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Studies on acute toxicity of cartap hydrochloride (50% SP) a carbamate impact on freshwater fish, *Labeo rohita* (Hamilton)

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Abstract: The acute toxicity tests for 24, 48, 72 and 96 h to the freshwater fish, *Labeo rohita* were conducted with a cartap hydrochloride (50% SP) commercial grade, chemical structure, *S, S'*- (2-dimethylamino-trimethylene) bis (thiocarbamate) a carbamate systemic insecticide, to both static and continuous flow-through methods. The LC_{50} values obtained were 0.4152, 0.4053, 0.3948 and 0.3851ppm respectively for 24, 48, 72 and 96 h for static, 0.3132, 0.2964, 0.2821 and 0.2732 ppm respectively for continuous flow-through method. The fish exposed to chemical showed erratic swimming, loss of balance, surfacing and convulsions in a dose dependent manner. Cartap was reported as moderately toxic to freshwater fish, *Labeo rohita* and producing altered behavioral pattern in dose dependent manner. These changes were in agreement with the earlier reports. The results obtained in all were discussed with the available literature. The Regression values and the 95% confidence limits of the LC_{50} values for each test were also calculated for different time periods by using Statistical Package for the Social Sciences (SPSS) software.

Key Words: Cartap hydrochloride; acute toxicity tests; *Labeo rohita*

INTRODUCTION

Acute toxicity tests has been historically played an important role in assessing the effect of human activities on animals and such tests have wide applicability in evaluating the toxicities of various types and mixture of pollutant in fish and other aquatic species (Craddock, 1977). The parameters of short-term (toxicity) exposure are the most common measures of toxicity (Cowell *et al.,* 1972; Krebs and Burns, 1977). The importance of potential damage to aquatic ecology by effluent has been advocated and demonstrated (Sprague, 1969), informing through various toxicity tests used in the management of water pollution as, to estimate environmental effect of waste, to compare the toxicity of different toxicants in animal, to regulate the amount of discharge pollutant (Buikema *et al.,* 1982).

Cartap is a pesticide that was first introduced into the market in Japan in 1967. Its commercial names include Padan, Kritap, AG-Tap, Thiobel and Vegetox. Its basic chemical structure is S, S-[2-(dimethylamino)-1, 3-propanediyl] dicarbamothioate (Fig.1). It is commonly used as a hydrochloride ($C_7H_{15}N_3O_2S_3HCl$). Cartap is essentially a contact and stomach poison. It is used for the control of chewing and sucking pests and results in insect paralysis. It has been categorized as a high-effectiveness, low-toxicity, and low-residue pesticide used in rice and sugarcane fields (Hari Boorugu and Anugrah Chrispal, 2012).

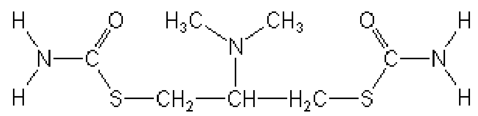

Figure 1: Chemical structure of Cartap hydrochloride

In the present study acute toxicity tests for 24, 48, 72 and 96 h to the fish, *Labeo rohita* were conducted with a cartap hydrochloride (50% SP) commercial grade a carbamate systemic insecticide, in both static and continuous flow-through methods. The static, 1/10th of 96 h LC_{50} value was taken as sub-lethal concentration to study the behavioral alterations and physiological alterations (As per the recommendations of committee on toxicity studies-Anon, 1975).

MATERIALS AND METHODS

The capture freshwater major carp fish, *Labeo rohita* (Hamilton) with a size range of 8-9 cm, weight 21.9 ± 2 g, irrespective of their sex, have been chosen as the test organism in the present study. The fish were obtained from the Kuchupudi village, Tenali mandal, Guntur, Andhra Pradesh, India which is 30 km away

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from the Acharyana Nagarjuna University. The fish were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water for two weeks at a room temperature of 28±2°C. During the period of acclimatization fish were fed daily with fish meal on an average of 3% of their body weight. Feeding was stopped one day prior to the experimentation. All the precautions laid down by APHA (1998) were followed. Cartap hydrochloride (50% SP) commercial grade was purchased from Mangalagiri, Guntur District. The stock solution was made with water as solvent. Controls were maintained for each experiment and they were added with the quantity of water equal to the highest concentration used in the test (APHA, 1998). The hydrographical properties of water were estimated by the modified methods Golterman and claimo (1969) method. Finney's probit analysis (Finney, 1971) as reported by Roberts and Boyce (1972) was followed to calculate the LC_{50} value. The respective probit values for respective Percent mortalities were taken from Fisher and Yates (1938). The 95% confidence limits of the LC_{50} values for each test were also calculated for different time periods by using SPSS software.

