Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 08, Issue, 09, pp.5793-5802, September, 2017

RESEARCH ARTICLE

TOXICITY EVALUATION OF THE ORGANOPHOSPHATE COMPOUND CHLORPYRIFOS ON TARGET AND NON-TARGET ORGANISMS WITH REFERENCE TO MICE

***[1]Muniya Naik, M., [2]Jayasankar, A., [3]Narasimha Rao, C., [3]Srineetha, U., [4]Sudhakara Reddy, M., [5]Udaykiran, V. and [5]Sivasankar, R.**

[1]S.G.Govt.Degree College, Piler, Chittoor(Dt), A.P.
[2]P.V.K.N.Govt.College, Chittoor (Dt), A.P
[3]Govt.College for men (A), KADAPA (Dt), A.P
[4]Reader in Department of Zoology, Loyola Degree College, Pulivendula, A.P
[5]Dept.Of Zoology, S.V.U. Tirupathi, A.P

ARTICLE INFO

Article History:
Received 10th June, 2017
Received in revised form
09th July, 2017
Accepted 26th August, 2017
Published online 27th September, 2017

Key words:

Chlorpyrifos,
LD_{50},Toxicologyevolution,
Wistar strain mice.

ABSTRACT

Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization. Chlorpyrifos exposure may lead to acute toxicity at higher doses, Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system and also skin and eye irritant. Healthy wistar strain mice of the same age group 100±10 days and weight 75±10 grams were selected as experimental animals for the present study. The organophosphate compound chlorpyrifos provided by Nagarjuna Agri Chem Limited, East Godavari District, Andhra Pradesh, was chosen as the toxic chemical. Acetone was used as the vehicle for administering chlorpyrifos. The route of administration followed was oral intubation method.In the present study the toxicity of chlorpyrifos in Mice was determined, to ascertain the LD_{50} and subacute doses. The animals were exposed to different concentrations of chlorpyrifos, showed no mortality up to 28 mg/kg body weight, 16.66% mortality at 32 mg/kg body weight, 25% mortality at 42 mg/kg body weight, 33.33% mortality at 52 mg/kg body weight, 50% mortality at 62 mg/kg body weight, 66.66% mortality at 72 mg/kg body weight, 83.33% mortality at 82 mg/kg body weight, 100% mortality at 92 mg/kg body weight and 102 mg/kg body weight observed. The LD_{50} value obtained from the sigmoid curve is 62 mg/kg body weight for 48 hours.During the present investigation the appearance of signs and symptoms were observed in between 20-150 minutes, except lacrimation, and salivation which were occurred between 10-20 minutes following the chlorpyrifos administration.

INTRODUCTION

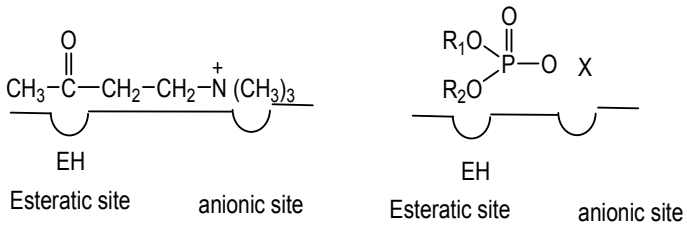
Toxicology, although an old branch of science, is a branch of medical science that deals with the nature, effects and properties and the detection of poisons. It is defined as any harmful effect of chemical or a drug on a test organism. Extremely high concentrations of these chemicals are more toxic to the biological systems. Evaluation of toxicity of a chemical therefore is necessary to know, because it would help us to know its potentiality so, that it could be possible to desire more powerful formulations. The term toxicity and hazard have been defined as a determinable value which is the capacity of a substance to produce injury and an indeterminable value which is the probability that injury will result from the use of substance in the proposed quantity and manner (Cartson, 1962).Toxicity means the intrinsic capacity of a chemical substance or a mixture of substances to induce injury. Hazard means the observed toxic manifestation(s) induced by a known quantity of a substance under known exposure conditions. The term is frequently used interchangeably with "intrinsic toxicity" (OECD, 2000). The toxicity of the chemical is also influenced by many biotic and abiotic factors such as size (Heit and Fingerman, 1977; Brazinskii*et al.*, 1979; JayanthaRao, 1982), nutritional status (Pal and Kushwah, 1981; Das and Garg, 1981), species specificity (Gouda *et al.*, 1981; Li and Choa, 1981; Jacob *et al.*, 1982; Janardhan*et al.*, 1987; Surendranath*et al.*, 1987ab) and chronobiology of the animal (Uttaman*et al.*, 1979), increase in animal density (Holden, 1973) and sex of the animal (Klein *et al.*, 1983; Chanh*et al.*, 1981). Every pesticide may vary greatly in its toxicity and persistence. Since the evaluation of toxicity of a test chemical is a sensitive

