Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Toxicology and Pharmacology 22 (2006) 20–26
ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY ETAP
www.elsevier.com/locate/etap

Effects of water quality and fish size on toxicity of methiocarb, a carbamate pesticide, to rainbow trout

Ilhan Altinok [a,*], Erol Capkin [a], Siyami Karahan [b], Muhammet Boran [a]

[a] *Karadeniz Technical University, Surmene Faculty of Marine Sciences, Department of Fisheries Technology Engineering, 61530 Surmene, Trabzon, Turkey*
[b] *Kirikkale Universitesi, Veteriner Fakültesi, 71451 Kampus, Kirikkale, Turkey*

Received 21 June 2005; accepted 19 November 2005
Available online 18 January 2006

Abstract

The acute toxicity of methiocarb in juvenile rainbow trout (*Oncorhynchus mykiss*, 3.25 ± 0.79 g) was evaluated in glass aquaria under static conditions. Nominal concentrations of methiocarb in the toxicity test ranged from 1.25 to 7.50 mg L^{-1}. The concentrations of methiocarb that killed 50% of the rainbow trout within 24-h (24-h LC_{50}), 48-h LC_{50}, 72-h LC_{50}, and 96-h LC_{50} were 5.43 ± 0.19, 5.04 ± 0.18, 4.95 ± 0.19, and 4.82 ± 0.21 mg L^{-1} (95% confidence limits), respectively. Mortality of fish increased with increasing water temperature. Increasing alkalinity from 19 mg L^{-1} as $CaCO_3$ to 40, 60, or 90 mg L^{-1} as $CaCO_3$ significantly decreased mortality of fish. Total hardness ranging from 50 mg L^{-1} as $CaCO_3$ to 147 mg L^{-1} as $CaCO_3$ did not affect mortality of fish exposed to methiocarb. Fish exposed to methiocarb had histological alterations such as lamellar edema, separation of epidermis from lamellae, and lamellar fusion. Methiocarb exposed fish had necrosis between molecular and granular layer of cerebellum where Purkinje cells present. Results indicate that alkalinity, temperature, and fish size affect methiocarb toxicity of rainbow trout.

Keywords: Mesurol; Acute toxicity test; Pesticide; Alkalinity; Hardness; *Oncorhynchus mykiss*

1. Introduction

Pesticides can enter water through surface runoff, leaching, and/or erosion. Also, drift, evaporation, and wind erosion can carry pesticide residues into the atmosphere that can lead to contamination of surface waters via precipitation (Dubus et al., 2000; Hamers et al., 2001). Whether they are dissolved in water or carried by sediment, pesticides that are carried off-site can contaminate surface waters (Willis and McDowell, 1982; Capel et al., 2001). Improper cleaning or disposal of containers, as well as mixing and loading pesticides in areas where residues or run-off are likely to threaten surface waters, are other potential sources of contamination. The extent to which a pesticide runs off an agricultural field is determined by the unique combination of climatic, soil, and management factors that characterize each field, crop, and year combination (Wauchope, 1978; Weber et al., 1980; Leonard, 1988; Willis and McDowell, 1982).

Methiocarb [mesurol; 3,5-dimethyl-4-(methylthio) phenyl methylcarbamate] has been used since the 1960s as pesticide for a variety of invertebrate pests and also used as a bird repellent on fruit crops (Hayes and Laws, 1991). In water, methiocarb breaks down to methiocarb sulphoxide and methiocarb sulphone, which are also toxic to aquatic animals (Menzie, 1974; Hayes and Laws, 1991). Methiocarb is an insecticide that inhibits acetylcholinesterase activity in the nervous system (Ecobichon, 1996; Hoffman et al., 1996; Taylor, 1996a,b).

