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Acute toxicity assessment of Chlorpyriphos 20 % EC to *Clarias batrachus* (Linnaeus, 1758)

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Abstract
Acute toxicity of Chlorpyriphos 20 % EC to *Clarias batrachus* (Linnaeus, 1758) has been assessed to understand their toxicity level, 95 per cent confidence limits, Safe or harmless and safe dischargeable concentrations. The experiment was design up to 96 hours and; $LC_{10,}$ LC_{50} and LC_{99} values were calculated for 24, 48, 72 and 96 by Probit analysis statical method. The experimental water of hardness 560 ± 5 mg/l and pH 7.4 ± 0.3 were used during bioassay test. The upper and lower confidence limit was also estimated for LC_{50} values of 24, 48, 72 and 96 hours. It has been reported that the safe or harmless concentration were too high as compared to safe dischargeable concentration. Behavioral response in the test specimen has been also observed during course of bioassay. Results indicates that Chlorpyriphos 20 % EC were found to be acutely toxic to *Clarias batrachus*, since their $LC_{10,}$ LC_{50} and LC_{99} values were noticed in ppb.

Keywords: acute toxicity, LC50 values, chlorpyriphos 20 % EC and *Clarias Batrachus*

Introduction
Chlorpyriphos ((0, 0-Diethyl – 0 – 3, 5, 6 – trichloro-2-pyridyl – Phosphorothioate)) is a broad spectrum organophosphate based pesticide, commonly known as Dursban. It is employed to damage the pest on fruit, nut, grain, cotton field and vegetable crops and also on lawns and ornamental plants (USEPA, 1984; GL. Berg,1986) [1, 2] Chlorpyriphos is extremely toxic to freshwater fish, aquatic invertebrates such as estuarine and marine organisms (USEPA, 1989) [3]. It inhibits the activities of cholinesterase enzyme at even very low concentration during acute toxicity tests of fish (New York State Department of Environmental Conservation, 1986) [4]. It has been reported that an active metabolite Chlorpyriphos oxon is formed which is responsible for its acute systemic toxicity (Gennady *et al*., 2001) [5]. It has been noticed that in India, about 70% of the synthetic pesticides utilised in agricultural system enters into aquatic ecosystem and mainly affects non-target organisms specially fishes (Bhatnagar and Bana) [6]. Since, world most food is supplied from fish product, therefore the health of fishes is needed to to secure (Tripathi *et al*., 2002) [7].
In view of this, the study has been aimed to assessed the acute toxicity of Chlorpyriphos 20 % EC to *Clarias batrachus* (Linnaeus, 1758) to know their LC_{50} values, Safe or harmless concentration and Safe dischargeable concentration and also to aware the people from their harmful effect in aquatic bodies.

Materials and Methods
Experimental fish
The experimental fish, *Clarias batrachus* (10.90 ±1.30 cm) were collected from local sources. It was acclimatized separately in plastic tank of 250 litres capacity for 10 days. Healthy fishes of approximately equal sizes were selected for the bioassay tests.

Toxicant solution
The stock solution of Chlorpyriphos 20% EC were prepared by using the formula, $N_1V_1 = N_2V_{2,}$ Where, N_1 = Concentration of selected pesticide, V_1 = Volume of selected pesticide, N_2 = Required concentration of pesticide to be prepared, V_2 = Volume of solution required for application. The series of different concentrations (in ppb) of selected pesticides were prepared by using the stock solution with the help of micropipette and it was based on the progressive bisection of intervals on logarithmic scales (APHA, 2005) [8].

Exposure system
The static bioassay test for Chlorpyriphos 20% EC to *Clarias batrachus* were conducted in 5 litre glass containers for the evaluation of short-term toxicity (96 hrs). The experimental water of hardness 560±5 mg/l and pH 7.4±0.3 were used during the course of bioassay.

