Original Article

Acute Toxicity of an Organophosphate Insecticide Chlorpyrifos to an Anuran, *Rana cyanophlyctis*

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ABSTRACT

Background: Chlorpyrifos is an organophosphate pesticide that elicits broad-spectrum insecticidal activity against a number of important arthropod pests. Determining the insecticides' toxicity to amphibians can give us a better understanding regarding the role of toxicants in amphibian declines. This information would be beneficial to assess their ecological relevance at environmental concentrations. The present study assessed toxicity of chlorpyrifos to an anuran *Rana cyanophlyctis*.

Methods: For the determination of LC_{50} values for chlorpyrifos, four-day static renewal acute toxicity test was used. Five replicates each containing ten frogs were subjected to each concentration of chlorpyrifos (2, 4, 6, 8, 10, 12, 14 and 16 mg/L) for the test. Mortality of the frog at different exposure periods (24, 48, 72 and 96 h) was subjected to Probit analysis with the POLO-PC software (LeOra Software) to calculate the LC_{50} and 95% confidence level.

Results: The LC_{50} values of chlorpyrifos for the frog *R. cyanophlyctis* at 24, 48, 72, and 96 h were 8.252, 7.254, 6.247 and 4.993mg/L, respectively.

Conclusion: Mortality has been noticed in chlorpyrifos treated frogs related to the decline in amphibian population. Therefore, chlorpyrifos should not be used near water reservoirs.

Keywords: Amphibian, Anuran, Chlorpyrifos, LC_{50}, Organophosphate, Toxicity.

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INTRODUCTION

In past two decades, biologists have gathered information regarding global amphibian declines [1-3]. Numerically, about 2000 of roughly 6300 described species of amphibians are seriously threatened [4]. Several hypotheses have been proposed for such dramatic declines in amphibian populations. The decline of the world's amphibian populations is now gaining scientists' great concern [1-3]. The International Union for Conservation of Nature [5] released the Global Amphibian Assessment in 2004 that provided globally comprehensive assessment of all described amphibian species. Among amphibian population, 32.5% were listed as vulnerable, endangered, or critically endangered, 7.4% species were listed as critically endangered, and about 43% were experiencing some form of population decline [6]. For such amphibian population decline, several causes have been given as follows: habitat loss [5]; climate change [7]; UV-B radiation [8, 9]; infectious disease [10]; contaminants [11-13]; non-native predators [14]; and a combination of factors [15, 16]. The role of pesticides in amphibian population decline has been reported [12, 13, 17].

Chlorpyrifos [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridil) phosphor- rothioate], is a member of organophosphate class of pesticides that elicits broad-spectrum insecticidal activity against a number of important arthropod pests [18-20].

Toxicological experiments conducted in laboratory play a useful role in establishing baseline sensitivity of amphibians to contaminants because other environmental stressors can be controlled. Determining the insecticides' toxicity to amphibians can give us a better understanding regarding the role toxicants in amphibian declines. This information would be beneficial to assess their ecological relevance at environmental concentrations.

The objectives of this study were to determine the chronic median lethal

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concentrations (LC_{50}) and sublethal effects of chlorpyrifos to an anuran *Rana cyanophlyctis.*

MATERIALS AND METHODS

For experiments, laboratory reared *R. cyanophlyctis* (both sexes, body wt. 14.34±0.45 g) were selected (2012; Gorakhpur, India). There was no significant difference ($P>0.05$) between the mean weights of the frogs used in the experiments. Since metabolic activity changes with size and affects the parameters should have been measured, individuals of almost same weight range were used. Frogs were kept in all-glass aquaria and acclimatized to the laboratory conditions (under natural photoperiod 11.58-12.38 h and temperature 27.2±1.4 °C) for at least two wk. Each aquarium contained dechlorinated tap water. The physicochemical characteristics of the tap water were pH 7.20 ± 60.1: dissolved oxygen 7.95 ± 60.25 mg/L and hardness as $CaCO_3$ 167.06 ± 65.61 mg/L. During acclimatization, the frogs were fed daily with live insects, 2-3 times per day. Water was renewed daily after cleaning the fecal matter. All care was taken to avoid giving stress to the frogs. Feeding was stopped 24 h before and during the experimental period to avoid the excretory substances to influence the toxicity test solutions.

For the determination of LC_{50} values for chlorpyrifos, four-day static renewal acute toxicity test [21] was used. Five replicates each containing ten frogs (kept in glass aquarium containing 30 L of the test solution) were subjected to each concentration of chlorpyrifos (2, 4, 6, 8, 10, 12, 14 and 16 mg/L) for the test. Chlorpyrifos (trade name coroban) was firstly dissolved in acetone and then desired volume of the solution was mixed with tap water to obtain the above-mentioned toxicant concentrations. A control group with five replicates (each containing 10 frogs) kept in 30 L tap water (containing equal volume of acetone as used for preparation of chlorpyrifos solution) was also run. The solutions of all the aquaria (control and experimental) were renewed daily. Precautions were taken to remove the dead frog immediately because dead animals deplete dissolved oxygen which greatly affected toxicity data [22].

