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Acute toxicity and Histopathological changes in freshwater fish *Cirrhinus mrigala* exposed to chlorantraniliprole

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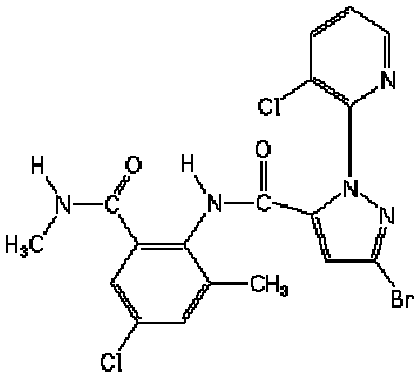
Abstract

Fish was exposed to different concentrations of chlorantraniliprole for different hrs, percent mortality and behavioral responses were studied. The 96 hrs LC_{50} value of test toxicant to the fish was found to be 16.465mg/l^{-1}. Behavioral patterns were observed in during exposure period, test organism showed normal behavior in control group but jerky movements, hyper secretion of mucus, opening and closing of mouth for gasping, losing scales, hyperactivity were observed experimental group. The toxic effects of the environmental contaminant chlorantraniliprole, on the gill and liver of freshwater fish *L.rohita* were determined by light microscope. The fish were exposed to sub lethal concentrations ($1/10^{th}$ 96 hrs of LC50) for 15 days. Gills were found to be the most seriously affected organ compared to liver, because of the direct contact with the toxicant. Also the liver is an important organ which breakdown chemicals and as a result, hepatocytic alterations was prominent often among those that are damaged by chlorantraniliprole. No histopathological changes were observed in control group.

Keywords: Mortality, value, exposure, behavior, gill and losing scales

1. Introduction

Aquatic ecosystem is the final sink for the many chemicals used in industry and agriculture has a global problem, the continuous release of these chemicals impair water quality and become unsuitable for aquatic organisms due to their persistence, bioaccumulation, toxicity and biomagnification in food chain and ecological balance [1, 2]. 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. 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).



Chemical structure of chlorantraniliprole

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 [5-7], and possibly ways to mitigate adverse effects [3] 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 [15]. Histopathological investigations on different tissues of fish are valuable tools for toxicology studies and monitoring water pollutions. In histopathology, we can provide information about the health status and functionality of different organs of fish. Tissues injuries and damages in organs can result in the reduced survival, growth and fitness, the low reproductive success or increase of susceptibility to pathological agents. In the present study, an attempt has been made to analyse the toxicity of the chlorantraniliprole 18.5% suspension concentrate (SC) on the freshwater fish *Cirrhinus mrigala.*

2. Materials and Methods

The freshwater fish *Cirrhinus mrigala* size 10-12 cm and weight 14-16.5 g were brought from a local fish farm at Nandivelugu. The fish were acclimatized to the laboratory conditions at 28 ± 2 ^{0}C for 12 days. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. Pesticide was purchased from local market in Guntur of Andhra Pradesh, India. 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., and mortality, log concentrations, LC values, 95% Confidence limits were shown in Table 2,3,and 4.The probit mortality and log concentration linear curve shown in Figure 1 and 2.

The containers of the test media are of 15 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 90%.Basing on the pilot experiments, the experiments were conducted to determine the toxicity in different concentrations; 1-20 mg/l^{-1} for 1hr to 96 hrs with compound Anthralinic diamides, in static system.

The data of each concentration was pooled up to calculate the LC_{50} values. The un-weighted regression method of probit analysis and SPSS v20.0was used to calculate the LC_{50} values [3]. According to [4] the sample water is clear, colorless and odorless. The following results are in mg/l^{-1}.

Table 1: Physico-chemical analysis of water

Turbidity	8 silica units
Electrical conductivity at 28^{o}C	816 Micro ohms/cm
pH at 28^{o}C	8.1
i) Phenolpthalene	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^{o}C

Tissues like gill, liver were isolated from control and experimental fish. Physiological saline solution (0.85% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 hours, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut at 6µ thickness, stained with Ehrlich hematoxylin Eosin (dissolved in 70% in alcohol) [8] and were mounted in *Canada balsam*. The slides were viewed through microscope and photographs were taken with Canon camera.

