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Acute toxicity of trichlorophon on two ornamental fish: tiger barb (*Systomus tetrazona*) and glowlight tetra (*Hemigrammus erythrozonus*)

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ABSTRACT

Objective: To determine the acute toxicity of trichlorophon (an organ phosphorus compound used in agricultural fields and a common disinfectant used for fish diseases) towards two ornamental fish *Systomus tetrazona* and *Hemigrammus erythrozonus*.
Methods: In this research, two aquarium fish were exposed to different concentrations of trichlorophon (5, 10, 20 and 40 mg/L) for 96 h and physicochemical properties of water used for these experiments were stable and every mortality was recorded daily for 96 h. LC_{50} of 24 h, 48 h, 72 h and 96 h were attained by probit analysis software SPSS version 16.
Results: The 96-h LC_{50} of trichlorophon for *Systomus tetrazona* and *Hemigrammus erythrozonus* were 8.74 and 8.88 mg/L, respectively.
Conclusions: Eventually, toxicity values indicated that trichlorophon has the same toxicity towards the studied species and the lower value of LC_{50} for studied species in comparison with that of most species can be stated.

1. Introduction

Tiger barb [*Systomus tetrazona* Bleeker, 1855 (*S. tetrazona*)] is an ornamental freshwater fish of tropical cyprinid fish and its maximum length was reported to reach 4 cm. The tiger barb is one of over 70 species of barb with commercial importance in the aquarium fish trade[1]. They live in tropical climates and prefer water with a pH 6.0-8.0, a water hardness of 0.891-3.388 mmol/L and a temperature range of 20-26 °C. The discovery of them in swamp lakes subjects to great changes in water level, suggesting that they are of a wide tolerance to water quality fluctuations and also feeds on worms, small crustaceans and plant matter with the average lifespan of six years[2,3].

Glowlight tetra [*Hemigrammus erythrozonus* Durbin, 1909 (*H. erythrozonus*)] is a small freshwater fish of Characidae family and live in tropical condition with temperature 24-28 °C, pH 6.0-8.0 and 0.891-2.139mmol/L[2]. It is a popular aquarium fish that the maximum total length was reported to reach 4 cm[3]. The glowlight tetra is a peaceful and shoaling fish and it is an omnivore and eats small live, frozen, dry and flake foods in the aquarium.

Trichlorophon is an organophosphate insecticide used to control some agriculture pests and used for treating domestic animals for the control of internal parasites and also for the control of parasites of fish in designated aquatic environments. Its chemical name is dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate and is currently registered under the trade names Dylox 420, Dipterex, Neguvon pour-on cattle insecticide, and Dylox 80%[4].

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It is classified by the U. S. Environmental Protection Agency as a general use pesticide and in toxicity class II-moderately toxic and is a selective insecticide, meaning it kills selected insects but not targets towards many or most other organisms. Most trichlorophon-containing products used in agriculture (such as Dylox 420) are applied several times throughout the year, thus prolonging the exposure period to nontarget organisms. Although in this case, trichlorophon is not applied directly to water bodies, detrimentes as a result of volatilization following application may be significant depending on environmental conditions[5]. Trichlorophon is a pale clear, white, or yellow crystalline solid with an ethyl ether odor. It is solid at room temperature and highly soluble in water, with a solubility of 154 000 mg/L at 25 °C. Its melting point has been reported at 83-84 °C[6]. Trichlorophon is useful for the treatment of fish parasites such as parasitic copepods, monodigenetic and digenetic flukes, fish lice (*Argulus*) and leeches. As well clout may be effective for at least partially exposed nematodes such as camalanus worms. Organ phosphorous compounds can entere into the fish body through the gills, food, water and skin; after the toxin enters into fish body, it is absorbed by the blood and displaced in blood through the albumin. Signs of poisoning in fish are dependent on the amount of toxin[7].

Due to the importance of trichlorophon use in order to disinfect and eliminate the parasites in aquatics, this study was conducted to determine the acute toxicity of trichlorophon on two aquarium species: tiger barb (*S. tetrazona*) and glowlight tetra (*H. erythrozonus*).

2. Materials and methods

The purpose of this research was to determine the toxicity of trichlorophon on 2 aquarium fish based on ethical considerations. Lethal experiments were conducted using 35 tiger barb and 70 glowlight tetra. They were acclimatized for 2 weeks. Before the test, fish were fed twice daily with BioMar feed at 2% of body weight. Physicochemical properties of water used for these experiments were stable as follows: (21±1) °C, 7-9 mg/L dissolved oxygen, pH 6.7-7.9 and 2.098 mmol/L total hardness. Each aquarium was equipped with aeration system and water physicochemical conditions were similar in all aquariums.

Experiments were performed according to the Organization for Economic Co-operation and Development standard method[8], to determine the 96-h LC_{50} of these species. First, preliminary experiments were conducted in small level to obtain a lethal concentration of this poison on both fish species because the data on trichlorophon toxicity of these species was not found. Second, base on this information, 4 concentrations of trichlorophon (5, 10, 20 and 40 mg/L) with 3 replacement were considered for each species.

According to the method used (static-renewal test condition), all water in the tanks were exchanged daily containing the same concentration of trichlorophon in order to avoid the effects of metabolites and waste organic matter of fishes. Dead fish were removed from the water and mortality rates were recorded at time 0, 24, 48, 72 and 96 h. Acute toxicity tests was carried out according to method of Hotos *et al.*[9], the nominal concentration of trichlorophon estimated to result in 50% mortality of fishes within 24 h (24-h LC_{50}), 48 h, 72 h and 96 h was attained by probit analysis by software SPSS version 16 and the degree of toxicity was determined[10,11].

