

**ACUTE TOXICITY OF CHLORANTRANILIPROLE TO FRESH WATER FISH CTENOPHARINGODON IDELLA (VALENCIENNES, 1844)**

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**ABSTRACT**

Pesticidal pollution constitutes the most dangerous health hazard apart from creating adverse effects on fish production. The aim of the present study was to determine the acute toxicity of chlorantraniliprole to the fresh water fish Grass carp (*Ctenopharingodon idella*), experimental fish were exposed to different concentrations of chlorantraniliprole. The 96h LC50 value of chlorantraniliprole on the fish was found to be 11.008mg/l. The variation in the LC values is due to its dependence upon various factors viz., sensitivity to the toxicant, its concentration and duration of exposure. Increase in opercular movement was initially observed but later decreased with increase of exposure period. They slowly became lethargic, restless, and secreted excess mucus all over the body. Intermittently some of the fish were hyper excited resulting in erratic movements. An excess secretion of mucous in fish forms a non-specific response against toxicants, thereby probably reducing toxicant contact. Further study needs the processes by which these chemicals affect physiology and pathological changes and of fish and their bio-concentration and bio-accumulation in fish tissues.

**Keywords:** Health hazard, concentration, LC values, bioconcentration and physiology

**INTRODUCTION**

Pesticidal pollution constitutes the most dangerous health hazard apart from creating adverse effects on fish production. As the fishes are economically important non-target organisms, they are quite sensitive to a wide variety of toxicants and are used as pollution indicator in the water-quality management. The basic mechanism of action for most pesticides is proved to be an alteration in the transfer of a signal along a nerve fiber and across the synapse from one nerve to another or from nerve to a muscle fiber. The signal is transferred across the synapse to the next nerve cell by the release of neurotransmitters such as acetylcholine (AChE). The biochemical processes represent the most sensitive and relatively early events of pollutant damage. Thus, it is important that pollutant effects be determined and interpreted in biochemical terms, to delineate mechanisms of pollutant action, and possibly ways to mitigate adverse effects [1]. Many of workers have been used the acute toxicity tests of pesticides on fish to acquire rapid estimates of the concentrations that caused direct, irreversible harm to test organism [2].

In the present study, an attempt has been made to analyse the toxicity of the chlorantraniliprole 18.5% SC on the fresh-water fish Grass carp (*Ctenopharingodon idella*). The result is expressed as the lethal dose (LD) in the case of terrestrial organism and as lethal concentration in the case of aquatic organisms. Since some members of population may prove to be excessively susceptible and others may prove to be very resistant to the dose or the concentration of the toxicant that affects 50% of the population under consideration is expressed as LD<sub>50</sub> or LC<sub>50</sub> values, which is statistically calculated on the basis of the observed percentage of mortality at different concentrations of the pesticides.

**MATERIALS AND METHODS**

The fresh water fish Grass carp (*Ctenopharingodon idella*) size 10-15 cm and weight 20 g were brought from a local fish farm at Nandivelugu. The fish were fed daily with commercial fish pellets and allowed to acclimate for 15 days. The fish were acclimatized to the laboratory conditions at 28 ± 2°C, if in any batch; mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. For acute toxicity study the Insecticide chlorantraniliprole 18.5% SC (Suspension concentrate), (Trade name as Coragen) was purchased from local market in Guntur of Andhra Pradesh, manufactured by DuPont Canada company

Agricultural products Ontario. The water used for acclimatization and conducting experiments was clear unchlorinated ground water and the hydrographic conditions of water are shown in the Table 1. The containers of the test media are of 10 liter capacity, where in each test five containers were used and each container consisted of 10 fish. The mortality rate was taken into consideration and while taking the data, dead fish was removed immediately. Pilot experiments were conducted to choose the mortality range between 10% and 100%. Basing on the pilot experiments, the experiments were conducted to determine the toxicity in different concentrations (0, 1, 4, 6, 8, 10, 10.5, 11, 11.5, 12 and 12.5 mg/l) for 24, 48, 72, and 96h with chlorantraniliprole in static system.

**Physico-Chemical analysis of water**

The sample of water was analyzed according to guidelines [3]. The sample water is clear, colorless and odorless; the following results are in mg/l.

**Table 1: Chemical analysis of water used for experiments**

Turbidity	:	8 silica units
Electrical conductivity at 28°C	:	816 micro ohms/cm
pH value at 28°C	:	8.1
Alkalinity:		
i. Phenolphthalein	:	Nil
ii. Methyl orange	:	472
Total Hardness (as CaCO <sub>3</sub> )	:	232
Non-carbonate Hardness (as CaCO <sub>3</sub> )	:	Nil
Calcium Hardness (as N)	:	Nil
Sulphate (as SO <sub>4</sub> )	:	Trace
Chloride (as Cl)	:	40
Fluoride (as F)	:	1.8
Iron (as Fe)	:	Nil
Dissolved oxygen	:	8-10 ppm
Temperature	:	28 ± 32°C

**Statistical Analysis**

The data of each concentration was pooled up to calculate the LC<sub>50</sub> values. The un-weighted regression method of probit analysis [4], and SPSS v20.0 were used to calculate the LC<sub>50</sub> values.