Preliminary or screening tests
It has been conducted by using different concentrations of Chlorpyriphos 20% EC (higher concentration in the beginning and lower concentrations at latter) to know the critical concentration range for tested fish. The test range for Chlorpyriphos 20% EC was taken between the highest and lowest concentrations for the full-scale bioassay within a specified period of exposure, i.e. 24, 48, 72 and 96 hrs.

Full scale bioassay test
There are 10 acclimatized test fishes, *Clarias batrachus* were transferred to these containers (5 litre) after about 20 minutes of the preparation of toxicant solutions and were placed in three rows and each container was labelled with the details of the experiment (concentration, replicate number, date and time of the experiment). However, the proper controls were run simultaneously. The test solutions were changed after each 24

hrs by fresh toxicant solutions and the experiments were ruined for 96 hrs. It has been carefully observed the numbers of tested fishes died in each concentration of toxicant solution at the time intervals of 24, 48, 72 and 96 hrs and were removed from the test solution. Now, the Probit analysis statical methods (Finney, 1971) [9] were used to calculate the $LC_{10,}$ LC_{50} and LC_{99} values and; the 95 percent confidence limits at different concentrations and time intervals (24, 48, 72 and 96 hrs) for experimental pesticides and the presumable safe and dischargeable concentrations of Chlorpyriphos 20% EC for *Clarias batrachus* were also calculated by standard formula, proposed by Hart *et al*., 1945 [10]. Behavioural changes in the test specimen, if any, were noticed carefully after introduction in to the different concentrations of Chlorpyriphos 20% EC solution.

Results and Discussion

The $LC_{10,}$ LC_{50} and LC_{99} values were noticed for 24 hours durations as 194.015, 288.482 and 459.963 ppb whereas; these values were estimated for 48 hours durations as 178.611, 258.429 and 403.317 ppb. The $LC_{10,}$ LC_{50} and LC_{99} values for 72 hours durations were calculated as 138.788, 232.706 and 403.192 ppb and these values were recorded as 157.136, 215.063 and 320.217 ppb for 96 hours durations (Table-1). The lower and upper confidence limit and; their ratio were estimated for 24 hours as 259.454, 355.873 and 1.376 ppb and; for 48 hours as 232.218, 290.775 and 1.252 ppb respectively. However, the lower and upper confidence limit and their ratio were analyzed as 193.735, 262.910 ppb and 1.357 for 72 hours and 187.350, 235.300 and 1.255 ppb respectively for 96 hours (Table-2). The Safe or harmless concentrations and safe dischargeable concentrations of Chlorpyriphos 20 % EC for *Clarias batracus* were noticed as 69.4520 and 1.0565 ppb respectively (Table-3).

Table 1: Median lethal concentrations (LC_{50}'s), LC_{10} and LC_{99} of Chlorpyriphos 20 % EC (in ppb) for 24, 48, 72 and 96 hrs to *Clarias batracus*.

Duration (hrs)	LC_{10}	LC_{50}	LC_{99}
24	194.015	288.482	459.963
48	178.611	258.429	403.317
72	138.788	232.706	403.192
96	157.136	215.063	320.217

Table 2: 95 per cent confidence limits for 24, 48, 72 & 96 hrs LC_{50}'s of Chlorpyriphos 20 % EC for *Clarias batracus.*

Duration (hrs)	*Clarias batracus*		
	LCL	UCL	R
24	259.454	355.873	1.376
48	232.218	290.775	1.252
72	193.735	262.910	1.357
96	187.350	235.300	1.255

Table 3: Safe or harmless and safe dischargeable concentrations of Chlorpyriphos 20 % EC for *Clarias batracus*.

