MORTALITY OF *RANA TIGRINA* IN DIFFERENT CONCENTRATIONS OF FENVALERATE, TECHNICAL AND COMMERCIAL GRADE AT 24, 48 AND 72 HOURS OF EXPOSURE.

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ABSTRACT

The chemical pesticides are influencing much on the populations of non-target organanisms like fishes and amphibians. Frogs have served as important model organisms throughout the history of science and the importance of frogs ecologically, economically and academically has prompted me to take up the study. In the present study Healthy frogs, Rana tigrina weighing 50 ± 3 gms were collected from the pond, acclimated to the laboratory conditions in large glass aquaria with water. The frogs were divided into groups of ten animals. They were exposed to different pesticide concentrations of fenvalerate, both commercial and technical grade, according to biomass ratio as suggested by Doundroff *et al*., (1951). In The percent study Frogs were exposed to different concentrations of commercial and technical grade of fenvalerate for 24, 48 and 72 hours. The present findings also agree with the previous reports that the commercial grade pesticides are relatively more toxic than the technical grade pesticides (***Pickering et al***., 1962). From all the observations, it is inferred that the LC_{50} values were not constant for a group of insecticides or any particular group of animals and it varies from species to species with alteration in the chemical and physical factors.

Key words: Fenvalerate (both commercial and technical grade), LC_{50}, *Rana Tigrina* (Indian Bull Frog),

INTRODUCTION:

The life style of modern man has been greatly improved by chemicals. Through the use of chemical substances, we can increase food production, cure diseases and control the pests. Every corner of the world and every part of our life has been benefited by chemicals. With the development of new formulations, the synthesis of pesticides has come into the picture replacing the lower toxic chemicals. When new insecticides are developed, they must undergo extensive testing before they can be made available to the public. With the recognition that fish, crabs and other non-target organisms are very sensitive to a wide variety of pesticide chemicals, it has now become a normal practice to test all new chemicals for their toxicity. This assessment of lethality or toxicity would help in knowing the potentiality of a chemical. So that new and more powerful formulations may be speeded up in the manufacture of pesticides.

Due to the indiscriminate use of hard and persistent pesticides, the thought of using plant extracts as insecticides namely pyrethroids also tend to affect the biology of non-target species along with that of pests (Hoyt *et al.,* 1978; Chari, 1980; Elliott *et al.*, 1978). Toxicity of a chemical can be influenced by physical factors (Herzberg *et* al., 1978) and biological factors (Braginski *et al.*, 1979; Jayantha Rao, 1982), nutritional status (Das and Godg, 1981; Pal and Kushwah, 1981), species specificity (Gouda *et al.*, 1981) and Chronobiology (*Uttaman et al.*, 1979) of the animal. It also depends on the developmental stages of the animal (*Salama et al.*, 1980) and also duration to which the animal is exposed (Abel, 1980; Gouda *et al.*, 1981; Jacob *et al*, 1982). Hence in order to arrive at a precise dosage level, it is necessary to plan a systematic model to conduct toxicity studies (NIN special report, 1982; NTP, 1982).

Increase in the number of synthetic pyrethroid insecticides introduced into the environment, detrimental effects of fish population and the frequency of mortality are apparently increased (Metcalf, 1980). Entry of pesticides into fish is largely through the gills (Broadburry *et al.*, 1986). Fresh water fauna since the former inhabit a confirmed water pocket (*Brown*, 1978). There are several reports on insecticides being slightly toxic or non-target to the amphibians in fields. Lackery and Stenle (1945) observed the application of DDT was safe for frogs and their tadpoles except in pools where the water was less than 3 inches deep. Field studies showed DDT was to lethal frogs and toads (Logier, 1949).

Endrin, aldrin and endosulphan, when applied as mosquito larvicides, were lethal to tadpoles of Bull Frog (Mulla, 1963). Lindane and BHC (technical grade) were found to be the least toxic compounds to frogs (Kaplan and Overpeck, (1964). Cole and Casida (1983) worked on the effect of deltamethrin on frog Rana pipens pipens. Martian *et al.*, (1983) on carbaryl with relation to survival, growth and size of metamorphosing Rana tigrina. The use of frog as test species in toxicological studies in relation to pollution is increasing. In this respect mention may be made of some investigators who have contributed to the knowledge of toxicological studies on frog. Mathur *et al*. (1981), Verma *et al*. (1989), Vijaya joseph and Jayantha Rao (1990,), Noor Alam (1991), Abbasi and Soni (1991). Kanumadi and Sidapur (1992) reported the effects of exposure of mercuric chloride in Rana cyanophlyctis.