****Corresponding author: Muniya Naik,***
S.G.Govt.Degree College, Piler, Chittoor(Dt), A.P.

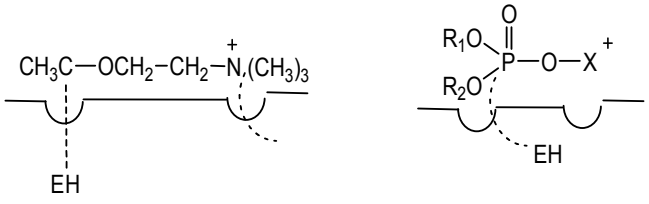
phenomenon, which can be influenced by several factors such as size (JayanthaRao, 1982) animal weight, its developmental stage, time of exposure and temperature. Thus, various factors influence the LD_{50} values. These factors are manifold and dependent upon the given set of experimental conditions (Russell and Overstreet, 1987). The LD_{50} values can also significantly vary between animals of the same basic strain obtained from different suppliers (Russell and Overstreet, 1987), according to the purity of the chemical (HO *et al.,* 1983) and the sex difference (Overstreet *et al.,* 1979). It has also been observed that the LD_{50} differs between water deprived and non-deprived animals (Russell *et al.,* 1986). In general the rat and mouse are normally used for oral toxicity test, because they have digestive system geared to the same sort of diet as man in many respects, has similar metabolic responses (McEwen and Stephenson, 1979). Generally the lethality of a chemical (or) pesticide to a particular animal species is expressed in terms of mortality and time. In the case of terrestrial animals it is expressed in terms of lethal dose (LD) as mg/kg weight of animals. In aquatic animals the lethality is expressed in terms of lethal concentration (LC) as mg/litre (or) parts per million (ppm) (or) parts per billion (ppb) while conducting the toxilogical studies with the administration of toxicants. Usually the toxicity of a chemical is expressed as LD_{50}, since greater confidence may be attached to the estimates in the middle ranges rather than at the extremes. The LD_{50} is the concentration of a chemical (or) pesticide required to kill 50% of a group of animals with in a given time interval. Toxicity evaluation of a pesticide will be highly useful in the final evaluation of designing safe level (or) tolerable level of pollution to the environment, and this will help in establishing limits and levels of acceptability by the biotic components.

Mechanism of Toxicity

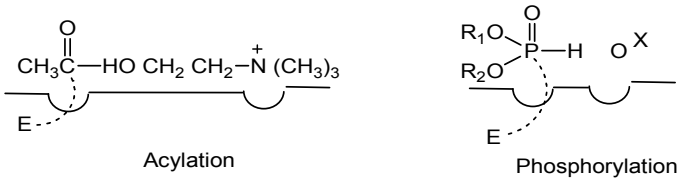
The most conspicuous feature of all Organophosphates (OP) compounds is that they are structurally complementary to the target enzyme molecule, cholinesterase. OP compounds mimic the gross molecular shape of the natural substrate of cholinesterase, acetylcholine. Cholinesterase has two active sites viz., esteratic and anionic sites. The process of OP inhibition of cholinesterase is essentially analogous to the early stage of acetylcholine hydrolysis. The inhibition of the enzyme is carried out in four steps.



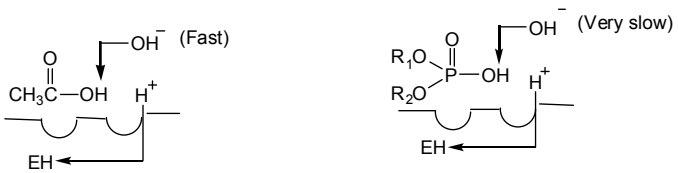
STEP 1



STEP 2



STEP 3 and 4



(Matsumura, 1985)

Step 1: Coulombic binding of acyl carbon or phosphorous atom to the esteratic site, and in the case of acetylcholine, to that of cationic nitrogen moiety.

Step 2: A hydrogen atom is transferred from the enzyme (esteratic site) to the choline part of acetylcholine (or O-X part for the OP compound) by hydrogen bond formation and then by cleavage and rearrangement of the C-O-C or P-O-X part of the substrate. This step is known as acylation and phosphorylation.

Step 3: The acylated or phosphorylated enzyme can undergo further rearrangement by the action of ambient water to form hydroxylated complex.

Step 4: The analogy between acetylcholine and OP compounds almost fails. This process of deacylation occurs very rapidly, whereas dephosphorylation is very slow. This particular step makes OP compounds powerful cholinesterase inhibitors.

Acute Toxicity of Chlorpyrifos

Chlorpyrifos is moderately toxic to humans (U.S. Environmental Protection Agency, 1989). Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system and also skin and eye irritant (Occupational Health Services, Inc. 1986). While some organophosphates are readily absorbed through the skin, studies in humans suggest that skin absorption of chlorpyrifos is more limited (Hayes and Laws, 1990). Skin which has come in contact with this material should be washed immediately with soap and water and all contaminated clothing should be removed. The acute dermal LD_{50} for chlorpyrifos in male and female rats is greater than 2,000 mg/kg (The Dow Chemical Company, 1986). Three hundred and nineteen human exposure incidents were reported by the Pesticide Incident Monitoring System (PIMS) from 1970 through 1981, most resulting from inhalation and dermal exposure. Three human deaths were caused by chlorpyrifos and/or chlorpyrifos combined with other active ingredients (U.S. Environmental Protection Agency, Sept, 1984). Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, cholinesterase impairment, or liver malfunction are at increased risk from exposure to chlorpyrifos. The organophosphate insecticides are cholinesterase inhibitors which may be absorbed through all routes of exposure. When toxic amounts are inhaled, the first effects are usually respiratory and may include bloody or runny nose, coughing,

chest discomfort, difficult or short breath, and wheezing due to constriction or excess fluid in the bronchial tubes. (U.S. Environmental Protection Agency, Dec, 1984). Since chlorpyrifos is absorbed through the skin, especially through cuts and scratches, dermal contact should be avoided (U.S. Environmental Protection Agency, Sept, 1984). In addition to causing inhibition of cholinesterase, acute exposure to chlorpyrifos may cause skin irritation. Absorption through the skin may result in systemic intoxication, or general poisoning in a bodily system. (Occupational Health Services, Inc. 1986). The amount (dose) of a material that causes death in one-half (50%) of the test population, when it is given on a short-term basis by mouth is referred to as its oral lethal dose (LD_{50}). The oral LD_{50} for chlorpyrifos in rats is 82 to 270 milligrams per kilogram (mg/kg) (Leo, 1978; U.S. Environmental Protection Agency, Sept, 1984; Berg, 1986). This indicates that it takes 82 to 270 mg ofchlorpyrifos for each kg of body weight to kill 50% of the experimental animals tested (Gosselin, 1984; Occupational Health Services, Inc. 1991). The LD_{50} for chlorpyrifos in mice is 60mg/kg, 1000mg/kg in rabbits, 32 mg/kg in chickens, 500 to 504 mg/kg in guinea pigs, and 800 mg/kg in sheep (Berg, 1986; Gosselin, 1984; Hartley and Kidd, 1983; Occupational Health Services, Inc. 1991). The dermal LD_{50} in rats is greater than 2000mg/kg (The Dow Chemical Company, 1986), and 1000 to 2000mg/kg in rabbits (Berg, 1986; Hartley and Kidd, 1983).

