



Investigation of acute toxicity of beta-cypermethrin on guppies *Poecilia reticulata*

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Abstract

Beta-cypermethrin, a synthetic pyrethroid pesticide and potential toxic pollutant, contaminating aquatic ecosystems was investigated in the present study for acute toxicity. Guppy fish (*Poecilia reticulata*) was selected for the bioassay experiments. The experiments were repeated three times and the 48-h LC₅₀ was determined for the guppies. The static test method of acute toxicity test was used. Water temperature was regulated at 22 ± 1 °C. In addition, behavioral changes at each beta-cypermethrin concentration were observed for the individual fish. Data obtained from the beta-cypermethrin acute toxicity tests were evaluated using the probit analysis statistical method. The 48-h LC₅₀ value for guppy was estimated as 21.4 µg/l.

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1. Introduction

Synthetic analogs of the pyrethrins, extracts from the ornamental *Chrysanthemum cinerariaefolium*, have been developed to circumvent the rapid photodegradation encountered with the insecticidal natural pyrethrins. The pyrethroids are widely used in field pest control, as household pesticides, and as veterinary and human pediculicides, and are among the most potent insecticides known (Smith and Stratton, 1986). The widespread use of these pesticides consequently leads to the exposure of manufacturing workers, field applicators, the ecosystem and finally the public to the possible toxic effects of these pesticides.

Many products containing cypermethrin are classified as “restricted use pesticides” by the US EPA be-

cause of cypermethrin’s toxicity to fish. Cypermethrin is classified as a toxicity class II (moderately toxic) chemical. Some cypermethrin formulations are designated as toxicity class III (slightly toxic) (URL 1).

Pyrethroids have been reported to be extremely toxic to fish, some beneficial aquatic arthropods (for example, lobster and shrimp); and to honey-bees in laboratory tests. The 96-h LC₅₀ of cypermethrin in rainbow trout was 8.2 µg/l and in bluegill sunfish, 1.8 µg/l (Bradbury and Coats, 1989; URL 1). Bradbury and Coats (1989) also reviewed pyrethroid toxicology in mammals, birds, amphibians and both terrestrial and aquatic invertebrates. Toxicity is highly dependent on stereochemical structure. Most products however, are mixtures of isomers. Several larvicides and adulticides including resmethrin and permethrin were evaluated for toxicity to standard test (in-house cultures) and resident organisms to measure effects of mosquito control pesticides to non-target organisms (Milam et al., 2000).

Pyrethroids are especially advantageous for use in northern climate zones, since they exhibit a negative

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temperature coefficient of toxicity. They are also considered as relatively non-persistent, therefore are not expected to biomagnify through the food chain. Maximum bioconcentration factors ranged from 698X for whole fish (deltamethrin) to 6090X (bifenthrin); cypermethrin's bioconcentration factor was 444X for whole fish using ^{14}C -cyclopropyl labeled cypermethrin and 468X using ^{14}C -benzyl labeled cypermethrin (URL 2). Toxic effects of pyrethroids on non-target organisms have been reviewed and reported to be in the ppb toxicity range (Smith and Stratton, 1986). In both the laboratory and field, adsorption of pyrethroids substantially reduces toxicity. Cypermethrin has been classified "immobile" by the US EPA (URL 2). Therefore in the field most of the affected organisms showed rapid recovery. The environmental fate and effects of synthetic pyrethroid insecticides have been summarized (Hill, 1989).

Due to their lipophilicity, pyrethroids have a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of fish to aqueous pyrethroid exposures. Fish seem to be deficient in the enzyme system that hydrolyzes pyrethroids. In mice and rats the main reaction involved in the metabolism of deltamethrin, cypermethrin or cyhalothrin is ester cleavage mainly due to the action of carboxyesterase. Metabolism in fish is largely oxidative (Demoute, 1989). Edwards et al. (1987) found that cypermethrin was metabolized and eliminated significantly more slowly by fish than by mammals or birds, which may explain this compound's higher toxicity in fish compared to other organisms. Fish make intimate contact with the surrounding water through the gills. The potential hazard to fish is due to its heavy use in many aquatic larvicidal programs. Synergistic interactions between the active ingredient and other components of the formulation should be taken into consideration when evaluating toxicity.

This study was conducted to determine the acute toxicity of beta-cypermethrin, a synthetic pyrethroid pesticide, to the guppy (*Poecilia reticulata*) using the static test system.

2. Materials and methods

Male, adult guppies were obtained from a local breeder in Ankara and brought to the laboratory within 30 min in plastic bags with sufficient air. The plastic bags were placed into the maintenance aquarium for about 30–35 min for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium water. Test chambers were glass aquaria of about 25 l capacity. Temperature was regulated at 22 ± 1 °C by using heaters. At the dosing instance air was turned off; it was on at all times, otherwise. The

water was continuously aerated for several days before putting the fish in, to remove chlorine.

