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Investigation of Acute Toxicity of Alpha-Cypermethrin on Adult Nile Tilapia (*Oreochromis niloticus* L.)

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Abstract

Alpha-cypermethrin, which is a synthetic pyrethroid pesticide and potential toxic pollutant, contaminating aquatic ecosystems was investigated in the present study for acute toxicity. Nile tilapia (*Oreochromis niloticus*) adults were selected for the bioassay experiments. The experiments were repeated three times and the 96-h LC_{50} value was determined for the adults. The static test method of acute toxicity test was used. Water temperature was regulated at 24±1°C. In addition, behavioural changes at each alpha-cypermethrin concentration were observed for the individual fish. Data obtained from the alpha-cypermethrin acute toxicity tests were evaluated using the probit analysis statistical method. The 96-h LC_{50} value for Nile tilapia was estimated as 5.99 µg/L.

Keywords: bioassay, acute toxicity, alpha-cypermethrin, tilapia; *Oreochromis niloticus*, behavioral changes.

Introduction

Alpha-cypermethrin is an active pyrethroid, which intensively controls a wide range of pests in agriculture and animal breeding. Alpha-cypermethrin is highly toxic to aquatic invertebrates. The 24- and 48-h EC_{50} (immobilization) values for *Daphnia magna* are 1.0 and 0.3 µg/L, respectively, and the 24-h LC_{50} value for *Gammarus pulex* is 0.05 µg/L. Alpha-cypermethrin is highly toxic to a number of aquatic arthropod taxa, but is of lower toxicity to molluscs. The short-term toxicity of the compound can be reduced by formulation of the product as an oil-enhanced suspension. Although spray drift may result in toxic effects on aquatic invertebrates, the rapid loss of alpha-cypermethrin from the water gives potential for recovery (URL 1).

Alpha-cypermethrin is highly toxic to fish. The 96-h LC_{50} values range between 0.7 and 350 µg/L (URL 1). 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 adsorbed 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 (Grayson *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 96-h LC_{50} 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 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).

Moore and Waring (2001) reported even low levels of cypermethrin in the aquatic environment having a significant long-term effect on Atlantic salmon populations through disruption of reproductive functions. They studied the effect of cypermethrin on olfactory mediated reproductive endocrine function where pheromones released by reproductively mature female salmonids are detected by the olfactory systems of the males and that this results in an increase in the levels of plasma

reproductive steroids and expressible milt. Salmon milt and ova were mixed in the presence of sub-lethal concentrations of cypermethrin and the subsequent development of the embryos was monitored. Fertilization was reduced in the cypermethrin exposed group.

Sub-lethal exposure of cypermethrin (1/10 and 1/50 of 96-h LC_{50} at 0.139 ppm) altered the biochemical, haematological parameters and enzymes of organs tissue and exerted stress on *Labeo rohita* (Indian major carp) fingerlings. Plant extracts may be useful in counteracting some of these effects (Das and Mukherjee, 2003). However, no data is present in the open literature on early life stages such as larvae. Philip *et al*. (2002) examined the carbohydrate metabolism of *Labeo rohita* subjected to sub-lethal concentration of cypermethrin under in vitro conditions. They reported that the fish adopted a compensatory mechanism to derive energy during pyrethroid toxicosis. Davit *et al*. (2004) studied the effect of the sub-lethal concentration of cypermethrin on the metabolic profile of *Cyprinus carpio*. They reported a decrease in soluble proteins, while amino acids and activities of protease, aspartate and alanine aminotransferase increased with the administration of the toxicant. They interestingly noted that ammonia content decreased but urea and glutamine increased with the dosage. Wend Rasch *et al*. (2003) investigated the effect of cypermethrin upon the composition of crustacean, rotifer, periphyton and phyhoplankton communities and observed that the effects started from the toxicant concentration of 0.13 µg/L. Stephenson *et al*. (2003) carried out acute toxicity tests with cypermethrin on *Tilapia nilotica* and found that cypermethrin was much more toxic than other commercially available pesticides such chlorfenvinphos and carbofuran.

This study investigates the toxic effects of alpha-cypermethrin on Nile tilapia, *Oreochromis niloticus* adults by the determination of 96-h LC_{50} values and evaluates behavioural changes of the fish exposed to different concentrations.

