J. Agric. Sci. Mansoura Univ., 33 (1): 509 - 518, 2008

THE ACUTE ORAL TOXICITY OF CHLORPYRIFOS AND CYPERMETHRIN AND THEIR MIXTURE ON ALBINO RATS

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ABSTRACT

The overall purpose of this work is to throw some light on the acute oral toxic effects of three commercial formulations of chlorpyrifos (i.e. Dursban, Pyriban and Chlorzan) 48% E.C. and cypermethrin (Sparkil 25% E.C.) and the mixture of Dursban and Sparkil on adult male and female albino rats. All the tested formulations were administered in corn oil to the rats and the median lethal doses (LD_{50} values) were determined after 24, 48, 72 and 96 hours (hrs) of treatment.

The obtained results revealed that the oral LD_{50} values of the tested insecticide formulations (Dursban, Pyriban, Chlorzan and Sparkil) on adult male and female rats were (125, 115), (100, 94), (140, 103) and (387, 251) mg/Kg body weight (b.w.) after 24 hrs, respectively. The 48hrs oral LD_{50} values of the fore mentioned formulations were (112, 90), (100, 75), (126, 81) and (230, 206) mg/Kg b.w., respectively.

On the other hand, the acute oral toxicity of the mixture of Dursban and Sparkil was considerably higher than the acute toxicity of each of them since the acute oral LD_{50} values were 102 and 75 mg/Kg b.w. for male and female rats after 24hrs, respectively.

INTRODUCTION

Pesticides are a broad group of heterogeneous chemicals that have a significant public health benefit by increasing food production productivity and decreasing food-borne and vector-borne diseases. However, depending on the agent and the exposure, they may pose human health risks (Weiss *et al.,* 2004). Acute pesticide poisonings are an important cause of morbidity and mortality. Although available data were inadequate to quantify with certainty the extent of the problem (WHO, 1986 and 1990), some estimates suggested that each year worldwide there were 3 million acute sever pesticide poisonings with 220000 human deaths (USDA, 1994).

Acute toxicity study detect short-term effects of high chemical doses (Paget, 1970). The simplest acute toxicity study employs LD_{50} value determination. The toxicity of chemicals has been classified by Matsumura (1995) according to their oral LD_{50} values, giving simple approximate expression of the degree of toxicity.

Much work has been done on the insecticides belonging to organophosphorus, pyrethroid and chlorinated groups (Khan and Sarwar, 2003).Chlorpyrifos is a broad range organophosphorus (OP) insecticide widely used in agriculture and indoor disinfestation (Worthing and Walker, 1987). Chlorpyrifos is classified as a moderately hazardous, class II, insecticide by the WHO (1997).

Synthetic pyrethroids, such as cypermethrin, have been considered among the safest pesticides available and are used worldwide (Igbedioh,

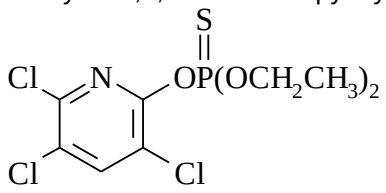
1991).Cypermethrin is classified by the WHO as a moderately hazardous, class II (WHO, 1995).

Thus, the present study was undertaken to determine the LD_{50} values of orally administered three formulations of chlorpyrifos (i.e. Dursban, Pyriban and Chlorzan 48% E.C.),one formulation of cypermethrin (Sparkil, 25% E.C..) and the mixture of Dursban and Sparkil on adult male and female albino rats.

MATERIALS AND METHODS

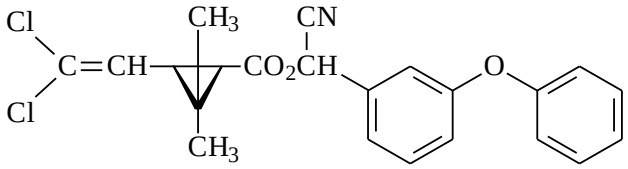
A-The Used Insecticides:

1. Chlorpyrifos: *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate.



The following three commercial formulations of chlorpyrifos, Dursban 48% EC (provided by Dow Agro Sciences Company), Pyriban 48% EC (provided by Helb Pesticides and Chemicals Company-Egypt) and Chlorzan 48% EC (provided by Kafr El-Zayat Pesticides and Chemicals Company-Egypt) were used.

2.Cypermethrin:(RS)-α-cyano-3-phenoxybenzyl(1RS)-*cis-trans*-3-(2,2-dichlo-rovinyl)-2,2-dimethylcyclopropanecarboxylate.



Cypermethrin, Sparkil 25% EC (provided by Helb Pesticides and Chemicals Company, Egypt), was used.

