On the other hand, kidneys of rats exposed to acute treatment of chlorpyrifos were highly affected than those exposed to aldicarb, where the first caused lysis of some focal areas of the proximal tubules and loss of normal architecture while other cells still having cuboidal cell lining with vesicular nuclei, Fig. 6. The effect of aldicarb was limited to the contraction of glomeruli after acute treatment (Fig. 6).

Comparatively under subchronic intoxication, chlorpyrifos caused significant elevation in ACP after 21, 28 days whereas aldicarb increased ACP since the day 7 of treatment. Both compounds increased ALP, AST, ALT, γ-GT and lipase significantly ($p < 0.001$) at all times of bleeding as compared to the control (Table 2). Statistically, these alterations were time-dependent and the maximal elevation was recorded at 28 day. Serum AST, ALT, γ -GT levels of rats treated with aldicarb were significantly higher ($p < 0.001$) than those treated with chlorpyrifos.

Furthermore, chlorpyrifos induced significant increase in AST concentration but did not cause significant elevation in ALT. These findings may be interpreted as being not only due to cellular injury, but also as a result of a precedent direct stimulation of tissue enzymes by the pesticides.

In view of these findings, increase in AST, ALT, γ-GT was found to be consistently correlated with liver parenchymal cells damage in mammals (Tietz, 1986) which may lead to leakage of enzymes from injured sites into the blood stream (Poovala et al., 1999; Rahman et al., 2001).

Secondly, such elevation was accompanied concomitantly with significant increase in serum bilirubin (total) after 14, 21, 28 days of treatment with chlorpyrifos in a time-dependent manner while, aldicarb induced significant elevation only after 28 of treatment ($p < 0.01$). Furthermore, total protein was significantly decreased after 21 and 28 days of treatment with both compounds.

Table 2: Toxicological effects of subchronic treatment of chlorpyriphos and aldicarb at 1/10 LD$_{50}$ for 28 days and liver and kidney function tests (data are expressed as mean±SE).

Treatment	Time Intervals (Days)											
	7			14			21			28		
	Control	gp(a)	gp(b)	Control	gp(a)	gp(b)	Control	gp(a)	gp(b)	Control	gp(a)	gp(b)
LFTs												
ACP	3.25	3.55	3.89	3.29	3.72	3.95	3.30	3.78	3.94	3.24	3.80	4.21
	±0.03	±0.12	±0.09*	±0.05	±0.08	±0.06**	±0.04	±0.05*	±0.05**	±0.05	±0.06**	±0.05**
ALP	36.98	42.18	45.27	38.14	47.15	49.27	39.95	46.11	48.45	42.00	51.33	51.88
	±0.56	±0.61*	±0.47**	±0.72	±0.70*	±0.61**	±0.67	±0.81*	±0.74**	±0.96	±1.28**	±0.40**
AST	26.45	37.41	40.38	29.75	40.15	42.18	28.10	45.45	49.27±	26.25	49.68	52.25±
	±0.52	±0.65	±0.73**	±0.69	±0.65**	±0.75	±0.63	±0.73***	0.71***	±0.62	±1.77*	0.65***
ALT	18.75	20.65	19.95	18.82	21.89	22.75	17.95	22.15	23.08	18.75	23.63	25.00
	±0.33	±0.25	±0.39	±0.29	±0.42	±0.53	±0.33	±0.42*	±0.39**	±0.45	±1.00*	±0.42**
γ-GT	8.65	10.35	11.15	9.11	11.35	12.15	9.15	11.74	12.65	9.75	12.65	13.63
	±0.29	±0.33*	±0.27*	±0.33	±0.27*	±0.31*	±0.42	±0.33**	±0.37*	±0.37	±0.67**	±0.32**
Bilirubin	0.47	0.52	0.43	0.43	0.58	0.47	0.45	0.63	0.52	0.49	0.71	0.61
	±0.03	±0.05	±0.03	±0.04	±0.03*	±0.03	±0.02	±0.03*	±0.04	±0.03	±0.03***	±0.03**
Total	6.95	6.87	6.82	7.25	7.58	6.95	7.29	6.21	6.35	7.21	6.29	6.24
Protein	±0.10	±0.08	±0.06	±0.06	±0.05	±0.04*	±0.06	±0.08*	±0.11*	±0.04	±0.06*	±0.06*
Lipase	40.18	53.75	48.85	40.25	59.88	53.47	42.75	64.60	66.85	39.00	72.83	70.88
	±0.48	±0.32**	±0.28*	±0.54	±0.61**	±0.53**	±0.61	±1.05**	±0.88**	±0.65	±2.83***	±0.35***
KFTs												
Urea	10.95	25.73	23.85	11.42	29.72	36.35	12.51	33.25	31.17	12.75	33.67	32.75
	±0.35	±0.65**	±0.43**	±0.32	±0.28***	±0.56***	±0.62	±0.59***	±0.81**	±0.45	±0.82***	±0.75***
Creatinine	0.23	0.37	0.40	0.25	0.47	0.49	0.23	0.73	0.68	0.24	0.73	0.75
	±0.03	±0.04	±0.03**	±0.03	±0.04**	±0.04**	±0.03	±0.05***	±0.07**	±0.02	±0.03***	±0.02***

