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Acute toxicity of trichlorofon on four viviparous fish: *Poecilia latipinna*, *Poecilia reticulata*, *Gambusia holbrooki* and *Xiphophorus helleri* (Cyprinodontiformes: Poecilidae)

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PEER REVIEW

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Comments
This study was conducted to determine the acute toxicity of trichlorofon on four viviparous aquarium species. The paper is concise, the methodology is scientifically sound and the results are clear.
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ABSTRACT

Objective: To determine toxicity and safety margins of trichlorofon in different species as trichlorofon toxicity has a large variation in the susceptibility of different species.
Methods: In this research, four viviparous aquarium fish were exposed to trichlorofon for 96 h. LC_{50} of 24 h, 48 h, 72 h and 96 h were attained by probit analysis software SPSS Version 16. Fish samples were exposed to different concentrations of trichlorofon (5, 10, 20 and 40 mg/L) for 96 h and mortality were recorded, separately.
Results: The 96 h LC_{50} of *Poecilia latipinna*, *Poecilia reticulata*, *Gambusia holbrooki* and *Xiphophorus helleri* were 9.80, 9.80, 9.95 and 7.99 mg/L, respectively.
Conclusions: According to the results of this research, LC_{50} values indicated *Xiphophorus helleri* was the most resistant and *Gambusia holbrooki*, with a few differences, was the most sensitive species compared with the fishes examined.

KEYWORDS
Aquarium fish, Toxicity, Thriclorophon, *Poecilia latipinna*, *Poecilia reticulata*, *Gambusia holbrooki*, *Xiphophorus helleri*

1. Introduction

Poeciliidae family is a member of Cyprinodontiformes order. This family is benthopelagic and lives in brackish and freshwater. Members of this family are viviparous. Sailfin molly [*Poecilia latipinna* (*P. latipinna*)], guppyor millions fish [*Poecilia reticulata* (*P. reticulata*)], eastern mosquitofish [*Gambusia holbrooki* (*G. holbrooki*)] and red swordtail [*Xiphophorus helleri* (*X. helleri*)] from Poeciliinae subfamily are studied here[1–3].

Red swordtail feeds on worms, crustaceans, insects and plant matter[4], and are used for genetics research[5]. The red varieties of this species are very popular aquarium fishes[3]. Eastern mosquitofish feeds on small terrestrial insects usually in the drift and amongst aquatic plants, actively selecting very small prey and also frequently feeds on mosquito larvae[6,7]. Guppy has a wide salinity range but requires fairly warm temperatures (23–24 °C) and quiet vegetated water for survival[8], also this species is one of the most popular aquarium fishes with many standardized varieties that is used in genetics research[9], and is a very popular and widely available species in the aquarium trade. Sailfin molly mainly feeds on alga and animal material such as rotifers, crustaceans and aquatic insects[10].

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Trichlorofon is an organophosphorus pesticide used to control cockroaches, crickets, silverfish, bedbugs, fleas, cattle grubs, flies, ticks, leaf miners, and leaf–hoppers. It is applied to control of parasites of fish in designated aquatic environments[11]. It is highly soluble in water, with a solubility of 154 000 mg/L at 25 °C and is both toxic to fish and man's nervous system[12,13], and is also toxic to snails, clams and aquatic insects[14]. Trichlorofon is a pale clear, white, or yellow crystalline solid powder with an ethyl ether odor. Trichlorofon is solid at room temperature and its chemical name is dimethyl 2,2,2–trichloro–1–hydroxyethylphosphonate[15]. Organophosphorus pesticides work by interfering with the nervous system of insects, a mechanism that also affects the human nervous system when people are exposed. Other health effects of individual organophosphorus pesticides are different. Some of them are highly acutely toxic while some cause development or reproductive harm, and some are known or suspected as endocrine disruptors[16].

Trichlorofon is a common disinfectant and therapeutic agent in fisheries–related activities. It is necessary to determine its toxicity in different species because trichlorofon toxicity has a large variation in susceptibility of different species. Toxicity values of trichlorofon were reported for some invertebrates and fish species such as goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), bluegill sunfish [*Lepomis macrochirus* (*L. macrochirus*)], fathead minnow [*Pimephales promelas* (*P. promelas*)], *Daphnia pulex*, *Pteronarcella badia*, *Hexagenia* sp., *Acroneuria pacifica* and others[12]. Considering a large variation of trichlorofon toxicity in susceptibility of different species, this study was conducted to determine the acute toxicity of trichlorofon on four viviparous aquarium species: *P. latipinna*, *P. reticulata*, *G. holbrooki* and *X. helleri*.