Test chambers were filled with 20 l of tap water. Characteristics of this aquarium water were as follows; temperature 22 ± 1 °C, dissolved oxygen 7.2–7.9 mg/l and conductivity 0.212–0.260 mS.

Following the preliminary experiment, all determinations were repeated three times. Groups of experimental animals, each consisting of 10 individuals, were selected at random and placed into aerated aquaria. After 48 h of adaptation, the different concentrations of beta-cypermethrin in acetone were added to the experimental aquaria. During the last 24 h of adaptation, and throughout the duration of the experiment, animals were not fed. Mortality was assessed at 24, 48, 72 and 96 h after the start of the tests. Dead individuals were removed immediately. Behavioral changes were followed closely.

Technical grade (99%) beta-cypermethrin was from the Insecticide Testing Laboratory of Hacettepe University, Ankara. Composition was essentially the same as reported by Pap et al. (1996): (1*R cis*)*S* + (1*S cis*)*R* and (1*R trans*)*S* + (1*S trans*)*R* enantiomer pairs of cypermethrin in a 4:6 ratio. Beta-cypermethrin stored at +4 °C was prepared by 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 of 15, 20, 30, 45, and 50 µg/l. 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. The bioassay system was as described in standardized methods (OECD, 1993; APHA, AWWA, WEF, 1998) and the national regulation (Turkish Official Gazette, 1991). LC₅₀ and 95% confidence limits were calculated by a computer program (US EPA, 1999).

3. Results

The calculated 48-h acute LC₅₀ value (95% confidence limits) of technical beta-cypermethrin, dissolved in acetone, using a static bioassay system to adult, male guppies *P. reticulata* was 21.4 µg/l (15.6–26.4). Control mortality was zero. The results show that beta-cypermethrin is highly toxic to fish. The selected species is as recommended by the reference/standard methods (Turkish Official Gazette, 1991; OECD, 1993; APHA, AWWA, WEF, 1998). Results are in Table 1 and Fig. 1.

Observations of behavioral response of guppies were conducted at 1–8, and every 12 h during the acute toxicity tests. The control group showed normal behavior during the test period. The changes in behavioral response started 1 h after dosing in the highest two beta-cypermethrin concentrations tested. In others, changes started 2 h after dosing. The 15 µg/l (lowest) concen-

Table 1
Acute 48-h toxicity of technical beta-cypermethrin in adult male guppies (*P. reticulata*)

Point	Concentration (µg/l)	95% confidence limits	Slope ± SE	Intercept ± SE
LC 1.00	6.7017	1.74–10.73	4.62 ± 1.18	−1.14 ± 1.66
LC 5.00	9.4129	3.39–13.60		
LC 10.00	11.2819	4.84–15.49		
LC 15.00	12.7489	6.13–16.94		
LC 50.00	21.3735	15.62–26.41		
LC 85.00	35.8324	28.71–57.12		
LC 90.00	40.4917	31.77–71.57		
LC 95.00	48.5320	36.52–100.98		
LC 99.00	68.1655	46.68–195.75		