Treated gp(a): gp of rates treated with 1/10 LD$_{50}$ of chlorpyriphos
Treated gp(b): gp of rates treated with 1/10 LD$_{50}$ of aldicarb
LFTs: Liver function tests   KFTs: Kidney function tests
*, **, ***: Significance at p<0.05, 0.01, and 0.001 respectively.

Such hypoproteinemia is probably attributed to inhibited hepatic synthesis of blood proteins as a result of insecticide-protein interaction or due to stimulated protein catabolism to provide extra-energy requirements to overcome the stress in the polluted medium. Additionally, loss of protein from damaged kidneys could have contributed further to such hypoproteinemia (Shaker et al., 1988). Also, increased evidence of general pesticide protein interactions manifested as inhibited protein synthesis and augmented proteolysis, for possible utilization of their products for metabolic purposes, has been suggested by Casida et al., 1983. Moreover, depletion of liver soluble structural proteins concomitant with increased aminotransferases activity indicating utilization of proteins for energy production.

The present results are in agreement with results of other studies (Enan et al., 1982; Vodela and Dalvi, 1997). Exposure to both Ops and carbamates decreased the level of total serum proteins accompanied with alteration in the level of serum enzymes and amino acids (Gomes et al., 1999).

In the current investigation, both compounds caused elevation in serum lipase activity (Table 2); reflecting an interference with lipid metabolism, which in accordance with findings of Kozlowska et al., 1988, where the lipoprotein lipase activity in adipose tissues was slightly raised.
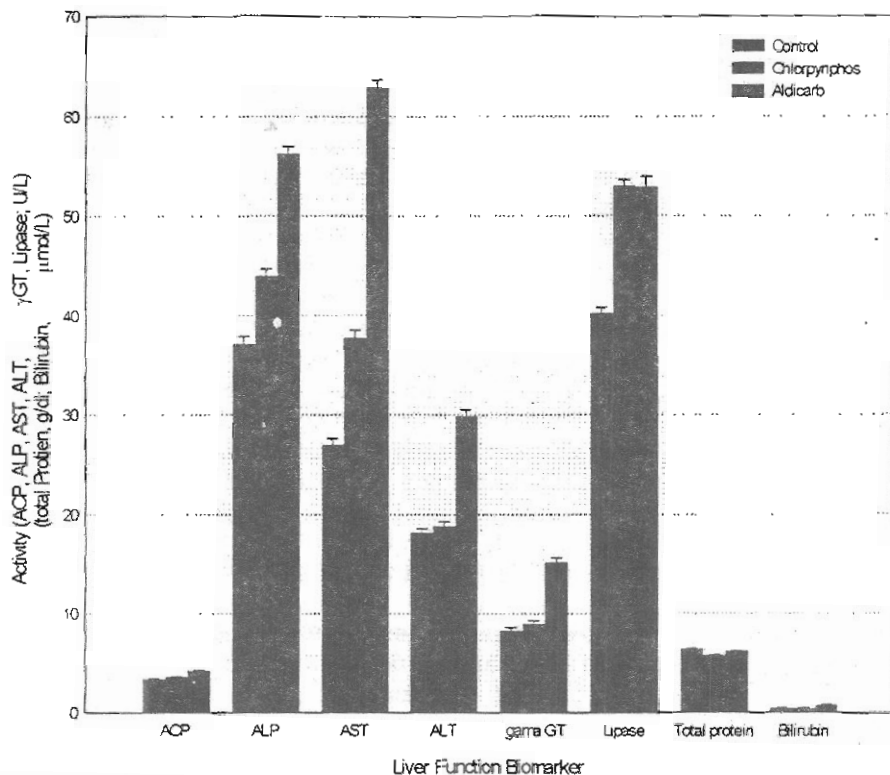
Figure (1): Acute toxicity of chlorpyriphos and aldicarb on liver function biomarkers after 24 hours of exposure.