Safe or harmless (as ppb)	Safe dischargeable (as ppb)
69.4520	1.0565

In present investigation, LC_{50} values of Chlorpyriphos 20 % EC to *Clarias batrachus* (Linnaeus, 1758) were noticed as 288.482, 258.429, 232.706 and 215.063 ppb for 24, 48, 72 and 96 hrs respectively (Table-1). However, it has been estimated the 96 hrs LC_{50} for Chlorpyriphos methyl as 4.89 µM to *Poecilia reticulata* (Selvi *et al*., 2005) [11]. The effect of Chlorpyrifos toxicity to behavioural and morphological manifestation of fry fish of *Channa punctatus* were also studied and observed the mortality data through probit analysis as 0.365, 0.328, 0.269, and 0.253 µl/l at 24th, 48th, 72nd, and 96th hrs respectively (Devi and Mishra, 2013) [12]. Further, they also recorded the behavioural response of fish such as abnormal opercular movement, vertical hanging, convulsions, swimming erratically, loss of balance and lateral flexure, with tail beat were shown and finally fish became lethargic and settled at the bottom and their belly turned up before death, which were also shoes conformities to the changes in behavioral patterns observed during present investigation. The acute toxicity of Chlorpyriphos were also studied in the fish, *Cyprinus carpio* under static condition and determined their LC_{50}'s as 5.28 ppm at the end of 21 day (Ramesh and Saravanan, 2008) [13]. The acute toxicity of Chlorpyriphos-methyl were also studied on larvae of Nile tilapia, *Oreochromis niloticus* and the behavioural changes at each Chlorpyriphos-methyl concentration of the individual fish were also observed (Gul, 2005) [14].The 96 hr LC_{50} values of for methyl parathion (MP) and Chlorpyriphos were noticed (CPF) for *Poecilia* as 8.48 ppm/l (5.98 - 10.89) and 0.176 ppm/l (0.313 -0.224) respectively and also noted the changes in behavioural pattern such as oxidative stress-induction potential in brain, liver and gills (Sharbidre *et al*., 2011)[15]. It has been also found that low soluble concentrations of Chlorpyriphos affect mating behaviour, number of offspring and offspring survival of guppy, *Poecilia reticulata* (De Silva and Samayawardhena, 2005) [16].

It has been reported that the 96-h LC_{50} values for Chlorpyriphos 50 % + Cypermethrin 5% EC to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters, 1859) as 13.396, 18.845, 261.866 and 106.255 ppb respectively (Nageshwar et al., 2014) [17]. Further, safe dischargeable concentrations were recorded from 1.044 to 1.069 ppb whereas, safe or harmless concentration were noticed between 4.381 to 82.205 ppb for various test specimen of *Poecilia reticulata* (Nageshwar *et al.,* 2014) [17]. The 24, 48, 72 and 96 hrs LC_{50}'s of Chlorpyriphos 20 % EC for juveniles, males, females and mixed population of *Poecilia reticulata* were recorded as: 7.009, 14.575, 130.777 and 51.924 ppb respectively [18]. Whereas, the presumable safe or harmless concentrations for Chlorpyriphos 20 % EC were noticed as: 1.987, 4.962, 41.821 and 17.08 ppb respectively for juveniles, males, females and mixed population and the safe dischargeable concentrations of Chlorpyriphos 20 % EC for the juveniles, males, females and mixed population were estimated as: 1.103, 1.042, 1.063 and 1.062 ppb respectively (Nageshwar *et al.,* 2015) [18]. The LC_{50} values for Chlorpyriphos to *L. irrorata* have also been reported as 0.01251, 0.00549 and 0.00510 ppm for 24, 48 and 72 hrs respectively (Vidyarani *et al.,* 2010) [19]. According to these author the behavioural pattern of *L. irrorata* such as slow swimming, lying the body by the side at the bottom of the aquarium, sluggish and imbalance movement prior to death of the fish, reddish colour of the gills, which might be due to

haemorrhage in the gills were approximately similar to the observation noticed in present investigation.

Results exhibits that Chlorpyriphos is highly toxic to the studied fishes and also exhibits conformities with the findings of previous authors in in context of approximately all the studied parameters.

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