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

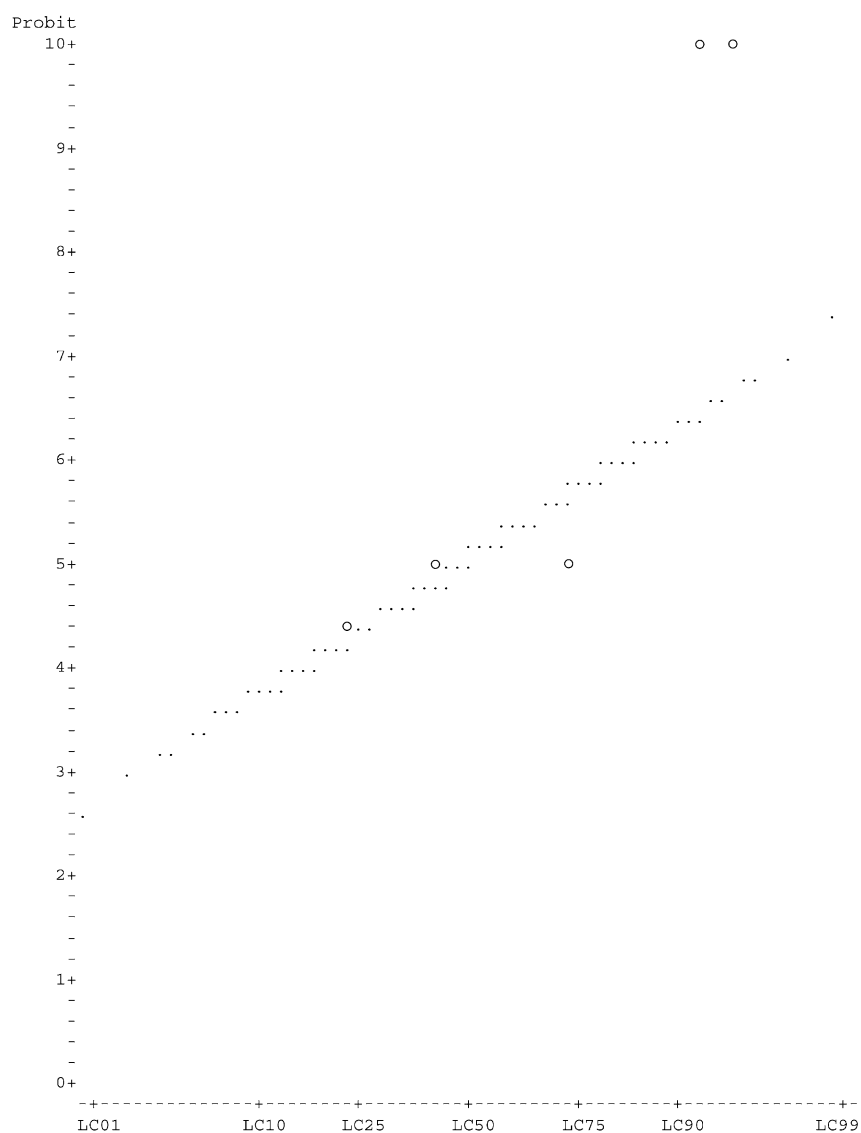


Fig. 1. Plot of adjusted probits and predicted regression line for beta-cypermethrin to guppies (*P. reticulata*).

tration had less general activity when compared with the control group. Other changes were loss of equilibrium, color darkening and staying motionless at a certain location generally at mid-water level for prolonged periods.

The highest concentration of 50 µg/l showed all responses at high levels. The loss of equilibrium, hanging vertically in water, rapid gill movement, erratic swimming, swimming at the water surface, staying vertically and “gulping for air”, prolonged and motionless laying down on the aquarium bottom, color change to yellow in the abdominal area, turning around its axis, gathering at one corner on the surface of the aquarium, backbone defects such as bending, enlargement of the eyes, and keeping the gills in open position for prolonged periods were other responses observed at all concentrations above 15 µg/l.

4. Discussion

The 48-h LC₅₀ value of beta-cypermethrin in guppies was found to be 21.4 µg/l in the present work and therefore we report beta-cypermethrin to be highly toxic to fish. The USDA National Agricultural Pesticide Impact Assessment Program’s EXTOWNET document (URL 1) reports beta-cypermethrin acute toxicity to fish in laboratory tests, in the average range LC₅₀ value of 1.8 (bluegill)–8.2 (rainbow trout) µg/l. Our results are in good agreement with the reports of other investigators using fish species; i.e. the acute LC₅₀ values are in the µg/l range (Shires, 1985; Smith and Stratton, 1986; Edwards et al., 1987; Bradbury and Coats, 1989; Reddy and Yellamna, 1991; Reddy et al., 1991; Reddy and Philip, 1994).

Shires (1985) exposed rainbow trout (*Salmo gairdneri* Richardson) to an emulsion concentrate formulation of WL85871 (FASTAC, a Shell registered trade mark of a mixture of the (1*R* *cis*)*S* and (1*S* *cis*)*R* isomers of cypermethrin). The formulation was 100 g/l EC. Analysis of water samples after the removal of suspended solids indicated that only 50% of the cypermethrin residues was present in the aqueous phase, the rest being associated with the particulate material. Cypermethrin concentration of about 2–5 µg/l was toxic to the rainbow trout.

Smith and Stratton (1986) have compiled the toxic effects of cypermethrin on fish species as follows (LC₅₀; µg/l): Atlantic salmon (*Salmo salar*) 96-h 2.0; *cis*-cypermethrin to rainbow trout (*S. gairdneri*) 96-h 6.0; *cis*-cypermethrin to mosquito fish (*Gambusia affinis*) 24-h 9.0 and 48-h 8.0; *cis*-cypermethrin to desert pupfish (*Cyprinodon macularius*) 24-h 10.0 and 48-h 6.0.

Edwards et al. (1987) found *cis*- and *trans*-cypermethrin brain levels associated with toxic signs as 0.25

and 0.17 µg/g trout brain, respectively. They attributed the high toxicity of pyrethroids to fish to a combination of three factors: a sensitive central nervous system (cf. mammal), rather slow hydrolytic detoxification, and the route of exposure (direct absorption via the gills into the bloodstream).