The elevation of circulating levels of lipids may be due to several ways including increase of production by liver and other tissues, release from damaged cell membranes, decreased hepatic excretion, blocked conversion of cholesterol to sex steroids and finally thyroid dysfunction (Guyton and Hall, 1996). Additionally, both compounds led to inhibition of AChE activity either after acute or subchronic exposure. The effect of aldicarb was more pronounced than chlorpyrifos after 24 hr of exposure (39.47% and 30.89%; respectively). This neurotoxic effect was time dependent with a maximal inhibition at 28 day of exposure (Table 3). Such findings agree with earlier reports which stated that carbamates are able to reversibly inhibit AChE depending upon the time of exposure and adverse effects seem to occur in humans and other mammalian species when the AChE level drops below 70% of normal levels (Extoxnet, 1993). Furthermore, it was documented that aldicarb metabolites are more active than the parent compound in their AChE inhibition so that metabolism by the liver serves as activation rather than deactivation (WHO, 1991).

In a supportive study, feeding mice for 90-d a diet mixed with chlorpyrifos residues in stored soybeans led to considerably inhibited plasma

and RBCs-AChE activity by 78 and 46%; respectively, and significantly affected blood picture, liver and kidney functions during the feeding period (Zayed et al., 2003). In a similar study for 13 week, inhibition of brain AChE was slightly more than 60% (Yano, 2000).

Subchronic treatment of chlorpyrifos led to hypertrophy of hepatocytes which were amalgamated with no cellular boundaries (Fg. 4), while aldicarb caused complete lysis of nucleus, vaculated cytoplasm, and hypertrophic hepatocyted with no sinusoid (Fig. 4). Furthermore, vacuolation, necrosis and lysis of hepatocytes particularly at the peripherals were noticed. Such injury in hepatocytes could be enhanced by elevation in Kupffer cell function after insecticides, treatment as supported by Vidella et al., 1997.

In a similar situation, Drusban® injection i.p. at ½ LD_{50} resulted in significant increase in serum AST, ALT, ALP activity and the treated animals showed liver necrosis of mid-zonal type and fatty change at the periphery accompanied with necrosis of some of the seminiferous tubules of the testes and cloudy swelling of the convoluted tubules of the kidney (Mikhail et al., 1979). Also, ultrastructural changes in the liver, kidney, lung and the heart in addition to elevated bactericidal activity of neutrophils resulted from dermal application of chlorpyrifos (Nurelle D 550 EC) (Latuszynska, et al., 1999).

Table 3. Neurotoxic effect of tested pesticides expressed as % inhibition of serum AChE activity after acute and subchronic exposure.

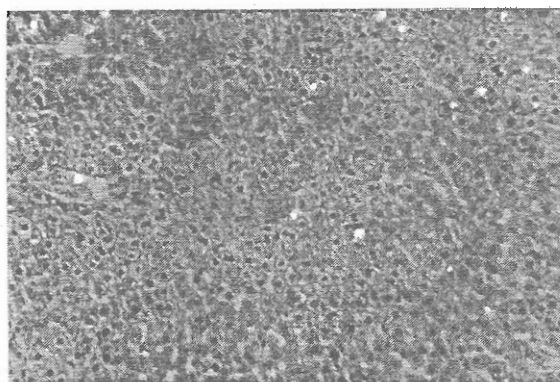| Treatment | % inhibition of AChE | | | | |
| | Acute dosing | Subchronic dosing/days | | | |
		7	14	21	28
Chlorpyrifos	30.89	13.88	12.37	28.94	37.59
Aldicarb	39.47	17.64	20.88	33.13	48.24

Fig (2): Section in normal liver of control rats showing normal hepatocytes with vesicular nuclei and acidophilic cytoplasm, the hepatic sinusoid with the lining endothelial cells and Von kupffer cells are in between the hepatocytes (X 400).

(A)　　　　　　　　　　　　　　(B)
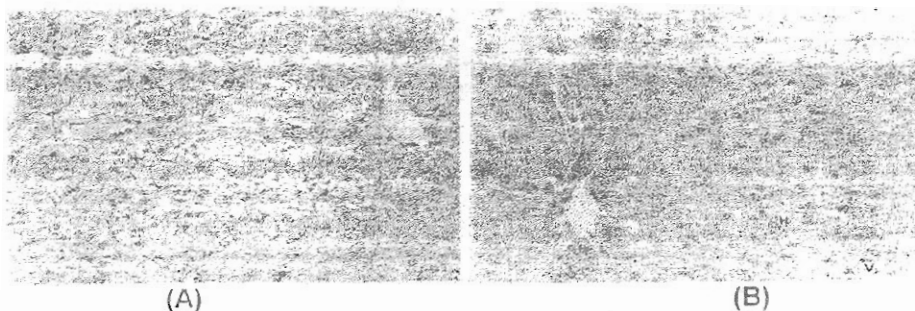
Fig (3): Sections in livers of rats exposed to acute treatment of chlorpyrifos and aldicarb showing loss of normal architecture of hepatocytes with enlargement of hepatocytes as common features (X 400).

