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# Investigation of acute toxicity of chlorpyrifos-methyl on Nile tilapia (*Oreochromis niloticus* L.) larvae

Ali Gül *

*Faculty of Education, Department of Biology, University of Gazi, Teknikokullar, 06500 Ankara, Turkey*

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**Abstract**

Chlorpyrifos-methyl, a wide-spectrum organophosphorus insecticide and potential toxic pollutant contaminating aquatic ecosystems, was investigated for acute toxicity. Larvae of the freshwater fish Nile tilapia (*Oreochromis niloticus* L.) were selected for the bioassay experiments. The experiments were repeated three times and the 96 h $LC_{50}$ was determined for the larvae. The static test method for assessing acute toxicity was used. Water temperature was maintained at 25 ± 1 °C. In addition, behavioral changes at each chlorpyrifos-methyl concentration were observed for the individual fish. Data obtained from the chlorpyrifos-methyl acute toxicity tests were evaluated using Finney's probit analysis statistical method. The 96 h $LC_{50}$ value for Nile tilapia larvae was calculated to be 1.57 mg/l.


*Keywords:* Chlorpyrifos-methyl; Nile tilapia; *Oreochromis niloticus*; FQPA; Behavioral effects; Larvae

## 1. Introduction

Chlorpyrifos-methyl [CAS 5598-13-0, *O,O*-dimethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate, formula: $C_7H_7Cl_3NO_3PS$] is a wide spectrum insecticide of the organophosphorus group (URL 1; URL 2). It is hydrolyzed more rapidly at higher pH and undergoes rapid photodecomposition in UV light. It is mainly effective against rice stem borer, aphids, cutworms, plant and leaf hoppers, mole crickets and some moths, and stored grain pests. Currently, the main use is on stored grain. Mode of action is by cholinesterase inhibition. Maximum residue limits have been recommended by the joint FAO/WHO Meeting on pesticide residues (URL 2).

Chlorpyrifos-methyl was initially registered in 1985; but it is being evaluated currently with other organophosphate pesticides under the FQPA Tolerance Reassessment Program and the Interim Risk Management Decision (TRED) by the United States Environmental Protection Agency (US EPA, 2001; US EPA, 2002). Any significant data gaps, including developmental neurotoxicity studies and chemical-specific occupational exposure studies are required to be filled for all organophosphate pesticides under FQPA to evaluate their safety. Registrants of chlorpyrifos-methyl (i.e. Dow Agrosciences) have requested voluntary cancellation of their products rather than develop the additional data required by EPA to complete the toxicology data base. When the cumulative risk assessment for the organophosphate pesticides has been completed, EPA will issue its final tolerance reassessment decision for chlorpyrifos-methyl. The international regulatory status

* Tel.: +90 312 2126470; fax: +90 312 2228483.
*E-mail addresses:* aligul@gazi.edu.tr, ali@gef.gazi.edu.tr

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is: "Not Listed" under UNEP Persistent Organic Pollutants, UNEP Prior Informed Consent Chemicals, WHO Obsolete Pesticide lists, and "Unlikely to be Hazardous" under WHO Acute Hazard Ranking, where the parent chemical chlorpyrifos is "II, Moderately Hazardous" (URL 3). While EPA and Dow are discussing the phase-out process and alternatives to chlorpyrifos-methyl, it still can be used on stored barley, oats, rice, sorghum and wheat (URL 4; URL 5). Hazard characterization and human health risk assessment including dietary risk can be found in the EPA web sites (URL 6; URL 7). In a recent review on the microbiological, biological, and chemical weapons of warfare and terrorism, Greenfield et al. (2002) estimated the lethal dose of the parent compound, chlorpyrifos liquid, to be approximately 15 000 000 μg/person (estimated from published rat $LD_{50}$; assuming 75 kg person), drawing attention to health risks of this group of agricultural insecticides.

It is toxic to non-target organisms such as shrimps, crabs, fish and *Daphnia* ($LC_{50}$ = 1.11 ppb/48 h) (URL 8). Although most of the possible ecological risk of chlorpyrifos exist in the open literature, there is very little published on the aquatic toxicity of chlorpyrifos-methyl on early life stages of fish. The Columbia Environmental Research Center Acute Toxicity Database (URL 9) reports 96 h technical (97%) chlorpyrifos toxicity to 0.30 g Lake trout as 0.227 mg/l in a static test system at 12 °C.

The aim of this study is to provide fish larvae acute toxicity data for chlorpyrifos-methyl to contribute to filling the ecological risk data gap. This work was conducted to determine the acute toxicity of chlorpyrifos-methyl, an organophosphothionate, to a standard freshwater test species, Nile tilapia (*Oreochromis niloticus* L.) larvae using the static test system.

## 2. Materials and methods

Nile tilapia larvae (av. wt. 0.0125 g ± 0.00202 S.D.; av. length 0.995 cm ± 0.0099 S.D.) were obtained by artificial reproduction from a local breeder. Test aquaria had an approximate capacity of 5 l. Temperature was maintained at 25 ± 1 °C (24.5–26.2) by using heaters. The tanks were aerated at all times except during the time of dosing. The water was continuously aerated for several days to remove chlorine prior to putting the tilapia larvae into the tanks.

Test chambers were filled with 3 l of tap water. Water quality characteristics were as follows; temperature 25 ± 1 °C, dissolved oxygen 6.4–6.7 mg/l (both before and after the tests), calcium hardness 31.6 mg/l, conductivity 0.189–0.205 mS and pH 6.7–6.9.

The range finding tests showed the following mortality: 0.7 mg/l dose no mortality; 1.66 mg/l 7 out of 10; 3.33 mg/l 10 out of 10. Following the preliminary experiments, all determinations were repeated three times. Groups of larvae, each consisting of 10 individuals, were selected at random and placed into the aerated aquaria. After 48 h of adaptation, different concentrations of chlorpyrifos-methyl in acetone were added to the aquaria. During the 48 h of adaptation, and throughout the duration of the experiment, the larvae 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 (92%) chlorpyrifos-methyl was obtained from the Insecticide Testing Laboratory of Hacettepe University, Ankara (source: Shenzen Co. Ltd., China). Technical chlorpyrifos-methyl was stored at +4 °C until stock solution preparation. Stock solution was prepared by bringing chlorpyrifos-methyl to room temperature then weighing a certain amount (cf. below) and diluting it in acetone (all manipulations carried out in darkness) to give the stock material. Dosing solutions were prepared from this stock by diluting with acetone to give the dosing concentrations of 1.0, 1.2, 1.3, 1.5 and 1.7 mg/l. The dosing volume did not exceed 0.2 ml. Control group received acetone at the maximum acetone volume used (0.2 ml) in the dilution of the dosing concentrations. 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 LC50 software program, version 1.00 computer program developed by EPA (US EPA, 1999).

## 3. Results

The calculated 96 h acute $LC_{50}$ value (95% confidence limits) of technical chlorpyrifos-methyl, dissolved in acetone, using a static bioassay system for Nile tilapia larvae was 1.57 mg/l (1.43–1.98). Control mortality was zero. Results are depicted in Table 1.

Observations of behavioral response of tilapia larvae 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. The two lowest concentrations of 1.0 and 1.2 mg/l had similar behavior with the control group. Observed behavioral changes/effects were in the 1.3, 1.5 and 1.7 mg/l concentration aquaria: the larvae were turning around their axis, but in an upside-down position, with very slow motion in comparison with the controls. Color fading and no response when gently touched with a glass rod was noted. In the highest two concentrations all mentioned above responses were observed, plus a loss of equilibrium (swimming to the water surface and then falling vertically to the aquarium bottom).