The assessment of toxicity of insecticides involve the determination of LC_{50} (lethal concentration that produces 50% mortality in the test species). Lethality is expressed as parts per million (ppm) or parts per billion (ppb) or mg/L. Some workers also represent these LC values as Tlm (medium Tolerance Limit) of the fish or animal. Thus, different workers have adopted different methods for assessing the toxicity of pesticides. Finney (1964) described the "probit method" for determination of LC_{50}. Pickering *et al*. (1962) reported the LC_{50} value of different pesticides to different species of fish exposed to 24, 48 and 96 hours. With the above background, an effort has been made to evaluate the toxicity of synthetic pyrethroid namely fenvalerate to an Amphibian, Rana tigrina (Daudin) under strictly controlled conditions. The present toxicity tests show that toxicity tests were conducted in static waters to determine LC_{50} values with commercial and technical grade of fenvalerate on Rana tigrina.

Animals were starved for 24 hours prior to the exposure and during the exposure to pesticide. Only actively moving frogs of the same size were selected from the collection for testing. Preliminary screening tests were conducted to obtain the range of lethal concentrations and based on these results, the experiments were carried out. They were divided into groups of 10 animals each as suggested by Wuhramann and Worker (1950) and exposed to different concentrations of fenvalerate, both commercial and technical grade pesticide solutions. Mortality rate was noted for 24, 48 and 72 hours. This was repeated six times in both commercial and technical grade stock solutions.

MATERIAL AND METHODS:

1. Test species:- *Rana tigrina (Daudin)* (Indian Bull Frog):

Phylogenic status

Phylum	:	Chordata
Sub-phylum	:	Verterbrata
Division	:	Gnathostomata
Class	:	Amphibia
Sub-class	:	Lissamphibia (living amphibians)
Order	:	Anura or Sailentia
Sub-order	:	Diplasciocoela
Family	:	Ranidae
Genus	:	Rana
Species	:	Tigrina.

2. Procurement of the experimental animal:

Rana tigrina is commonly known as Indian Bull Frog. They are occurring near the tanks and ponds in and around Tirupati (A.P.). Besides experimental frogs other species of frogs were also collected and their morphological features were studied. For the present study, the locally available frog, *Rana tigrina* was selected.

3. Maintenance of frog:

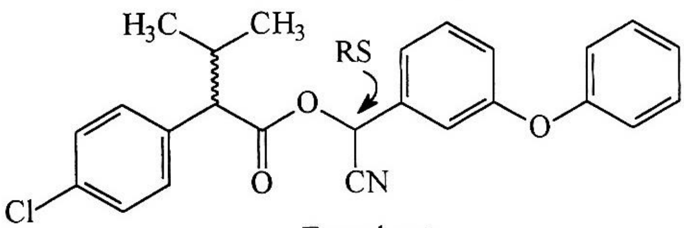
Healthy frogs, *Rana tigrina* weighing 50 ± 3 gms were collected from the pond, acclimated to the laboratory conditions in large glass aquaria with water (Temperature 27 ± 2°C; P^H 7.0 ± 0.2, light period – 12 hours) for 7 days. They were fed with cockroaches and earthworms *ad libitum*, with change of water daily.

4. Selection of the test chemical:

Fenvalerate is a pyrethroid insecticide extensively used in agricultural crops such as rice, wheat, sorghum, pulses, groundnut, and vegetables and on cotton to kill the stem borers, leaf folders, fruit borers and head borers (Dedachanji and Patil, 1983). Besides its usefulness against agricultural pests, the synthetic pyrethroids have also been proved effective against household, industrial pests, public health, veterinary and pests of stored grains. Because of their extensive use, accumulation and increased susceptibility, they are affecting several non-target organisms like fishes and other aquatic organisms (Clark *et al*., 1985). Fenvalerate is largely used in India, because of its high photostability, high degradability and application at minimal doses.