　　A: chlorpyrifos treatment caused absence of hepatic sinusoid, vaculated and lysed hepatocytes, presence of small dark nucleus with vaculated cytoplasm,

　　B: aldicarb treatment caused disappearance of hepatic sinusoid.

(C)　　　　　　　　　　　　　　(D)
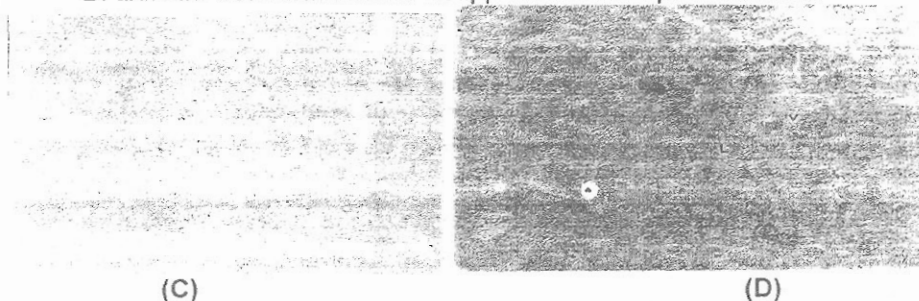
Fig (4): Sections in livers of rats exposed to subchronic treatment (X 400).

　　C: chloropyrifos treatment caused hypertrophy of hepatocytes and amalgamation with no cellular boundaries.

　　D: aldicarb treatment caused complete lysis of nucleus, vaculation of cytoplasm and hypertrophy of hepatocytes with no sinusoid.

In most cases, the affection of the hepatic-renal nexus either cells or membranes may be due to the toxic effect of the tested pesticides since its effect was previously reported to disrupt cell membranes leading to degeneration of cell texture (Melendez and Lopez, 1998) in type, dose and time-dependent manner.

Statistical analysis revealed that serum urea and creatinine of rats exposed to the tested pesticides were significantly increased in a time-dependent manner as compared to control (Table 2). Kidneys showed signs of renal damage and nephropathy after exposure to both pesticides and a significant association ($p < 0.01$) was observed between the length of exposure and level of damage. Renal damage and nephropathy marked by contracted glomeruli, extra-vasation of the blood, lumen obliteration of renal tubules as well as loss of normal architecture due to cell lysis were noticed after treatment with both pesticides (Fig. 7).

Elevation in urea and creatinine levels in the serum supports the occurrence of insecticide induced protein catabolism, impairement of renal function, renal insufficiency, reduction in glomerular filtration followed by increased blood urea nitrogen and creatinine and renal failure due to tubular necrosis (Mohssen, 2001). Many other studies support these findings (Akhtar et al., 1996; Melendez and Lopez, 1998; Shobha and Prakash, 2000; Verplanke et al., 2000; Mohssen, 2001).

Shobha and Prakash (2000) stated that poisoning with Ops and carbamates induced oxidative stress and resulted in renal tubular damage, which cause glucose leakage. According to our findings, it seems that chlorpyrifos can highly modify the concentration of endogenous antioxidants leading to development of oxidative stress and organ damage in a higher trend than aidicarb as illustrated in the way of liver and kidney damage exerted by chlorpyrifos compared with aldicarb although the later induced greater changes in some biochemical parameters than chlorpyrifos. Interestingly, aldicarb oxime, intermediate in the metabolism of aldicarb, was found to produce no alteration in body weight and no change in weight, gross or histopathology of the liver, spleen, lungs, thymus, kidneys or brain (NTP, 2005).

Furthermore, there is a great guess that disruption of renal tubular cell membrane could affect renal permeability of electrolytes such as Na^+ and Ca^{2+}, hence, the current work studied the potential changes in Ca^{2+} level. Both pesticides were found to affect serum Ca^{2+} as compared to control in a significant decreasing manner (Table 4). Ca^{2+} is known as a second messenger and plays an essential role in cell signaling cascades. However, the mechanism of cell signaling in case of subchronic exposure to chlorpyrifos or aldicarb received very little concerns and need to be further studied.