Methiocarb is one of the most frequently used pesticides to control *Balaninus nucum*, *Palomena prasina*, *Lymantria dispar*, *Xyleborus dispar*, *Agelastica alni*, and *Obera linearis*. Suggested application rate of methiocarb is 150 g/acre (Bayer Crop Science AG, Frankfurt, Germany). Other than target pests, methiocarb can affect non-targeted species such as aquatic animals. In general, methiocarb is moderately toxic to aquatic organisms and the toxic concentration depends on the fish species (Johnson and Finley, 1980). However, no information is available about the effects of water hardness, alkalinity, and temperature on the toxicity of methiocarb.

In this study, acute toxicity of methiocarb to rainbow trout was determined. The objectives were (1) to determine acute toxicity

* Corresponding author. Tel.: +90 462 7522805; fax: +90 462 7522158.
E-mail address: ialtinok@ktu.edu.tr (I. Altinok).

1382-6689/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2005.11.002

(LC_{50}) of methiocarb concentration to rainbow trout at 24, 48, 72, and 96 h of exposure, (2) to determine effects of water alkalinity, hardness, and temperature on methiocarb toxicity, and (3) to determine the susceptibility of different size of fish to methiocarb toxicity.

2. Materials and methods

2.1. Experimental animals

Juvenile rainbow trout *Oncorhynchus mykiss* (3.25 ± 0.8 g; 6.3 ± 0.5 cm; mean ± S.D.) were obtained from Karadeniz Technical University, Faculty of Marine Sciences and held in two closed recirculating systems (200 L) for at least 15 days to acclimate to laboratory conditions prior to experiments. During the acclimation period, about 20% of the water in each recirculating system was replaced daily. Throughout the acclimation period and subsequent periods of methiocarb exposure, fish were held under a photoperiod of 12-h light:12-h dark. During acclimation, fish were fed 5% body weight twice a day with commercial trout pellets. Fish were not fed 48 h before exposure and during the toxicity tests.

2.2. Water quality

During exposure to methiocarb, water quality characteristics (temperature, pH, total hardness, alkalinity, unionized ammonia, nitrite, and dissolved oxygen) in each treatment were measured daily. Total ammonia was measured by an indophenol method, and nitrite was measured by an azo method. Total hardness and total alkalinity were measured by titration method. Dissolved oxygen concentration was measured by Winkler method (Boyd and Tucker, 1992). Water temperature and pH were determined with a glass electrode (Thermo Orion, Beverly, MA, USA).

2.3. Acute methiocarb toxicity experiments

Experiment was conducted based on description by Altinok (2004). Briefly, the fish were examined and determined to be free of external parasites before the exposure (AFS-FHS, 2003). After acclimation, fish from one of the acclimation tanks were randomly transferred to 1 of 21 glass aquaria containing 25 L of static water (20 fish per aquarium). Test solutions of methiocarb were prepared from a commercial formulation containing 50% active ingredient (Bayer Crop Science AG). Nominal concentrations tested were 0 (control), 1.25, 2.5, 3.75, 5.0, 6.25, and 7.5 mg L^{-1} and triplicate aquaria were designated for each concentration. Methiocarb was dissolved in 500 mL distilled water and then added in aquaria. Control tanks received 500 mL distilled water. This study was conducted under OECD Guideline No. 203 under static-renewal test conditions (OECD, 1992). Fifty-six percent of the test solution was renewed each day. During the 96 h acute toxicity experiment, water in each aquarium was aerated and had the following characteristics: dissolved oxygen 6.34 ± 0.45 mg L^{-1}, temperature 15.6 ± 0.4 °C, pH 7.59 ± 0.14, total hardness 34.3 ± 2.0 mg L^{-1} as $CaCO_3$, alkalinity 23.2 ± 1.8 mg L^{-1} as $CaCO_3$, unionized ammonia 10 ± 3 ng L^{-1} and nitrite 8.3 ± 5.0 μg L^{-1}. Fish were considered dead when gill opercula and body movement ceased, and they were removed immediately. Fish mortality was recorded 0, 6, 8, 12, 18, 24, 48, 72, and 96 h after exposure to methiocarb. At the end of the 96 h toxicity tests, survivors were transferred to flow through tanks to observe further effects of methiocarb for during 2 months. At the end of the 2 months of observation, 10 fish were sampled for histology as described in Section 2.8.

