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Acute Toxicity of Alpha-Cypermethrin on Tilapia (*Oreochromis niloticus* L.) Larvae

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Alpha-cypermethrin is a non-systemic insecticide with contact and stomach action. It consists of the active isomer of the pyrethroid insecticide cypermethrin and is highly effective against a wide range of chewing and sucking insects. It is also active against mosquitoes, flies and other insect pests in public and animal houses (URL 1). In fish aquaculture cypermethrin is used against lice infestations (Das and Mukherjee 2003).

Alpha-cypermethrin is practically non-toxic to birds but is highly toxic to fish and aquatic invertebrates. This is mainly because it is metabolized and eliminated significantly more slowly by fish than mammals or birds. In general, the hypersensitivity of fish to pyrethroid intoxication is partly due to species specific differences in pyrethroid metabolism, but principally to the increased sensitivity of the piscine nervous system to these pesticides. It is also highly toxic to bees and causes no mutagenic effects (URL 2).

Alpha-cypermethrin is not soluble in water and is taken into extensively and rapidly in water-sediment systems. Mean biota-sediment accumulation factors (BSAFs) were 0.08 for *Daphnia magna* and *Chironomus tetans* in 13% organic carbon sediments (Maund et al. 2002).

Alpha-cypermethrin is classified as a Schedule 6 poison in the Standard for the Uniform Scheduling of Drugs and Poisons. The 24-h LC_{50} value of alpha-cypermethrin for 20.0 μg/L for silver barb and 4.50 μg/L for common mirror (Whalon et al. 1990). In general, for the pyrethroids, lethality varies inversely with water temperature, particularly between 10°C and 20°C.

Bradbury and Coats (1989) have reviewed the toxicology of pyrethroids in mammals, birds, fish, amphibia and invertebrates (terrestrial and aquatic) and cited the 96-h LC_{50} for cypermethrin toxicity as 2.2 μg/L for *Tilapia nilotica*, 0.9-1.1 μg/L for carp (*Cyprinus carpio*), 1.2 μg/L for brown trout (*Salmo trutta*), 0.5 μg/L for rainbow trout (*Salmo gairdneri*), and 0.4 μg/L for *Scardinius erythropthalmus*. Polat et al. (2002) found the 48-h LC_{50} value of beta-cypermethrin in male guppies as 21.4 μg/L. Başer et al. (2003) studied the acute toxic effects of permethrin on guppies and reported 48-h LC_{50} value as 245.7 μg/L

Stephenson (1983) has compiled 96-h LC_{50} of cypermethrin on fish species as follows; 2.8 µg/L for rainbow trout (*Oncorhynchus mykiss*), 1.2 µg/L for fathead minnow and 0.93 µg/L for *Pimephales promelas* (juvenile). In another study Stephenson (1982) investigated the toxic effect of cypermethrin on various fish such as *Cyprinus carpio, Scardinius erythrophthalmus, Salmo gairdneri, Salmo trutta* and *Tilapia nilotica* and found that the LC_{50} values ranged between 0.4-2.2 µg/L.

This study investigated the toxic effects of alpha-cypermethrin on tilapia, *Oreochromis niloticus,* larvae by the determination of 96-h LC_{50} values and evaluated behavioral changes of the larvae exposed to different concentrations.

MATERIALS AND METHODS

Tilapia larvae were obtained from A. Çağlan Karasu Benli of the Faculty of Agriculture, Ankara University in Ankara. The specimens (av. wt. 0.054±0.001 g; av. length 0.79±0.03 cm, 3-4 days old) were transported to the laboratory in appropriately aerated plastic bags. The plastic bags were placed into the maintenance aquarium for about 30-35 minutes for acclimatization, then the larvae were allowed to swim into the aquarium water. Test chambers were made of plastic with 20 liters capacity. Temperature was regulated at 23±1^{0}C by using heaters. At the time of dosing air was turned off; it was on at all times otherwise.

Technical grade (98%) alpha-cypermethrin was obtained from the Insecticide Testing Laboratory of Hacettepe University, Ankara (source: Hockley Ltd. Int., UK) and stored at +4^{0}C until stock solution preparation. The stock solution was prepared by bringing alpha-cypermethrin to room temperature, then weighing a certain amount and diluting it in acetone to give the stock material. Dosing solutions were prepared from this stock by diluting with acetone to give the dosing concentrations 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 µg/L. Dead larvae were removed immediately and behavioral changes at each concentration were recorded. The dosing volume never exceeded 0.2 ml. Control group received acetone at the maximum acetone volume (0.2 ml) used in the dilution of the dosing concentrations.

Groups of larvae, each consisting of 10 individuals, were selected at random and placed into aerated test chambers. After 48 hours of adaptation, different concentrations of alpha-cypermethrin (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 µg/L.) in acetone were added to the experimental aquaria. During the adaptation period, and throughout the duration of the experiment, animals were not fed. Mortality was assessed at 24, 48, 72 and 96 hours after the start of the tests. Dead individuals were removed immediately. Following the preliminary experiment, all determinations were repeated three times. Water quality parameters were pH:7.2±0.1, dissolved oxygen 7.6±0.5 mg/L, temperature 23±1, conductivity 0.225±0.010 mS. The measurements were taken daily.

