

Investigation of acute toxicity of fenitrothion on peppered corydoras (*Corydoras paleatus*) (Jenyns, 1842)

Rabia Sarikaya, Mahmut Selvi, Figen Erkoç *

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

Received 16 July 2003; received in revised form 20 February 2004; accepted 21 April 2004

Abstract

Fenitrothion, as an organophosphothionate insecticide, is a contact insecticide and selective acaricide, also used as a vector control agent for malaria in public health programs. A 96 h LC₅₀ value of fenitrothion, a potential toxic pollutant contaminating aquatic ecosystems, was determined on the adult peppered corydoras (*Corydoras paleatus*). The experiments were repeated three times. The static test method of acute toxicity test was used. Water temperature was regulated at 23 ± 1 °C. In addition, behavioral changes at each fenitrothion concentration were observed for the individual fish. Data obtained from acute toxicity tests were evaluated using the Probit Analysis Statistical Method. The 96 h LC₅₀ value for peppered corydoras was estimated as 3.51 mg/l.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Fenitrothion; Acute toxicity; Peppered corydoras; *Corydoras paleatus*; Bioassay; Behavioral effects

1. Introduction

Fenitrothion [CAS Number: 122-14-5, *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] is a contact-acting organophosphorus pesticide which inhibits acetylcholinesterase (AChE) activity, thus disrupting the nervous system. In view of its broad-spectrum action, it is widely used against insect pests and mites. Most fenitrothion applied in Europe is used in agriculture, but it is also used in conjunction with pyrethroids to protect stored grain against insect damage, and in a number of domestic ant and fly killers. It is effective against a wide range of pests, i.e. penetrating, chewing and sucking insect pests (for example, coffee leafminers, locusts, rice stem borers, wheat bugs, flour beetles, grain beetles, grain weevils) on cereals, cotton, orchard fruits, rice, vegetables, and forests. It may also be used as a fly,

mosquito, and cockroach residual contact spray for farms and public health programs. Fenitrothion is also effective against household insects and all of the nuisance insects listed by the World Health Organization, as well as an effective vector control agent for malaria. Fenitrothion is non-systemic, and non-persistent.¹

It is preferred in some countries where parathion has been banned. Products containing fenitrothion must bear the signal word “Caution” on their label. Fenitrothion was introduced in 1959 both by Sumitomo Chemical Company and Bayer Leverkusen and later by American Cyanamid Company.

Fenitrothion is used as a conventional chemical insecticide to control locusts and grasshoppers causing significant damage during the short rainy season and require frequent control measures in the Sahel (Arthur et al., 2003). Field toxicity as a locust control agent in Madagascar showed vegetation-dwelling and flying insects were moderately affected by fenitrothion (Peveling

* Corresponding author. Tel.: +90-312-212-6470/3741; fax: +90-312-222-8483.

E-mail address: erkoc@gazi.edu.tr (F. Erkoç).

¹ <http://ace.orst.edu/info/extoxnet/pips/fenitrot.htm>

et al., 1999). In studies using laboratory animals, fenitrothion generally has been shown to be of moderate to high acute toxicity by the US EPA. However, high acute risk to freshwater invertebrates is expected from a single application.² The acute toxicity of fenitrothion to mammals is considered to be low in the range LD₅₀: 250 to >3000 mg/kg. Chronic symptoms in humans include: general malaise, fatigue, headache, loss of memory and ability to concentrate, anorexia, nausea, thirst, loss of weight, cramps, muscular weakness and tremors. At sufficient dosage produces typical cholinergic poisoning. Oral LD₅₀ for chickens was reported as 28 mg/kg. It is highly toxic to species such as the honeybee, spider mites and aquatic invertebrates such as *Daphnia* (Fawell and Hedgecote, 1996).² It was shown to be significantly toxic in chronic tests on *Daphnia magna*, another standard aquatic test species (Sakai, 2002).

Literature LC₅₀ values for fish toxicity vary among species. In general fenitrothion is considered moderately toxic to fish.^{1,2} The 96 h LC₅₀ was 1.7 ppm for brook trout, 3.8 ppm for bluegill sunfish, various North American fish species in the range 2–12 µg/l, 2.1 mg/l and 2.6 mg/l for *Oryzias latipes* and mullet *Mugil cephalus*. For 48 h acute toxicity: carp 2.0–4.1 mg/l, *Gambusia* 2.6 mg/l (0.25–0.60 g sized fish). Potential hazard to birds and aquatic organisms was the criterion based on the classification of fenitrothion as “Restricted-Use Pesticide”.^{1,3} Fenitrothion is readily degraded by microorganisms found in sludge, soil and sediment by dealkylation, hydrolysis, oxidation and reduction. Photolysis is also important in degradation.^{2,3}

Fenitrothion neurotoxicity to honeybees was also shown by the inhibition of acetylcholinesterase activity; the percentage inhibition exceeded 60% at 0.2 nmol/bee (Bendahou et al., 1999). Efficiency of fenitrothion against the isopod parasite, *Ceratothoa gaudichaudii* and its safety to the host fish, *Salmo salar* were studied by Sievers et al. (1995). Fenitrothion was found toxic to fish at the tested concentrations. However, it was not effective to two sizes of parasites.