3. Results

In the present investigation the test organism *Cirrhinus mrigala,* 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 fish chlorantraniliprole in static test 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. The toxicity of chlorantraniliprole to *Cirrhinus mrigala* exposed for 96 hrs showed 10% mortality at 14.183 mg/l^{-1} and 100% mortality was observed at 19.114mg/l^{-1}. The mortality rate increased with increase in the concentration of toxicant (Table.1-3). When percent kill was plotted against log concentration of pesticide chlorantraniliprole, a straight line was obtained (Figure. 1). The percent kill after transforming to probit mortality was plotted against log concentration of chlorantraniliprole using probit method of Finney, 1971. Thus the average 96 hrs LC_{50} value determined by the above Probit methods was 16.465mg/l^{-1} (Table 5). The upper and lower 95% confidence intervals were found to be 16.076mg/l and 16.866 mg/l^{-1} respectively.

Table 2: Estimation of chlorantraniliprole in fish *C.mrigala*

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	pesticide concentration	35.901	7.591	4.729	.000	21.022	50.779
	Intercept	-43.675	9.236	-4.729	.000	-52.911	-34.440

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

Table 3: Chi-Square Tests

PROBIT Pearson Goodness-of-Fit Test	Chi-Square	df[b]	Sig.
	1.542	5	.908[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 and mortality data of Fish *C.mrigala*

Number	Pesticide concentration	Number of Subjects	Observed Responses	Expected Responses
1	1.176	10	1	.731
2	1.190	10	2	1.732
3	1.204	10	3	3.276
4	1.217	10	5	5.132
5	1.230	10	6	6.910
6	1.243	10	8	8.291
7	1.255	10	10	9.177
8	Control	10	0	-----

Table 5: Lethal concentration and confidence limits for fish *Cirrhinus mrigala*

Point	95% Confidence Limits for pesticide concentration			95% Confidence Limits for log(pesticide concentration)[a]		
	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
.010	14.183	12.717	14.871	1.152	1.104	1.172
.020	14.433	13.096	15.063	1.159	1.117	1.178
.030	14.594	13.341	15.187	1.164	1.125	1.181
.040	14.716	13.529	15.282	1.168	1.131	1.184
.050	14.816	13.682	15.360	1.171	1.136	1.186
.060	14.902	13.814	15.427	1.173	1.140	1.188
.070	14.978	13.931	15.486	1.175	1.144	1.190
.080	15.046	14.035	15.540	1.177	1.147	1.191
.090	15.108	14.131	15.589	1.179	1.150	1.193
.100	15.166	14.219	15.635	1.181	1.153	1.194
.150	15.406	14.588	15.830	1.188	1.164	1.199
.200	15.600	14.882	15.993	1.193	1.173	1.204
.250	15.768	15.133	16.139	1.198	1.180	1.208
.300	15.920	15.356	16.279	1.202	1.186	1.212
.350	16.063	15.558	16.418	1.206	1.192	1.215
.400	16.200	15.743	16.559	1.210	1.197	1.219
.450	16.333	15.915	16.708	1.213	1.202	1.223
.500	16.465	16.076	16.866	1.217	1.206	1.227
.550	16.598	16.227	17.037	1.220	1.210	1.231
.600	16.735	16.372	17.224	1.224	1.214	1.236
.650	16.877	16.512	17.430	1.227	1.218	1.241
.700	17.028	16.652	17.660	1.231	1.221	1.247
.750	17.193	16.796	17.921	1.235	1.225	1.253
.800	17.378	16.949	18.224	1.240	1.229	1.261
.850	17.597	17.123	18.592	1.245	1.234	1.269
.900	17.875	17.336	19.074	1.252	1.239	1.280
.910	17.944	17.387	19.193	1.254	1.240	1.283
.920	18.018	17.442	19.324	1.256	1.242	1.286
.930	18.100	17.503	19.469	1.258	1.243	1.289
.940	18.192	17.570	19.633	1.260	1.245	1.293
.950	18.297	17.647	19.823	1.262	1.247	1.297
.960	18.422	17.737	20.048	1.265	1.249	1.302
.970	18.576	17.847	20.329	1.269	1.252	1.308
.980	18.783	17.994	20.711	1.274	1.255	1.316
.990	19.114	18.226	21.328	1.281	1.261	1.329