Fig (5): Section in normal kidney of control rats showing normal architecture (X 400).
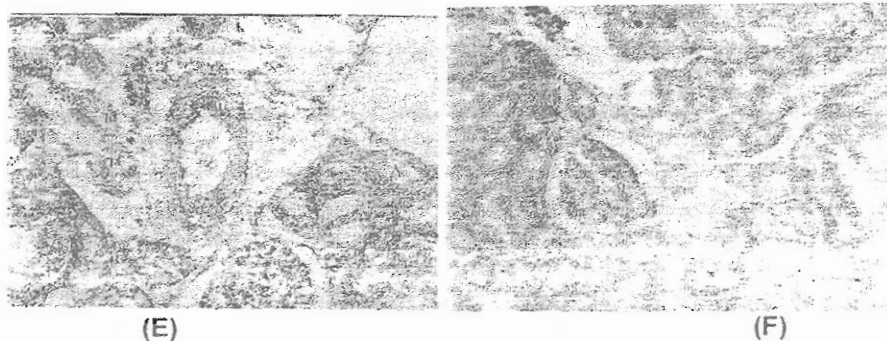
(E) (F)

Fig (6): Sections in kidneys of rats exposed to acute treatment of chlorpyrifos and aldicarb (X 400).
   E: chlorpyrifos treatment caused lysis of some focal areas of the proximal tubules, loss of normal architecture.
   F: aldicarb treatment caused contration of glomeruli.
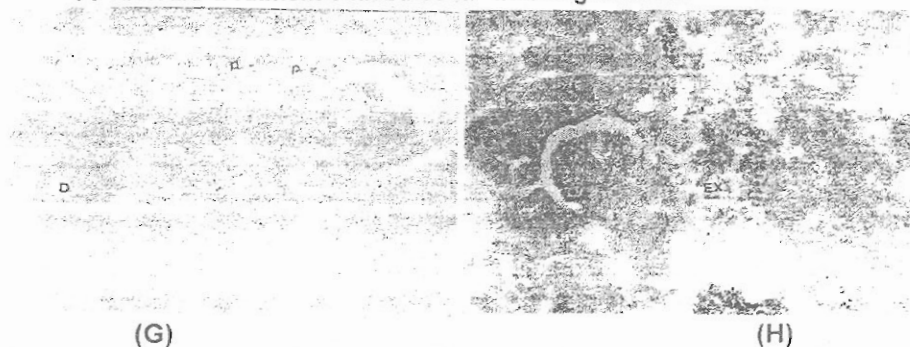

(G) (H)

Fig (7): Sections in kidneys of rats exposed to subchronic treatment (X 400).
   G: chlorpyrifos treatment caused obliteration of the lumen of some proximal convoluted tubules and loss of their architecture.
   H: aldicarb treatment caused extra-vasation of the blood.

Table 4: Effect of different dosing regimes of chlorpyriphos and aldicarb on serum Ca^{+2} level at the end of each treatment (data are expressed as mean±SE).

Treatment		Exposure	
		Acute	Subchronic
		Ca^{+2} Level (mg/dl)	
Control		6.99±0.05	7.11±0.05
Chlorpyriphos:	(49.0 mg/Kg)	6.71±0.06	6.35±0.04**
	(14.8 mg/Kg)		
Aldicarb:	(0.35 mg/Kg)	6.33±0.04*	6.54±0.03**
	(0.10 mg/Kg)		

*, **, ***: Significance at p<0.05, 0.01, and 0.001 respectively.

Based on the above mentioned results, it seems like liver appears to be more sensitive than the renal tissues to the effect of the tested pesticides.

Recently, it was documented that zinc treatment can protect hepatocytes from the marked disruptions in the membranous organelles and narrowing/blocking of biliary channels, which was otherwise a common observation following chlorpyrifos treatment (Goel and Dhawan, 2001). Furthermore, high doses of melatonin and a combination of vitamins E plus C considerably can reduce the toxic effect of chlorpyrifos on kidney tissues of exposed rats since chlorpyrifos increases lipid peroxidation and decreases the antioxidant potential by increasing oxidative stress (Oncu et al., 2002).

The current study contributes somehow with the other researches to identify the safety factors for interindividual variability that could help setting public health standards for repeated (subchronic and/or chronic) exposure of humans to low levels of chlorpyrifos as Op compound and aldicarb as carbamate. Further work is needed to address the potential implications of these effects for cardiovascular, mutagenic and metabolic disorders that may emerge long after the end of exposure to aldicarb and chlorpyrifos.

