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Chemosphere 60 (2005) 93–96

CHEMOSPHERE

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Investigation of acute toxicity of chlorpyrifos-methyl on guppy *Poecilia reticulata*

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Received 3 May 2004; received in revised form 19 August 2004; accepted 29 November 2004
Available online 26 January 2005

Abstract

Static bioassays were made to determine acute toxicity of chlorpyrifos-methyl, a wide spectrum organophosphorus insecticide and potential toxic pollutant of aquatic ecosystem, Guppy fish (*Poecilia reticulata*). Bioassays were made at a regulated temperature of 22 ± 1 °C and were repeated three times. Lethal doses of the insecticides were determined using LC50 software programme of U.S. EPA based on Finney's Probit Analysis statistical method. The 96 h LC_{50} value and 95% confidence limit of chlorpyrifos-methyl for Guppy was estimated as 1.79 (1.47–2.10) mg/l. The fish exposed to chlorpyrifos-methyl exhibited behavioral changes in the form of neurotoxin toxicity: less general activity than control group, loss of equilibrium, erratic swimming and staying motionless at a certain location generally at mid-water level for prolonged periods. The 1 mg/l (lowest) concentration had similar behavior (NOEC) with the control group.

Keywords: Chlorpyrifos-methyl; Acute toxicity; Guppy; *Poecilia reticulata*; Bioassay; FQPA; Behavioral effects

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 poorly soluble in water (4 mg/l), readily soluble in organic solvents such as acetone, benzene and chloroform and stable under normal storage conditions. It is hydrolyzed more rapidly at higher pH and undergoes rapid photodecomposition in UV light. The insecticide is mainly effective against rice stem borer, aphids, cutworms, plant and leaf hoppers, mole crickets and some moths; stored grain pests. Present main use is on stored grain. Mode of action is cholinesterase inhibition (Boone and Chambers, 1997; Levin et al., 2003). Maximum residue limits of the insecticide have been recommended by the joint FAO/WHO Meeting on Pesticide Residues (URL 2).

Chlorpyrifos-methyl was initially registered in 1985 but is being evaluated with other organophosphate pesticides under FQPA Tolerance Reassessment Progress and Interim Risk Management Decision (TRED) by the United States Environmental Protection Agency (U.S. EPA, 2001, 2002). The significant data gaps including developmental neurotoxicity studies are required for all organophosphate pesticides under FQPA to evaluate their safety to children, and chemical-specific occupational exposure studies. Registrants of chlorpyrifos-methyl (Dow Agrosciences) have requested voluntary cancellation of their products rather than develop the additional

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doi:10.1016/j.chemosphere.2004.11.093

data requested by EPA to complete the toxicology data base. EPA will issue its final tolerance reassessment decision for chlorpyrifos-methyl as soon as the cumulative risk assessment for the insecticide is completed. Present international regulatory status is: ''Not Listed'' under UNEP Persistent Organic Pollutant, UNEP Prior Informed Consent Chemical, 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, for now, it can still 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 and URL 7). Recently in a review on the microbiological, biological, and chemical weapons of warfare and terrorism, Greenfield et al. (2002) estimated lethal dose of the parent compound chlorpyrifos liquidas approximately 15 000 000 μg/person, drawing attention to health risks of this group of agricultural insecticides.

It is toxic to non-target organisms such as shrimps, crabs, fish (96 h LC_{50} for rainbow trout: 12.6 ppb) and *Daphnia* (48 h LC_{50} for daphnia: 1.11 ppb) (URL 8). Although most of the information on possible ecological risk of chlorpyrifos exist in the open literature, there is very little published on the aquatic toxicity of chlorpyrifos-methyl.

The Columbia Environmental Research Center Acute Toxicity Database (URL 9) reports 96 h chlorpyrifos-methyl toxicity to various standard fish test species in static test system as:

Rainbow trout (1.40 g) technical grade 301 μg/l at 13 °C
Brook trout (1.15 g) technical grade 200 μg/l at 12 °C
Brook trout (1.15 g) 61% emulsifiable concentrate 100 μg/l at 12 °C
Fathead minnow (0.90 g) technical grade 678 μg/l at 18 °C.

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. The aim of this study is to provide fish acute toxicity data for chlorpyrifos-methyl to contribute to the significant ecological risk data gap. Therefore, static bioassays were made to determine LC_{50} value of chlorpyrifos-methyl to Guppy (*Poecilia reticulata*).

2. Materials and methods

Male, adult specimens of Guppy 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 time of dosing 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 6.3–6.6 mg/l and conductivity 0.189–0.207 mS, French hardness 21–24 FS°, total ammonia 0.017–0.021 mg/l, nitrite 0.008–0.009 mg/l, nitrate 0.11–0.16 mg/l.

Technical grade (92%) chlorpyrifos-methyl was obtained from the Insecticide Testing Laboratory of Hacettepe University, Ankara (source: Shenzen Co. Ltd., China) and 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 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 1, 1.5, 2, 2.5, 3 mg/l. 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 chlorpyrifos-methyl 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.

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. Each bioassay was repeated three times. The bioassay system was as described in standardized methods (OECD, 1993; APHA, 1998) 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 (U.S. 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 to adult, male Guppy *Poecilia reticulata* was 1.79 mg/l (1.47–2.10). Control mortality was zero. Results are in Table 1 and Fig. 1.