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A study on median lethal concentration and behavioural responses of cichlid fish, *Etroplus maculatus* (Bloch, 1795) exposed to organophosphorus insecticide, chlorpyrifos

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ABSTRACT *The present study was carried out to evaluate the median lethal concentration and the behavioural responses of one of the organophosphorus insecticide, chlorpyrifos on cichlid fish, Etroplus maculatus. Fish were exposed to seven different concentrations of chlorpyrifos (3, 4, 5, 6, 7, 8 and 9 µg/ L) for 96 h maintaining control groups. Propylene glycol was used a vehicle to dissolve chlorpyrifos and so it was maintained as positive control. The median lethal concentration (LC50) for 96 h, determined by probit analysis was 6.61 µg/ L that killed 50% of the test animal. Chlorpyrifos-induced behavioural changes were continuously monitored throughout the study. Propylene glycol did not caused mortality or behavioural changes where the specimens remained active during the treatment period. However, chlorpyrifos-treated fishes showed jerky vertical movement, loss of equilibrium, surface swimming, scoliosis, darkening of skin and bulging of eyes. It is concluded that chlorpyrifos caused acute toxicity and behavioural modifications in Etroplus maculatus.*

KEYWORDS : Chlorpyrifos, Acute toxicity, LC50, Etroplus maculatus, Behaviour

INTRODUCTION

The United Nations Environment Programme defined pesticide as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides are commonly employed to protect crops from various pathogens and nowadays its widespread use is a common event (Omitoyin *et al*, 2006). These chemicals may reach other ecological compartments like water bodies such as lakes and rivers through rains and winds and target many aquatic organisms away from the primary target and only 0.1% reaches the specific target (Rand and Petrocelli, 1984). Changes in the chemical composition of natural aquatic environments due to toxicant exposure may affect the freshwater fauna, particularly fish. Effect of pesticide on fish and its consumption by human have important significance in the evaluation of adverse effects of pesticides to human health (Begum and Vijayaraghavan, 1996).

Organophosphates (OPs) have become the most widely used class of insecticides in the world replacing the persistent and problematic organochlorine compounds (Halappa and David, 2009). Chlorpyrifos (O, O-Diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate), which was introduced in 1965 belongs to a group of broad-spectrum organophosphorus insecticide known as phosphorothioates used globally for crop protection and pest control. In India, chlorpyrifos is the second largest selling organophosphate agrochemical (Mathur and Tannann, 1999). Chlorpyrifos when exposed to humans moves to all parts of the body and it breakdown to form a more toxic compound, chlorpyrifos oxon, which bind permanently to major metabolic enzymes. The unbounded form of chlorpyrifos is readily excreted through feces and urine within a few days of exposure. Chlorpyrifos when released into the soil takes weeks to years for breaking down into its metabolites, as the soil temperature and pH level has been found to affect its level of persistence in the soil. Numerous studies have reported that chlorpyrifos has been shown to affect the central nervous system, cardiovascular system, respiratory system and reproductive system by exposing to different laboratory animals as rat, mice and dogs.

It is well known that fish are the most important non-target victims of pesticide over exposure as they have an important role in food chain. Several acute toxicity studies on the effect of chlorpyrifos have been reported in various species of fishes. The median lethal concentration of chlorpyrifos determined in Bluegill sun fish, *Lepomis macrochirus* has been reported as 3.3 ppb/ L, in rainbow trout, *Oncorhynchus mykiss* it is 3 ppb/ L, in channel catfish, *Ictalurus punctatus* is 13.4 ppb/ L, in lake trout, *Salvelinus namaycush* at pH 6.0 is 140 ppb/ L, at pH 7.5 is 98 ppb/ L and at pH 9.0 is 205 ppb/ L while for cutthroat trout, *Salmo clarki*, at pH 7.5 is 18.4 ppb/ L at pH 9.9 is 5.4 ppb/ L, in fathead minnow, *Pimephales promelas* is 203 ppb/ L, in golden shiner, *Notemigonus crysoleucas* is 35 ppb/ L, in juvenile and adult *Oreochromis niloticus* is 98.67 µg/ Land 154.01 µg/ L, respectively, whereas in *Poecila reticulata* it is 0.176 ppm/ L, in mosquito fish, *Gambusia affinis* is 297 mg/ L and in common carp is 580 µg/ L, which reveals that chlorpyrifos can be rated as highly toxic to fish (Barron and Woodburn, 1995; Deb and Das, 2013). In the present study the cichlid fish, *Etroplus maculatus* was selected as an experimental model as they are the common fish of India because of their low-cost culture and are widely used as a good biomonitoring tool in ecotoxicological studies. The median lethal concentration of chlorpyrifos in freshwater cichlid fish, *Etroplus maculatus* for 96 h exposure period was determined along with alterations in behavioral pattern.