REFERENCES

Akhtar, N.; Kayani, S.; Ahmad, M. and Shahab, M. (1996). Insecticide-induced changes in secretory activity of the thyroid gland in rats. J. Appl. Toxicol., 16:397-400.

ANZFA [Australia New Zealand Food Authority] (2001). The 19th Australian Total Diet Survey, ANZFA, Canberra.

Bebe, F. and Panemangalore, M. (2003). Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats. J. Environ. Sci. Health B., 38(3):349-363.

CCINFO [Canadian Centre of Occupational Health Database]. 1991.

Carleton, H.; Druy, R.; Willingaton, E. and Coneron, S. (1967). Histological Techniques, 4th Ed., pp. 125-137. Oxford Univ Press, N.Y.

Casarett, L. (1980). The Basic Science of Poisons. In: Casarett and Doull's Toxicology. Doull, J.; Klaassen, C. and Amdur, M. (Eds.), 3rd Edition, Macmillan Publishing Company, New York.

Casida, J.; Gammon,D., Glickman, A. and Lawrence, L. (1983). Mechanisms of selective action of pyrethroid insecticides. Annu. Rev. Pharmacol., 23: 413-418.

Costa, C.; Catania, S. and Silvari, V. (2003). Gentoxicity and activation of organophosphate and carbamate pesticides by cytochrome P-450 2D6. G. Ital. Med. Lav. Ergon., 25 Suppl (3): 81-87.

Dalvi, R.; Dalvi, P. and Lane, C. (2004). Cytochrome P-450-mediated activation and toxicity of chlorpyrifos in male and female rats. Vet. Hum. Toxicol., 46(6): 297-299.

Ellman, G.; Courtney, K.; Andres, V. and Featherstone, R. (1961). New and rapid colorimetric determination of acetyl cholinesterase activity. Biochem. Pharmacol.,7:88-95.

Enan, E.; El-Sebae, A.; Enan, O. and El-Fiki, S. (1982). In vivo interaction of some prganophosphorus insecticides with different biochemical targets in white rats. J. Environ. Sci. Health B., 17: 549-570.

Extoxnet (author unknown) (1993). Extension Toxicology Network, (Online). Available at: http://ace.ace.orst.edu/info/extoxnet/pips/aldicarb.p93.

Gaines, T. (1969). Acute toxicity of pesticides. Toxicol. Appl. Pharmacol., 14: 515-534.

Goel, A. and Dhawan, D. (2001). Zinc supplementation prevents liver injury in chlorpyrifos-treated rats. Biol. Trace Elem. Res., 82(1-3)185-200.

Gomes, J.; Dawodu, A.; Lloyd, O.; Revitt, D. and Anilal, S. (1999). Hepatic injury and distributed amino acid metabolism in mice following prolonged exposure to organophosphorus pesticides. Hum. Exp. Toxicol., 18(1): 33-37.

Green, M., Heumann, M., Wehr, H., Foster, L., Williams, L., Polder, J., Morgan, C., Wagner, S., Wanke, L. and Witt, J. (1987). An outbreak of watermelon-borne pesticide toxicity. Am. J. Public Health, 77(11): 1431-1442.

Guyton, A. and Hall, J. (1996). Text Book of Medical Physiology. W.B. Saunders Company, Philadelphia, 9th Edition, pp:1017-1022.

Hirsch, G.; Mori, B.; Morgan, G.; Bennett, P. and Williams, B. (1987). Report of illnesses caused by aldicarb-contaminated cucumbers. Food Addit. Contam., 5(2): 155-165.

Kang, H.; jeong, S.; Cho, J.; Kim, D., Park, J. and Cho, M. (2004). Chlorpyrifo-methyl shows anti-androgenic activity without estrogenic activity in rats. Toxicol., (2-3): 219-230.

Kevekordes, S.; Gebel, T.; Pav, K.; Edenharder, R. and Dunkelberg, H. (1996). Genotoxicity of selected pesticides in the mouse bone-marrow micronucleus test and in sister-chromatid exchange test with human lymphocytes *in vitro*. Toxicol. Lett., 89(1):35-42.

Kozlowska, A.; Sadurska, B. and Szymczyk, T. (1988). Effect of dichlorvos on the activity of lipoprotein lipase from adipose tissue, on plasma lipids and postheparin activity in rats. Arch. Toxicol., 62: 227-229.

Latuszynska, J.; Luty, S.; Halliop, J.; Przylepa, E.; Tochmann, A.; Obuchowska, D. and Korczak, E. (1999). Studies of toxicity of dermal-absorbed nurelle D 550 EC preparations. Ann. Agric. Environ. Med., 6(2):151-159.

