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STATISTICAL ANALYSIS IN BEHAVIOURAL OF THE *GAMBUSIA AFFINIS* FOLLOWING SUBLETHAL EXPOSURE TO CHLORPYRIFOS

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ABSTRACT

Gambusia affinis were exposed to different concentrations (0.245 to 0.300 mg/L) of an organophosphate pesticide, chlorpyrifos for 96 hrs. The acute toxicity (LC_{50}) of chlorpyrifos by static renewal (semi-static) bioassay test was found to be 0.284 mg/L. One-third (0.0946 mg/L) and one-sixth (0.0473 mg/L) of the 96 hrs LC50 were selected as sublethal concentrations for sub-acute studies. The fish were exposed to both the sublethal concentrations for 15, 30 and 45 days and were allowed to recover in toxicant free medium. Behavioural responses and morphological deformities were studied in the experimental periods. Fish in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom. The fish were found under stress, but mortality was insignificant in both the sublethal concentrations. Caudal bending was the main morphological alteration during the exposure periods. The behavioural and morphological changes may be due to the inhibition of acetylcholinesterase (AChE) activity. Inactivation of AChE activity results in excess accumulation of acetylcholine (ACh) in cholinergic synapses leading to hyperstimulation and cessation of neuronal transmission (paralysis). Impaired behavioural responses and morphological deformities were observed even under recovery periods. This may be a consequence due to the inhibition of brain and muscular AChE activity by chlorpyrifos-oxon via biotransformation of bioaccumulated chlorpyrifos in the tissues.

KEYWORDS: Acute toxicity (96 hrs LC_{50}), Behavioural anomalies, Caudal bending, Chlorpyrifos, *Gambusia affinis*.

INTRODUCTION

The mosquitofish, *Gambusia affinis* is live-bearing freshwater minnows related to guppies (Family: Poeciliidae). They are native to the North and Central America, and had been introduced to suitable warm waters around the world .The mosquitofish can thrive a wide variety of water types, being very tolerant to high water temperatures, a wide range of salinities, pH and very low dissolved oxygen levels. [1, 2] The mosquitofish are considered the main predators of mosquitoes. In most habitats, fish can feed on a wide variety of preys ranging from floating larvae of mosquitoes, other aquatic insects, crustacean to zooplankton and algae [3, 4]. Organophosphates (OPs) have become the most widely used class of insecticides in the world replacing the persistent and problematic organochlorine compounds. Exposure of aquatic ecosystems to these insecticides is difficult to assess because of their short persistence in the water column due to low solubility and rapid degradation. However, monitoring of these insecticides is important, because they are highly toxic to aquatic organisms. Fish are ideal sentinels for behavioural assays of various stressors and toxic chemical exposure due to their 1) constant, direct contact with the aquatic environment where chemical exposure occurs over the entire body surface, 2) ecological relevance in many natural systems [5], 3) ease of culture, 4) ability to come into reproductive readiness [6] and 5) long history of use in behavioural toxicology. Behaviour provides a unique perspective linking the physiology and ecology of an organism and its environment [7]. Behaviour is both a sequence of quantifiable actions, operating through the central and peripheral nervous systems and the cumulative manifestation of genetic, biochemical and physiologic processes essential to life such as feeding, reproduction and predator avoidance. Behaviour allows an organism to adjust to external and internal stimuli in order to the best meet the challenge of surviving in a changing environment. Conversely, behaviour is also the result of adaptations to environmental variables. Thus, behaviour is a selective response that is constantly adapting through direct interaction with physical, chemical, social and physiological aspects of the environment. Selective evolutionary processes have conserved stable behavioural patterns in concert with morphologic and physiologic adaptations. This stability provides the best opportunity for survival and reproductive success by enabling organisms to efficiently exploit resources and define suitable habitats. [7].

Chlorpyrifos is a synthetic organophosphate (OP), non-systemic, broad-spectrum insecticide and acaricide, acting as a cholinesterase inhibitor, with contact, stomach and respiratory action. Commercial manufacture of chlorpyrifos started in 1969, since then chlorpyrifos has been used for many purposes.