## RESULTS

In the present investigation the fish Grass carp (*Ctenopharingodon idella*) has shown differential toxicity level with the function of period. This shows that the more is the duration period the less is the concentration required. The observed percentage of mortality of Grass carp (*Ctenopharingodon idella*) for chlorantraniliprole in static tests and observed LC values and 95% confidence limits in static tests for different hours were shown in Tables 3, and 4. The probit parameters estimation, mortality rate and toxicant concentration and obtained results were shown in (Table 2, and Figure 1, respectively. Table 5) showing the 96h LC<sub>50</sub> value for the experimental fish. Control mortality value was zero in the experimental fish; the estimated 96h LC<sub>50</sub> value by using probit analysis as 11.008mg/l. The 95% Confidence limits for the LC<sub>50</sub> value of 96h were 10.686 to 11.3687mg/l, respectively. The mortality was increased, as the concentration of the toxicant was increased (Table 3 and 4, figure1).

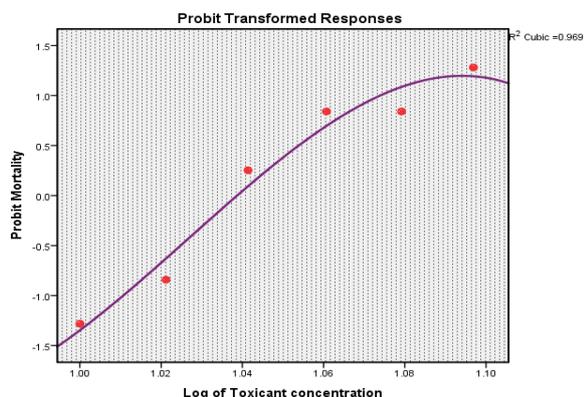
**Table 2: Parameters Estimates for the probit analysis**

parameter	Estimate	Std. Error	Z	Sig.	Lower bound	Upper bound
toxicant concentration	32.28	7.133	4.5	P<0.001	18.304	46.265
PROBIT <sup>a</sup> Intercept	33.63	7.418	4.5	P<0.001	41.049	26.213

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

**Table 2: The relationship between the chlorantraniliprole concentration and mortality rate of Grass carp (*Ctenopharingodon idella*) for the 24h, 48h, 72h, and 96h exposure**

Concentration (mg/l)	Mortality			
	24h	48h	72h	96h
10	0	0	1	1
10.5	1	2	3	4
11	2	3	4	6
11.5	6	5	6	7
12	8	6	7	9
12.5	8	8	9	10
Control	0	0	0	0



**Fig.1: Probit line graph of acute toxicity of chlorantraniliprole on fish Grass carp (*Ctenopharingodon idella*)**

## DISCUSSION

The toxicity of a pesticide could vary from species to species. These results have important implications for ecological risk assessments, particularly those that focus on the toxicity of individual chemicals as

the basis for estimating impacts to imperiled aquatic species. Although the importance of multiple stressors is widely recognized in aquatic Ecotoxicology [5], pesticide mixtures continue to pose major challenges for natural resource agencies [6]. These challenges include the data gaps that exist for many individual chemicals, experimental design difficulties (e.g., near-insurmountable factorial complexity for large numbers of chemicals), poorly understood pathways for chemical interaction, potential differences in response among species, and the need for more sophisticated statistical tools for analyzing complex data.

The effects observed provide strong evidence of the capacity for mixtures of similarly acting chemicals to behave in an additive manner according to the principles of concentration addition. The effects of this mixture were consistent with concentration addition at low-effect concentrations, but a divergence occurred with increasing effect level, with the predicted effects exceeding those that were observed. This was attributed to the limitations of the experimental design rather than being the result of a real deviation from additivity [7].

The variation is due to differential tolerance of animals to pesticide exposure [8-9] reported that toxic effect of the organophosphate pesticide phosphamidon in thiourea medium, on the fresh water fish, *Sarotherodon mossambica*, the LC<sub>50</sub> values of phosphamidon treatment such as 5.0869, 4.0598, 3.0520 2.3784 for 24, 48, 72, 96 respectively and phosphamidon in 0.03% thiourea medium such as 5.1105, 3.5650, 2.4940, 1.7330 for 24, 48, 72, 96. [10] reported the 96h LC<sub>50</sub> value of a neem biopesticide (Triology) on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112ppm. The 96hrs LC<sub>50</sub> values of diazinon on different fishes reported from tenth to several tens of mg/L. A value of diazinon 96 hrs LC<sub>50</sub> was 0.8 mg/l for guppy (*Poecilia reticulata*) and for zebra fish (*Brachydanio rerio*) was 8 mg/L. Which were all previously described by earlier workers [11], in crayfish [12-13] has estimated LC<sub>50</sub> value as 4 ppm for organophosphate quinolphos when exposed to *Oreochromis mossambicus*. In the present study, the 96h LC<sub>50</sub> value of chlorantraniliprole (Coragen) on the fish Grass carp found to be 11.008 mg/l. The variation in the LC values is due to its dependence upon various factors viz., sensitivity to the toxicant, its concentration and duration of exposure.