Results and Discussions

The ground water used for acclimatization of fish and experimental purpose was clear and unchlorinated. The hydrographical properties of water were estimated by the modified methods of Golterman and Claimo (1969) and are presented in Table 1.

The results of the present work i.e., the observed percentage mortality of fish under exposure to cartap hydrochloride (50% SP) for 24, 48, 72 and 96 h to fresh water fish, *Labeo rohita* in static and continuous flow through systems. The Percent mortality for cartap hydrochloride were compared with the other animals and different pesticides LC_{50} values. The Percent mortality and probit mortality increased with the increasing concentration of cartap hydrochloride.

The 24, 48, 72 and 96 hrs LC_{50} values of cartap hydrochloride (50% SP) commercial grade formulation in static and continuous flow through systems for the fish *Labeo rohita* are:

Static: 0.4152 mg/L, 0.4053 mg/L, 0.3948 mg/L and 0.3851 mg/L

C.F.M: 0.3132 mg/L, 0.2964 mg/L, 0.2821 mg/L and 0.2732 mg/L

Table 1: Physico-chemical properties of water used for experiments.

Parameter (units)	Testing water
Temperature (°C)	28 ± 2
Turbidity (silica units)	8
Electrical conductivity at 28°C (μmhos/cm)	816
pH at 28°C	8.2
Dissolved oxygen (mg/l)	8-10
Alkalinity (mg/l as $CaCO_3$)	
i) Phenolpthalene	Nil
ii) Total	472
Total Hardness (mg/l as $CaCO_3$)	320
Calcium Hardness (mg/l as $CaCO_3$)	80
Magnesium Hardness (mg/l as $CaCO_3$)	40
Nitrite – N (mg/l)	Nil
Sulphate –S (mg/l)	Trace
Chloride (mg/l)	40
Fluoride (mg/l)	1.8
Iron (mg/l)	Nil

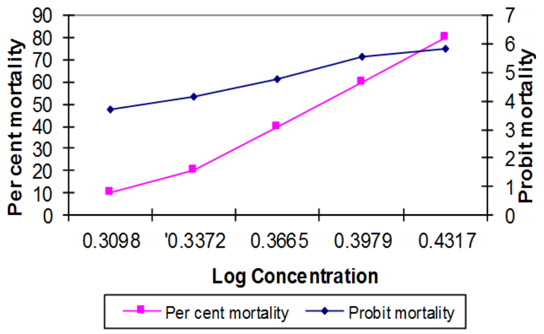

Figure 2: Static 24 h Percent Mortality and Probit Mortality of the fish *Labeo rohita* exposed to cartap hydrochloride (50% SP).

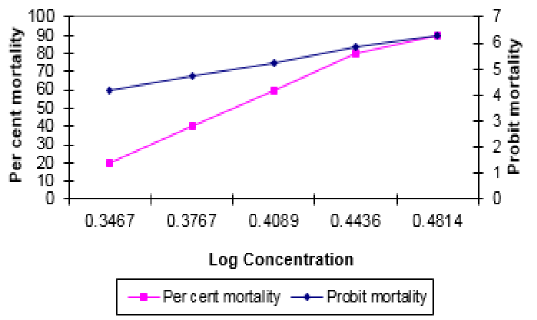

Figure 3: Static 48 h Percent Mortality and Probit Mortality of the fish *Labeo rohita* exposed to cartap hydrochloride (50% SP).