Effects on Non-target Species

Effects on Birds: Chlorpyrifos is moderately to very highly toxic to birds (U.S. Environmental Protection Agency, 1989). Its oral LD_{50} in pheasants is 8.41 mg/kg, 112 mg/kg in mallard ducks, 21.0 mg/kg in house sparrows, and 32 mg/kg in chickens (Hartley and Kidd, 1983; Toxnet, 1975-86; U.S. Environmental Protection Agency, 1989). The LD50 for a granular product (l5G) in bobwhite quail is 108 mg/kg (U.S. Environmental Protection Agency, 1989). Two one-generation reproductive studies resulted in NOEL's of 125 ppm (the highest dose tested) for bobwhite quail and 25 ppm for mallard ducks. At 125 ppm, mallards laid significantly fewer eggs (U.S. Environmental Protection Agency, 1989). There was no evidence of changes in weight gain, or in the number, weight and quality of eggs produced by hens fed dietary levels of 50 parts per million (ppm), or about 5.12 mg/kg, of chlorpyrifos (Hayes, 1982). Bird deaths have not been observed in repeated mosquito control efforts (Hartley and Kidd, 1983).

Effects on Aquatic Organisms: Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms (U.S. Environmental Protection Agency, 1989). Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide (New York State Department of Environmental Conservation, 1986). Precautions and restrictions are being imposed by EPA to decrease potential hazards (U.S. Environmental Protection Agency, Sept, 1984). Application of concentrations as low as 0.01 pounds of active ingredient per acre may cause fish and aquatic invertebrate deaths (U.S. Environmental Protection Agency, 1989). Chlorpyrifos toxicity to fish may be related to water temperature. Its 96-hour LC50 varied in rainbow trout from 7.1 micrograms per liter (μg/l) to 51 μg/l at three different temperatures (Schimmel, 1983). The 24-hour LC_{50} for chlorpyrifos in goldfish is 180 μg/l, and less than 1,000 μg/l in mosquito fish (Meister, 1992). The 96-hour LC_{50} for chlorpyrifos in mature rainbow trout is 9 μg/l, 98 μg/l in lake trout, 806 μg/l in goldfish, 10 μg/l in bluegill, and 331.7μg/l in fathead minnow (U.S. Environmental Protection Agency, Sept, 1986). Due to its high acute toxicity and its persistence in sediments, chlorpyrifos may represent a hazard to sea bottom dwellers (Schimmel, 1983). Smaller organisms appear to be more sensitive than larger ones (U.S. Environmental Protection Agency, Sept, 1986). Aquatic and general agricultural uses of chlorpyrifos may be extremely poisonous to wildlife and honeybees (U.S. Environmental Protection Agency, Sept, 1984; Hartley and Kidd, 1983). Treated areas should not be used for grazing, nor should the chemical be used when bees are actively collecting pollen or nectar (Berg, 1986; Morse, 1987; Thomson, 1982). Studies indicate that with continuous exposure over time, chlorpyrifos may accumulate to toxic levels in test animals (Thomson, 1982). Since the toxicity of a chemical depends upon many biotic and abiotic factors. The general conditions such as temperature, humidity, food and water supply etc., were maintained constant to the maximum possible extent during experimentation.

Dose-Response Relationship

The dosage of any compound is always a decisive factor in determining its effects (Hayes, 1975). Hence it is important to measure the toxicity i.e., the determination of the dose or concentration or dose at which toxicant produces harmful response on a target organism. Dose refers to a stated quantity or concentration of a substance to which an organism is exposed. It is most commonly expressed as the amount of test substance per unit weight of test animal (e.g. mg/kg body weight) (OECD, 2000). Dosage is a general term comprising the dose, its frequency and the duration of dosing. Dosage is properly applied to any rate or ratio involving a dose. Dosages often involve the dimension of time (e.g. mg/kg/day), but the meaning is not restricted to this relationship (Hayes, 1991). The basic assumptions which underlie and support the concept are: (a) the observed response is a function of the concentration at a site; (b) the concentration at a site is a function of the dose, and (c) response and dose are causally related (Eaton and Klaassen, 1996). The existences of a dose-response relationship for a particular biological or toxicological response (effect) provide a defensible conclusion that the pressure is a result of exposure to a known substance. The purpose of an acute toxicity is to establish the degree of toxicity of a new chemical entry. Repeated-dose (subacute) toxicity studies are designed to examine the adverse effects resulting from repeated exposure to a chemical at lower doses than used in acute studies. Repeated-dose studies to test the substance is often incorporated in to the diet or added to the drinking water. Ample work has been done on the insecticidal properties of organophosphates and organochlorides (Aldridge, 1981, O'Brien, 1967, Cutkomp *et al.,* 1982) while scanty work is available on the toxicity evaluation of pyrethroids to insects in general (Shinoda *et al.,* 2001) and mammals in particular. In the present investigation a toxicological evaluation was made using chlorpyrifos which is widely used for crop protection against insect pests.

MATERIALS AND METHODS

Species: Mice.

Pesticide: Chlorpyrifos Technical (95.30%) was obtained from Nagarjuna Agri. Chem Limited, RavulapalemMandal, East Godavari District, A.P., India.

Concentration selected: Tenth fold ($1/10^{th}$) lower concentration of LD_{50} was selected for sublethal treatment to the experimental mice.

Course of study: Single, double and multiple doses with 48 hours interval.

Route of administration: Oral.

Tissues selected: Heart, liver, kidney, muscle, intestine, testes and blood.

Pesticide stock solution: Stock solution of chlorpyrifos was prepared in acetone. Working pesticide test solutions were prepared by diluting the stock solution with distilled water.