B-Experimental animals:

Albino-rats, (*Rattus norvegicus*) obtained from Helwan Farm of Egyptian Organization for Vaccine and Biological Preparations, and ranging in weight from 180-250 gm were used for experimentation. Rats were fed on standard chow diet obtained from El-Salam Factory for Dry Ration-El Marg - Egypt ; and water was freely provided *ad libitum.*

Before reaching the adult stage, male and female rats were separated and placed in stainless steel cages, with different capacities i.e. 35 x 25 x 20 cm and 30 x 20 x 20 cm, (5 males or females/cage). All animals were observed and allowed to acclimatize to the laboratory conditions for a minimum 2 weeks prior to admission into experiments. At the beginning of each experiment ,unhealthy animals were excluded; and animal body weight was measured to the nearest gram. The total numbers of rats used in the present studies were 175 adult male and 175 adult female rats.

C-Experimentation:

The acute oral LD_{50} values of Dursban, Pyriban, Chlorzan and Sparkil and a mixture of Dursban and Sparkil(i.e.5compounds) on adult male and female rats were determined. The total number of rats for each sex was divided as : 5 compounds × 6 doses for each compound plus the control (i.e.7 treatments) × 5 rats for each treatment = 175 rats . Prior to dosing ,animals were deprived of food for 15-18 hrs overnight but given free access to water . Each insecticide was dissolved in corn oil (1 ml/kg b.w. rat), while untreated animals were given 1 ml corn oil/kg b.w. . Oral dosing was carried out using a plastic syringe attached to a curved stainless steel animal intubation needle with spherical ball tip.

The tested doses of Dursban, Pyriban or Chlorzan were 50, 75, 100, 125, 150 and 175 mg/kg body weight (b.w.). In the case of Sparkil, the doses were 100, 200, 300, 400, 500 and 600 mg/kg b.w. .

For determination of LD_{50} values of a mixture of Dursban and Sparkil, the doses were (15+48.375), (18+58.05), (21+67.725), (24+77.4), (30+96.75) and (36+116.1) mg/kg b.w. for male rats and (15+31.375), (18+37.65), (21+43.925), (24+50.2), (30+62.75) and (36+75.3) mg/kg b.w. for female rats which representing 12.5, 15, 17.5, 20, 25 and 30% of the obtained LD50 value (24 hrs) for Dursban or Sparkil , respectively.

The animals had been observed and the mortality rate was recorded in each group with the next 24, 48, 72 and 96 hrs. The LD_{50} values were determined according to the “Probit analysis” technique described by Finney (1971).

RESULTS AND DISCUSSION

The estimated LD_{50} values are shown in tables (1 and 2) and Figs. (1 and 2). It was observed that all rat mortality was occurred within 48 hrs from treatment .

The LD_{50} values of Dursban 48% E.C. for male rats were 125 mg/kg b.w. after 24 hrs post treatment , and 112 mg/kg after 48 or 72 or 96 hrs post treatment . While the values of LD_{50} for female rats were 115 mg/kg after 24hrs and 90 mg/kg after 48 hrs.

Table (1):The oral LD_{50} values of the tested insecticide formulations for male albino rats after 24 and 48 hrs of treatment.

Duration / Insecticides	24 hrs				48 hrs			
	LD_{50} values mg/kg	Confidence limits (mg/kg)		Slope	LD_{50} values mg/kg	Confidence limits (mg/kg)		Slope
		Lower	Upper			Lower	Upper	
Dursban	124.73	111.43	139.64	14.985	111.72	98.4	126.77	12.593
Pyriban	100.14	87.54	114.76	13.632	100.14	87.54	114.76	13.632
Chlorzan	139.5	125.3	155.6	15.323	126.19	112.2	141.25	15.019
Sparkil	386.57	262.42	568.85	3.082	229.64	171.4	306.2	6.689
Mixture (1+4)	102.33	75.34	138.995	3.555	102.33	75.34	138.995	3.555

Table (2):The oral LD_{50} values of the tested insecticide formulations for female albino rats after 24 and 48 hrs of treatment.

Duration / Insecticides	24 hrs				48 hrs			
	LD_{50} values mg/kg	Confidence limits (mg/kg)		Slope	LD_{50} values mg/kg	Confidence limits (mg/kg)		Slope
		Lower	Upper			Lower	Upper	
Dursban	114.65	102.1	128.53	9.328	89.71	78.16	103.04	13.034
Pyriban	94.35	82.22	108.39	12.862	75.15	63.97	88.31	12.519
Chlorzan	102.62	89.33	117.76	12	80.78	69.024	94.41	12.681
Sparkil	251.37	190.55	331.13	5.589	206.06	153.11	277.33	7.655
Mixture (1+4)	75.05	57.02	98.62	4.026	75.05	57.02	98.62	4.026

The LD_{50} value for Pyriban was 100 mg/kg at all time intervals after dosing of male rats, while it was 94 mg/kg after 24 hrs of dosing and 75 mg/kg at 48 hrs for female rats.