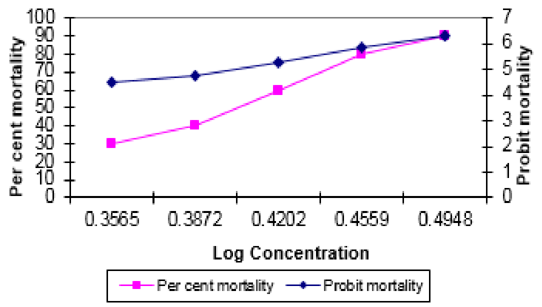

Figure 4: Static 72 h Percent Mortality and Probit Mortality of the fish *Labeo rohita* exposed to cartap hydrochloride (50% SP).

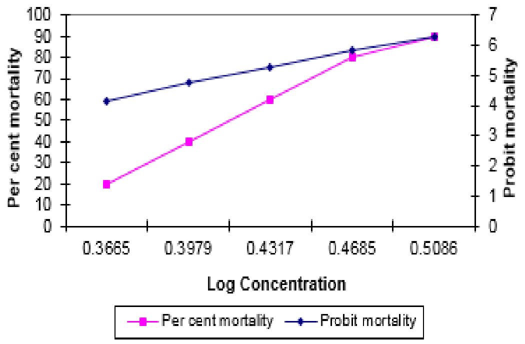

Figure 5: Static 96 h Percent Mortality and Probit Mortality of the fish *Labeo rohita* exposed to Cartap Hydrochloride (50% SP).

In general, *Labeo rohita* is sensitive towards the test toxicant. In the present study the results of static method LC_{50} values of water soluble, cartap hydrochloride (50% SP) were 0.4152 mg/L, 0.4053mg/L, 0.3948 mg/L and 0.3851 mg/L for 24, 48, 72 and 96 h to *Labeo rohita*. The similar results were also observed in different chemical compounds of carbamate group. Mayes *et al.,* (1984) found the 96 h LC_{50} to Triclopyr, 12.5 mg/L for adult (90 days) juvenile, Fathead minnow (*Pimephales*). In static method Kim *et al.,* (2008) described the 96 h LC_{50} values to cartap, 1.38 mg/L for embryo; 0.356 mg/L for Fry (0.5 cm) and 0.354 mg/L for Adult (2cm) Japanese medaka (*Oryzias latipes*). The results of static 24, 48, 72 and 96 h, Percent Mortality and Probit Mortality were graphically represented in Figure 2 to Figure 5.

Kim *et al.,* (2008) calculated the static, 96 h LC_{50} values to Cypermethrin, 0.111 mg/L for Embryo; 0.0385 mg/L for fry (0.5 cm) and 0.0308 mg/L for adult (2 cm) Japanese medaka (*Oryzias latipes*). Lamal *et al.,* (1999) found the 96 h LC_{50} values in static method to Dieldrin, 0.006 mg/L for fry (Yolk sac) and 0.0170 mg/L for fry (5 day and 37 day old) African catfish (*Clarias gariepinus*). Lamal *et al.,* (1999) found the 96 h LC_{50} values in static method to Dieldrin, 0.007 mg/L for fry (yolk sac) and 0.00495 mg/L for fry (37-day old) Nile tilapia (*Oreochromis niloticus*). Koprucu and Aydn (2004) calculated the 48 h LC_{50} value in static method to Deltamethrin, 0.213 µg/L for embryo stage of Common carp (*Cyprinus carpio*). Rajbir Kaur and Anish Dua (2014) studied toxicity in fingerlings of *Labeo rohita* exposed to geometric concentrations (6.25 to 100%) of municipal wastewater, the behavioral and morphological alterations were observed. In the present study, the continuous flow-through method LC_{50} values of cartap hydrochloride (50% SP) were 0.3132 mg/L, 0.2964 mg/L, 0.2821 mg/L and 0.2732 mg/L for 24, 48, 72 and 96 h to. The similar toxicity results of thiocarbamates were also observed in the early studies of Mayes *et al.,* (1987), they calculated the 96 h LC_{50} value in flow-through method to Picloram, 2.02mg/L for embryo larval of Rainbow trout (*Salmo gairdner*). Kumargaguru and Beamish (1981) observed the 96 h LC_{50} values in flow-through method to Permethrin, 0.00317mg/L for juvenile (1g); 0.00643 mg/L for juvenile (5g) and 0.287 mg/L for juvenile (50g) of Rainbow trout (*Salmo gairdneri*).