At different exposure periods (24, 48, 72 and 96 h), the mortality of the frog was subjected to Probit analysis with the POLO-PC software (LeOra Software) to calculate the LC_{50} and 95% confidence level.

Ethical Consideration

The Ethics Committee was informed about the research work and the use of the frogs. As such, there was no ethical committee disapproval because the research work included the use of frogs bred and cultured in laboratory.

RESULTS

The percent mortality of *R. cyanophlyctis* after exposure to various concentrations of chlorpyrifos for 24, 48, 72, and 96 h is shown in Figures 1- 4. The LC_{50} (50% Lethal Concentration) values of chlorpyrifos (Table 1) for the frog *R. cyanophlyctis* at 24, 48, 72, and 96 h were 8.252, 7.254, 6.247 and 4.993mg/L, respectively. The slope functions and upper and lower confidence limits for *R. cyanophlyctis* are shown in Table 1.

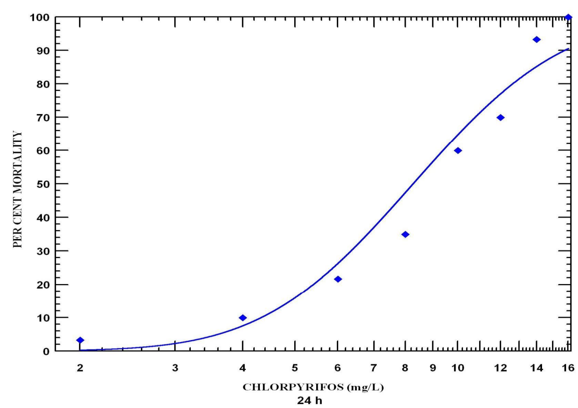

Figure 1. Per cent mortality of the frog *Rana cyanophlyctis* after 24 h exposure to different concentrations of chlorpyrifos.

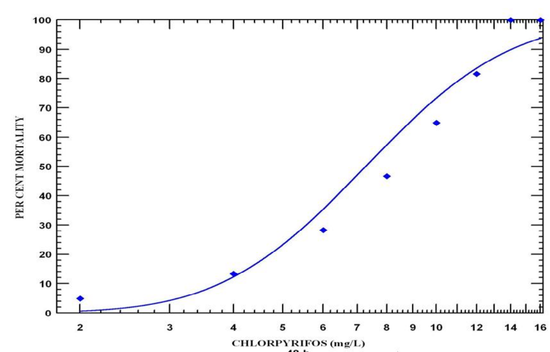

Figure 2. Per cent mortality of the frog *Rana cyanophlyctis* after 48 h exposure to different concentrations of chlorpyrifos.

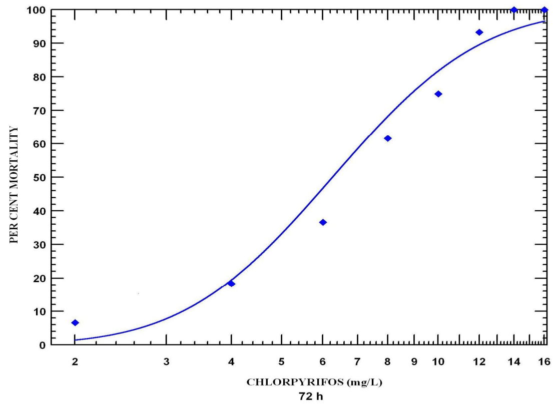

Figure 3. Per cent mortality of the frog *Rana cyanophlyctis* after 72 h exposure to different concentrations of chlorpyrifos.

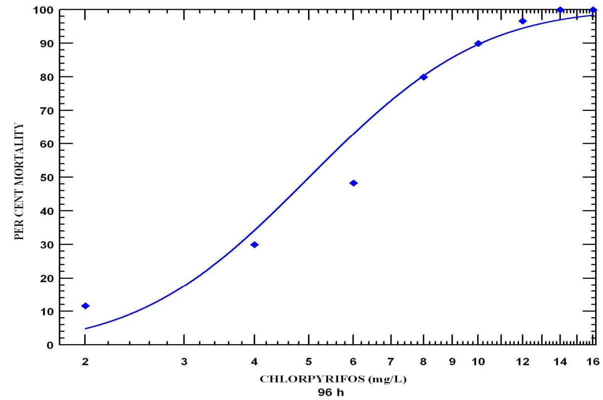

Figure 4. Per cent mortality of the frog *Rana cyanophlyctis* after 96 h exposure to different concentrations of chlorpyrifos.