2. Materials and methods

Lethal experiments were conducted using 70 specimens of *P. latipinna*, 70 of *P. reticulata*, 35 of *X. helleri* and 150 of *G. holbrooki* that are all aquatic models related to human health. They were acclimatized for 2 weeks. Physicochemical properties of water used for these experiments were: (21±1) °C temperature, 7 to 9 mg/L dissolved oxygen, 6.7 to 7.9 pH and 210 mg/L total hardness. Before the test, fish were fed twice daily with BioMar feed at 2% of body weight.

Experiments were performed according to the OECD standard method[17], to find the 96 h LC_{50} of these species. Because the data on trichlorofon toxicity of these species was not found, first, preliminary experiments were conducted in small level to obtain a lethal concentration of this poison on each fish species, and then base on this information, four concentrations of trichlorofon (5, 10, 20 and 40 mg/L) with three replacement were considered for each species. Each aquarium was equipped with aeration system and water physicochemical conditions were similar in all aquariums.

According to the method used (Static–renewal test condition), all water tanks were exchanged with daily water replacement containing the same concentration of trichlorofon, 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 were carried out according to Hotos and Vlahos[18]. The nominal concentration of trichlorofon estimated to result in 50% mortality of fishes within 24 h, 48 h, 72 h and 96 h was attained by probit analysis by software SPSS Version 16 and degree of toxicity was determined[19].

3. Results

No fish died during the acclimation period before exposure and also no control fish died during acute toxicity tests. The mortality of sailfin molly, guppy, eastern mosquitofish and red swordtail was examined during the exposure times at 24, 48, 72 and 96 h with the rates in 5, 10, 20 and 40 mg/L, respectively (Table 1).

Table 1

The mortality rate of fishes exposed to acute toxicity of trichlorofon.

Group	Concentration (mg/L)	Number	No. of mortality			
			24 h	48 h	72 h	96 h
	5	14	0	1	1	3
	10	14	1	2	3	4
P. reticulata	20	14	2	3	6	10
	40	14	3	6	0	14
	Control	14	0	0	0	0
	5	30	1	2	3	6
	10	30	2	4	6	10
G. holbrooki	20	30	6	10	14	20
	40	30	10	16	22	30
	Control	30	0	0	0	0
	5	7	0	0	1	2
	10	7	0	1	2	3
X. helleri	20	7	1	3	3	4
	40	7	2	5	7	7
	Control	7	0	0	0	0
	5	14	0	0	1	3
	10	14	0	2	3	5
P. latipinna	20	14	2	4	6	9
	40	14	4	6	13	14
	Control	14	0	0	0	0

The result of this study indicated 96 h LC_{50} of trichlorofon was 9.80, 9.95, 7.99 and 9.80 mg/L for *P. reticulate*, *G. holbrooki*, *X. helleri* and *P. latipinna*, respectively (Table 2). These results indicated *X. helleri* is more sensitive than that of the others, exposed to trichlorofon.

Table 2
Lethal concentration of trichlorofon to the four viviparous aquarium species.

Group	Point	Concentration (mg/L)			
		24 h	48 h	72 h	96 h
	LC_1	–	–	–	–
	LC_{10}	20.15	6.86	0.84	3.65
	LC_{30}	46.58	28.10	15.69	7.28
P. reticulata	LC_{50}	64.88	42.82	25.97	9.80
	LC_{70}	83.18	57.53	36.25	12.32
	LC_{90}	109.60	78.78	51.10	15.96
	LC_{99}	146.07	108.10	71.59	20.98
	LC_1	–	–	–	–
	LC_{10}	12.79	3.81	–	2.77
	LC_{30}	35.17	22.48	14.62	7.01
G. holbrooki	LC_{50}	50.67	35.41	24.85	9.95
	LC_{70}	66.16	48.34	35.09	12.89
	LC_{90}	88.54	67.01	49.87	17.13
	LC_{99}	119.42	92.78	70.27	22.99
	LC_1	–	–	0.02	–
	LC_{10}	17.73	2.86	4.64	2.92
	LC_{30}	39.29	15.59	7.98	5.92
X. helleri	LC_{50}	54.22	24.41	10.30	7.99
	LC_{70}	69.15	33.22	12.62	10.06
	LC_{90}	90.71	45.95	15.96	13.05
	LC_{99}	120.46	63.52	20.58	17.17
	LC_1	–	–	–	–
	LC_{10}	21.09	7.42	3.15	3.65
	LC_{30}	39.95	27.76	12.48	7.28
P. latipinna	LC_{50}	53.01	41.84	18.94	9.80
	LC_{70}	66.07	55.92	25.40	12.32
	LC_{90}	84.93	76.25	34.73	15.96
	LC_{99}	110.95	104.31	47.60	20.98

LC_{50} values indicated *X. helleri* was the most resistant and *G. holbrooki*, with a few differences, was the most sensitive species compared with the fishes examined.