Selection of sublethal treatment to the experimental model: As the acute oral LD_{50} value of chlorpyrifos was determined, tenth fold lower ($1/10^{th}$) concentration was selected as sublethal to study the effect of chlorpyrifos. Healthy adult mice were divided into four groups having ten animals each. The second, third and fourth groups of animals were termed as experimental animals. To the animals of second group single dose of pesticide (i.e. on 1^{st} day) was administered orally by gavage method. To the third group of animals double doses were given i.e. on 1^{st} and 3^{rd} day. Similarly multiple doses i.e., 1^{st}, 3^{rd}, 5^{th} and 7^{th} day were given to the fourth group of animals. The first group of animals was considered as controls.

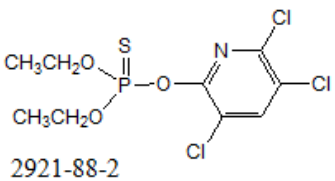
Procurement of experimental animals: Healthy wistar strain mice of the same age group 100±10 days and weight 75±10 grams were selected as experimental animals for the present study. The mice were collected from Indian Institute of Science (I.I.Sc.), Bangalore. Prior to experimentation the animals were acclimatized according to the instructions given by Behringer (1973).

Maintenance of animals: The mice were maintained at laboratory conditions in the animal house at 25±2°C with a photoperiod of 12hrs light and 12hrs darkness throughout the course of the present study. The mice were fed with standard pellet diet supplied by SaiDurga feeds and foods, Bangalore and water *ad libitum*.

Isolation of tissues: The control and experimental animals after the stipulated period (i.e. on 9^{th} day) were sacrificed and the tissues were isolated, cleaned in physiological saline and processed immediately for microscopic analysis. The tissues were also quickly isolated under ice cold conditions and stored in deep freezer at -80ºC for biochemical analysis.

Pesticide selection: Chlorpyrifos, an organophosphate insecticide was selected for the present investigation. Chlorpyrifos O, O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate with 95.30% purity was used as the test chemical for the present study. Technical grade chlorpyrifos was obtained from Nagarjuna Agri. Chem Limited, Ravulapalem Mandal, East Godavari District, A.P., India. Chlorpyrifos has a wide applicability and safety compared to other compounds of its class. Hence this pesticide was selected for the present study.

The following are the specifications of chlorpyrifos used in the present study.

Generic name	:	Chlorpyrifos
Chemical name	:	O,O-diethylO-(3,5,6-trichloro-2-pyridyl) Phosphorothioate
Synonym(s)	:	Phosphorothioic acid O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester; chlorpyrifos-ethyl; chlorpyrifos
Registered trade name (s)	:	Dowco 179; ENT 27311; Dursban; Lorsban; Pyrinex; DMS-0971
Chemical formula	:	$C_9H_{11}Cl_3NO_3PS$
Chemical structure	:	
Identification numbers	:	
CAS Registry	:	2921-88-2
NIOSH RTECS	:	TF6300000
EPA Hazardous Waste	:	059101

EPA Hazardous Waste	:	059101
OHM / TADS	:	7800025
DOT/UN/NA/IMCO	:	NA 2783 Chlorpyrifos
HSDB	:	389
Molecular Weight	:	350.57
Color	:	White granular crystals White to tan Amber solid cake with amber oil Colorless crystals
Physical state	:	Crystalline solid
Melting point	:	41-42°C
Boiling point	:	Decomposes at approximately 160°C
Density at 43.5°C	:	1.398 g/cm^3
Odor	:	Mild mercaptan
Solubility	:	
Water at 20°C	:	0.7 mg/L
Water at 25°C	:	2 mg/L
Organic solvent (s)	:	79% w/w in iso octane 43% w/w in methanol Readily soluble in other organic solvents
Partition coefficients	:	
Log K_{ow}	:	4.82
Log K_{oc}	:	3.73
Vapor pressure at 20°C	:	1.87 x 10^{-5} mm Hg
Vapor pressure at 25°C	:	1.87 x 10^{-5} mm Hg
Henry's law constant at 25°C	:	1.23 x 10^{-5} atm-m^3 / mol
Conversion factors (25°C)	:	1 ppm = 14.3 mg/m^3 1 mg/m^3 = 0.070 ppm
Flammability limits at 25°C	:	No data

(CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America / International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substance Data Bank; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials / Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances).

Sub acute (Tolerable) Daily Dose Evaluation

After determining the LD_{50} dose, sub acute oral doses ranging from ½nd LD_{50} to 1/10th LD_{50} were administered to five batches of mice having ten animals in each batch. 1/10th LD_{50} was selected as sub lethal and administered to mice as single, double and multiple doses with an interval of 48 hrs for biochemical studies. After stipulated time the animals were sacrificed and different tissues were isolated for further investigations.

Toxicity dose response studies were conducted according to the Probit analysis (Finney, 1971) and the LD_{50} value was determined in mice by chlorpyrifos. Further experiments were carried out by treating the mice to sub lethal doses.

Statistical treatment of the data

The mean, standard deviation (SD), percent change and one – way analysis of variance (ANOVA) (Steel and Torrle, 1960) were performed using the SPSS package programming

techniques on "Intel Core 2Duo Processor" personnel computer. Probability values less than 0.05 were considered significant (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

The data was computed according to probit analysis (Finney, 1971), and LD50 values were determined. The animals were exposed to different concentrations of chlorpyrifos, showed no mortality up to 28 mg/kg body weight, 16.66% mortality at 32 mg/kg body weight, 25% mortality at 42 mg/kg body weight, 33.33% mortality at 52 mg/kg body weight, 50% mortality at 62 mg/kg body weight, 66.66% mortality at 72 mg/kg body weight, 83.33% mortality at 82 mg/kg body weight, 100% mortality at 92 mg/kg body weight and 102 mg/kg body weight observed (Table 1).