The LD_{50} values of Chlorzan in male rats were 140 mg/kg at 24 hrs after treatment and 126 mg/kg after 48 hrs of dosing . For female rats, the LD_{50} value was 103 mg/kg at 24 hrs after treatment and 81 mg/kg at 48 hrs post treatment . These finding indicated clearly that chlorpyrifos was more toxic to female rats than to male rats.

The above findings for chlorpyrifos are coincided with those obtained by other investigators, the acute oral LD_{50} values for rats were 97 mg/kg (Drummond *et al.,* 1967). Gains *et al.* (1969) reported that the oral LD_{50} values of chlorpyrifos ranged from 82-163 mg/kg for rats. The acute oral toxicity of chlorpyrifos is considered moderate, with acute oral LD_{50} values in rats ranging from 118 to 245 mg/kg (Mc Collister *et al.,* 1974), and the acute oral LD_{50} value in rats is 150 mg/kg, which is considered moderate (Dow Chemical Company, 1982). While, Worthing and Walker (1987) recorded an oral LD_{50} values of chlorpyrifos of 136-169 mg/kg for rats, and WHO (1997) reported that oral LD_{50} value was 135 mg/kg b.w. rats.

On the other hand, the LD_{50} values for Sparkil were 387 mg/kg at 24hrs after dosing and 230 mg/kg at 48 hrs after dosing in male rats. While they were 251 mg/kg at 24 hrs and 206 mg/kg at 48 hrs after treatment for female rats. Also, these findings indicated that females generally were more sensitive than males.

On the basis of the obtained LD_{50} values shown in Tables (1 and 2) cypermethrin (Sparkil 25% EC) has been found to be more toxic to female than to male rats. The findings in the present investigation gain support by the observations made by Worthing and Walker (1983) who reported that the acute oral LD_{50} values of cypermethrin in rats were between 251 to 4123 mg/kg b.w., and the EPA (1989) reported oral LD_{50} values of 187 to 326 mg/kg in male rats and 150 to 500 mg/kg in female rats. Also, Gupta (1990) found that there was no difference between the two sexes in rats when treated with cypermethrin . However, the LD_{50} values of cypermethrin varies from 367 to 2000 mg/kg in female rats depending on the ratio of cis / trans-isomers present (Ray, 1991) . While Putintseva *et al.* (1995) cited that the LD_{50} value of Fury (zeta-cypermethrin) taken by mouth was 385 mg/kg for rats .

Fig. (1): Determination of median lethal doses (LD_{50} values) of the tested insecticides on adult male albino rats dosed orally at different times.

A: Ld-p line after 24 hrs.

B: Ld-p line after 48,72 and 96 hrs.

Fig. (2): Determination of median lethal doses (LD_{50} values) of the tested insecticides on adult female albino rats dosed orally at different times.
A: Ld-p line after 24 hrs.
B: Ld-p line after 48,72 and 96 hrs.

Several factors influence acute LD_{50} values, such as chemical concentration, vehicle used for drug administration, formulation (cis/trans ratio), temperature; sex, age and strain of the animals used and interlaboratory variation (Coombs *et al.,* 1979); route of exposure is critical in assessing the potential of a toxicant. Some of these factors probably contributed to the variation of the oral LD_{50} values.

The obtained results indicated that chlorpyrifos formulations were more toxic to rats than cypermethrin formulation . This may be due to the synthetic pyrethroids were weakly toxic to mammals when administered orally due to rapid detoxification (Matsui and Yamamoto, 1971 and WHO, 1989). Several signs of intoxication were observed in all cypermethrin treated groups. Death was observed due to respiratory failure and convulsions preceded death. In addition, the cypermethrin toxicity in rats was characterized by salivation followed by jerking movements of limbs and rolling convulsions (Coombs *et al.,* 1979; He *et al.,* 1989 and Ray and Forshaw, 2001). These symptoms were thought due to the action of cypermethrin on nerve cell axons in the peripheral and central nervous systems (WHO, 1989).

It was observed that all rats had signs of intoxication, which varied in severity according to the dose administered; there was full recovery of surviving rats (Lyaniwura and Okonkwo, 2004).

Finally, the LD_{50} value obtained for the mixture of Dursban and Sparkil was 102 mg/kg at all time intervals after insecticide administration to male rats, however, this value was 75 mg/kg for female rats at the same time intervals after treatment. On the basis of LD_{50} values, the mixture was found to be more toxic to female than to male rats.