Bradbury and Coats (1989) have reviewed the toxicology of pyrethroids in mammals, birds, fish, amphibians, and invertebrates (terrestrial and aquatic) and cited 96-h cypermethrin toxicity (LC₅₀; µg/l) to carp (*Cyprinus carpio*) 0.9–1.1; brown trout (*Salmo trutta*) 1.2; rainbow trout (*S. gairdneri*) 0.5; *Scardinius erythrophthalmus* 0.4; *Tilapia nilotica* 2.2 µg/l.

Reddy and Yellamna (1991) reported significant changes in carbohydrate metabolism in liver, brain and gill tissues of *Tilapia mossambica* (a freshwater teleost) exposed to sublethal concentration of 0.04 ppm cypermethrin. They calculated 24-h LC₅₀ as 0.2 ppm. A decrease in glycogen and pyruvate levels and an increase in lactate content was observed in all tissues. Aerobic glycolytic pathway increased; mitochondrial metabolism decreased due to oxygen uptake and pyruvate mobilization. Reddy et al. (1991) also investigated effects of sublethal cypermethrin concentration of 0.04 ppm on lipid metabolism of the brain, liver and gill tissues of *T. mossambica* and found increases in total lipid, lipase and free fatty acids; decrease in glycerol content leading to simultaneous operation of lipogenesis and lipolysis during cypermethrin stress. Phospholipid levels dropped, while cholesterol content increased in all the tissues.

Reddy and Philip (1994) used a sublethal concentration of 20 µg/l of 96% technical cypermethrin (RS) to expose male *C. carpio* for 6, 12, 24 and 48 h and studied acetylcholinesterase (AChE) and ATPase inhibition in vivo to elucidate mechanism of toxicity. The activity of AChE was inhibited with an elevation of acetylcholine (ACh) content in gill, brain, liver and muscle. Both Mg²⁺ and Na⁺–K⁺ dependent ATPases were inhibited, probably leading to a decrease in nerve impulse transmission and impairment of ionic regulation and salt uptake from the medium.

Rainbow trout exposed to 10 µg/l *cis*-cypermethrin exhibited toxic signs of gill flailing and hyperactivity, followed by loss of buoyancy and trim control. The time taken for half of the dosed group to exhibit the toxic effects (TE₅₀) has been reported as median time at which 50% of the treated individuals were showing initial toxic signs was 2.5 h for *cis*- and 2.0 h for *trans*-isomer (Edwards et al., 1986).

US EPA states cypermethrin’s bioconcentration factor as 444X for whole fish using ¹⁴C-cyclopropyl labeled cypermethrin and 468X using ¹⁴C-benzyl labeled cypermethrin (URL 2). Although under field conditions cypermethrin is considered to pose less risk due to its high

adsorption to soil, these data should be considered when assessing possible/potential ecosystem risks.

Examining cypermethrin toxicity to other aquatic organisms, the work of Clark et al. (1987) reported the cypermethrin 96-h LC₅₀ for grass shrimp (*Palaemonetes pugio*) as 0.016 µg/l. The 24-h topical and aqueous LD₅₀ 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 for topical and aqueous (Siegfried, 1993). The author concluded that exposure of aqueous organisms to pyrethroids may also secondarily induce an osmotic imbalance that contributes to their toxicity.

It is interesting to note that only a few studies on the acute toxicity to fish of one of the most toxic pyrethroids, namely beta-cypermethrin, are available in the open literature.

5. Conclusion

Beta-cypermethrin is a highly toxic synthetic pyrethroid pesticide widely used in agriculture. Special attention is drawn to its heavy use in mosquito control programs which necessitates in-depth sub-chronic and chronic toxicity tests to fish species and to non-target species to be undertaken. In addition, potential risk from beta-cypermethrin metabolites should be investigated to get a more complete picture in terms of toxicity. The low toxicity of beta-cypermethrin to mammals may be misleading at this point in terms of ecotoxicology and lead to extrapolation problems to aquatic species. Delistraty (2000) in the study of examining relationships among physicochemical properties and acute toxicity endpoints of 231 chemicals in rats and trout concluded that trout aquatic LC₅₀ was predicted from rat inhalation LC₅₀ with moderate success. Therefore such data are useful in ecological risk assessment but there are limitations and uncertainties. Further work with toxicity testing methods directly on fish will be very useful in assessing possible ecological risk assessment 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 insufficient.

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URL 2: <http://www.epa.gov/scripoly/sap/1999/february/pyreth.pdf>.