Fenvalerate (Sumcidin (R) (5-5602 OMS – 2000) a synthetic pyrthroid compound both commercial (Fenvalerate EC 20) and Technical grade, 93.7% (wt/vol) supplied as gratis by Rallis India Limited, Bangalore (India) was used. The following are the physico-chemical properties of fenvalerate used in the present study.

1. Common name:- Fenvalerate
2. Commercial name:- (R.S.) Cyano-3-phenoxy benzyl (IR, IS) 2-(4-Chlorophynyl) 3-methyl-Butgrate.
3. Structural formula:-



Fenvalerate

4. Synonyms:- Fenvalerate (Bis, Iso)
5. Trade names:- Sumicidin, Belmark and Pydrin
6. Empirical formula :- C_{25} H_{22} Cl No_3
7. Molecular weight :- 419.9
8. Physical weight :- Viscous liquid

9. Density :- 1.17 gm/ml. at 23°C
10. Vapour pressure: - 2.8 x 10 mm Hg . at 23°C
11. Boiling point:- 300°C /37 mm hg
12. Solubility in water :- 1 mg. in liter of water at 23°C
13. Solubility in solvents :- Acetone, Cyclohexane, Ethanol, Xylene and Chloroform
14. Odour:- Odourless
15. Colour :- Light brown
16. Courtesy:- Rallis India Agrochemical Division, Navi, Mumbai
17. Partition coefficient:- 1.03 x 10 5 (n-octyl alcohol/H2 O) at 23°C
18. Stable:- stable is most organic solvents except alcohols and inorganic mineral dilutents unstable in alkaline media.

5. Preparation of Stock Solution:

The active ingredient of commercial grade fenvalerate EC 20 and Technical grade 93.7% of fenvalerate was used for present investigation. A stock solution of fenvalerate was prepared by dissolving the fenvalerate in Acetone. Available literature indicates that low levels of acetone are harmless to the biological system (Pickering *et al*., 1962). The quantity of acetone used was found to be non-toxic to non-target animals and it was biologically safe in the preparation of stock solution of pesticides (Jagannatha Rao, 1982). One gram of commercial grade fenvalerate EC 20 dissolved in 200 ml of water to make 1000 ppm of stock solution. One gram of technical grade of fenvalerate (93.7%) is dissolved in minimal quantity of acetone and this was made upto 937 ml with water to make 1000 ppm of stock solution. Fresh stock solution was prepared for experimental use.

6. Toxicity evaluation:

Toxicity was evaluated in static waters. Animals were starved 24 hours prior to the exposure. During the exposure to pesticide, only actively moving frogs of same size were selected from the collection for testing. Preliminary screening tests were conducted to obtain the range of lethal concentrations and based on these results, the experiments were carried out.

The frogs were divided into groups of ten animals. They were exposed to different pesticide concentrations of fenvalerate, both commercial and technical grade, according to biomass ratio as suggested by Doundroff *et al*., (1951). For LC_{50} studies seven concentrations viz 29, 30, 31, 32, 33, 34 and 35 ppm in commercial grade and seven concentrations viz 35, 40, 45, 50, 55, 60 and 65 ppm in technical grade of fenvalerate were used. The mortality was observed at 24, 48 and 72 hours and the data was computed according to Finney (1964) and LC_{50} value was determined in static water for 24, 48 and 72 hours.

7. Statistical treatment of the data: - The standard deviation, mean and the test of significance were calculated following the method of Pillai and Sinha (1968).

RESULTS AND DISCUSSIONS:

Frogs were exposed to different concentrations of commercial grade of fenvalerate for 24, 48 and 72 hours. At 24 hours the results showed no mortality upto 30 ppm; 20% mortality at 31 ppm; 40% mortality at 32 ppm; 60% mortality at 33 ppm; 80% mortality at 34 ppm; 100% mortality at 35 ppm **(Table 1)**. At 48 hours in commercial grade of fenvalerate the results showed no mortality at 29 ppm; 30% mortality at 30 ppm; 40% mortality at 31 ppm; 60% mortality at 32ppm; 80% mortality at 33 ppm; 90% mortality at 34 ppm; 100% mortality at 35ppm **(Table 3).** At 72 hours commercial grade of fenvalerate the results showed 10% mortality at 29ppm; 30% mortality at 30 ppm; 50% mortality at 31 ppm; 60% mortality at 32 ppm; 80% mortality at 33 ppm; 90% mortality at 34 ppm; 100% mortality at 35 ppm (**Table 5**).

