

Median lethal concentrations (LC₅₀) of Chlorantraniliprole and its effects on behavioral changes in Freshwater Fish *Labeo Rohita*

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ABSTRACT

The aim of the present study was to determine the acute toxicity of chlorantraniliprole insecticide to the fresh water fish *Labeo rohita*. Environmental protection has attracted the attention of the wide cross-section of people all over the world which has now become a global issue amongst scientists and researchers working in this area. Experimental fish were exposed to different concentrations of chlorantraniliprole between range 0, 1, 2,3,4,5,6,8,10,12 and 13mg/L⁻¹ for 96 hrs in test container. The 96hrs LC₅₀ value of chlorantraniliprole (Coragen) on the fish was found to be 12.7mg/L⁻¹. 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. 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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1. INTRODUCTION

Environmental protection has attracted the attention of the wide cross-section of people all over the world which has now become a global issue amongst scientists and researchers working in this area. 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.

Coragen 18.5% SC is a new chemical insecticide product, the active substance is chlorantraniliprole. It is applied for use in apples against codling moth apple fruit moth and free leaf living larvae. Chlorantraniliprole belongs to the Diamides (Ryanodine receptor modulators). The mode of action is different to most other commercial insecticides. Identity and physical/chemical data (Product name: Coragen 18.5% SC.

Active substance: chlorantraniliprole; Formulation: Suspension concentrate; CAS number 500008-45-7, IUPAC-name:3-bromo-4'-chloro-1-(3-chloro-2-pyridyl)-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide).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 *Labeo rohita*. 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 LD50 or LC₅₀ values, which is statistically calculated on the basis of the observed percentage of mortality at different concentrations of the pesticides.

2. RESEARCH METHOD

The fresh water fish *Labeo rohita* size 7-8 cm and weight 9-10 gm were brought from a local fish farm at Nandivelugu. The fish *Labeo rohita* were acclimatized to the laboratory conditions at $28 \pm 2^\circ\text{C}$ for 15 days. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. Insecticide was purchased from local market in Guntur of Andhra Pradesh. 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.

2.1. Acute toxicity tests

The containers of the test media are of 30 liter capacity, where in each test five containers were used and each container consisted of 30 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 90%. Basing on the pilot experiments, the experiments were conducted to determine the toxicity in different concentrations (0,1,2,3,4,5,6,8,9,10,12 and 13mg/L¹ for 24,48,72, and 96 hrs with compound Anthralinic Diamides, in semi-static system.

The data of each concentration was pooled up to calculate the LC₅₀ values. The un-weighted regression method of probit analysis and SPSS v20.0 was used to calculate the LC₅₀ values [3]. According to [4],[5], the sample water is clear, colorless and odorless. The following results are in mg/L⁻¹.

Table 1. Chemical analysis of water used for experiments

Chemical analysis of water	
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 ₃)	: 232
Non-carbonate Hardness (as CaCO ₃)	: Nil
Calcium Hardness (as N)	: Nil
Sulphate (as SO ₄)	: Trace
Chloride (as Cl)	: 40
Fluoride (as F)	: 1.8
Iron (as Fe)	: Nil
Dissolved oxygen	: 8-10 ppm
Temperature	: $28 \pm 32^\circ\text{C}$

3. RESULTS AND ANALYSIS

In the present investigation the test species, *Labeo rohita* 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 *Labeo rohita* for chlorantraniliprole in static tests continuous for different hours and different concentrations were shown in Table 2, 3, 4 & 5. The observed LC values and 95% confidence limits in static tests for LC₂₅ (10.798-12.018), LC₄₅ (12.290- 12.822), LC₇₅ (13.485-14.346), and LC₉₆ (15.073-16.909) were shown in Table 5.

The toxicity of a pesticide could vary from species to species. The variation is due to differential tolerance of animals to pesticide exposure.[6];[7] reported that toxic effect of the organophosphate pesticide phosphamidon in thiourea medium, on the fresh water fish, *Sarotherodon mossambica*, the LC₅₀ 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.[8],

reported the 96h LC₅₀ value of a neem biopesticide (Triology) on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112ppm. The 96hrs Lc50 values of diazinon on different fishes reported from tenth to several tens of mg/L⁻¹. A value of diazinon 96 hrs LC50 was 0.8 mg/L⁻¹ for guppy (*Poecilia reticulata*) and for zebra fish (*Brachydanio rerio*) was 8 mg/l⁻¹. Which were all previously described by earlier workers in crayfish[9];[11], have estimated LC₅₀ value as 4 ppm for organophosphate quinolphos when exposed to *Oreochromis mossambicus*.

Table 2. Parameter Estimates for chlorantraniliprole vs. *Labeo rohita*

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
probit a	concentration	18.926	2.165	8.743	.000	14.683	23.169
	Intercept	-20.922	2.382	-8.783	.000	-23.304	-18.540

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

Table 3. Chi-Square Test of probit analysis

probit	Chi-Square	df ^b	Sig.
Pearson Goodness-of-Fit Test	3.766	7	.806 ^a

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.