4. Discussion

Trichlorofon, in both technical and formulated forms, is highly toxic to many aquatic species such as *Daphnia*, stoneflies, crayfish and several freshwater fish species. Toxicity in the field can be affected by many factors including temperature, pH, and water hardness, which may have different effects on species. In some species, temperature differences of 10 °C can result in differences of 7 to 60 fold in observed 96 h LC_{50} values, also effects of changing pH from 6.5 to 8.5 resulted in changes of 13 to 20 fold in several species[19,20]. Generally, toxicity increased with higher temperature and higher pH. Researches did not show a potential for trichlorofon accumulation in fish[21]. An estimated bioaccumulation of trichlorofon is reported for aquatic organisms exposed to trichlorofon. This suggests that the potential for bioconcentration is low[22]. This is supported by the rapid rate of transformation and low octanol-water partition coefficient reported for trichlorofon. These factors indicate that trichlorofon will have a low persistence in water and is unlikely to partition to the lipids of aquatic organisms. Trichlorofon is highly toxic to freshwater fish[13]. Toxicity values available from the literature ranged from a 96 h LC_{50} of 234 μg/L for bluegill sunfish (*L. macrochirus*) to a 96 h TLm of 180 000 μg/L for fathead minnow (*P. promelas*)[14,15].

Trichlorofon is readily soluble in water and degrades rapidly in alkaline pond water (pH: 8.5). Approximately 99% of applied trichlorofon was broken down within 2 h. It was stable in the same pond water kept under acidic (pH: 5.0) conditions for 2 h. The major breakdown product of trichlorofon in water is dichlorvos (DDVP). Trichlorofon was shown to persist at detectable levels for 526 d in water at 20 °C[17].

Estimated LC_{50} (96 h) values are 0.18 mg/L in *Daphnia*, 0.01 mg/L in stoneflies, 7.80 mg/L in crayfish, 1.40 mg/L in rainbow trout, 2.50 mg/L in brook trout, 0.88 mg/L in channel catfish and 0.26 mg/L in bluegill[18]. Also, other researches indicated that trichlorofon in 1 mg/L concentration has a significant effect on *Hypophthalmichthys molitrix* blood serum biochemical parameters[19]. Organophosphate compounds caused vacuolization of the cytoplasm, deformation of liver cells nucleus and eventually destroy these cells. However, mode of action of organophosphate pesticide is different[20].

The toxicity of trichlorofon on examined fishes increased with increasing concentration and exposure time. Toxicity values of trichlorofon were reported for several invertebrates and fish species that some fishes including goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), bluegill sunfish (*L. macrochirus*), and fathead minnow (*P. promelas*). Toxicity values available from the literature ranged from a 96 h LC_{50} of 234 μg/L for *L. macrochirus* to 8 570 μg/L for *Anguilla rostrata*[12], whereas this research indicated 96 h LC_{50} *G. holbrooki* is 9 950 μg/L, so *G. holbrooki* is not a very sensitive species to trichlorphon. The importance of the findings was that trichlorofon is more toxic poison and because of the high sensitivity of freshwater aquarium fish to trichlorphon, the side of caution must be applied. Our result indicated that *X. helleri* is more sensitive than other species, however lethal range was much closed. This information could provide the maximum acceptable dose of poisons in the water medium for environmental policies after considering human health, since poisons accumulation in aquatic animals and affecting human health via food chain.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

This study tested the effects of trichlorofon, an organophosphate pesticide on toxicity to various viviparous fish species. The fish were exposed to different concentrations of the compound for 96 h in a static-renewal test and the LC_{50} was calculated.

Research frontiers

Organophosphate pesticides affect various aquatic species as a result of unintentional contamination of the waterways. The results of this study provide information on the effects of this compound on selected viviparous fish species that are important in the ornamental fish industry.

Related reports

Researches did not show a potential for trichlorofon accumulation in fish. An estimated bioaccumulation of trichlorofon is reported for aquatic organisms exposed to trichlorofon. This suggests that the potential for bioconcentration is low.

Innovations and breakthroughs

This present study has implications to proper husbandry of the fish to ensure that the water in the rearing systems should be free of organophosphate pesticides.

Applications

The results have significant impact on the ornamental fish industry and have important implications to basic fisheries science and fish toxicology.

Peer review

This study was conducted to determine the acute toxicity of trichlorofon on four viviparous aquarium species. The paper is concise, the methodology is scientifically sound and the results are clear.

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