Klaverkamp *et al.,* (1977) calculated the 24h and 96 h LC_{50} value in flow-through method to fenitrothion, 3.4 mg/L (24 h) and 2.0 mg/L (96 h) for fingerlings (9.2 cm) and observed 0 Percent mortality at 34 mg/L for embryo 3 days before hatch, 0.5 days sac-fry, 6-9 days sac-fry and100 Percent mortality at 34 mg/L for sac-fry (10-11 days) of Rainbow trout (*Salmo gairdneri*). Adelman *et al.,* (1976) observed the 96 LC_{50} values in flow- through method to Pentachlorophenol, 0.198 mg/L for 4 weeks; 0.230 mg/L for 7 weeks; 0.222 mg/L for 11 weeks and 0.190 mg/L for 14 weeks of fat head minnow (*Pimephales promelas*). These toxicity results of carbamates were similar to present study. The results of flow-through 24, 48, 72 and 96 h, Percent Mortality and Probit Mortality were graphically represented in Figure 6 to Figure 9.

The effects of pollutants are generally characterized on survival, reproduction or growth due to physiological alteration in the animal. The physical, chemical and biological components of the environment play an important role in manifestation of biological response to pollutants. The toxicity of particular pollutants depends upon many factors such as animal weight (Pickering, 1968), developmental stages (Kamaldeep and Joor, 1975), period of exposure and temperature, pH, hardness of water and dissolved content of the medium (Mc leese, 1974 and Brungs *et al.,* 1977).

WHO (1977) has set the recommended maximum acceptable daily intake level for cartap hydrochloride as 0.05 mg/Kg weight. The world Health Organisation as a guideline (1978) has classified cartap hydrochloride as a moderately hazardous technical product.

In the present study, it was observed that the static LC_{50} values are higher, compared to the continuous flow-through values. The toxicity may be influenced by exposure conditions, formulation, source and size of fish and water quality. Borthwick *et al.,* (1985) and Mayer (1987) reported that the static LC_{50} values were 2-5 times lower in flow through systems in marine fishes.

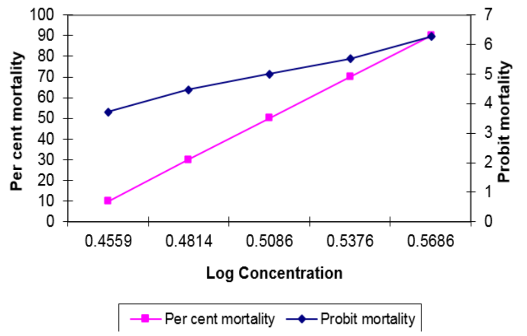

Figure 6: Continuous flow through 24 h Percent Mortality and Probit Mortality of the fish, *Labeo rohita* exposed to cartap hydrochloride (50% SP).

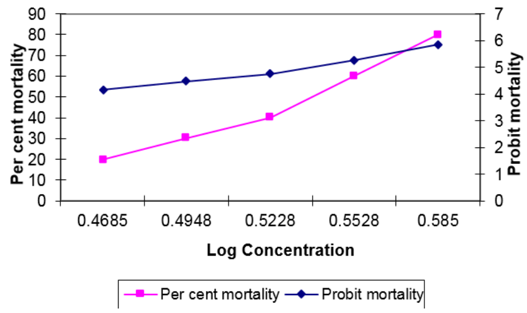

Figure 7: Continuous flow through 48 h Percent Mortality and Probit Mortality of the fish *Labeo rohita* exposed to cartap hydrochloride (50% SP).

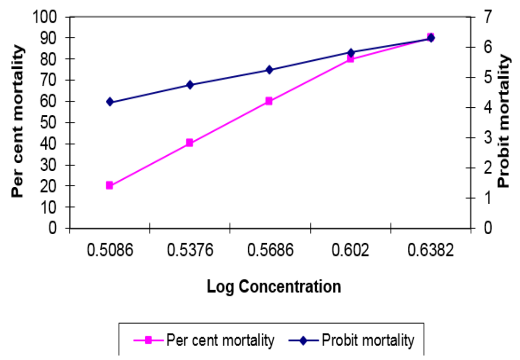

Figure 8: Continuous flow through 72 h Percent Mortality and Probit Mortality of the fish *Labeo rohita* exposed to cartap hydrochloride (50% SP).