3. Results

No fish died during the acclimation period before exposure and also no fish with control treatment died during acute toxicity tests. Caused by poisoning, stimulation, excitement and feeding decreases in samples were observed. The mortality of tiger barb and glowlight tetra was checked during the exposure times at 24, 48, 72 and 96 h with the trichlorophon concentrations of 5, 10, 20 and 40 mg/L (Table 1).

Table 1
The mortality rate of fishes exposed to acute toxicity of trichlorophon.

Fish	Concentration (mg/L)	No. before treatment	No. of mortality			
			24 h	48 h	72 h	96 h
S. tetrazona	5	7	0	0	1	1
	10	7	0	2	2	3
	20	7	1	3	4	5
	40	7	2	4	6	7
	control	7	0	0	0	0
H. erythrozonus	5	14	0	2	3	4
	10	14	2	3	4	6
	20	14	3	5	7	10
	40	14	4	10	14	14
	control	14	0	0	0	0

Control: Without trichlorophon.

The result of this study indicated that 96-h LC_{50} of trichlorophon was 8.74 and 8.88 mg/L for tiger barb and glowlight tetra, respectively (Table 2). These results indicated that tiger barb is more sensitive than glowlight tetra with a few differences exposed to trichlorophon.

Table 2
Lethal concentration of trichlorophon.

Fish	Point	Concentration (mg/L)			
		24 h	48 h	72 h	96 h
S. tetrazona	LC_1	-	-	-	1.02
	LC_{10}	23.84	-	0.01	4.49
	LC_{30}	46.41	18.76	9.54	7.00
	LC_{50}	62.05	34.80	16.15	8.74
	LC_{70}	77.69	50.85	22.75	10.49
	LC_{90}	100.27	74.02	32.29	13.00
	LC_{99}	131.42	105.99	45.45	16.47
H. erythrozonus	LC_1	-	-	-	-
	LC_{10}	11.10	0.36	1.31	2.83
	LC_{30}	37.55	15.65	7.72	6.40
	LC_{50}	55.88	26.23	12.16	8.88
	LC_{70}	74.20	36.82	16.59	11.36
	LC_{90}	100.66	52.11	23.00	14.93
	LC_{99}	137.16	73.21	31.85	19.87

4. Discussion

Trichlorophon is moderately toxic by ingestion or dermal absorption. As among all organophosphates, trichlorophon is readily absorbed through the skin. Skin sensitivity can result from dermal exposure. Trichlorophon decreases activity of the cholinesterase enzyme which is necessary for normal nervous system function. Trichlorophon primarily affects the nervous system through inhibition of cholinesterase, which is required for proper nerve functioning, as well as other target organs include liver, lungs and bone marrow (blood-forming tissue). Pure trichlorophon is reported to be less toxic than the technical material[12,13]. Toxicity in the field can be affected by many factors including temperature, pH and water hardness, which may have different effects across species[14-17].

Trichlorophon degrades rapidly in alkaline pond water (pH 8.5). Almost 99% of applied trichlorophon was broken down within 2 h. It was stable in the same pond water kept under acidic (pH 5.0) conditions for 2 h. In the present study, alkalinity was almost neutral, so its effect was notable on experiment. The major breakdown product of trichlorophon in water is dichlorvos (DDVP; 2,2,-dichlorovinyl dimethyl phosphate)[18]. This insecticide was shown to persist at detectable levels for 526 d in water at 20 °C[19]. Biotransformation in soil and hydrolysis in water are the primary methods of trichlorophon transformation[20]. The transformation to DDVP in water occurs by dehydrochlorination. The rate of transformation increases at greater water alkalinities[21]. Trichlorophon can be transformed to DDVP through photolysis. DDVP causes the inhibition of cholinesterase at a rate 100 times that of trichlorophon[22]. For freshwater fish, the 96-h LC_{50} of DDVP ranged from 200 μg/L for lake trout (*Salvelinus namaycush*) to 8 900 μg/L for walking catfish (*Clarias batrachus*)[23-25].

An estimated bioconcentration factors of 3 is reported for aquatic organisms exposed to trichlorophon. This suggests that the potential for bioconcentration is low[20]. This is supported by the rapid rate of transformation and low octanol-water partition coefficient reported for trichlorophon. These factors indicate that trichlorophon will have a low persistence in water. Some researchers reported a halflife time of 57 h (2.5 d) for trichlorophon in water; to this end, in this study, it was necessary to replace the water with constant concentration of trichlorphon. Therefore, bioaccumulation and biomagnification of trichlorophon are unlikely to occur[23].

Canadian Water Quality Guidelines reported the concentration of the trichlorophon for the protection of freshwater life was 0.009 μg/ L for long term exposure and 1.1 μg/ L for short-term exposure[5]. In this regard, Nazifi *et al*. indicated that when silver carp was exposed to 1 μg/L trichlorophon, compared to the control group, there was a statistically significant difference in total protein, albumin, blood urea nitrogen, sodium, phosphorus, alanine transaminase, aspartate transaminase and alkaline phosphatase[22,26].

As the International Programme on Chemical Safety reported, the results of present study also found that trichlorophon was moderately to highly toxic to freshwater fish. Toxicity values available from the literature ranged from a 96-h LC_{50} of 234 μg/L for bluegill sunfish (*Lepomis macrochirus*) to a 96-h LC_{50} of 180 000 μg/L for fathead minnow (*Pimephales promelas*)[21].

In the present study, toxicity values indicated that trichlorophon has the same toxicity with the studied species. Values of LC_{50} of trichlorophon obtained in this research compared with the corresponding values that have been published in the literature for other species of fish showed difference in different species and even in different times, but it can be stated that the value of LC_{50} for studied species is lower in comparison with that of the most species and confirmed that the species in this study is sensitive to lower trichlorophon doses.

Conflict of interest statement

We declare that we have no conflict of interest.

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