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Assessment of Acute Toxicity of Chlorpyriphos 20% EC to the Guppy, *Poecilia reticulata* (Peters, 1859)

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Abstract: The experimental routine static bioassay test have been conducted in laboratory condition to screen the acute toxicity of Chlorpyriphos 20% ECto juveniles, males, females and mixed population of *Poecilia reticulata*(Peters, 1859) to evaluate the LC_{50} values and the 95% confidence limits for different concentrations and time intervals (24, 48, 72 and 96 hrs) by Probit analysis statistical method, using experimental water of hardness 560±5 mg/l and pH 7.4±0.3. The changes in behavioural pattern in the studied fishes were also observed during the course of bioassay test. The presumable safe concentration were ranges from 1.98-41.82 ppb however, safe dischargeable concentrations were estimated between 1.04-1.10 ppb. Results indicate that all the specimen of *Poecilia reticulata*are extremely sensitive (juveniles>males> mixed population> females) to this pesticide since their LC_{50} values were recorded too low.

Key words: Toxicity · Chlorpyriphos 20% EC · *Poecilia reticulata* and LC_{50}Values

INTRODUCTION

Chlorpyriphos is commonly known as Dursban. It is a broad spectrum pesticide, based on organophosphate agrochemical. It is most toxic for fish and is more toxic than organochlorinepesticides [1]. Chlorpyriphos is an emulsifiable, contact and stomach poison, used for the control of flies, mosquitoes, cockroaches, bedbugs and ants on a many crop [2]. The acute systemic toxicity of Chlorpyriphos is due to its production of active metabolite Chlorpyriphos Oxon [3]. Chlorpyriphos is highly toxic to aquatic invertebrates, freshwater fish and marine organisms [4]. Chlorpyriphos kills insects by affecting the normal function of the nervous system [5] by inhibiting the breakdown of acetylcholine [6]. Chlorpyriphos binds to the active site of the cholinesterase enzyme, which prevents breakdown of acetylcholine in exposed insects [7].

In view of this, an attempt has been made to screen the acute toxicity of Chlorpyriphos 20% EC to juveniles, males, females and mixed population of a freshwater fish, *Poecilia reticulata* (Peters, 1859) to analysed the LC_{50} values, the 95 percent confidence limits, presumable safe and safe dischargeable concentrations for better management of aquaculture.

MATERIALS AND METHODS

Experimental Fish: The experimental fish, *Poecilia reticulata*were collected from local sources (Udaipur, Rajsthan). The juveniles, males and females were acclimatized separately in plastic tank of 250 litres capacity for 10 days before starting the experiment. During acclimatization they were fed rice bran and oil cake (1:1). During the bioassay tests, the fishes were not provided any food supplement to avoid excretory waste products and change in metabolic rate, which may influence the toxicity of the test solution. Healthy, juveniles (1.0±0.2cm), males (2.8±0.2cm) and females (3.9±0.3 cm) 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 adding the stock solution into the measured diluents water with the help of micropipette. The series of

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different concentrations of selected pesticides used in the full-scale static bioassay tests were based on the progressive bisection of intervals on logarithmic scales [8].

Exposure System: The experimental routine static bioassay for the evaluation of short-term toxicity (96 hrs) for Chlorpyriphos 20% EC to the juveniles, males, females and mixed population of *Poecilia reticulata* were conducted in 1 litre glass jar containing experimental water of hardness 560±5 mg/l and pH 7.4±0.3.

Preliminary or Screening Tests: The screening tests with different concentrations of Chlorpyriphos 20% EC were made by maintaining higher concentration of it in the beginning and later lower concentrations were tested to investigate the critical concentration range for each tested fish. The test range for Chlorpyriphos 20% Ecfor the full-scale bioassay was taken between the highest and lowest concentrations at which mostof the tested fishes died or survived within a specified period of exposure, i.e. 24, 48, 72 and 96 hrs.