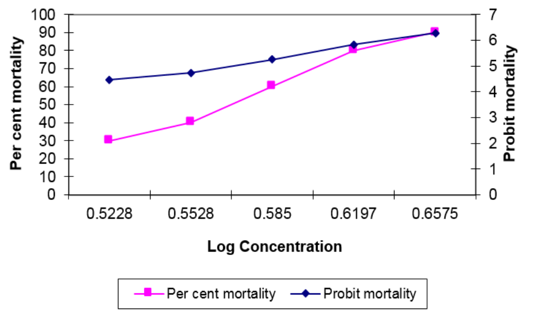

Figure 9: Continuous flow through 96 h Percent Mortality and Probit Mortality of the fish *Labeo rohita* exposed to cartap hydrochloride (50% SP).

The size of the fish has been reported to influence toxicity in static tests, possibly because absorption of exposure concentration by fish (Barron *et al.,* 1993). The continuous flow-through system LC_{50} values were low when compared to the static values. This is due to the constant maintenance of concentration in flow-through system. The static values are higher because of bioaccumulation, pesticide absorption to toxicant chamber walls and degradation of toxic effect of the compound.

The results of Regressions values and 95 % Confidence Levels for static and Continuous flow through methods for 24 h, 48 h, 72 h and 96 h exposed periods were given in the table 2 and table 3.

Table 2: Regression values for toxicant of cartap hydrochloride (50% SP) exposed to *Labeo rohita* at different exposed periods.

Hours of exposure	Static method Régression équation Y= (y-bx) +bx	Flow-through method Régression équation Y= (y-bx) +bx
24	y = 0.561x + 3.114 R^2 = 0.986	y = 0.617x + 3.147 R^2 = 0.994
48	y = 0.534x + 3.653 R^2 = 0.997	y = 0.414x + 3.651 R^2 = 0.973
72	y = 0.470x + 3.907 R^2 = 0.987	y = 0.534x + 3.653 R^2 = 0.997
96	y = 0.534x + 3.653 R^2 = 0.997	y = 0.470x + 3.907 R^2 = 0.987

Table 3: The 95% Confidence Levels of cartap hydrochloride (50% SP) exposed to fish *Labeo rohita* at different exposed periods in Static and flow-through methods.

S.No	Exposure periods	95 % Confidence levels: Static method Lower	Static method Upper	Flow-through method Lower	Flow-through method Upper
1	24 h	0.3834	0.5066	0.2707	0.3493
2	48 h	0.3311	0.4489	0.2607	0.3393
3	72 h	0.3211	0.4389	0.2307	0.3093
4	96 h	0.3111	0.4289	0.2207	0.2993

A behavioral response was the first response of an organism to the environmental perturbations. The behavior of an organism represents the final interrelated result of a diversity of biochemical and physiological processes. Behavior is considered a promising tool in ecotoxicology. In the present investigation, when fish, *Labeo rohita* were exposed to Sub-lethal concentration (static 96 h, LC_{50}) of cartap hydrochloride for 6 days, several behavioral changes were observed which include erratic swimming movements and they appeared to be in distress. During the second day, onwards opercular movements were quicker and the fish surfaced more frequently gasping for air but subsequently this breathing difficulty seemed to have subsidized. Hyper excitation, loss of equilibrium, flaring of gills, increase in production of mucus from the gills, darting movements and hitting against the walls of test tanks were noticed in the test fish, *Labeo rohita*. The reddish spots on surface scales and scales falling were also observed at experimental period. The morphological and behavioral changes exhibited by the fish can be taken as useful parameter as a bio-indicator in assessing the extent of aquatic pollution.

Conclusions

This type of study can be useful to compare the sensitivity of various species of aquatic animals and potency of effluent using LC_{50} values and to derive safe concentration. Changes in behavior of fish, *Labeo rohita* due to toxicant stress can be used as a biological indicator of pollution as biological early alarm system. Since majority of pesticide residues are known to bio-accumulate in the lipid tissues of fish and other animals, and transfer via food chain to the human bodies, the grave risk to the health of the people who consume these fish seems to be considerable. Though we cannot avoid the use of pesticides, measures should be taken for the conservation of the water quality and also the aquatic resources.

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