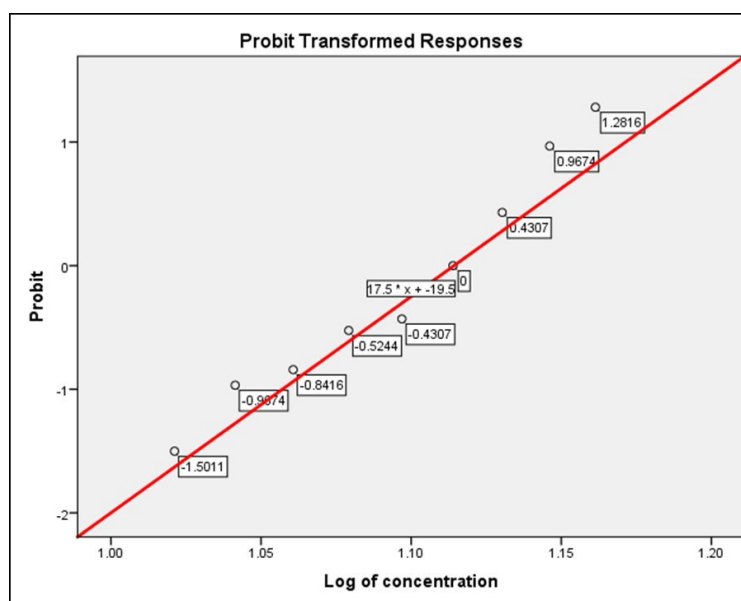
b. Statistics based on individual cases differ from statistics based on aggregated cases.

Table 4. Log concentration, observed responses in fish *Labeo rohita*

Number	concentration	Number of exposed fish	Observed Responses	Expected Responses	Residual
1	1.021	30	2	1.662	.338
2	1.041	30	5	3.381	1.619
3	1.061	30	6	5.955	.045
4	1.079	30	9	9.286	-.286
5	1.097	30	10	13.074	-3.074
6	1.114	30	15	16.915	-1.915
7	1.130	30	20	20.435	-.435
8	1.146	30	25	23.379	1.621
9	1.161	30	27	25.651	1.349

Table 5. Confidence Limits for fish *Labeo rohita*

Probability Probit point	95% Confidence Limits for concentration			95% Confidence Limits for log(concentration) ^a	
	Concentration	Lower Bound	Upper Bound	Concentration	Lower Bound
LC1	9.606	8.855	10.127	.983	.947
LC2	9.930	9.238	10.409	.997	.966
LC3	10.141	9.489	10.594	1.006	.977
LC 4	10.303	9.681	10.735	1.013	.986
LC 5	10.436	9.841	10.851	1.019	.993
LC 6	10.551	9.978	10.952	1.023	.999
LC 7	10.653	10.100	11.041	1.027	1.004
LC 8	10.745	10.210	11.122	1.031	1.009
LC 9	10.830	10.311	11.196	1.035	1.013
LC10	10.908	10.404	11.265	1.038	1.017
LC15	11.238	10.798	11.557	1.051	1.033
LC 20	11.508	11.117	11.800	1.061	1.046
LC 25	11.744	11.393	12.018	1.070	1.057
LC 30	11.960	11.642	12.222	1.078	1.066
LC 35	12.164	11.872	12.420	1.085	1.075
LC 40	12.361	12.086	12.619	1.092	1.082
LC 45	12.555	12.290	12.822	1.099	1.090
LC 50	12.748	12.487	13.033	1.105	1.096
LC 55	12.945	12.679	13.256	1.112	1.103
LC 60	13.147	12.871	13.493	1.119	1.110
LC 65	13.360	13.065	13.749	1.126	1.116
LC 70	13.588	13.268	14.030	1.133	1.123
LC 75	13.839	13.485	14.346	1.141	1.130
LC 80	14.123	13.727	14.710	1.150	1.138
LC 85	14.461	14.009	15.151	1.160	1.146
LC 90	14.899	14.368	15.729	1.173	1.157
LC 91	15.007	14.456	15.873	1.176	1.160
LC 92	15.125	14.551	16.031	1.180	1.163
LC 93	15.256	14.657	16.206	1.183	1.166
LC 94	15.403	14.775	16.405	1.188	1.170
LC 95	15.573	14.912	16.635	1.192	1.174
LC 96	15.774	15.073	16.909	1.198	1.178

Figure 1. The graph showing linear curve between probit mortality of fish against log concentration in *Labeo rohita* on exposure to chlorantraniliprole

In the present study, the 96hrs LC₅₀ value of chlorantraniliprole (Coragen) on the fish *Labeo rohita* was found to be 12.7mg/L⁻¹. 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.

3.1. 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 chloroanthraniliprole the fish 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 [12];[13]. 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. 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 [14];[15], 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.[16], reported that fish in sub lethal concentration were found under stress but that was not fatal. [17] Reported that the abnormal changes in the fish exposed to lethal concentration cypermethrin are time dependent. [17] 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 [5];[18];[19]. 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 assement of the hazards that a contaminant may pose in natural systems.

4. CONCLUSION

Chlorantraniliprole and the formulations, is acutely toxic to fresh water fish *Labeo rohita* 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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