In accordance with the obtained LD_{50} values of the mixture on adult male and female rats, we found that this mixture was more toxic to female rats, (LD_{50} value of 75 mg/kg) than to male rats (LD_{50} value of 102 mg/kg), and the LD_{50} values decreased in comparable with the LD_{50} values which obtained from each insecticide alone to both sexes of rats.

These findings may be due to the fact that simultaneous contact with substances that inhibit detoxification processes, which called synergists, can increase the acute toxic effects of a pyrethroid. High levels of some synergists (organophosphorus and carbamate compounds) can block esterase enzymes that degrade pyrethroids by cleaving the molecule at the double bond between a carbon and an oxygen atom. Other synergists (piperonyl butoxide and sulfoxide) block the mixed function oxidases (MFO), enzymes which oxidize and detoxify a wide variety of compounds (Casida, 1980; Gaughan *et al.*, 1980 and Williamson, 1989).

The sex differences in susceptibility to toxicants and drugs in rats have been well documented. In general, female rats are more susceptible on a per kilogram basis, particularly to organophosphorus insecticides (Matsumura, 1975). While Bradbury and Coats (1989) reported that the acute toxicities of some pyrethroids differ for male and female rats and mice.

Also, it was reported that the formulation of insecticides affects its toxicity. El-Sebae *et al.* (1978) found that the acute 24 hrs LD_{50} value was 60 mg/kg for the formulated chlorpyrifos versus 140 mg/kg for the technical grade compound.

Since the technical grade of a pyrethroid is usually formulated (mixed with carriers, solvents, etc.) for use in commercial pest control, the toxicity of these other ingredients must be taken into consideration when assessing the toxicity of a formulated product (Williamson, 1989).

Formulated pesticides seem to be more toxic than their active ingredients alone (El-Sebae *et al.,* 1982); and the WHO (1991) emphasized that the final toxic classification of any pesticide is related to its formulation form. This may be due to the effects of additives in the formulation form of pesticides which increase its penetration through its target organism causing more toxicity than the pure active ingredient materials of the pesticides (Abou-Zeid *et al.,* 1993).

The use of organic solvents and surfactants in the formulation appears to enhance toxicity, probably as a result of increased rate of penetration through its target tissue of organisms causing more toxicity than the pure active ingredient materials of the pesticides (El-Sebae *et al.*, 1978, 1982; WHO, 1991 and Abou-Zeid *et al.,* 1993).

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السمية الحادة لمبيدي الكلوربيريفوس و السيبرميثرين و مخلوطهما على الفئران البيضاء

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يهدف هذا البحث إلى تقدير السمية الحادة لثلاثة تجهيزات تجارية لمبيد الكلوربيريفوس ٤٨% مركز قابل للاستحلاب(EC) وهى الدورسبان والبيريبان والكلورزان وتجهيزة تجارية واحدة لمبيد السيبرميثرين ٢٥%مركز قابل للاستحلاب وهى سبارکل وكذلك مخلوط كل من الدورسبان وسبارکل وذلك عن طريق حساب قيم الجرعات النصفية القاتلة (LD_{50} values) لهذه التجهيزات على ذكور و إناث فئران التجارب البيضاء بعد فترات ٢٤و ٤٨و ٧٢و٩٦ ساعة من المعاملة عن طريق الفم .

دلت النتائج أن الموت للفئران فى جميع المعاملات يحدث خلال ٤٨ ساعة من المعاملة وقدرت قيم ال LD_{50} للتجهيزات الثلاث لمبيدالكلوربيريفوس وهى الدورسبان والبيريبان والكلورزان بعد٢٤ساعة من المعاملة لذكور وإناث الفئران هى (١٢٥ , ١١٥) و (١٠٠ , ٩٤) و(١٤٠ , ١٠٣) مجم/كجم ولمبيد سبارکل(٣٨٧ , ٢٥١) مجم/كجم على الترتيب.

وبعد ٤٨ ساعة من المعاملة كانت قيم ال LD_{50} هى(١١٢ , ٩٠) و (١٠٠ , ٧٥) و (١٢٦ , ٨١) مجم/كجم لتجهيزات مبيد الكلوربيريفوس وكانت(٢٣٠ , ٢٠٦) مجم/كجم لمبيد سبارکل على الترتيب وهذه القيم ظلت كما هى بعد فترات ٧٢ و ٩٦ ساعة.

أعطى مخلوط الدورسبان وسبارکل سمية أعلى من سمية كل مبيد على حده حيث كانت قيم ال LD_{50} للمخلوط هى ١٠٢ و ٧٥ مجم/كجم على ذكور وإناث الفئران بعد ٢٤ ساعة من المعاملة على الترتيب ولم يحدث موت بعد ذلك .