Frogs were exposed to different concentrations of technical grade of fenvalerate for 24, 48 and 72 hours. At 24 hours the results showed no mortality upto 35 ppm; 20% mortality at 40 ppm; 30% mortality at 45 ppm; 50% mortality at 50 ppm; 70% mortality at 55 ppm; 90% mortality at 60 ppm; 100% mortality at 65 ppm (**Table 2**). At 48 hours the results showed no mortality upto 35 ppm; 20% mortality at 40 ppm; 40% mortality at 45 ppm; 60% mortality at 50 ppm; 80% mortality at 55 ppm; 90% mortality at 60 ppm; 100% mortality at 65 ppm (**Table 4**). At 72 hours of technical grade fenvalerate the results showed 20% mortality at 35 ppm; 40% mortality at 40 ppm; 60% mortality at 45 ppm; 80% mortality at 50 ppm; 90% mortality at 55 ppm; 100% mortality at 60 and 65 ppm (**Table 6**).

Synthetic pyrethroids are relatively safe for mammalian and avian species. Oral LD_{50} for rats and mice range from 100 to 200 mg/kg body weight (Casida *et al*., 1983). Acute oral toxicity of greater than 4000 have been reported for three pyrethroids in three species of birds (Smith and Stratton, 1986) indicating that avian species are also highly resistant to pyrethroid intoxication. Birds have longer capacity to eliminate the pyrethroids than mammals, which are themselves more efficient than fish, which have difficulty on rapidly degrading pyrethroids. The high toxicity of pyrethroids in fish is partly due to poor ability to metabolise them. Owing to their lipophilicity, fenvalerate was found to concentrate in the fat of fish (Bradburry *et al*., 1986).

Earlier studies on frogs with various insecticides showed different LC_{50} values. LC_{50} value of deltamethrin to frog, Rana pipens pipens is 0.13 mg/kg (Cole and Loretta, 1983). Organochlorine insecticides were evaluated for their toxicity on tadpoles by Sander (1970). These reports showed that frog and their tadpoles are considerably less susceptible to insecticides poisoning than fish. It can be said that the tolerable limits of frogs are beyond the tolerable capacity of fish. Resistance of insecticides in frogs may be attributed to the nature of skin, which was also opined by Murhead – Thomson (1971).

In the present study, The concentration of commercial grade fenvalerate required to produce mortality is considerably low compared to the technical grade fenvalerate indicating that commercial grade of fenvalerate is more detrimental than the technical grade of fenvalerate. Thus the commercial samples are more toxic than technical samples of fenvalerate. The relatively high toxicity of commercial grade of fenvalerate can be due to the other ingredients present in the commercial samples which act as emulsifiers. Thus it can be presumed that the ingredients other than the actual pesticide may also contribute towards synergism by enhancing the toxicity of the actual fenvalerate. The present findings also agree with the previous reports that the commercial grade pesticides are relatively more toxic than the technical grade pesticides (Pickering *et al*., 1962). From all the observations, it is inferred that the LC_{50} values were not constant for a group of insecticides or any particular group of animals and it varies from species to species with alteration in the chemical and physical factors.

CONCLUSION:

Toxicity evaluation was conducted on the frog, *Rana tigrina* with commercial and technical grade at 24, 48 and 72 hours. The concentration response studies showed LC_{50} value for the both commercial and technical grade, in tables 1, 5, (Commercial), 3, 4, 6 (Technical). Chronic stress impairs the metabolic functions in different ways. In an environmentalist's point of view sublethal chronic stress studies are more helpful to understand the pollution hazards and protection of non-target species. Duration of chronic stress also would be very important to understand the time courses of damage to the tissues and metabolism of the animal (*Jayantha Rao*, 1982).

