J.Agric.&Env.Sci.Alex.Univ.,Egypt Vol.4 (1)2005

IN VIVO GENOTOXIC EFFECTS OF TWO ORGANOPHOSPHORUS AND ONE CARBAMATE INSECTICIDES ON WHITE ALBINO RATS

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ABSTRACT

The present study describes the *in vivo* genotoxic effects of certain formulated forms of chlorpyrifos; chlorpyrifos-methyl; and methomyl as compared with the carcinogen compound, benzidine. Results showed that all of the tested insecticides decreased significantly the mitotic activity and chlorpyrifos was the most detrimental insecticide. The tested insecticides as well as benzidine increased significantly the chromosomal aberration, micronucleated polychromatic erythrocytes in rat bone marrow cells. Also, the tested compounds caused significant increase in the percentage of total aberration in rat spermatocytes and significant increase in DNA content of rat liver.

INTRODUCTION

Genotoxic effects are considered among the most serious of the possible potential side effects of agriculture pesticides. Data on genotoxicity and carcinogenicity of the organophosphorus (OP) and carbamate insecticides are rather controversial, depending on the genetic or the assay used (Moretti *et al.*, 1997). Because of these controversial results, this study aimed to further evaluate the potential genotoxic activities of sub-lethal doses chloropyrifos, chlorpyrifos-methyl and methomyl in a variety of *in vivo* short-term tests using male white rats .

MATERIAL AND METHOIDS

1. Tested animals:

Albino white male rats (*Rattus novegicus var. albus*), weighting 100-120 g obtained from Animal Health Research Center (Cairo) were used as test animals . Healthy male rats were utilized for the experiments. Four animals were used per each dose of each tested compound.

Test chemicals and treatments:

Chlorpyrifos-methyl (EC 50%) , O, O- Dimethyl- O- (3, 5, 6- trichloro- 2- pyridinyl) phoshorothioate; **Chlorpyrifos (** EC 48%) , O, O- Diethyl- O- (3, 5, 6- trichloro- 2- pyridinyl) phoshorothioate; **Methomyl** (W.P. 90%) , S- Methyl- N- (methyl carbamoyl) thio-acetimidate and **Benzidine** (98% a.i.) 1, 1' - biphenyl- 4, 4'- diamine.

Two sub-lethal doses, 0.04 and 0.1 LD_{50}, from each of chloropyrifos (3.84 and 9.6 mg a.i./kg b.w.); chloropyrifos-methyl (120 and 300 mg a.i./kg b.w.); methomyl (0.68 and 1.7 mg a.i./kg b.w.) (Anonymous,2005) and the standard carcinogen benzidine (12.36 ; 30. g mg a.i. / kg b.w.) were given daily via oral route to rats for 90 days. The control animals received equal volumes (of water or corn oil 0.5 ml /rat).

Chromosomal abnormalities in rat bone-marrow cells:

In all experiments 2-3 hours before killing, rats were injected with 0.6 mg/kg b.w. colcemid and animals were killed by decapitation after 24h. from last treatment. Bone marrow was taken in a centrifuged tube containing 0.075 M KCl. Chromosomes from bone-marrow cells were prepared following the method of Yosida and Amano (1965). Slides were stained with 10% Giemsa at pH 6.8 (Schmid, 1973). Hundred well spread metaphases were scored per dose and comparison with a negative control was also statistically tested.

Mitotic activity (cell cycle):

Investigation the effects of the compounds used upon cell proliferation were carried out. Three thousand cells were counted and the mitotic activity was estimated according to Driss-Ecole *et al.*, (1994).

Micronucleus test of polychromatic erythrocytes:

Bone marrow was flushed into a test tube containing calf serum (3ml) and then centrifuged at 1500 rpm. Smears were made on the slides, air dried and stained with May-Grunwald Giemsa method (Schmid, 1973; 1975 &1976). Micronuclei were identified as dark-blue staining bodies in the cytoplasm of polychromatic erythrocytes. Four thousands polychromatic erythrocytes per animal were scored by using a special hand counter. The frequently of micronucleated cells was expressed as a percent of micronucleated cells based on the total polychromatic erythrocytes percent. The obtained data from this study were analyzed according to Hart and Pederson (1983).