The bioassay system was as described in standardized methods (APHA AWWA WEF 1998; OECD 1993) and the national regulation (Turkish Official Gazette 1991). The selected species is also as recommended in these references. The static test method for assessing acute toxicity was used. LC_{50} and 95% confidence limits were calculated by a computer program (US EPA 1999). Data obtained from the alpha-cypermethrin acute toxicity tests were evaluated using Finney's probit analysis statistical method.

RESULTS AND DISCUSSION

The 96-h LC_{50} value (95% confidence limits) of alpha-cypermethrin was 3.42 µg/L (3.11-3.73) (Table 1). There was one death fish 24-h later at the highest dose (5 µg/L). The LC_{50} value for 48-h was 6.42 µg/L and for 72-h was 4.29 µg/L. No mortality was observed in the control group during the experiment. The changes in behavioral response started 1-2 hours after dosing, depending on the concentration of toxicant. The fish started to display intense activity one hour after exposure. They left themselves to water currents and made sudden movements at a concentration of 5 µg/L. The fish gave a response when the aquaria walls were tapped at a toxicant concentration of 1 µg/L. They gave no such response at higher concentrations and made movements such as spinning around their own axis. There was no mortality within the first two hours at 5 µg/L. Two larvae died by the end of the second hour at this concentration.

The 96-h LC_{50} value of alpha-cypermethrin in tilapia (*Oreochromis niloticus*) larvae was found to be 3.42 µg/L in our work. This shows that alpha-cypermethrin is highly toxic to fish. Yılmaz et al. (2004) reported that behavioral changes of male guppies manifested themselves starting at an alpha-cypermethrin concentration of 8 µg/L. It is clearly evident that larvae, the early life-stage, is far more sensitive than adults in terms of response to toxicants. Behavioral changes due to alpha-cypermethrin exposure in our work are similar to those reported by Polat et al. (2002) for beta-cypermethrin. The authors reported 48-h LC_{50} value of beta-cypermethrin in male guppies as 21.4 µg/L. Our results are in agreement with these data. As can be seen from the results, early life-stages are more sensitive than adult fish. Edwards et al. (1986) reported acute cypermethrin toxicity in rainbow trout as: gill flailing, hyperactivity, loss of buoyancy and inability to remain upright. However, published experimental work on alpha-cypermethrin toxicity to fish is quite limited.

These results are in agreement with the results of other workers. Smith and Stratton (1986) report the toxic effects (LC_{50}) of *cis*-cypermethrin on various fish species as follows: 2.0 µg/L (96-h) for Atlantic salmon (*Salmo salar*), 6.0 µg/L (96-h) for rainbow trout (*Salmo gairdneri*), 9.0 µg/L (24-h) and 8.0 µg/L (48-h) for mosquito fish (*Gambusia affinis*) and 10.0 µg/L (24-h) and 6.0 µg/l (48-h) for desert pupfish (*Cyprinodon macularius*).

Examining cypermethrin toxicity to other aquatic organisms, the work of Clark et al. (1987) reported the cypermethrin 96-h LC_{50} for grass shrimp (*Palaemonetes*

pugio) as 0.016 µg/L. The 24-h topical and aqueous LD_{50} values for selected terrestrial and aquatic insects, when exposed to technical grade cypermethrin (99.4% purity), were in the range 0.30-49 ng/mg body weight and 1.3-9.8 µg/L, respectively (Siegfried 1993). The author concluded that exposure of aqueous organisms to pyrethroids may also secondarily induce an osmotic imbalance that contributes to their toxicity.

Data produced using only model ecosystems for ecological risk assessment have limitations and uncertainties. Further work with toxicity testing methods directly on early-life stages of fish will be very useful in assessing the possible ecological risk of these pesticides. To overcome discrepancies and potential synergistic effects from the components of the pyrethroid formulations, toxicity tests with formulations must be included together with active ingredient tests. Using only the pyrethroid active ingredient in the tests is not sufficient. In addition, potential risk from alpha-cypermethrin metabolites should be investigated to get a more complete picture in terms of toxicity.

Table 1. Acute 96-h toxicity of technical alpha-cypermethrin on tilapia *(Oreochromis niloticus)* larvae

Point	Concentration (µg/L)	95% Confidence Limits	Intercept ± SE	Slope ± SE
LC 1.00	2.08	1.46-245	-0.722 ± 1.81	10.71 ± 2.13
LC 5.00	2.40	1.84-2.73		
LC 10.00	2.60	2.08-2.91		
LC 15.00	2.74	2.26-3.03		
LC 50.00	3.42	3.11-3.73		
LC 85.00	4.28	3.90-5.03		
LC 90.00	4.51	4.08-5.45		
LC 95.00	4.87	4.34-6.15		
LC 99.00	5.64	4.86-7.77		

Note. Control group (theoretical spontaneous response rate) = 0.0000

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