MATERIALS AND METHODS

Experimental animal:

Healthy adult freshwater cichlid fish, *Etroplus maculatus* weighing 3.5 ± 0.5 g and length 6 ± 0.3 cm were collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India and acclimated to the laboratory conditions for 15 days before experiment. During acclimatization and in all experimental tests, specimens were fed with standard fish pellets. Fishes were maintained in large cement tank containing dechlorinated water and well aeration. The physiochemical features of the tap water were estimated as per APHA (1998) by maintaining water temperature at 28 ± 2°C, dissolved oxygen at 8.5 and pH was maintained at 7.6 throughout the experiment.

Experimental design:

After acclimatization, fishes were randomly selected and distributed in nine 40 L capacity tanks. Every tank was retained with ten animals and consisted of two control groups – positive (propylene glycol as vehicle solvent) and negative control, and 7 treatment groups with different concentrations, i.e 3, 4, 5, 6, 7, 8 and 9 µg/ L of chlorpyrifos for 96 h as exposure period. Mortality and the behavior of specimens were monitored throughout the study.

Determination of median lethal concentration:

The acute LC_{50} value of chlorpyrifos for 96 h was determined by probit analysis according to the method of Finney (1971) with 95% confident limit. Seven different concentrations (3, 4, 5, 6, 7, 8 and 9 µg/ L) were maintained in 40 L of water by adding the toxicant dissolved in 400 µl of propylene glycol. The control groups were also maintained simultaneously with an addition of vehicle solvent and maintained as positive control and another group without toxicant or vehicle solvent was sustained as negative control. Concentration at which 50% fish mortality occurred after 96 h of toxicant exposure was selected as median lethal concentration (LC_{50}-96 h). The body weight of animal in each treatment groups were recorded as soon as the mortality was observed and at the end of 96 h. The behavioral changes were monitored throughout the study in all treatment groups and compared to control groups.

Statistical analyses:
Data were analyzed using SPSS 19.0 statistical analysis software with probit analysis as statistical method. Differences in body weights against control groups were analysed using students t-test and the data are presented as mean ± SD for ten animals per group. MS Excel 2007 was used to find regression equation (Y = mortality of fish; X = concentrations of toxicant), the LC_{50} was derived from the best-fit line obtained.

RESULTS
Percentages of mortality observed in different concentrations of chlorpyrifos during 96 h of exposure period were shown in Table 1. Negative and positive controls did not showed mortality throughout the study and the animal remained active. Similarly at 3 and 4 µg/ L concentrations did not showed any lethality whereas at 5 µg/ L concentration showed mortality of one animal at the end of 96 h. Chlorpyrifos at 6 µg/ L killed four animals among the ten exposed at the end of 96 h, whereas at 7µg/ L showed mortality of six animals after 65 h. Concentrations of 8 µg/ L and 9µg/ L showed 100% mortality at 26 and 24 h, respectively. Computation of median lethal concentration by probit analysis showed 6.61 µg/ L as LC_{50}-96 h (Table 2). R^2 value, a measure of goodness of fit of linear regression was computed in MS Excel to determine the linear relationship and the result obtained was $r^2 = 0.795$, which denotes the best fit line and the regression equation obtained was Y = 1.222X – 2.794 (Figure 1).