The computation of percent mortality against different log concentrations of the pesticide given a typical sigmoid curve (Fig.1). The LD50 value obtained from the sigmoid curve is 62 mg/kg body weight for 48 hours. The probit mortality of the Mice was calculated from percent mortality, when the probit mortality was plotted against log concentrations of the pesticide a straight line was obtained (Fig.2). The LD50 value obtained from this straight line graph is 62 mg/kg body weight, and 1/10th of LD 50 was 6.2mg/kg, i.e sub lethal dose. After the administration of chlorpyrifos mice were lost their normal activity when compared to controls . Chlorpyrifos is a broad spectrum insecticide, a chemical used to kill a wide variety of insects. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, as well as on lawns and ornamental plants (U.S. Environmental Protection Agency, Sept, 1984; Berg, 1986).

Table 1. Mortality of Mice administered with different doses of chlorpyrifos for 48 hrs

S.No	Dose mg/kg	Log. Concentration	Number of Animals exposed	Number of Animals died	Percent kill	Probit kill
1.	32	1.5051	12	2	16.66	4.05
2.	42	1.6232	12	3	25.00	4.33
3.	52	1.7160	12	4	33.33	4.56
4.	62	1.7924	12	6	50.00	5.00
5.	72	1.8573	12	8	66.66	5.44
6.	82	1.9138	12	10	83.33	5.95
7.	92	1.9638	12	12	100.00	8.09
8.	102	2.0086	12	12	100.00	8.09

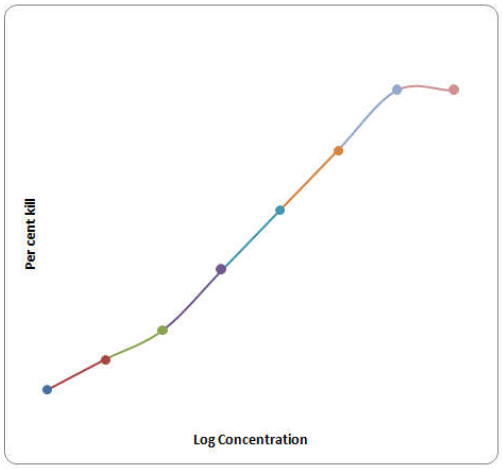


Fig. 1. Sigmoid "Graded Response" curve showing the relation between the log concentrations of chlorpyrifos and percent mortality of the mice

Table 2. Toxicity of Chlorpyrifos in different animals

S. No.	Animal	LD_{50} value mg/kg wt.	Route	Reference
1.	Rats	82-270	Oral	Leo (1978); U.S. Environmental Protection Agency (1984) (Sept), Berg, ed. (1986).
2.	Rats	>2000	Dermal	The Dow chemical Company (1986)
3.	Rats	135	Oral	Vasantha Raj David and Kumarswami (1996)
4.	Rats	150	Oral	Pimental (1971)
5.	Mice	60	Oral	Berg ed (1986); Gosselin (1984); Hartley and Kidd eds. (1983); Occupational Health Services Inc., (1991).
6.	Rabbits	1000	Oral	Berg ed(1986);Gosselin(1984); Hartley and Kidd eds. (1983); Occupational Health Services Inc., (1991).
7.	Chickens	32	Oral	Berg ed(1986); Gosselin(1984); Hartley and Kidd eds. (1983); Occupational Health Services Inc., (1991).
8.	Guinea Pig	500-504	Oral	Berg ed(1986);Gosselin(1984); Hartley and Kidd eds. (1983); Occupational Health Services Inc., (1991).
9.	Sheep	800	Oral	Berg ed(1986);Gosselin(1984); Hartley and Kidd eds. (1983); Occupational Health Services Inc., (1991).
10.	Rabbits	1000-2000	Dermal	Berg ed. (1986); Hartley and Kidd, eds. (1983).

There have been a number of works reported on toxicity of chlorpyrifos in different animals. The LD50 values of organophosphate compound chlorpyrifos in different animals are summarized (Table 2).

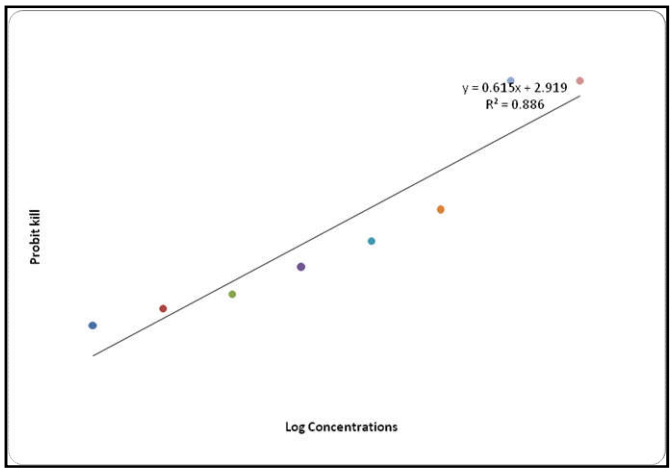


Fig. 2. Graph showing straight line relation between the log concentration of chlorpyrifos and probit kill mortality of the mice

In the present study the obtained LD50 value is 62 mg/kg body weight. This LD50 value is differed from the LD50 values of chlorpyrifos reported by Vasantha Raj David and Kumaraswamy, (1996) and Pimental (1971). Vasantha Raj David and Kumaraswamy, (1996) have reported this value as 135 mg/kg body weight, while Pimental (1971) has reported as 150 mg/kg body weight. Various factors influence the LD50 values (Table 1 to 6). These factors are manifold and dependent up on the given set of experimental conditions (Russell and Overstreet, 1987). The LD50 values can also significantly vary between animals of the same basic strain obtained from different suppliers (Russell and Overstreet, 1987), according to the purity of the chemical (Ho and Hoskins, 1986) and to the sex differences (Overstreet et al., 1979). The differences in LD50 value between water-deprived and non-deprived were also observed. So the differences of LD50 obtained in the present investigation from the reported LD50 values by other investigators may be due to one (or) more factors as listed above. The toxic signs and symptoms of chlorpyrifos toxicity appear to be mainly cholinergic. In buffalo calves Bubarisbubaris the toxic manifestations, as observed in symptoms and signs were hyper salivation,