Materials and Methods

The test fish, Nile tilapia *(Oreochromis niloticus* L.) adults were obtained from the Fisheries Unit of Fisheries and Aquaculture Department of Ankara University. The specimens (av. wt. 25.03±5.35 g; av. length 11.05±1.05 cm) 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, and then the larvae were allowed to swim into the aquarium water. Test chambers were of plastic with a capacity of about 20 litres. Temperature was regulated at 24±1°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 +4ºC until stock solution preparation. The concentration of stock solution in bracket was prepared by bringing from alpha-cypermethrin to room temperature, then weighing 1020 mg alpha-cypermethrin (98%) and diluting it in 100 ml acetone to give the stock material (10 mg/ml). 200 µg/L was taken from stock solution and added to 20 litre aquaria as given 10 µg/L concentration. Experimental solutions were prepared from this stock by diluting with acetone to give the dosing concentrations of 5, 6, 7, 8 and 10 µg/L. During the experiment, dead fish were removed immediately and behavioural changes at each concentration were recorded. The dosing volume never exceeded 0.2 ml. Control group received acetone at the maximum acetone volume used in the dilution of the dosing concentrations. Test tanks were filled with 20 to litres of tap water. Some characteristics of this aquarium water were; temperature 24±1ºC, dissolved oxygen 6.9±0.2 mg/L, conductivity 0.182–0.197 mS/cm, alkalinity HCO_3 32 mg/L, calcium hardness 31.6 mg/L, NH_3-N 0.02 mg/L, NO_3-N 1.02 mg/L, NO_2-N 0.001 mg/L and pH 6.8.

Groups of fish, each consisting of 8 individuals were selected at random and placed into aerated test chambers. After 48 hours of adaptation, different concentrations of alpha-cypermethrin 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. Behavioural changes were followed closely.

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. LC_{50} and 95% confidence limits were calculated by a computer program (US EPA 1999).

Results

The number of fish deaths at a various concentrations and times were showed in Table 1. The data obtained from acute toxicity test of alpha-cypermethrin on tilapia adults were evaluated according to Finney's Probit Analysis Method and 96-h LC_{50} value (95% confidence limits) of alpha-cypermethrin was 5.99 µg/L (5.40-6.51) (Table 2, Figure 1).

No mortality was observed in the control group during the experiment. The changes in behavioural response started 1-2 hours after dosing, depending on the concentration of toxicant. The fish started to

Table 1. The number of fish deaths at a various concentrations and times

Concentration (µg/L)	Number exposed	Number responding for 24 hours	Number responding for 48 hours	Total dead fish (96-h)
5	8	-	-	-
6	8	2	4	6
7	8	1	5	6
8	8	8	-	8
10	8	8	-	8

Table 2. Acute 96-h toxicity of technical alpha-cypermethrin on tilapia *(Oreochromis niloticus)*

Point	Concentration (µg/L)	95% Confidence Limits	Intercept±SE	Slope±SE
LC 1.00	4.43	2.89-5.05	-8.79±4.00	17.75±5.07
LC 5.00	4.84	3.52-5.37		
LC 10.00	5.07	3.89-5.57		
LC 15.00	5.24	4.17-5.70		
LC 50.00	**5.99**	5.40-6.51		
LC 85.00	6.85	6.33-8.22		
LC 90.00	7.07	6.51-8.78		
LC 95.00	7.41	6.75-9.71		
LC 99.00	8.09	7.19-11.79		

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

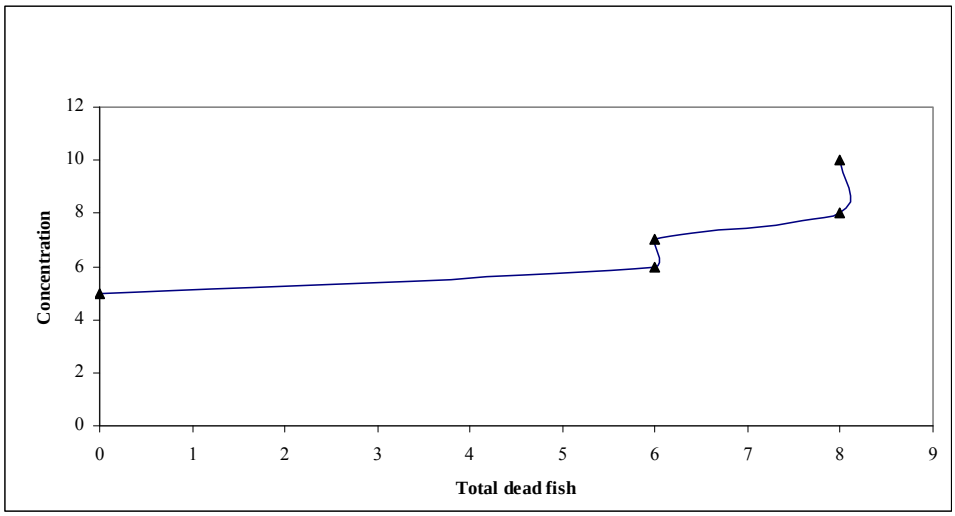

Figure 1. The number of fish deaths at a various concentrations

display intense activity one hour after exposure. They left themselves to water currents and made sudden movements at a concentration of 6 µg/L. The fish gave response when tapped on the aquaria walls at a toxicant concentration of 7 µg/L. They gave no such response at higher concentrations and made movements such as somersaulting around their own axis.