Full Scale Bioassay Test: Now, the toxicity of Chlorpyriphos 20% ECwas measured by testing various concentrations in the known range, based upon preliminary exploratory test. The test containers (1 litre glass jars) filled with one litre toxicant solution were placed in three rows and each container was labelled with the details of the experiment such as concentration, replicate number, date and time of the experiment. The acclimatized juveniles, males and females of *Poecilia reticulata*were transferred to these jars after about 20 minutes of the preparation of test solutions. The bioassays for juveniles, males, females and mixed population of *Poecilia reticulata* were conducted for selected pesticides. Ten acclimatized test specimens of fish were placed in each experimental glass jars. Proper controls were run simultaneously. The test solutions were renewed after each 24 hrs by fresh toxicant solutions. The experiments were continued for a period of 96 hrs. The number of tested fishes died in each concentration of toxicant solution were observed carefully and recorded at the time intervals of 24, 48, 72 and 96 hrs. The dead fishes were removed from the test solution after knowing the exact mortality, which was observed by their body movements. The LC_{50}'s and the 95 percent confidence limits were estimated statistically at different concentrations and time intervals (24, 48, 72 and 96 hrs) for selected pesticides by Probit Analysis methods [9]. Presumable safe and dischargeable concentrations of Chlorpyriphos 20% EC for juveniles, males, females and mixed population of *Poecilia reticulata*were calculated by the formula of Hart *et al*. [10]. Behavioural changes if any in the exposed juveniles, males and females of *Poecilia reticulata* were also observed carefully after introduction in to the various concentrations of Chlorpyriphos 20% EC.

RESULTS

The LC_{50} values for Alphamethrin 10 EC to the juveniles, males, females and mixed population of *Poecilia reticulata*for the time intervals of 24, 48, 72 and 96 hrs have been summarized in Table 1 and compared in Figure 1. The 24, 48, 72 and 96 hrs LC_{50}'s for juveniles and males were recorded as: 9.832, 8.070, 7.485 and 7.009 ppb; and 19.558,17.982, 15.768 and 14.575 ppb respectively, however, these values for females and mixed population for the same time intervals were noticed as: 178.687, 157.806, 145.768 and 130.777 ppb; and 72.535, 64.257, 57.814 and 51.924 ppb respectively. The order of sensitivity for the studied fishes toChlorpyriphos 20 % EC was noticed as: females <mixed population < males < juveniles.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,

Table 1: Median lethal concentrations (LC_{50}'s) of Chlorpyriphos 20 % EC (ppb) for 24, 48, 72 and 96 hrs to juveniles, males, females and mixed population of*Poecilia reticulata*

	LC_{50}'s of Chlorpyriphos 20 % EC (ppb)			
Duration (hrs)	Juveniles	Males	Females	Mixed population
24	9.832	19.558	178.687	72.535
48	8.070	17.982	157.806	64.257
72	7.485	15.768	145.768	57.814
96	7.009	14.575	130.777	51.924

Table 2: Safe or harmless and safe dischargeable concentrations of Chlorpyriphos 20 % EC for juveniles, males, females and mixed population of *Poecilia reticulata*

Concentrations (as ppb)	Juveniles	Males	Females	Mixed population
Safe or harmless	1.987	4.962	41.821	17.08
Safe dischargeable	1.103	1.042	1.063	1.062

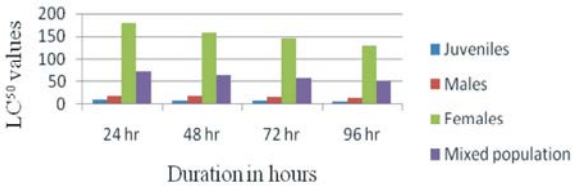

Fig. 1: Median lethal concentrations (LC_{50}'s in ppb) of Chlorpyriphos 20 % EC for 24, 48, 72 and 96 hrs to juveniles, males,females and mixed population of *Poecilia reticulata*.

Table 3: 95 percent confidence limits for 24, 48, 72 and 96 hrs LC_{50}'s of Chlorpyriphos 20 % EC for the juveniles, males, females and mixed population of *Poecilia reticulata*

Duration	Juveniles			Males			Females			Mixed population		
(hrs)	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R
24	8.181	49.379	*6.035	16.747	37.565	2.243	160.440	216.357	1.348	63.854	103.185	1.615
48	7.172	9.567	1.333	15.221	33.616	2.208	138.729	180.999	1.304	55.844	81.235	1.454
72	6.589	8.522	1.293	13.572	18.662	1.375	119.040	167.378	1.406	47.805	68.234	1.427
96	6.230	7.727	1.240	12.335	16.544	1.341	104.796	145.795	1.391	43.461	57.802	1.329

UCL = Upper Confidence Limits; LCL = Lower Confidence limits; R = Confidence Ratio (UCL/LCL)

males, females and mixed population. However, 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 (Table2). The 95 per cent confidence limits and their ratio for 24, 48, 72 and 96 hrs LC_{50}'s of Chlorpyriphos 20 % EC for the juveniles, males, females and mixed population of *Poecilia reticulata* were also estimated and summarized (Table 3). Behavioural response were also noticed in studied fishes such as; Fish showed more excitement, Whirling movements in males and females, rapid opercular movement at high concentration, loss of equilibrium, Profuse secretion of mucous like substance, Dorsal side of the skin appeared slightly dark in females, reddish colour of the gills and fish settled down on bottom with belly upside after death.