4. Discussion

The results showed that chlorantraniliprole was toxic to the fish than other pesticides according to previous studies and toxicity of the pesticides was both time and concentration dependent, thus accounting for differences in LC_{10}-LC_{99} values obtained at different concentrations and times of exposure. The test result of the 96 hrs LC_{50} of *C.mrigala* exposed to chlorantraniliprole obtained in the present study was slightly higher than the 96 hrs LC_{50} value of 11.008mg/l^{-1} estimated by [15, 9] for Grass carp (*Ctenopharyngodon idella)* and crayfish for the same pesticide, respectively. In the present study, the LC_{50} value of chlorantraniliprole (Coragen) on the fish *C.mrigala* was found to be 16.465mg/l^{-1}. The variation in the lethal concentration values is due to its dependence upon various factors like sensitivity to the toxicant, its concentration and time of exposure. After the determination of 96 hrs LC50 value of chlorantraniliprole to fish, then fish was exposed for 15 days to sub lethal concentrations (1/10th of LC50; i.e 1.64mg/l^{-1}).At the end of the exposure period, fish were randomly selected for histopathological examination.

5. 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 pesticide 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 [13, 14]. 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 hyper stimulation.

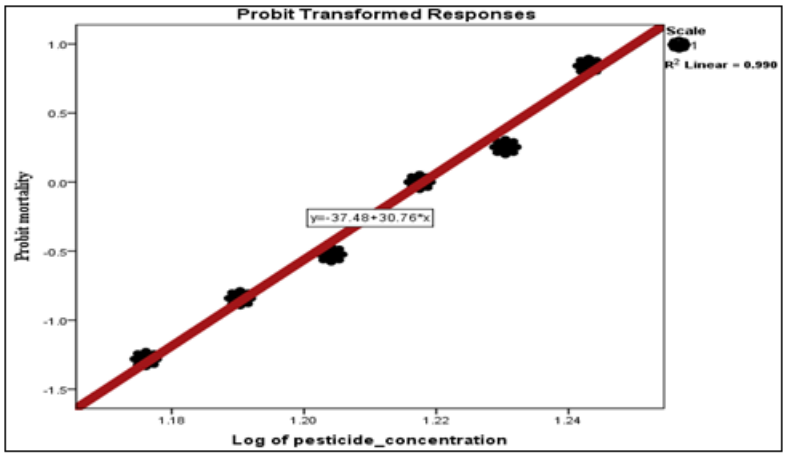


Fig 1: The graph showing linear curve between probit mortality of fish against log concentration in *Cirrhinus mrigala* on exposure to chlorantraniliprole

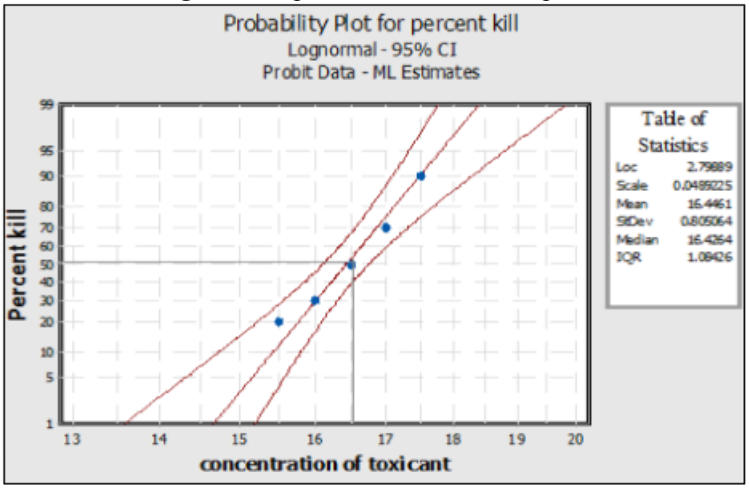


Fig 2: The graph showing sigmoid curve between percent mortality and concentration of pesticide in *Cirrhinus mrigala* on exposure to chlorantraniliprole

And inhibition of AChE activity is a typical characteristic of organophosphate compounds [15, 16]. 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. 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 [9, 10]. 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.