Table 3. Acute oral toxicity of some organochlorine pesticides to Mice

S. No.	Pesticide	Exposure time	LD_{50} mg/kg body weight	Reference
1.	Endrin	48 hrs	3-6	CFTRI Annual Report (1980)
2.	Isodrin	-do-	3-17	Nayar and David (1968)
3.	Dieldrin	-do-	40	-do-
4.	Heptachlor	-do-	40	CFTRI Annual Report (1980)
5.	Aldrin	-do-	60	Anil Kumar (1994)
6.	Endosulfan	-do-	55-220	CFTRI Annual Report (1980)
7.	Gama BHC (Lindane)	-do-	200	-do-
8.	Chlordane	-do-	283	-do-
9.	DDT	-do-	300-500	-do-
10.	Alpha BHC	-do-	500	-do-
11.	BHC mixed isomer	-do-	600-1200	-do-
12.	Delta BHC	-do-	1000	-do-
13.	Beta BHC	-do-	6000	-do-

Table 4. Acute oral toxicity of some OP compounds to Mice

S. No.	Pesticide	Exposure time	LD_{50} mg/kg body weight	Reference
1.	Parathion	48 hrs	13	Pimental(1971)
2.	Azinphos methyl	-do-	16	-do-
3.	Monocrotophos	-do-	21	-do-
4.	Phosphamidon	-do-	28	-do-
5.	Methylparathion	-do-	42	-do-
6.	Chlorpyrifos	-do-	150	-do-
7.	Dimethoate	-do-	215	-do-
8.	Fenthion	-do-	310	-do-
9.	Maled	-do-	430	-do-
10.	Trichloroform	-do-	500	-do-
11.	Fenitrothion	-do-	680	-do-
12.	Malathion	-do-	1650	-do-
13.	Temephos	-do-	5000	-do-

Table 5. Acute oral toxicity of some carbamate pesticides toMice

S. No.	Pesticide	Exposure time	LD_{50} mg/kg body weight	Reference
1.	Aldi carb	48 hrs	0.93	Pimental (1971)
2.	Carbofuran	-do-	14	-do-
3.	Mexacarbat	-do-	19	-do-
4.	Aldoxy carb	-do-	27	-do-
5.	Amino carb	-do-	50	-do-
6.	Isolan	-do-	54	-do-
7.	Pyrolan	-do-	62	-do-
8.	Bentiocarb	-do-	100	-do-
9.	Primicarp	-do-	147	-do-
10.	Dimetan	-do-	150	-do-
11.	Cartap	-do-	380	-do-
12.	Carboxyl	-do-	560	-do-
13.	Carbaryl (sevin)	-do-	850	-do-

Table 6. Acute oral toxicity of some synthetic pyrethroids in Mice

S. No.	Pesticide	Exposure time	LD_{50} mg/kg body weight	Reference
1.	Tefluthrin	48 hrs	25-35	ICI 1985
2.	Flucythrinate	-do-	67-81	Larson *et al.,* (1985)
3.	Fenfluthrin	-do-	85-120	-do-
4.	Deltamethrin	-do-	100-139	Casida*et al.,* (1983) Larson*et al.,* (1985)
5.	Fluvelarate	-do-	261-282	-do-
6.	Cypermethrin	-do-	310-500	Casida*et al.,* (1983)
7.	Fenvelarate	-do-	451-850	Larson*et al.,* (1985) Hiromori*et al.,* (1986)
8.	Cyfluthrin	-do-	500-800	Larson*et al.,* (1985)
9.	Tralomethrin	-do-	1070-1250	Larson*et al.,* (1985)
10.	Permethrin	-do-	5000	Miyamota, (1976)

lacrimation, muscular fasciculation inco-ordination, diarrhoea, prostration and dyspnoea (Sandhu and Malik, 1988). In other domestic animals also similar symptoms were observed (Nambaet al., 1971; Maddy and Riddelle, 1977). During the present investigation the appearance of signs and symptoms were observed in between 20-150 minutes, except lacrimation, and salivation which were occurred between 10-20 minutes following the chlorpyrifos administration. This was presumably due to the absorption rate of chlorpyrifos in to gastrointestinal tract or the time taken for chlorpyrifos to reach the brain. The other factors like affinity to lipids binding, PH, degree of ionization etc., might also play a role in the process of absorption of chlorpyrifos.

Conclusion

Pesticides play a significant role in everyday life of modern man. The use of these 'super chemicals' became indispensable for fighting with the pests of crops, stored foods, farm animals and also for counteracting the insect and other vectors of diseases. However, indiscriminate use of these chemicals has resulted in posing a serious threat to human health as they leave residues in the food and produce ill effects. This work is a modest attempt by the author towards an understanding the toxic potentials of chlorpyrifos in different tissues of mice. This study is far from being comprehensive, yet the author remains hopeful that the present study would contribute useful information to the existing knowledge on changes in metabolic activities and toxicology evolution during chlorpyrifos toxic stress.

Acknowledgement

Authors are highly thankful to Professor. P. Jacob Doss,(HOD). Department of Zoology, S.V.University. Tirupati,for their constant Encouragement, and also express deep sense of gratitude to Department of Zoology, Sri Dravidian University, Kuppam,-517 425. (A.P) for providing laboratory facilities to carry out this work and cooperation.

REFERENCE

Aldridge, W.N. 1981. Organophosphorus compounds molecular basis for the biological properties. *Sci. Prog. Oxf.,* 67; 131-147.

Behringer, M.P. 1973. Laboratory care of vertebrates, In: Techniques and materials in biology, McGraw Hill. Inc., New York, pp. 171-174.