Edith et al. (2003) investigated the, histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus pesticide in water and food. They examined the effect of contamination, through water or food, of a sublethal dose of the organophosphate methyl parathion in tissues that are responsible for absorption (gills, intestine) and metabolism (liver), in the freshwater fish *C. paleatus*. The researchers reported that, epithelial hyperplasia, edema and detachment occurred in gill respiratory lamellae, diminishing sooner

after contamination by food than after contamination through water.

This study was conducted to determine the acute toxicity of fenitrothion, an organophosphothionate, to the *C. paleatus* using the static test system.

2. Materials and methods

Technical grade (95%) fenitrothion was from the Insecticide Testing Laboratory of Hacettepe University, Ankara (source: Shenzen Co. Ltd., China). Technical fenitrothion was stored at +4 °C until stock solution preparation. Stock solution was prepared by bringing fenitrothion 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 2.5, 3.0, 3.5, 4.0 and 4.5 mg/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). The selected species is also as recommended in these references. LC₅₀ and 95% confidence limits were calculated by a computer program (US EPA, 1999).

Male, adult peppered corydoras 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 fish were allowed to swim into the aquarium water. Test chambers were glass aquaria of about 25 l capacity. Temperature was regulated at 23 ± 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 23 ± 1 °C, dissolved oxygen 5.9–6.1 mg/l and conductivity 0.200–0.245 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.

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 fenitrothion 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. Following the preliminary experiment, all determinations were re-

² www.epa.gov/pesticides/a-z, EPA reregistration eligibility decision (RED) document.

³ www.ag.uiuc.edu/~vista/pdf-pubs/iapm98/ch24.pdf

peated three times. Behavioral changes were followed closely.

3. Results and discussion

The calculated 96 h acute LC₅₀ value (95% confidence limits) of technical fenitrothion, dissolved in acetone, using a static bioassay system to adult, male peppered corydoras (*C. paleatus*) was 3.51 mg/l (3.21–3.82). Control mortality was zero. LC₅₀ estimates for 24, 48 and 72 h are given together with 96 h for comparison. The LC₅₀ values were all very close. Results are given in Tables 1 and 2. Actual experimental mortalities are depicted in Table 3. The results show that fenitrothion is highly toxic to fish; but it is less toxic to peppered corydoras than to most other species. Our results are close to the 48 h *Gambusia* toxicity, 2.6 mg/l, 96 h *Oryzias* and *Mugil* 2.1 and 2.6 mg/l, respectively.^{1,2}

Observations of behavioral response of peppered corydoras 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 2.5 mg/l

Table 3
Mortalities of peppered corydoras exposed to technical fenitrothion during 24, 48, 72 and 96 h

Concentration (mg/l)	Mortality (number of dead fish out of 10 fish exposed)			
	24 h	48 h	72 h	96 h
2.5	0	0	0	0
3.0	2	2	3	3
3.5	4	4	4	5
4.0	4	5	5	6
4.5	8	10	10	10

Ten fish were exposed in each aquarium, at each concentration.

(lowest) concentration had similar behavior with the control group. Observed behavioral changes/effects were typical 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. Fish exposed to 3.0 mg/l showed less general activity with occasional loss of equilibrium. Loss of equilibrium become more frequent in the 3.5 mg/l concentration. The 4.0 concentration group stayed motionless close to the water surface and

Table 1
Acute 96 h toxicity of technical fenitrothion in adult male peppered corydoras (*Corydoras paleatus*)

Point	Concentration (mg/l)	95% confidence limits	Slope ± SE	Intercept ± SE
LC 1.00	2.30	1.57–2.67	12.71 ± 3.01	−1.93 ± 1.66
LC 5.00	2.60	1.96–2.92		
LC 10.00	2.78	2.21–3.07		
LC 15.00	2.91	2.38–3.18		
LC 50.00	3.51	3.21–3.82		
LC 85.00	4.23	3.87–5.10		
LC 90.00	4.42	4.01–5.51		
LC 95.00	4.72	4.22–6.20		
LC 99.00	5.34	4.62–7.76		

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

Table 2
Comparison of the acute toxicity of technical fenitrothion in adult male peppered corydoras (*Corydoras paleatus*) for 24, 48, 72 and 96 h