Discussion

According to Finney's Probit Analysis Method the 96-h LC_{50} value of alpha-cypermethrin in tilapia adult (*Oreochromis niloticus*) was found to be 5.99 µg/L (5.40-6.51) in our work. This shows that alpha-cypermethrin is highly toxic to fish. Yılmaz *et al.* (2004) reported that behavioural changes of male guppies manifested themselves starting at alpha-cypermethrin concentration of 15 µg/L. The LC_{50} value of alpha-cypermethrin for guppies was 17.94 µg/L. Behavioural 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 such as gill flailing, hyperactivity, loss of buoyancy and inability to remain upright. However, published experimental work on alpha-cypermethrin fish toxicity 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*).

Reddy *et al.* (1991a) reported significant changes in carbohydrate metabolism in liver, brain and gill tissues of *Tilapia mossambica* exposed to sub-lethal concentration of 0.04 ppm cypermethrin. They calculated 24-h LC_{50} as 0.2 ppm and observed a decrease in glycogen and pyruvate levels and an increase in lactate content in all tissues. Anaerobic glycolytic pathway increased and mitochondrial metabolism decreased due to pyruvate mobilization. Reddy *et al*. (1991b) also investigated the effect of sub-lethal cypermethrin concentration of 0.04 ppm on lipid metabolism of the brain, liver and gill tissues of *Tilapia mossambica* and found an increase in total lipid, lipase and free fatty acids and a decrease in glycerol content leading to simultaneous operation of lipogenesis and lipolysis during cypermethrin stress. Phospholipid levels dropped and cholesterol content increased in all the tissues.

Sublethal effects of cypermethrin and carbofuran on haematological parameters and their complete recovery were studied in *Labeo rohita* as a function of exposure time by Adhikari *et al*. (2004). Their experiments showed that exposure of *Labeo rohita* to sublethal levels of cypermethrin and carbofuran resulted in significantly ($P<0.05$) lower values for erythrocyte count (RBC), haemoglobin content (Hb), and hematocrit compared with the control group. In contrast, there was a significant increase ($P<0.05$) in leukocyte count (TLC) in the pesticide-treated group. Mean cell volume (MCV) and mean cell haemoglobin (MCH) increased in response to both pesticides during their study.

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.

Lethal concentrations of cypermethrin, dissolved either in water or acetone, were determined for freshwater catfish *Heteropneustes fossilis* at different hours of exposure by static bioassays by Saha and Kaviraj (2003). They reported that, up to 48 hours, there was no difference between LC_{50} values of aqueous and acetone solubilized cypermethrin. Seventy-two-hour LC_{50} values of aqueous cypermethrin and acetone-solubilized cypermethrin to *Heteropneustes fossilis* were 0.67 and 1.27 microg/L, respectively. Lethal values remained unchanged beyond 72 hours. The fish exposed to even lower concentration of cypermethrin (0.5 microg/L) showed hyperactivity.

Greulich and Pflugmacher (2004) studied the uptake and effects of environmentally relevant concentrations of the pyrethroid insecticide cypermethrin on two different amphibian species, *Bombina variegata* and *Rana arvalis*. The uptake from water of C-14-labeled cypermethrin (0.4 mug/L) by eggs and tadpoles of *B. variegata* was investigated. After 24 hours of exposure, 153.9 ng cypermethrin/g fresh weight were found in embryos, thus indicating that the jelly mass of the eggs does not act as a sufficient physical barrier to protect embryos from exposure to this compound. Uptake of cypermethrin into tadpoles of both species and in all exposed individuals caused dose-dependent deformities; behavioral abnormalities such as twisting, writhing, and coordinated swimming; and mortality. The observed physical and behavioural abnormities caused by environmentally relevant concentrations of cypermethrin indicate that despite detoxication of the chemical via GST-system contamination of ponds by cypermethrin could result in adverse effects on the development of amphibian embryos and tadpoles.

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 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.

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