6. Histopathology

In the present Investigation some of the lesions were observed in gill and liver of the fish exposed to sub lethal concentrations of chlorantraniliprole, were shown in the Fig.5, the important histopatho changes was epithelial lifting, aneurysm,necrosis,degeneration of secondary lamellae no changes were observed in the gills and liver of control fish(Fig.3.&4). Liver exhibits degeneration of hepatocytes, severe necrosis and oedemas were observed in pesticide treated fish (Fig.6).

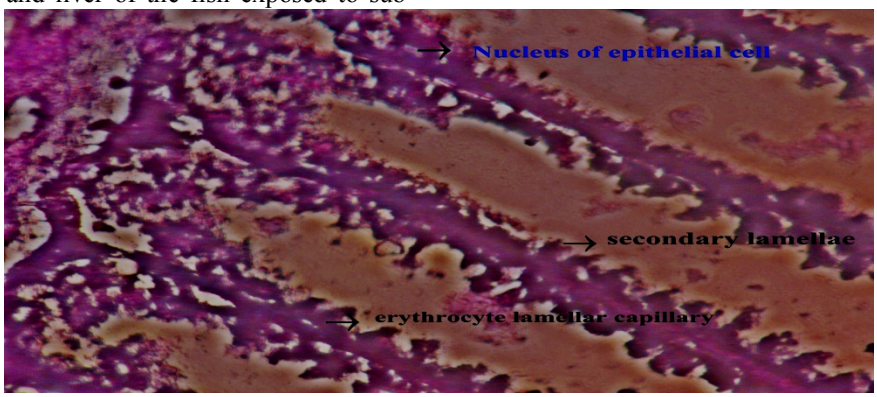


Fig 3: Control gill

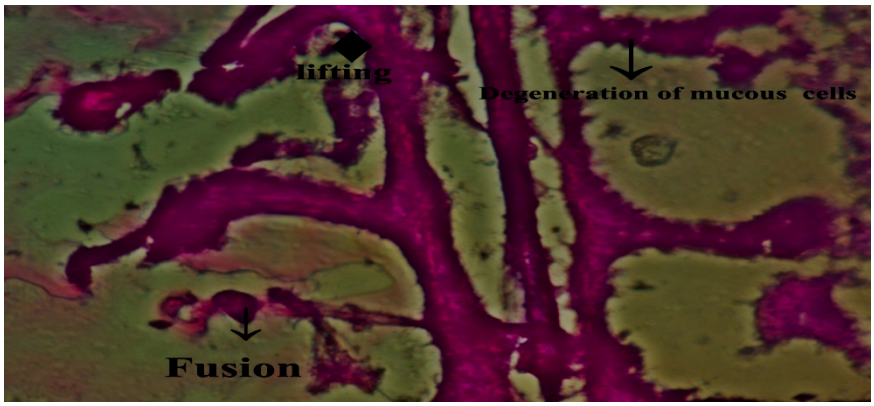


Fig 4: Exposed gill under chlorantraniliprole sublethal concentrations

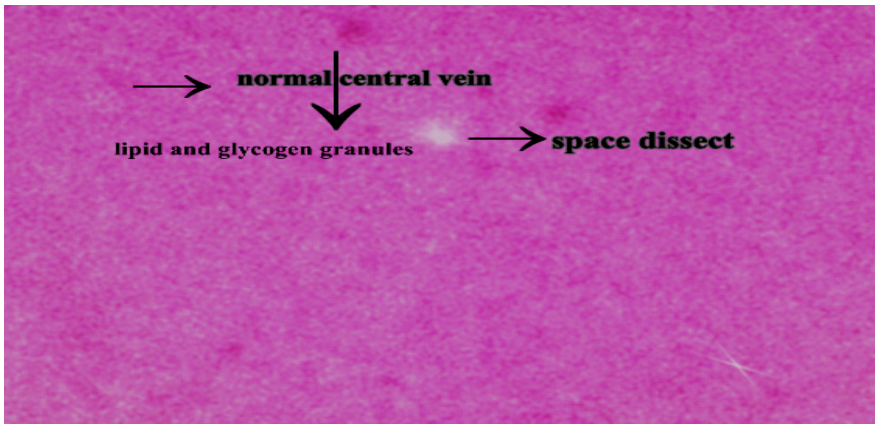


Fig 5: Control liver

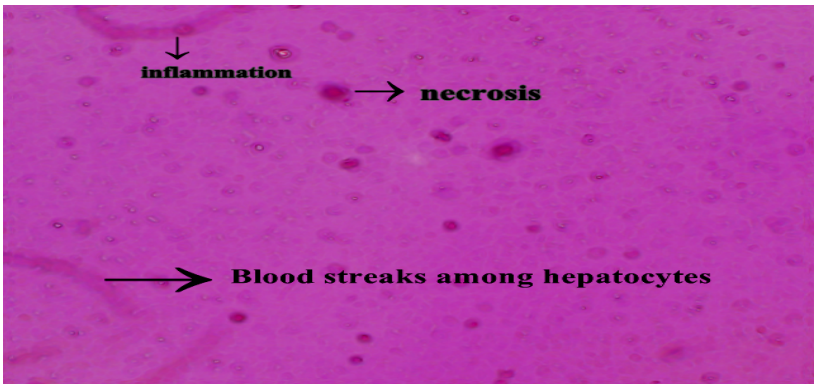


Fig 6: Exposed Liver under chlorantraniliprole sublethal concentrations

In this present investigation gills were found to be the most seriously affected organ compared to liver, because of the direct contact with the pesticide chlorantraniliprole. Similar findings were observed by[17] decreased carbohydrate activity in the secondary lamellae and also in the respiratory epithelium of the freshwater teleost *Channa punctatus* under exposure to the polluted water of Hussain Sagar and states that the degeneration of respiratory epithelium and damages of gill tissue causes a decrease in energy metabolism.

Hyperplasia of gill filaments, fusion of gill filaments due to separation of epithelium, necrosis of gill epithelium, degeneration of pillar cells, development of vacuoles in the epithelium are the pathological changes observed in chlorantraniliprole exposed fish. Similar