Berg, G.L. ed, 1986. "Farm chemicals handbook. Willoughby, OH: 1986. Meister Publishing Company.

Berg, G.L. ed. 1986. Farm chemicals handbook. Willoughby, OH: Meister Publishing Company.

Brazinskii, L.P., F. Kamaroski, Y. Porokhon-Iska, E.M. Pischolka, K. Yu and A.P. Rudenko, 1979. "Experimental DDT toxicosis of fish" Ozenn, Rechn, Rybn. Khoz, 144 : 86-92.

Cartson, S, 1962. Test methods and toxicity considerations under the federal Hazardous substances labeling". 1962, Act Proc. of 49th *Ann. Meeting Chem. Spec. Assam.*, New York.

Chanh, P.H., C. Navarro Delmasure, A. Chan and C. Phum Huu Martinez, 1981. "Toxicity of decamethrin depends on the solvent, the administration rates the sex and the animal species" IRCS. Med. Sci. Libr. Compord.9 (7): 565-570.

Cutkomp, L.K., Koch, K.B. and Desaiah, D. 1982. Inhibition of ATPases by chlorinated hydrocarbons In: Insecticide mode of action (ed. Coats JR) Academic Press, New York, pp.45-69.

Das, N. and A. Garg, "Effect of endosulfan in female rat grown on low protein and high protein cereal diet,1981, *Pestic. Biochem. Physiol.,* 5(1): 90-98.

Eaton, D.L. and D. Klaassen, 1996. Principles of toxicology. In: Casarett and Doull's Toxicology, The Basic Science or Poisons, 5th Edition, McGraw-Hill, New York. pp.13-33.

Environment, Health and Safety Publications, Series on Testing and Assessment No. 32 and Series on Pesticides No. 10. Organization for Economic Co-operation and Development, 2000, Paris.Pp.1-50.

Finney, D.J, Probit analysis, 1971, III Edition, Cambridge Univ. press, London, p.20.

Gosselin, R.E. 1984. "Clinical toxicology of commercial products. Fifth edition. Baltimore" M.D: Williams and Wilkins.

Gouda, R.K., N.K. Triphathy and C.C. Dass. 1981. "Toxicity of dimecron, sevin and lindaneto Anabas Seandens and Heteropneusterfosilis" *Comp. Physiol. Ecol.,* 6 (3): 170-172.

Hayes, A.W, "In principles and methods of toxicology" 1982. Ed. A.W. Hayes, Reven press, New York.

Hayes, W. J and E.R. Laws (ed.), 1990. "Handbook of Pesticide Toxicology" Vol.3, Classes of Pesticides. Academic Press, Inc. NY.

Hayes, W.J. Jr, 1975. Paper for the 14th annual meeting of the society of toxicology, William Virginia, 9-13.

Hayes, W.J, 1991. Dosage and other factors influencing toxicology. In: Hayes WJ and Laws ER (Eds). Handbook of Toxicology. Vol. 1, General Principles. Academic Press, San Diego, pp.40-41.

Heit and M. Fingerman, 1977. "The influence of size, sex and temperature on the toxicity of mercury to two species of gray fishes" *Bull Environ. Contam.Toxicol,*18 (5): 572-580.

Ho, I.K and B. Hoskins, 1986. Bicohemical and pharmacological aspects of neurotoxicity from and tolerance to organophosphate cholinesterase inhibitors. In: Hand book of toxicology. Eds. T.J. Haley, and W.O. Berndt. Hemisphere Publishing Corp., Washington.

Ho, I.K., S.P. Sivam and B. Hoskins, 1983. "Acute toxicity of DFP, Tabun, Sarin and Soman in rats: Lethality in relation to cholinergic and GABAergic enzyme activities" Fed. Proc., 42 : 656 (Abs).

Holden, A. V. 1973. "Effects of pesticides on fish. In: "Environmental pollution by pesticides", 1973, (Ed. Edwards, C.A. Plenum Press, New York, pp. 213-253.

Jacob, S.S., N.B. Nair and N.K.B. Subramanian, 1982. 'Toxicity of certain pesticides found in the habitat of larbiroraous fishes', A. linealus and M. cupanus. *Proc. Indian Acad.Sci. (Ani.Sci).,* 91(3) : 323 – 328.

Janardhan, A., A.B. Rao and P. Sisodia, 1987. "Sub-chronic toxicity of methyl benzimidazolecarbamate in rats" 1987. *Bull Environ Contam Toxiol,* 38 (5) : 890-8.

Jayantha Rao, K. 1982. "Effect of a Systemic Pesticide Phosphomidon on some aspects of metabolism in fresh water fish" Tilapia mossambica (peters). Ph.D., Thesis, S.V. University, Tirupati, A.P., India.

Klein, O., S.L. Meir and Sarakeran, 1983. "Comparative toxicology of several pyrethroids, Parathion and chlrpyriphos to adults of the spiny bolol worm", Eris isulerna (Lepidoptera, Natuidoe) in laboratory assays ISR. *J. Entomol.,* 16: 99-104.

Leo, A, 1978. "Report on the calculation of octanol / water log P values for structures in the USEPA files", Claremont, C.A.

Li, G.C and Y.C. Choa, 1981. "Study on the acute fish toxicities of commonly used Pesticides. *Nat. Sci. Counc;* 9(2): 146-152.

Maddy, K.T and L. C. Riddelle, 1977. Pesticide poisoning in domestic animals. Modern Veterinary Practice, 9: 913 – 20.

McEwen, F.L and G.R. Stephenson, "In the use and significance of pesticides in the environment" 1979, Johnny & son, New York.

Meister, R.T., Farm chemicals hand book'92. 1992, Meister Publishing Company. Willoughby, OH.

Morse, R.A. 1988. Bee poisoning. In New York State Pesticide recommendations. Forty-ninth annual pest control conference., Nov. 9, 10, 11. Cornell University, Ithaca, N.Y.