Analysis of primary spermatocytes:

After 24h. of the last 90 repetitive doses four males rats were killed by cervical dislocation. The testes were removed and meiotic chromosomal aberrations were made according to technique of Evans *et al*., 1964; Oued *et al.*, 1979 and Adler, 1984. Hundred well spread metaphases were scored per dose and comparison with a negative control was also statistically tested.

Liver DNA contents:

After killing insecticides – treated rats, as well as untreated rats, liver were removed and kept in deep freezer. DNA was extracted from the liver of rats according to the method of Sambrook *et al.*, (1989) with some modification as described by Abdel-Fattah (1995), and estimate the concentration of DNA by, the method of Charles (1970).

Statistical analysis :

The experimental design was a factorial CRD (Completed Randomized Design) with four replicates. Statistical analysis of data collected was carried out using a computer program.(Cohort Software, 1986).

RESULTS

Cell proliferation:

The results of the *in vivo* effects of the tested insecticides on the mitotic activity of rat bone marrow cells are presented in table (1). These results revealed that all of the tested insecticides and benzidine decreased significantly the mitotic activity of rat bone marrow cells in a dose-dependent manner. This effect was more pronounced in chlorpyrifos treatment .

Analysis of chromosomal aberrations:

Results in table (2) revealed that the tested insecticides , as well as benzidine caused significant increase in the percentage of total chromosomal aberration (CA) in rat bone marrow as compared with untreated control in a dose-dependent manner. The effect induced by chlorpyrifos was more pronounced than that caused by other insecticides .

Micronucleous test:

Results in table (3) revealed that the micronucleated polychromatic erythrocyte (MN) in rat bone marrow were adversely affected by the tested compounds, manifested as an increased of MN percentage, in a dose dependent manner. The carbamate insecticide, methomyl was the most potent insecticide in this respect.

Analysis of rat primary spermatocytes :

Results in table (4) showed that the tested insecticides, as well as, benzidine caused significant increase in the percentages of total aberration in rat spermatocytes in a dose dependent manner. The highest increased was noticed with chlorpyrifos .

Liver DNA content (μg/gm):

The Results of the in vivo effects of tested compounds on the content of DNA in the livers are presented in table (5) . It was observed that all of the tested compounds increased significantly of the liver DNA in a dose-dependent manner. The highest increased was noticed again with chlorpyrifos .

Table (1): In Vivo effect of tested insecticides on mitotic activity in bone marrow cells of treated rats .

Treatments	0.04 LD_{50}			0.10 LD_{50}		
	Total No. of examined cells	No. of dividing cells	Mitotic Index (MI%)	Total No. of examined cells	No. of dividing cells	Mitotic Index (MI%)
Control	3000	265	8.83	3000	265	8.83
Chlorpyrifos- methyl	3000	175	5.83*	3000	150	5.00**
Chlorpyrifos	3000	162	5.40**	3000	135	4.50**
Methomyl	3000	165	5.50*	3000	140	4.66**
Benzidine	3000	132	4.40**	3000	120	4.00**

Statistical difference from the control ;* significant at $P \leq 0.05$ & ** highly significant at $P \leq 0.01$.

Table (2)): In Vivo effect of tested insecticides on chromosomal aberration in bone marrow cells of treated rats .

Treatments	0.04 LD_{50}					0.10 LD_{50}				
	D	F	RCF	S	(1)Total aberration	D	F	RCF	S	(1)Total aberration
Control	2	2	1	2	7	2	2	1	2	7
Chlorpyrifos-methyl	8	10	6	18	42**	11	6	9	20	46**
Chlorpyrifos	10	11	12	19	52**	13	15	8	17	53**
Methomyl	10	11	10	10	31*	13	14	9	13	49**
Benzidine	9	10	1	20	40**	9	16	6	15	46**

D, Deletion; F, Fragment; S, Stickiness & RCF, Robertsonian centric fusion; (1) A number of 100 metaphases were counted; Statistical difference from the control ;* significant at $P \leq 0.05$ & ** highly significant at $P \leq 0.01$.