Marrs, T. and Dewhurst, I. (2000). Toxicology of Pesticides. In: Ballantyne B, Marrs TC, Syversen T (Eds): *General and Applied Toxicology*, 1993-2012. London-New York.

McCollister, S.; Kociba, R.; Humiston, C.; McCollister, D. and Gehring, P. (1974). Studies of the acute and long-term oral toxicity of chlorpyrifos (0,0-diethyl-0-(3,5,6-trichloro-2-pyridinyl)phosphorothioate). *Food Cosmet. Toxicol.* **12**, 45–61.

Melendez, C. and Lopez, H. (1998). Effect of cadmium and parathion on renal function in rat. Proc. West. Pharmacol. Soc., 41: 65-67.

Mikhail, T.; Aggour, N.; Awadallah, R.; Boulos, M.; El-Dessoukey, E. and Karima, A. (1979). Acute toxicity of organophosphorus and organochlorine insecticides in laboratory animals. Z. Ernahrungswiss, 18(4):258-268.

Mohssen, M. (2001). Biochemical and histopathological changes in serum creatinine and kidney induced by inhalation of Thimet (Phorate) in male Swiss albino mouse, Mus musculus. Environ. Res., 87: 31-36.

Montesissa, C.; Huveneers, M.; Hoogenboom, L.; Amorena, A.; De Liguoro, M. and Lucisano, A. (1994). The oxidative metabolism of aldicarb in pigs: in vivo-in vitro comparison. Drug Metabol. Drug interact., 11(2): 127-138..

NRA [National Registration Authority] (2000). Review of Chlorpyrifos. Commonwealth of Australia, accessed at www.nra.gov.au/chemrev/chemrev.shtml.

NTP [National Toxicology Program] (2005): Department of Health and Human Services. The Immunotoxicity of aldicarb oxime (CAS No. 1646-75-9) in female B6C3F1 mice. NTP Report Number IMM89025.

Nolan, R.; Rick, D.; Freshour, N. and Saunders, J. (1984) Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol. Appl. Pharmacol., 73, 8-15.

Oncu, M.; Gultekin, F.; Karaoz, E.; Altuntas, I. and Delibas, N. (2002). Nephrotoxicity in rats induced by chlorpyrifos-ethyl and ameliorating effects of antioxidants. Hum. Exp. Toxicol., 21(4):223-230.

Poovala, V.; Huang, H. and Salahudeen, A. (1999). Role of reactive oxygen metabolites in organophosphate-bidrin- induced renal tubular cytotoxicity. J. Am. Nephrol., 10: 1746-1752.

Qiao, D.; Seidler, F.; Padilla, S. and Slotkin, T. (2002). Developmental neurotoxicity of chlorpyrifos: what is the vulnerable period?. Environ. Health Perspect., 110(11):1097-1103.

Rahman, M.; Siddiqui, M. and Jamil, K. (2000). Acid and alkaline phosphatase activities in a novel phosphorothionate (RPR-11) treated male and female rats: Evidence of dose and time dependent response. Drug Chem. Toxicol., 23: 497-509.

Rahman, M.; Siddiqui, M. and Jamil, K. (2001). Effect of Vepacide (Azadirachta indica) on aspartate and alanine aminotransferase profiles in a subchronic study with rats. Hum. Exp. Toxicol., 20: 243-249.

Risher, J.; Franklin, L. and Stara, J. (1987). The toxicologic effects of the carbamate insecticide aldicarb in mammals: a review. Environ. Health Perspect., 72: 267-274.

Schalm, O. (1986). Veterinary Hematology. 4th Ed., pp. 21-36. Lea and Febiger, Philadelphia, USA.

Shaker, N.; Hassan, G.; El-Nouty, F.; Abo-Elezz, Z. and Abd-Allah, G. (1998). *In vivo* chronic effect of dimethoate and deltamethrin on rabbits. J. Environ. Sci. Health B., 23: 387-399.

Shobha, T. and Prakash,O. (2000). Glycosuria in organophosphate and carbamate poisoning. J. Assoc. Physicians India, 48: 1145-1160.

Snedecor, G. and Cochran, W. (1989). Statistical Methods. 14th Ed., pp. 593-598. Ames, Iowa State Univ. Press.

Tietz, N. (1966). A specific method for serum lipase determination. Clin. Chem. Acta, 13(3): 352-364.

Tietz, N. (1986). Textbook of Clinical Chemistry. Saunders, W. Co., Philadelphia, 1919.