Point	Concentration (mg/l) 24 h	Concentration (mg/l) 48 h	Concentration (mg/l) 72 h	Concentration (mg/l) 96 h
LC 1.00	2.24	2.46	2.31	2.30
LC 5.00	2.63	2.76	2.63	2.60
LC 10.00	2.86	2.94	2.82	2.78
LC 15.00	3.03	3.06	2.95	2.91
LC 50.00	3.87	3.66	3.61	3.51
LC 85.00	4.94	4.37	4.40	4.23
LC 90.00	5.23	4.56	4.61	4.42
LC 95.00	5.70	4.85	4.95	4.72
LC 99.00	6.69	5.45	5.64	5.34

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

later fell to the aquarium bottom in an uncontrolled manner. The highest concentration of 4.5 mg/l showed all responses at high intensities: the loss of equilibrium, hanging vertically in water, rapid gill movement, erratic swimming, sudden swimming motion in a spiral fashion, after long periods of motionlessness, prolonged and motionless lying down on the aquarium bottom and suddenly starting to move. Our results are in agreement with Sancho et al. (1998).

It is interesting to note that only a few studies on the acute toxicity of one of the most aquatic toxic organophosphates, namely fenitrothion, to fish exist in the open literature. Fenitrothion is a highly toxic insecticide widely used in agriculture. Here 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 fenitrothion metabolites should be investigated to get a more complete picture in terms of toxicity. Matsushita et al. (2003) reported amino-fenitrothion, an anaerobic degradation metabolite of fenitrothion, and other unidentified metabolites to be mutagenic in the *Salmonella* mutagenic assay.

The low toxicity of fenitrothion 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 fenitrothion formulations, toxicity tests with formulations must be included together with active ingredient tests. Using only the active ingredient in the tests is insufficient.

Acknowledgements

The authors would like to thank Dr. Oner Koçak, of Hacettepe University, for a generous gift of 95% fenitrothion used in the experiments.

References

- APHA, AWWA, WEF, 1998. Standard Methods for the Examination of Water and Wastewater. Washington, DC.
- Arthur, S., Thomas, M.B., Langewald, J., 2003. Field observations of the effects of fenitrothion and *Metarhizium anisopliae* var. *acidum* on non-target ground dwelling arthropods in the Sahel. *Biol. Cont.* 26, 333–340.
- Bendahou, N., Bounias, M., Fleche, C., 1999. Toxicity of cypermethrin and fenitrothion on the hemolymph carbohydrylates, head acetylcholinesterase, and thoracic muscle Na⁺, K⁺-ATPase of emerging honeybees (*Apis mellifera mellifera* L.). *Ecotoxicol. Environ. Saf.* 44, 139–146.
- Delistraty, D., 2000. Acute toxicity to rats and trout with a focus on inhalation and aquatic exposures. *Ecotoxicol. Environ. Saf.* 46, 225–233, doi:10.1006/eesa.1999.1906.
- Edith, F., Flavia, S., Silvia, R., Ana Cristina, C.V., Sandra, F., 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicol. Environ. Saf.* 54, 119–130.
- Fawell, J.K., Hedgecote, S., 1996. Derivation of acceptable concentrations for the protection of aquatic organisms. *Environ. Toxicol. Pharmacol.* 2, 115–120.
- Matsushita, T., Matsui, Y., Kazunori, I., Inoue, T., 2003. Contribution of metabolites to mutagenicity during anaerobic biodegradation of fenitrothion. *Chemosphere* 50, 275–282.
- OECD (Organization for Economic Co-operation and Development), 1993. OECD Guidelines for Testing of Chemicals. OECD, Paris.
- Peveling, R., Rafanomezantsoa, J.-J., Razafinirina, R., Tovonkery, R., Zafimaniry, G., 1999. Environmental impact of the locust control agents fenitrothion, fenitrothion-esfenvalerate and triflumuron on terrestrial arthropods in Madagascar. *Crop. Protec.* 18, 659–676.
- Sakai, M., 2002. Use of chronic tests with *Daphnia magna* for examination of diluted river water. *Ecotoxicol. Environ. Saf.* 53, 376–381.
- Sancho, E., Ferrando, M.D., Ten, A., Lleo, C., Andreu-Moliner, E., 1998. Sublethal bioconcentration of fenitrothion in the blood and brain of the European eel. *Bull. Environ. Contam. Toxicol.* 60, 809–815.
- Sievers, G., Palacios, P., Inostroza, R., Dölz, H., 1995. Evaluation of the toxicity of 8 insecticides in *Salmo salar* and the in vitro effects against the isopoda parasite, *Ceratothoa gaudichaudii*. *Aquaculture* 134, 9–16.
- Turkish Official Gazette (Resmi Gazete), 1991. Su Kirliliği ve Kontrolü Yönetmeliği Numune Alma ve Analiz Metodları Tebliği. Zehirlilik Seyreltme Faktörü (ZSF) Tayini. Tarih: 7.1.1991, Sayı: 20106.
- US EPA, 1999. LC50 Software Program, version 1.00. Center for Exposure Assessment Modeling (CEAM) Distribution Center.