Table 1. LC_{50} (50% Lethal Concentration) value, slope function and confidence limits for short-term exposure of chlorpyrifos at different intervals for the frog *R. cyanophlyctis*.

Exposure Periods	Effective dose (mg/L)	Limits(mg/L)* LCL	UCL	Slope Function	't' ratio	Hetero-geneity
24 h	LC_{10}=4.323	1.896	5.877	4.564	12.215	6.630
	LC_{50}=8.252	6.172	10.509	±		
	LC_{90}=15.752	11.976	31.534	0.374		
48 h	LC_{10}=3.757	1.730	5.142	4.485	12.619	6.104
	LC_{50}=7.254	5.381	9.121	±		
	LC_{90}=14.007	10.799	25.074	0.355		
72 h	LC_{10}=3.216	1.810	4.275	4.444	12.880	4.1064
	LC_{50}=6.247	4.866	7.559	±		
	LC_{90}=12.135	9.758	17.920	0.345		
96 h	LC_{10}=2.468	1.550	3.226	4.188	12.883	2.629
	LC_{50}=4.993	4.007	5.920	±		
	LC_{90}=10.101	8.359	13.462	0.325		

*The upper and lower confidence limits for LC_{50} values calculated at 0.05 levels.

DISCUSSION

The percentage mortality of the frogs increased in parallel with increasing the concentration of chlorpyrifos. This increase was also time-dependant. The LC_{50} value was reported of chlorpyrifos for larval amphibians. Totally, 96 h LC_{50} value was reported for chlorpyrifos as 2.41 mg/L for embryos of *Xenopus laevis* [23]. For larvae of *Ambystoma mexicanum* the 96 h LC_{50} value for chlorpyrifos has been reported as 1.36 mg/L [24]. Ninety six h LC_{50} value for chlorpyrifos for tadpoles of *Bufo bufo gargarizans* has been reported as 0.80 mg/L [25]. 5.174 mg/L chlorpyrifos was reported as 96 h LC_{50} for tadpoles of *R. dalmatina* [26]. Twenty-four h LC_{50} value were reported for chlorpyrifos as 3 mg/L for larvae of *Rana boylii* [27]. About 96 h LC_{50} for chlorpyrifos ranged from 1µg/L for *B. americanus* to 3 mg/L for *R. pipiens* [28]. Totally, 48 h LC_{50} value were studied for chlorpyrifos in five d post-hatch tadpoles of *B. melanostictus* and reported it as 1.47 ppm [29]. "Chlorpyrifos caused significantly high and dose-dependant mortality and the weekly LC_{50} (7 d–21 d) values ranged from 3003 µg/L to 462 µg/L" [30]. The acute LC_{50} value for chlorpyrifos *Rhinella fernandezae* tadpoles has been found [31] as 0.151 mg/L (in unpolluted area) and 0.293 mg/L (in area with high degree of anthropogenic disturbance).

Among lower vertebrates chlorpyrifos toxicity has been studied extensively in fishes. Ninety-six h LC_{50} value for chlorpyrifos has been reported as 3 ppb for *Oncorhynchus mykiss*, 3.3

ppb for *Lepomis macrochirus* and 13.4 ppb for *Ictalurus punctatus* [32]. About 96 h LC_{50} value for chlorpyrifos for *Heteropneustes fossilis* has been reported as 2.2. mg/L [33]. The 96 h LC_{50} value were reported for chlorpyrifos as 203 ppb for *Pimephalus promelas* and 35 ppb for *Notemigonus crysoleucas* [34]. "The 96 h LC_{50} value for chlorpyrifos for juvenile and adult *Oreochromis niloticus* has been determined as 98.67 µg/L and 154.01 µg/L, respectively" [35]. For chlorpyrifos 96 h, LC_{50} was reported as 0.176 ppm for *Poecilia reticulata* [36], 297 mg/L for *Gambusia affinis* [37], and 580 µg/L for *Cyprinus carpio* [38]. Acute static 96 h LC_{50} for several fingerling freshwater fishes indicate a broad range of sensitivity to chlorpyrifos 18 µg/L for *Salmo clerki*, 7.1 µg/L for *Salmo gairdneri*, 98 µg/L for *Salvenius namaycush*, 280 µg/L for *Ictalurus punctatus* and 2.4 µg/L for *Lepomis microchiras* [39].

In the present study, 96 h LC_{50} value for chlorpyrifos was 4.99 mg/L. Comparing the 95 h LC_{50} for other amphibians (mostly larval stage) and fishes, it appears that *R. cyanophlyctis* is more resistant to chlorpyrifos and may be considered as less sensitive to this pesticide.

CONCLUSION

Mortality has been noticed in chlorpyrifos treated frogs related to the decline in amphibian population. Therefore, chlorpyrifos should not be used near water reservoirs.

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