Table (3): In Vivo effect of tested insecticides on micronucleated polychromatic erythrocytes on bone marrow cells of treated rats .

Treatments	0.04 LD_{50}			0.10 LD_{50}		
	Total examined cells	Micronucleated polychromatic	(1)MN%	Total examined cells	Micronucleated polychromatic	MN%
Control	4000	25	0.62	4000	25	0.62
Chlorpyrifos-methyl	4000	90	2.25*	4000	115	2.87*
Chlorpyrifos	4000	110	2075*	4000	126	3.15*
Methomyl	4000	185	4.62**	4000	228	5.70**
Benzidine	4000	238	5.95**	4000	306	7.65**

(1)MN = Micronucleus; Statistical difference from the control ;* significant at $P \leq 0.05$ & ** highly significant at $P \leq 0.01$.

Table (4): In Vivo effect of tested insecticides on primary spermatocytes of treated male rats .

Treatments	0.04 LD_{50}						0.10 LD_{50}					
	D	F	S	E	I	(1)Total	D	F	S	E	I	Total
Control	0	1	0	1	0	2	0	1	0	1	0	2
Chlorpyrifos- methyl	3	4	4	10	3	24*	7	11	6	9	3	36**
Chlorpyrifos(F)	0	11	10	10	8	39**	2	9	11	12	10	44**
Methomyl (F)	9	5	0	5	6	25*	0	6	0	11	9	35**
Benzidine	9	7	6	15	5	42**	7	15	2	9	13	46**

D, Deletion; F, Fragment;E Exchange; S, Stickiness and I, Inversion; (1) A number of 100 metaphases were counted; ; Statistical difference from the control ;* significant at $P \leq 0.05$ & ** highly significant at $P \leq 0.01$.

Table (5): In Vivo effect of formulated insecticides on liver DNA content (µ g/g loiver) of treated male rats.

Treatments	0.04 LD_{50}	0.10 LD_{50}
Control	0.63 ± 0.07	0.63 ± 0.02
Chlorpyrifos-methyl(F)	1.57 ± 0.04*	1.61 ± 0.02*
Chlorpyrifos (T)	2.31 ± 0.08**	1.86 ± 0.02**
Methomyl (F)	1.01 ± 0.01*	1.24 ± 0.05*
$LSD_{0.05}$		

Each value is mean of three replicates ± SE ; Statistical difference from the control ;* significant at $P \leq 0.05$ & ** highly significant at $P \leq 0.01$.

DISCUSSION

Results of the present work revealed that two doses of the OP insecticides, chlorpyrifos (3.84 and 9.60 mg/kg) and chlorpyrifos-methyl (120 and 300 mg/kg), as repetitive oral treatments, induced significant decreases in the mitotic activity of rat bone marrow cells, MI, and significant increases in both total chromosomal aberration, CA, and micronucleated polychromatic erythrocytes, MN, of rat bone marrow cells. Also, these insecticides induced significant increases in DNA content of the livers and in chromosomal aberration of primary spermatocytes of treated rats. Therefore, these results revealed that the tested compounds have clastogenic activity upon somatic as well as germinal cells of treated rats. The genotoxic effect of chlorpyrifos was more pronounced than that of chlorpyrifos-methyl . The two OP insecticides contain the same structure except the substitution of ethyl groups in chlorpyrifos by methyl groups in chlorpyrifos-methyl which was less in its mammalian toxicity (LD_{50} of oral rats> 3000 mg/kg b.w.) and genotoxicity (in this work) than chlorpyrifos. This substitution may affect the intrinsic toxicity and genotoxicity and/or sensitivity of the structure to the metabolic systems in the treated rats.