Behavioral changes in the activities of *Etroplus maculatus* treated with different acute concentrations of chlorpyrifos were compared to that of control groups. It was noted that chlorpyrifos exposure showed jerky vertical movement, loss of equilibrium, surface swimming, scoliosis, darkening of skin and bulging of eyes (Figure 2). No significant changes were noticed on the body weight of the animal after different concentrations of chlorpyrifos exposure (Figure 3).

Table 1Percentage of fish mortality exposed to different concentrations of chlorpyrifos (n = 10)

Concentrations (µg/L)	Mortality (No. of animals)	Hour of mortality
Negative control	0	96 h
Positive control	0	96 h
3	0	96 h
4	0	96 h
5	1	96 h
6	4	96 h
7	6	65 h
8	8	26 h
9	10	24 h

Table 2 Probit analysis of 95% confidence limits for effective concentrations of chlorpyrifos in *Etroplus maculatus*

	95% Confidence Limits		
Prob	**Concentrations**	**Lower**	**Upper**
.01	3.72339	1.67943	4.65483
.02	4.06169	2.23345	4.90939
.03	4.27634	2.58318	5.07267
.04	4.43780	2.84512	5.19664
.05	4.56915	3.05732	5.29835
.06	4.68094	3.23723	5.38564
.07	4.77896	3.39436	5.46278
.08	4.86672	3.53451	5.53239
.09	4.94654	3.66148	5.59620
.10	5.02002	3.77789	5.65539
.15	5.32422	4.25418	5.90616
.20	5.56599	4.62421	6.11398
.25	5.77340	4.93339	6.30053
.30	5.95967	5.20260	6.47651
.35	6.13227	5.44324	6.64840
.40	6.29606	5.66231	6.82078
.45	6.45452	5.86459	6.99724
.50	6.61047	6.05373	7.18083
.55	6.76642	6.23286	7.37443
.60	6.92489	6.40501	7.58101
.65	7.08867	6.57338	7.80409
.70	7.26127	6.74164	8.04837
.75	7.44754	6.91438	8.32081
.80	7.65496	7.09811	8.63283
.85	7.89673	7.30344	9.00533
.90	8.20093	7.55199	9.48384
.91	8.27440	7.61077	9.60067
.92	8.35422	7.67416	9.72805
.93	8.44198	7.74336	9.86862
.94	8.54001	7.82008	10.02616
.95	8.65180	7.90695	10.20649
.96	8.78314	8.00823	10.41913
.97	8.94461	8.13175	10.68152
.98	9.15925	8.29453	11.03175
.99	9.49756	8.54848	11.58637

Figure 1 LC50-96 h exposure to chlorpyrifos in *Etroplus maculatus*

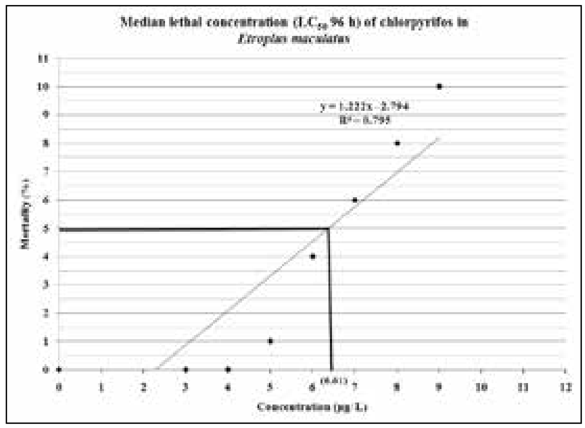


Figure 2 Chlorpyrifos-treated fish showing **A** - bulging of eyes; **B** - darkening of skin