Namba, T., C.T. Notte, J. Jackel and D. Grob, 1971. Poisoning due to organophosphorus insecticides; Acute and chronic manifestations. *American Journal of Medicine*, 50: 475-91.

New York State Department of Environmental Conservation (1986 Feb.). Draft Environmental Impact Statement on Amendments to 6 NYCRR Part 326 Relating to the restriction of the pesticides aldrin, chlordane, chlorpyrifos, dieldrin and heptachlor. Division of Lands and Forests. Bureau of Pesticides. Albany, NY.

O'Brien, R.D., 1967. In: Insecticides action and metabolism' Academic press, New York, London.

Occupational Health Services, Inc, "Material safety data sheet on chlorpyrifos"1986, Secaucus, N.J. OHS, Inc.

Occupational Health Services, Inc, MSDS for chlorpyrifos. OHS Inc., 1991 Secaucus, NJ. Hartley, D and H. Kidd, eds, The agrochemicals handbook" 1983, Nottingham, England: Royal Society of Chemistry.

Occupational Health Services, Inc, "Material safety data sheet on chlorpyrifos"1986, Secaucus, N.J. OHS, Inc.

OECD, "Guidance notes for analysis and evaluation of repeat-dose toxicity studies". OCED

Overstreet, D.H., R.W. Russell, H.C. Helps and M. Messenger, 1979. Selective breeding for sensitivity to the anticholinesterase DFP. Psychopharmacology, 65 : 15-20.

Overstreet, D.H., R.W. Russell, H.C. Helps and M. Messenger, 1979. "Selective breeding for sensitivity to the anticholinesterase DFP" Psychopharmacology,65 : 15-20.

Pal, S.K and H.S. Kushwath, "Dissipation of endosulfan residues from Berzizyphusmaritrana (lamk) fruits and leaves under arid conditions" 1981, Indian, *J. Entomon,* 42(1) : 148-151.

Pimental, "Ecological effects of Pesticides on non-target organisms". 1971. U.S. Government Printing Office Washington DC, p. 220.

Russell, R.W and D.H. Overstreet, "Mechanisms underlying sensitivity to organophosphorus anticholinesterase compounds"1987. Progress in Neurobiology, 28 : 97-128.

Russell, R.W and D.H. Overstreet. Mechanisms underlying sensitivity to organophosphorus anticholinesterase compounds. 1987, *Progress in Neurobiology*, 28 : 97-128.

Russell, R.W., R.A. Booth, S.D. Lauretz, C.A. Smith and D.J. Jenden, 1986. "Behavioral neurochemical physiological effects of repeated exposures to subsymptomatic levels of the anticholinesterase" soman. Neurobehaviroal. Toxicology and Teratology, 8: 675-685.

Sandhu, H.S and J.K. Malik, 1988. Acute toxicity of Monocrotophos and its effects on blood enzymes in buffalo calves. *Indian J. Anim. Sci.,* 58 (10): 1154-57.

Schimmel, S.C (1983 Jan.-Feb.). Acute toxicity, bioconcentration and persistence of AC 222. 705, Benthiocarb, Chlorpyrifos, Fenvalerate, Methyl parathion, and Permethrin in the estuarine environment. *In Journal of Agricultural and Food Chemistry.* The American Chemical Society.

Shinoda., Kuji, Lzudri, Taksuoki, Yamazuchi and Hiroshi, 2001. Studies on the insecticidal effect of vaporized pyrethroid on cockroaches, *Appl. Entomol.,* 18(4); 517-24.

Snedecor, G. W and W. G. Cochran, Statistical Methods, 1968, 6th Ed. Oxford and IBH Publishing Company, Calcutta, Bombay and New Delhi, pp.168-181.

Steel, R.D.G and J.M. Torrle, Principles and procedures of statistics with special reference to the biological sciences,1960, McGraw Hill Book Inc., New York, Toronto, London, XVI, 481.

Surendranath, P., T. Ramesh Babu and K.V. RamanaRao, 1987a, "Toxicity of Kelthane and its impact on behavioral responses of Penaeid prawn" Metapenaeusmonoceras., *Envion, Ecol*., 5(4): 782-785.

Surendranath, P., T. Ramesh Babu, K. SurendhraBabu and K.V. RamanaRao," Impact of Kelthane on respiratory potentials in Penaeid prawn" 1987b Penaeusindicus, Environ Ecol., 5(3): 593-597.

The Dow Chemical Company," Summary of acute dermal toxicity study on chlorpyrifos in Fischer 344 rats"1986 June 30, Dow Chemical, Indianapolis, IN.

Thomson, W.T., Insecticides, acaricides and ovicides. Agricultural Chemicals, Book I. Frenso, C.A. 1982, Thomson Publications.

U.S. Environmental Protection Agency, "Registration Standard (Second Round Review) for the registration of Pesticide Products Containing Chlorpyrifos"1989 June, Office of Pesticide Programs, US EPA, Washington, DC.

U.S. Environmental Protection Agency, "Registration Standard (Second Round Review) for the registration of Pesticide Products Containing Chlorpyrifos" 1989 June, Office of Pesticide Programs, US EPA, Washington, DC.

U.S. Environmental Protection Agency, "User's manual for the pesticide root zone model" (PRZM). 1984 Dec, Release 1, Athens, GA: Environmental Research Laboratory.

U.S. Environmental Protection Agency," Guidance for registration of pesticides containing chlorpyrifos as the active ingredient" 1984 Sept, Case # 0100.Office of Pesticide Programs. Washington, DC.

Uttaman, M., Y. Hemalatha and Y.S. Reddy, "The effect of BHC on the O2 consumption of the earthworm" 1979, Megascolexmauritii. A chronotoxicological approach. *Curr. Sci.,* 48(8) : 368-370.

Vasantha Raj David, B and T. Kumaraswami ,In: Elements of Economic Entomology,1996. popular Book Depot. 29, potters Street, Saidapet, Madras 600 015.

****