Tests with laboratory animals have shown that chlorpyrifos has the potential to cause genetic damage (Amer and Fahmy, 1982 and Amer and Aly, 1992). Micronuclei were also increased after

chlorpyrifos exposure in bone marrow cells from Chinese hamsters (Ni *et al.*, 1993). Chlorpyrifos can also cause genetic damage in organisms other than mammals (Patanik and Tripathy, 1992), and DNA damage in three species of bacteria (Garrett *et al.*, 1986). Formulated chlorpyrifos showed clastogenic potency with the *Tradesc antsa* micronucleus (Trad-MCN assay) at doses between 10 and 50 ppm (Rodrigues *et al.*, 1998).

Findings the present study support previous findings, that OP pesticides have genotoxic effects. Also, the genotoxic effects of chlorpyrifos were more pronounced than that of its methyl analogues, chlorpyrifos-methyl. There is no available literature about the genotoxicity of chlorpyrifos-methyl. Non genotoxic effects were not detected *in vitro* either in the salmonella/ microsome assay or in the SOS chromotest when bacterial tester strains were exposed to chlorpyrifos-methyl in the absence or presences of S9 mix (Ruiz and Marzin, 1997).

Very few reports could be found on the genotoxic effects of the carbamate insecticide, methomyl (Extoxnet, 1996). Amer *et al.*, (1996) reported that methomyl, induced significant increase in the percentage of chromosomal aberrations in treated mice. In the present study, the two doses (0.68 and 1.7 mg/kg) of methomyl induced significant decrease in the mitotic activity of rat bone marrow cells, significant increase in the percentages of total chromosomal aberration and in the number of micronucleated polychromatic erythrocytes of rat bone marrow. Also, the two doses of methomyl caused significant increase in the percentages of total aberrations in rat spermatocytes and increased the DNA content of the liver of treated rats. According these results, methomyl has genotoxic effects on somatic as well as germinal cells of treated rats.

In a number of assays methomyl was not mutagenic (US,EPA , 1987 and Bethesda,1995). Also, there was no evidence of carcinogenicity in either rats or dogs that ingested high doses of methomyl in 2-year feeding studies (US, EPA, 1987). Methomyl was not carcinogenic in 22-and 24-month studies with rats fed doses neither of up to 20mg/kg, nor in a two-year study with mice fed dietary doses of up to 93.4mg/kg/day (Baron *et al.*, 1991). A report of (Extoxnet, 1996) suggests that methomyl is not carcinogenic. The differences of the obtained results and the reported results may be due

to the differences in the used genetic systems and assays and/or may be due to the fact that commercial formulation of methomyl, which was used in our study, may contain additional hazardous additives. Therefore, it is important in assessing the real human hazard from pesticides to investigate not only the active principle, but also the commercial formulations used in agriculture.

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J.Agric.&Env.Sci.Alex.Univ.,Egypt Vol.4 (1)2005

الملخص العربي

تأثيرات السمية الجينية داخليا لاثنان من المبيدات الفوسفورية العضوية وواحد كربامات على ذكور الفئران البيضاء

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توضح الدراسة الحالية تأثيرات السمية الجينية للصورة التجارية للكلوربيريفوس ، الكلوربيريفوس-ميثيل ، الميثوميل مقارنة بالبنزيدين كمركب محدث للسرطان . وقد أظهرت النتائج أن كل المبيدات المختبرة قد أحدثت نقصا معنويا في معدل الانقسام الخلوي وكان الكلوربيريفوس أكثر المركبات تأثيرا . أحدثت المبيدات المختبرة والبنزيدين زيادة معنوية في كلا من الشذوذات الكروموسومية والأنوية الصغيرة في خلايا نخاع العظام للفئران. ةكذلك فإن المبيدات المختبرة قد أحدثت زيادة معنوية في النسبة المئوية لمجموع الشذوذات الكلية في الخلايا الأولية المكونة للحيوانات المنوية في الخصية وزيادة معنوية في محتوى الكبد من الـ د . ن . ا.