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Table 1: Mortality of ***Rana Tigrina*** in different concentrations of Fenvalerate, Commercial grade at **24 hours** of Exposure.

S.No.	Conc. Of Fenvalerate µg/l	Log. Conc. of Fenvalerate	No. of frogs exposed	No. of frogs killed	No. of frogs alive	% kill	Probit kill
1	29	1.4623	10	Nil	10	Nil	-
2	30	1.4771	10	Nil	10	Nil	-
3	31	1.4913	10	2	8	20	4.16
4	32	1.5050	10	4	6	40	4.75
5	33	1.5185	10	6	4	60	5.25
6	34	1.5314	10	8	2	80	5.84
7	35	1.5440	10	10	-	100	8.09

Table 2: Mortality of ***Rana Tigrina*** in different concentrations of Fenvalerate, Technical grade at **24 hours** of Exposure.

S.No.	Conc. Of Fenvalerate µg/l	Log. Conc. Of Fenvalerate	No. of frogs exposed	No. of frogs killed	No. of frogs alive	% kill	Probit kill
1	35	1.5440	10	-	10	-	-
2	40	1.6020	10	2	8	20	4.16
3	45	1.6532	10	3	7	30	4.48
4	50	1.6989	10	5	5	50	5.00
5	55	1.7403	10	7	3	70	5.52
6	60	1.7781	10	9	1	90	6.28
7	65	1.8219	10	10	-	100	8.09

Table 3: Mortality of ***Rana Tigrina*** in different concentrations of Fenvalerate **Commercial** grade at **48 hours** of Exposure.

S.No.	Conc. of Fenvalerate µg/l	Log. Conc. of Fenvalerate	No. of frogs exposed	No. of frogs killed	No. of frogs alive	% kill	Probit kill
1	29	1.4623	10	Nil	10	-	-
2	30	1.4771	10	3	7	30	4.48
3	31	1.4913	10	4	6	40	4.75
4	32	1.5050	10	6	4	60	5.25
5	33	1.5185	10	8	2	80	5.84
6	34	1.5314	10	9	1	90	6.28
7	35	1.5440	10	10	-	100	8.09

Table 4: Mortality of ***Rana Tigrina*** in different concentrations of Fenvalerate, **Technical** grade at **48 hours** of Exposure.

S.No.	Conc. of Fenvalerate µg/l	Log. Conc. of Fenvalerate	No. of frogs exposed	No. of frogs killed	No. of frogs alive	% kill	Probit kill
1	35	1.5440	10	-	10	-	-
2	40	1.6020	10	2	8	20	4.16
3	45	1.6532	10	4	6	40	4.75
4	50	1.6989	10	6	4	60	5.25
5	55	1.7403	10	8	2	80	5.84
6	60	1.7781	10	9	1	90	6.28
7	65	1.8219	10	10	-	100	8.09

Table 5: Mortality of ***Rana Tigrina*** in different concentrations of Fenvalerate, **Commercial** grade at **72 hours** of Exposure.

S.No.	Conc. of Fenvalerate µg/l	Log. Conc. of Fenvalerate	No. of frogs exposed	No. of frogs killed	No. of frogs alive	% kill	Probit kill

1	29	1.4625	10	1	9	10	3.72
2	30	1.4771	10	3	7	30	4.48
3	31	1.4913	10	5	5	50	5.0
4	32	1.5050	10	6	4	60	5.25
5	33	1.5185	10	8	2	80	5.84
6	34	1.5314	10	9	1	90	6.28
7	35	1.5440	10	10	-	100	8.09

Table 6: Mortality of ***Rana Tigrina*** in different concentrations of Fenvalerate**, Technical** grade at **72 hours** of Exposure.

S.No.	Conc. of Fenvalerate µg/l	Log. Conc. of Fenvalerate	No. of frogs exposed	No. of frogs killed	No. of frogs alive	% kill	Probit kill
1	35	1.5440	10	2	8	20	4.16
2	40	1.6020	10	4	6	40	4.75
3	45	1.6532	10	6	4	60	5.25
4	50	1.6989	10	8	2	80	5.84
5	55	1.7403	10	9	1	90	6.28
6	60	1.7781	10	10	Nil	100	8.09
7	65	1.8219	10	10	-	100	8.09