## Behavioral studies

In the present study of test organism showed normal behavior in control group but jerky movements, hyper secretion of mucus, opening mouth for gasping, losing scales, hyperactivity were observed experimental group. Behavioral characteristics are obviously sensitive indicators of toxicant effect. In toxic medium of chlorantraniliprole the fish Grass carp (*Ctenopharingodon idella*) sank to bottom of the test chamber and independency in swimming. Subsequently fish moved to the corners of the test chambers, which can be viewed as avoidance behavior of the fish to the toxicant. In the toxic environment fish exhibited irregular, erratic, darting swimming movements and loss of equilibrium followed by hanging vertically in water. The above symptoms are due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ensuing hyperstimulation. And inhibition of AChE activity is a typical characteristic of organophosphate compounds [14-15]. Increase in opercular movement was initially observed but later decreased with increase of exposure period. They slowly became lethargic, restless, and secreted excess mucus all over the body. Intermittently some of the fish were hyper excited resulting in erratic movements. An excess secretion of mucus in fish forms a non-specific response against toxicants, thereby probably reducing toxicant contact. It also forms a barrier between the body and the toxic medium, so as to minimize its irritating effect, or to scavenge it through epidermal mucus. Similar observations were made by [16] following RPR-V (a novel phosphorothionate pesticide) exposure to euryhaline fish, *Oreochromis mossambicus*.

Gulping air and swimming at the water surface (surfacing phenomenon) were observed also with mucus secretion on the body in both the lethal and sublethal exposure periods. [17-18] reported that fish in sub lethal concentration were found under stress but that was not fatal. [19], reported that the abnormal changes in the fish exposed to lethal concentration cypermethrin are time dependent,

[20] observed that the fish is exposed to cypermethrin, erratic swimming, hyper and hypoactive, imbalance in posture, increase in surfacing activity, opercular movement, gradual loss in equilibrium, spreading of excess of mucus all over the surface of the body. Fishes exhibited a number of behavioral changes when they were exposed to different concentrations. The opercular movement of fishes initially increases and then gradually decreases. Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Abnormal swimming and loss of balance was

caused by the deficiency in nervous and muscular coordination which may be due accumulation of acetylcholine in synaptic and neuromuscular junctions observed by [21]. It is necessary, to select behavioral indices for monitoring that relates to the organisms behavior in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural systems. Insecticide toxicity is influenced by physical factors like temperature and biological factors like size, Nutritional status and Species specificity.

**Table 3: Estimated lethal concentration values and confidence limits**

Probability	95% Confidence limits for		95% Confidence limits for log	
	Toxicant concentration Estimate	Upper bound	Lower bound	(Toxicant concentration) <sup>a</sup>
Probit .010(LC <sub>1</sub> )	9.325	9.842	0.97	0.91
0.02	9.508	9.984	0.978	0.93
0.03	9.626	10.075	0.983	0.94
0.04	9.716	10.145	0.987	0.95
.050(LC <sub>5</sub> )	9.79	10.203	0.991	0.95
0.06	9.853	10.253	0.994	0.96
0.07	9.908	10.297	0.996	0.96
0.08	9.958	10.338	0.998	0.96
0.09	10.004	10.375	1	0.97
.100(LC <sub>10</sub> )	10.046	10.409	1.002	0.97
0.15	10.224	10.556	1.01	0.98
0.2	10.367	10.68	1.016	0.99
0.25	10.491	10.794	1.021	1
0.3	10.604	10.903	1.025	1.01
0.35	10.71	11.012	1.03	1.01
0.4	10.811	11.124	1.034	1.02
0.45	10.91	11.241	1.038	1.02
.500(LC <sub>50</sub> )	11.008	11.367	1.042	1.03
0.55	11.107	11.502	1.046	1.03
0.6	11.209	11.648	1.05	1.04
0.9	12.062	13.094	1.081	1.07
0.91	12.113	13.188	1.083	1.07
0.92	12.168	13.291	1.085	1.07
0.95	12.378	13.685	1.093	1.07
0.96	12.472	13.863	1.096	1.08
.990(LC <sub>99</sub> )	12.995	14.882	1.114	1.09

**Table 4: Acute toxicity of chlorantraniliprole on fish Grass carp (*Ctenopharingodon idella*) during 96h**

Point	96h LC <sub>1</sub> (mg/l)	96h LC <sub>5</sub> (mg/l)	96h LC <sub>10</sub> (mg/l)	96h LC <sub>50</sub> mg/l	96h LC <sub>99</sub> (mg/l)	S.E
	9.325	9.790	10.046	11.008	12.995	7.133

## CONCLUSIONS

Chlorantraniliprole and the formulations, is less acutely toxic to fresh water fish Grass carp (*Ctenopharingodon idella*) than other chemical pesticides, based on acute toxicity data. Further study needs the processes by which these chemicals affect physiology and pathological changes and of fish and their bio-concentration and bio-accumulation in fish tissues

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