US EPA [US Environmental Protection Agency] (2000). Human Health Risk Assessment for Chlorpyrifos, US Environmental Protection Agency, Washington.

Verplanke, A.; Bloemen, L.; Brouwer, E.; Van Sittert, N. and Boogaard, P. (2000). Occupational exposure to cis-1,3-dichloropronene: Biological effect monitoring of kidney and liver function. Occup. Environ. Med., 57: 745-751.

Videla, L.; Troncose, P.; Arisi, A. and Junqueira, V. (1997). Dose-dependent effects of acute lindane treatment on kupffer cell function assessed in the isolated perfused rat liver. Xenobiotica, 27: 747-757.

Vodela, J. and Dalvi, R. (1997). Effect of chlorpyrifos on hepatic gamma-glutamyl transferase, serum cholinesterase and xenobiotic metabolizing enzyme activities in rats. Bull. Environ. Contam. Toxicol., 59: 796-801.

Wagner, S. (1983). Clinical Toxicology of Agricultural Chemicals. Noyes Data Corporation., Park Ridge, New Jersey.

Weil, C. and Carpenter, C. (1972). Aldicarb (A), aldicarb sulfoxide (ASO), aldicarb (ASO2) and a 1:1 mixture of ASO:ASO2. Two year feeding in the diet of rats. Unpublished report from Mellon Institute.

WHO [World Health Organization] (1975). Chlorpyrifos. WHO data sheets on pesticides, No. 18.

WHO [World Health Organization] (1991). Aldicarb. International Programme on Chemical Safety, (Environmental Health Criteria, 124).

Winnik, L.; Pach, D.; Gawlikowskii, T.; Targosz, D. and Hydzil, P. (1997). Multiorgan damage in acute oral carbamate poisoning. Przegl. Lek., 54(100:684-688.

Yano, B.; Young, J. and Mattsson, J. (2000). Lack of carcinogenicity of chlorpyrifos insecticide in a high-dose, 2-year dietary toxicity study in Fischer 344 rats. Toxicol. Sci., 53:135-144.

Zayed, S.; Farghaly, M. and El-Maghraby, S. (2003). Fate of ^{14}C-chlorpyrifos in stored soybeans and its toxicological potential to mice. Food Chem. Toxicol., 41(6): 767-772.

Abd-Allah, Salwa M.

التقييم النسيجى والسمى المقارن نتيجة التعرض *In Vivo* لمبيدى الكلوربيرفوس و الألديكارب فى ذكور فئران وستر

سلوى مصطفى عبد الله

قسم سمية المبيدات للثدييات – المعمل المركـزى للمبيـدات – مركـز البحـوث الزراعيـة – الإسكندرية

تم فى هذه الدراسة تقييم مؤشرات السمية بعد التعرض لمبيدى الكلوربيرفوس و الألديكارب وربطها مع الفحوص النسيجية والتركيبية الدقيقة للأعضاء التالفة. وقد تم تعاطى المبيدات المختبرة عن طريق الفم لذكور فئران وستر وبنمطين أحدهما حاد و الأخر تحت مزمن عند تركيزات تمثل ٣/١ و ١٠/١ من قيمة الجرعة النصف مميتة على التوالى. وتمت معاملة الفئران المتعرضة للسمية التحت مزمنة يوميا لمدة ٢٨ يوم ولوحظ أن أعراض التسمم وأنماط المرض والموت تعتمد على الجرعة.

وقد تميز تلف الكبد بارتفاع معنوى فى دلائل إنزيمات مصل الدم عند مستوى معنوية ($P < 0.001$) بعد التعرض الحاد والتحت مزمن وفقا لنمط يعتمد على نوع المبيد والجرعة وزمن التعرض. وقد أظهر مبيد الألديكارب تأثيرا كبيرا واضحا أكثر من مبيد الكلوربيرفوس سواء بعد التعرض الحاد أو التحت مزمن.

وأظهرفحص أنسجة الكلى علامات للتلف بعد التعرض لكلا المبيدين ولوحظ وجود إرتباط معنوى عند مستوى معنوية ($p < 0.01$) بين طول فترة التعرض ودرجة التلف. ومع ذلك أظهر الكبد حساسية أكبر للتأثير بهذين المبيدين حيث ظهرت دلائل ضمور وتلف واضح فى الكبد متلازمة مع المؤشرات البيوكيميائية. وتقدم الدراسة دليلا لكل من الخلل الوظيفى والتركيبى الدقيق الناتج عن التعرض الحاد والتحت مزمن لمبيدى الألديكارب والكلوربيرفوس.