Acute Toxicity of Chlorantraniliprole to Freshwater Fish *Channa punctatus* (Bloch)

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Abstract 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. The aim of the present study was to determine the acute toxicity of chlorantraniliprole insecticide to the fresh water fish *Channa punctatus* (Bloch). Experimental fish were exposed to different concentrations of chlorantraniliprole between range 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, 14, 15, 16 and 17 mg/L⁻¹ for 96 h in test container. The 96h LC₅₀ value of chlorantraniliprole on the fish was found to be 14.424 mg/L⁻¹. The variation in the lethal concentration 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.

Keywords Global Issue, Acute Toxicity, Chlorantraniliprole, Concentration And *Channa Punctatus*

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 [1].

In the present study, an attempt has been made to analyze the toxicity of the chlorantraniliprole 18.5% SC (Suspension concentrate) on the freshwater fish *Channa punctatus* (Bloch). 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.

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

2. Materials and Methods

The freshwater fish *Channa punctatus* size 12-13 cm and weight 18-20 g were brought from a local waterbodies at Nandivelugu, Guntur district of Andhra Pradesh, India. The fish were acclimatized to the laboratory conditions at

28±2°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. The stock solutions for the above were prepared by dissolving the chlorantraniliprole 18.5% SC in unchlorinated ground water according to Finney probit[3].

Table 1. Chemical analysis of water used for experiments

Ttt Turbidity	8 silica units
Electrical conductivity at 28°C	816 Micro ohms/cm
pH at 28°C	8.1
i) Phenolphthalene	Nil
ii) Methyl orange as CaCO ₃	472
Total Hardness	320
Calcium Hardness	80
Magnesium Hardnes	40
Nitrite nitrogen (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 ± 2°C

2.1. Acute Toxicity Tests

The containers of the test media are of 15 L 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 indifferent concentrations (0, 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 15, 16 and 17mg/L-1 for 96h with compound Anthracitic 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, 17] the sample water is clear, colorless and odorless. The following results are in mg/L-1.

3. Results and Discussion

In the present investigation the test species, *Channa punctatus* 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 *Channa punctatus* 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 were shown in Table 5.

Table 2. Parameter Estimates of the probit analyses for *Channa punctatus*

PROBIT a	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	concentration	28.676	5.227	5.486	.000	18.431	38.921
	Intercept	-33.238	6.037	-5.506	.000	-39.275	-27.201

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

Table 3. Chi-Square Test for 96 h LC₅₀ value of *Channa punctatus*

Probit	Chi-Square	df ^b	Sig.
Pearson Goodness-of-Fit Test	2.825	7	.901 ^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.

Number	Concentration	Number of exposed fish	Observed Responses	Expected Responses	Residual
1	1.079	10	0	.110	-.110
2	1.097	10	1	.373	.627
3	1.114	10	1	.978	.022
4	1.130	10	2	2.048	-.048
5	1.146	10	3	3.551	-.551
6	1.161	10	5	5.261	-.261
7	1.176	10	6	6.871	-.871
8	1.190	10	8	8.149	-.149
9	1.204	10	10	9.017	.983

Point	95% Confidence Limits for concentration			95% Confidence Limits for log(concentration) ^a		
	Concentration	Lower Bound	Upper Bound	Concentration	Lower Bound	Upper Bound
LC ₁	11.966	10.796	12.598	1.078	1.033	1.100
LC ₂	12.231	11.162	12.811	1.087	1.048	1.108
LC ₃	12.402	11.400	12.950	1.093	1.057	1.112
LC ₄	12.532	11.582	13.056	1.098	1.064	1.116
LC ₅	12.639	11.731	13.143	1.102	1.069	1.119
LC ₆	12.731	11.859	13.219	1.105	1.074	1.121
LC ₇	12.812	11.972	13.285	1.108	1.078	1.123
LC ₈	12.885	12.074	13.346	1.110	1.082	1.125
LC ₉	12.952	12.167	13.402	1.112	1.085	1.127
LC ₁₀	13.014	12.253	13.454	1.114	1.088	1.129
LC ₁₅	13.272	12.611	13.675	1.123	1.101	1.136
LC ₂₀	13.481	12.896	13.861	1.130	1.110	1.142
LC ₂₅	13.664	13.139	14.030	1.136	1.119	1.147
LC ₃₀	13.829	13.353	14.191	1.141	1.126	1.152
LC ₃₅	13.985	13.547	14.351	1.146	1.132	1.157
LC ₄₀	14.134	13.726	14.513	1.150	1.138	1.162
LC ₄₅	14.279	13.892	14.680	1.155	1.143	1.167
LC ₅₀	14.424	14.049	14.856	1.159	1.148	1.172
LC ₅₅	14.570	14.199	15.043	1.163	1.152	1.177
LC ₆₀	14.720	14.345	15.244	1.168	1.157	1.183
LC ₆₅	14.877	14.490	15.462	1.173	1.161	1.189
LC ₇₀	15.044	14.639	15.702	1.177	1.165	1.196
LC ₇₅	15.227	14.794	15.973	1.183	1.170	1.203
LC ₈₀	15.432	14.964	16.285	1.188	1.175	1.212
LC ₈₅	15.676	15.158	16.664	1.195	1.181	1.222
LC ₉₀	15.987	15.399	17.159	1.204	1.187	1.235
LC ₉₁	16.063	15.457	17.282	1.206	1.189	1.238
LC ₉₂	16.147	15.520	17.417	1.208	1.191	1.241
LC ₉₃	16.239	15.590	17.567	1.211	1.193	1.245
LC ₉₄	16.342	15.667	17.736	1.213	1.195	1.249
LC ₉₅	16.461	15.755	17.931	1.216	1.197	1.254
LC ₉₉	16.601	15.859	18.164	1.220	1.200	1.259

Figure 1. Probit line graph of acute toxicity of chlorantraniliprole to *Channa punctatus*

The toxicity of a pesticide could vary from species to species. The variation is due to differential tolerance of animals to pesticide exposure [5,6], reported that toxic effect of the organophosphate pesticide phosphamidon in thiourea medium, on the freshwater fish, *Sarotherodon mossambica*, the LC_{50} values of phosphamidon treatment such as 5.0869, 4.0598, 3.0520 2.3784 for 24, 48, 72, 96h respectively and phosphamidon in 0.03% thiourea medium such as 5.1105, 3.5650, 2.4940, 1.7330 for 24, 48, 72, 96 [7]. Reported the 96h LC_{50} value of a neem biopesticide (Triology) on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112ppm. The 96hrs LC_{50} values of diazinon on different fishes reported from tenth to several tens of mg/L^{-1} . A value of diazinon 96 hrs LC_{50} 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 [8-10], has estimated LC_{50} value as 4 ppm for organophosphate quinolphos when exposed to *Oreochromis mossambicus*.

In the present study, the 96h LC_{50} value of chlorantraniliprole on the fish *Channa punctatus* was found to be $14.424 mg/L^{-1}$. 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 *Channa punctatus* 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 [11, 12]. 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 [13, 14, and 19] 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.[14], reported that fish in sub lethal concentration were found under stress but that was not fatal. Reported that the abnormal changes in the fish exposed to lethal concentration cypermethrin are time dependent. [15, 20] Observed that the fish is exposed to cypermethrin, Rimon; 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 [16-18]. 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.

4. Conclusion

From the above acute toxicity investigation, we conclude that pesticide Chlorantraniliprole were highly toxic to fish *C.punctatus* based on the observed LC₅₀ values and had a impact on behavior and respiratory responses in fish *C.punctatus* in lethal concentrations.

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