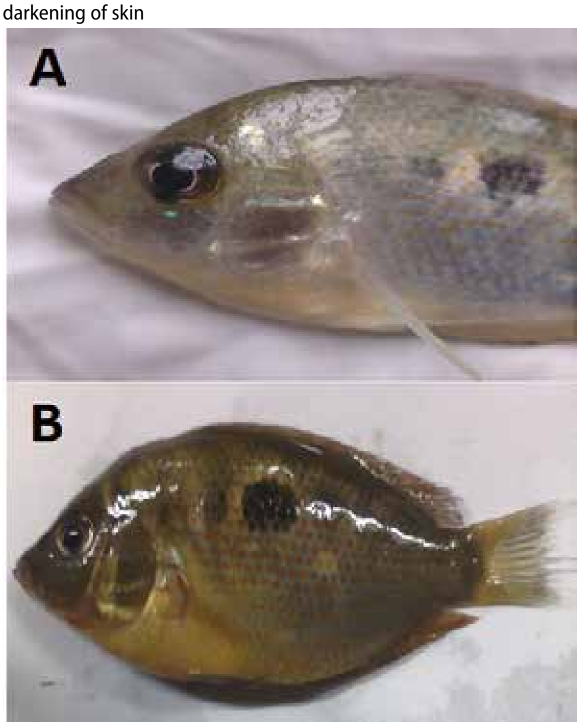
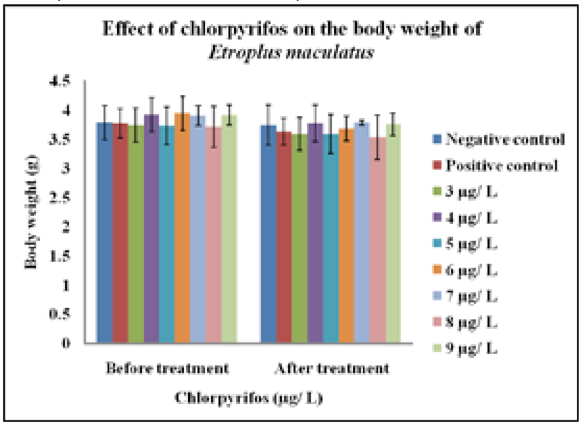


Figure 3 Acute toxicity effects of chlorpyrifos on the body weight of *Etroplus maculatus* after 96 h exposure



DISCUSSION
Chlorpyrifos, an organophosphate pesticide are widely used in agriculture against harmful insects. However, they find pathways to enter into aquatic ecosystems mainly through rainfall run-off and air drift so they are moderately persistent in aquatic environments, especially in freshwater and estuaries (Turner, 2003). Due to the lipophilic property of chlorpyrifos it accumulates in fatty tissues of animals to which they are exposed. Fish that are continuously exposed to low concentrations of contaminants has been shown to cause acute toxicity and behavioural modifications as well (Asifa and Chitra, 2015). The present study investigates the acute toxicity of chlorpyrifos at seven different concentrations in cichlid fish *Etroplus maculatus*. In the present study, based on the probit analysis the median lethal concentration of chlorpyrifos exposed for 96 h was determined to be 6.61 µg/ L that killed 50 % of test animal. No similar study was conducted to prove LC_{50}-96 h of *Etroplus maculatus* and the results also revealed that if concentration is increased the lethal rate also increases. The body weight of animal did not showed any significant changes when compared to control groups.

Behaviour and movement of the fish were also studied in the different concentrations of chlorpyrifos throughout the study. Behavioural toxicology is one of the biomonitoring tools that possess ecological significance to understand the toxicity effect of any contaminants. Both positive and negative control animals were very active with normal behavioural response and steady movement throughout the study and no mortality was noticed. On the other hand, fishes when exposed to the toxicant at all concentrations initially showed erratic motion, jerking movement, knocking at the walls of tanks, hit with each other, loss of equilibrium and caudal bending (scoliosis). At higher concentration, above 7 µg/ L, showed more aggressive behavior after an immediate exposure and after few hours they were tired, remained motionless at the bottom of tanks and undergone fatigue gradually. Among the apparent symptoms, eye ball bulging from the pupil and blackening of skin are noticed extensively irrespective to grading of concentrations.

CONCLUSION
Therefore, the use of pesticide chlorpyrifos near the aquatic ecosystems should be strongly monitored and regulated to avoid chlorpyrifos-induced toxicity in aquatic animals, especially fish as it forms the direct food source to human.

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