DISCUSSION

In present investigation, the 96 hrs LC_{50} values for juveniles, males, females and mixed population of *Poecilia reticulata*were recorded as 7.009, 14.575, 130.777 and 51.924 ppb respectively. However, Rao *et al*. [11] reported the 96 hr LC_{50} of Chlorpyriphos as 0.07 µM causes 88 % inhibition in brain and gill of *Oreochromismossambicus*. De Silva and Samayawardhena [12] studied the effects of Chlorpyriphos on reproductive behaviour in Guppy (*Poecilia reticulata*) and also found that low soluble concentrations of Chlorpyriphos affect mating behaviour, number of offspring and offspring survival of guppy.

Whereas, the 96 hrs $LC_{50}$for Chlorpyriphos methyl to *Poecilia reticulata*were reported as 4.89 µM [13]. Ramesh and Saravanan [14] have also studied the acute toxicity of Chlorpyriphos considering hematologicaland biochemical parameters for the fish, *Cyprinuscarpio* under static condition and at the end of 21 day and also determined their LC_{50}'s as 5.28 ppm. The LC_{50} values for Chlorpyriphos to *L. irrorata*reported as 0.01251, 0.00549 and 0.00510 ppm for 24, 48 and 72 hrs respectively [15]. Vidyarani *et al.* [15] also noticedbehavioural 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. The 96 hr LC_{50} values of methyl parathion (MP) and Chlorpyriphos (CPF) for *Poecilia*recorded 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 [16].

Devi and Mishra [17] have studied the effect of Chlorpyrifos toxicity to behavioural and morphological manifestation of fry fish of *Channapunctatus* and observed the mortality data through probit analysis as 0.365, 0.328, 0.269 and 0.253µl/l at 24^{th}, 48^{th}, 72^{nd} and 96^{th}hrs respectively. Further, they [17] reported the behavioural response of fish such as convulsions, swimming erratically, vertical hanging, coughing, loss of balance, abnormal opercular movement 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.However, the 96-h LC_{50} valuesfor Chlorpyriphos 50 % + Cypermethrin 5% EC to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters, 1859) were noticed as 13.396, 18.845, 261.866 and 106.255 ppb respectively [18]. According to these authors 18], the range of safe dischargeable concentrations (1.044-1.069 ppb) was estimated to low in comparison of safe or harmless concentration (4.381-82.205 ppb). Further, Wast *et al*. [19] have also analysed the 96-h LC_{50} values for Alphamethrin (Alpha-Cypermethrin) 10 EC,, separately (without Chlorpyriphos) to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters, 1859) as 5.531, 8.495, 32.439 and 15.202 ppb respectively, however they [19] found that the range of safe dischargeable concentrations (1.051-1.076 ppb) were very low as safe or harmless concentration (1.799-10.233 ppb) comparatively. Gul [20] studied the acute toxicity of Chlorpyriphos-methyl on larvae of Nile tilapia, *Oreochromis niloticus* and also recorded the

behavioural changes at each Chlorpyriphos-methyl concentration of the individual fish. The 96 hrLC_{50} value was estimated as 1.57 mg/L for Nile tilapia larvae. Whereas, the 96-hr LC_{50}were noticed as: 0.007-0.051 mg/l for rainbow trout (*Oncorhynchus mykiss*), 0.002-0.010 mg/l for bluegill sunfish (*Lepomis macrochirus*) and 0.12-0.54 mg/l for fathead minnows (*Pimephales promelas*) and the 48-hr LC_{50} for *Daphnia* as 1.7 µg/l [21].

Results of the present investigation are also in conformity with the findings of previous authors in context of LC_{50} values, behavioural response and other studied parameters. It also indicates that Chlorpyriphos 20 % EC are highly toxic to freshwater fishes *Poecilia reticulata.* Therefore, it is suggested to those, who using Chlorpyriphosas a pesticides, they should take consideration of both safe or harmless and safe dischargeable concentrations for better management of aquatic animals, particularly fishes.

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