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The major use of chlorpyrifos in farming is to protect corn, cotton and fruit trees against insects. Common carp is a prime cultured and very important staple freshwater fish generally found in rivers, ponds and reservoirs. Contamination of aquatic biota at sublethal levels by chlorpyrifos is common in our region affecting the non-target icthyofauna. Hence, the present study was undertaken to evaluate the aquatic toxicity of chlorpyrifos with special emphasis on behavioural responses of the fish, *Gambusia affinis* exposed to sublethal concentrations of commercial grade chlorpyrifos.

MATERIALS & METHODS

Healthy and active *Gambusia affinis* were procured from the Fisheries Department, Ajmer, India. Fish were brought to the laboratory in large aerated crates. Later they were acclimatized for week in laboratory condition. Test chambers were glass aquaria of about 50 liter capacity and fed with commercial dry feed pellets. The fish weighing (0.5-1.0 g), length (3.0-4.5 cm) were acclimatized to laboratory conditions for 20 d at 24±1°C and are held in 50 L glass aquaria containing dechlorinated tap water of the quality used in the test, whose physico-chemical characteristics were analyzed following the methods mentioned in APHA (2005) and found as follows, temperature 24±2°C, pH 7.1±0.2 at 24°C, dissolved oxygen 9.6±0.8 mg/L, carbon dioxide 6.3±0.4 mg/L, total hardness 23.4±3.4 mg as CaCO3/L, phosphate 0.39±0.002 µg/L, salinity, nil. Water was renewed every day and a 12-12 hrs. photoperiod was maintained during acclimatization and test periods. The fish were fed regularly with commercial fish food pellets during acclimatization and test periods, but feeding was stopped two days prior to exposure to the test medium for acute toxicity test only. Chlorpyrifos (20% EC: emulsifiable concentrate) was procured from the local market of Ajmer, Rajasthan, India. The expiry date of the test substance checked prior to initiation of the treatment was found suitable for the exposure. Required quantity of chlorpyrifos was drawn directly from this 20% EC using micropipette. In range finding test, fish were exposed in batches of ten (in 20 L of test medium) to varying concentrations (0.02530 to 0.03000 mg/L) of chlorpyrifos with six replicates for each test concentration along with the control sets. Water medium was replaced every 24 hr. followed by an addition of desired concentration of the test compound. Concentrations of the test compound used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality (Table 1). Mortality was recorded every 24 hrs. and the dead fish were removed when observed, every time noting the number of fish death at each concentration up to 96 hrs. Duncan's multiple range test, was employed for comparing mean mortality values after estimating the residual variance by repeated measures ANOVA [8] for arc sine transformed mortality data (dead individuals/initial number of individuals). The LC_{50} with 95% confidence limits for chlorpyrifos were determined for 96 hrs. by probit analysis. [9]

OBSERVATIONS

Study Periods and Toxicant Concentrations

Sublethal concentrations of one-third (0.0946 mg/L) and one-sixth (0.0473 mg/L) of the 96 hrs LC_{50} (0.284 mg/L) were selected for sub-acute studies. Fish were exposed to both the sublethal concentrations of chlorpyrifos for 15, 30 and 45 days. The control (exclusively toxicant free medium) and chlorpyrifos exposed fish were kept under continuous observation for study of behavioural responses and morphological deformities.

Table 1. Mortality of *Gambusia affinis* in different concentration of chlorpyrifos 96 hrs exposure periods

Conc. of chlorpyrifos (mg/L)	Log Conc.	No. of fish alive out of ten	% Corrected mortality	Probit kill
0.245	-0.610	10	0	---
0.255	-0.593	9	10	3.72
0.264	-0.578	8	20	4.16
0.274	-0.562	7	30	4.48
0.280	-0.552	6	40	4.75
0.284	-0.546	5	50	5.00
0.290	-0.537	4	60	5.25
0.294	-0.531	3	70	5.52
0.296	-0.528	2	80	5.84
0.298	-0.525	1	90	6.28
0.300	-0.522	0	100	---