changes were observed in rainbow trout exposed to sublethal concentrations of Monocrotophos, Vijayalakshmi, 1996 [17] to fenvalerate in *Labeo rohita* [18]. Liver is mainly involved in detoxification mechanism; liver cells are often among those that are damaged by pesticides etc, showed the fibrosis, large necrosis area, leukocyte infiltration, and the absence of melanomacrophages in the liver by Miranda [19].

Amminikutty and Rege (1977), [20] reported that rapid degeneration and vaculation of hepatocytes in liver tissue of fish widow tetra *Gymnocorymbus ternetzi* (Boulenger) eposed to thiodon and Agallol.A few other reports are available which deal with the other pesticides effect on histology. Cruz (1989), [21] reported that formalin treatment caused cloudy swelling haemorrhage, deposition of pigments and necrosis in liver of milk fish, *Chanos* fingerlings. Amalia Mitsoura *et al*.[22] reported moderate focal necrosis, granular glycogen, nuclei pyknosis, loss of the architecture structure, onion-like cells in fish *Cyprinus carpio* due to presence of microcystins.

7. Conclusion:

The results of the present study showed that chloroantraniliprole pesticide was moderately toxic to *C.mrigala*, based on acute toxicity data and acute toxicity tests have been recognized as the first step in determining the water quality parameters and reveal lethal concentrations that cause fish percentile mortality even at short time of exposure. In addition the results reinforce the high potential of histopathological alterations to reveal acute and chronic exposure of pesticides to fish.

8. Acknowledgements

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