RESULT & DISCUSSION

Acute toxicity (96 hrs LC_{50}) of chlorpyrifos for the fish, *Gambusia affinis* was found to be 0.284 mg/L. The upper and lower 95% confidence limits are presented in Table 2. Thus, chlorpyrifos can be rated as highly toxic to fish. No significant mortality was observed during the experimental periods in both the sublethal concentrations. Clark *et al.* reported 96 hrs LC_{50} of chlorpyrifos to channel catfish, *Ictalurus punctatus* and sheepshead minnow, *Cyprinodon variegatus* as 0.280 mg/L and 0.136 mg/L, respectively.[10] Chlorpyrifos toxicity reported by Rao *et al.* to mosquito fish, *Gambusia affinis* by semi-static method is 0.297 mg/L. We can infer from our results that chlorpyrifos is highly toxic to freshwater fish, *C. carpio* and comparison of the different LC50 values clearly indicates that the acute toxicity of chlorpyrifos varies with the fish species. [11] Vitellogenin induction and histo-metabolic changes in exposure of *Cyprinus carpio* to methyl paraben. In the present study, the control fish were active for feeding and alert to slightest of the disturbance with their well-synchronized movements.[12] The behaviour did not significantly vary between the control groups; therefore, these results were taken as standards for the entire experimentation. Gambusia affinis exposed to chlorpyrifos exhibited disrupted behaviour, localization to the bottom of test chamber and independency (spread out) in swimming. This followed loss of co-ordination and occupancy of twice the area to that of control group were the early responses of the Gambusia affinis following exposure to chlorpyrifos in both the sublethal concentrations. Subsequently, fish moved to the corners of the test chambers, which can be viewed as avoidance behaviour of the fish to chlorpyrifos.

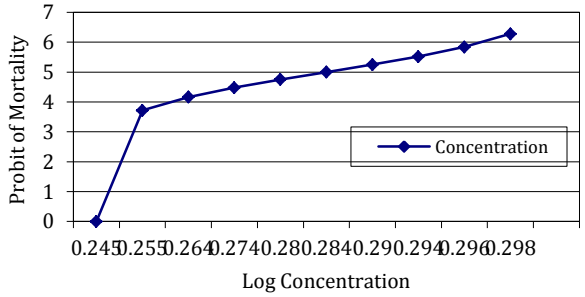

Figure 1. The plot for Probit vs. Log Concentration

Further, carp exhibited irregular, erratic and darting swimming movements and loss of equilibrium followed by hanging vertically in water. The above symptoms may be due to inhibition of acetylcholinesterase (AChE) activity leading to accumulation of acetylcholine (ACh) in cholinergic synapses due to hyper stimulation. Since, inhibition of AChE activity is a typical characteristic of organophosphate compounds indicated that the abnormalities in fish behaviour observed in exposure to OP insecticides (chlorfenvinphos, chlorpyrifos and diazinon) could be related to failure of energy production or the release of stored metabolic energy. [13-15].

Table2. Acute toxicity (96hrs LC_{50}) and confidence limits of chlorpyrifos to the *Gambusia affinis*

Pesticide	96 hrs LC_{50} (mg/L)	Confidence limits	
		Upper limit (1/3)	Lower limit (1/6)
Chlorpyrifos	0.284	0.0946	0.0473

CONCLUSION

The current study evidenced that chlorpyrifos is highly toxic and had a detrimental impact on the behavioural responses of *Gambusia affinis* at sublethal concentrations. It reduced/decreased the animals' ability to adapt to its environment by 1) increasing the time required to learn to escape or to avoid external noxious stimuli, 2) decrease the animal sensitivity to subtle changes in the environment, or 3) interfere with the animals' ability to retain previously learned behaviour. Thus, chlorpyrifos reduced instinctive behavioural response and affected morphological features. Impairments in behavioural responses even under recovery periods may be due to inhibition of brain AChE activity by chlorpyrifos-oxon via biotransformation of bioaccumulated chlorpyrifos in the tissues into their active oxygen analog (chlorpyrifos-oxon). These behavioural responses can be used as a tool in biomonitoring programme to monitor ecotoxicity risk of chlorpyrifos to the test species.

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