2.4. Effects of alkalinity on methiocarb toxicity

Twenty fish per aquarium were transferred after adjusting water alkalinity to 19.0 ± 2.8 (control), 41.1 ± 0.8, 61.4 ± 1.2, 90.1 ± 0.0, 111.1 ± 1.2, and 138.5 ± 1.2 mg L^{-1} as $CaCO_3$ (mean ± S.D.) by adding $NaHCO_3$. Total hardness was held constant at 30.8 ± 0.6 mg L^{-1} as $CaCO_3$. Triplicate aquaria were designated for each concentration. Methiocarb was dissolved in 500 mL distilled water and then added to aquaria for a final concentration of 5.43 mg L^{-1}. In Section 2.9, LC_{50} value of methiocarb for 24 h was calculated as 5.43 mg L^{-1} by probit analysis. Control tanks received 500 mL distilled water. During the 24 h acute toxicity experiment, water was aerated and its characteristics were: dissolved oxygen 7.62 ± 0.46 mg L^{-1}, temperature 15.4 ± 0.5 °C, unionized ammonia 14 ± 3.0 ng L^{-1}, and nitrite 1.14 ± 0.67 μg L^{-1}. Water pH was 7.58 ± 0.04, 7.94 ± 0.03, 8.13 ± 0.10, 8.26 ± 0.04, 8.36 ± 0.01, and 8.39 ± 0.01 in the alkalinity of 19.0 (control), 41.1, 61.4, 90.1, 111.1, and 138.5 mg L^{-1} as $CaCO_3$, respectively. Fish mortality was recorded 0, 6, 8, 12, 18, and 24 h after exposure to methiocarb.

2.5. Effects of hardness on methiocarb toxicity

Experiment was done as explained in Section 2.4 except water alkalinity was constant but hardness varied. Twenty fish per aquarium were transferred after adjusting water total hardness to 34 ± 0.7 (control), 50 ± 0.9, 74 ± 0.9, 99 ± 0.9, 124 ± 1.6, and 149 ± 0.9 (mean ± S.D.) mg L^{-1} as $CaCO_3$, by adding $CaSO_4$ and $MgSO_4$ (1:1). Total alkalinity was held constant at 17.3 ± 0.6 mg L^{-1} as $CaCO_3$. Triplicate aquaria were designated for each concentration. Each aquarium received methiocarb to reach final nominal concentration of 5.43 mg L^{-1}, except control tanks received distilled water. During the 24 h acute toxicity experiment, water characteristics were: dissolved oxygen 7.30 ± 0.67 mg L^{-1}, temperature 15.7 ± 0.3 °C, pH 7.7 ± 0.1, unionized ammonia 9 ± 3 ng L^{-1} and nitrite 0.44 ± 0.23 μg L^{-1}.

2.6. Effects of temperature on methiocarb toxicity

Experiment was done as explained in Section 2.3. Two different water temperatures (17.4 ± 0.26 and 14.9 ± 0.21 °C) were tested. Quadruplicate aquaria were designated for each temperature and each aquarium contained 20 fish. Final methiocarb concentration of each aquarium was adjusted to 5.43 mg L^{-1}. During the 24 h acute toxicity experiment, water characteristics were: dissolved oxygen 7.6 ± 0.7 mg L^{-1}, pH 7.6 ± 0.2, alkalinity 23.1 ± 1.7 mg L^{-1} as $CaCO_3$, hardness 34.5 ± 1.5 mg L^{-1} as $CaCO_3$, unionized ammonia 9.2 ± 2 ng L^{-1}, and nitrite 0.13 ± 0.05 μg L^{-1}.

2.7. Effects of fish size on methiocarb toxicity

Experiment was done as explained in Section 2.6. Two different fish sizes (small fish 3.25 ± 0.8 g and large fish 9.8 ± 2.8 g) were tested. Quadruplicate aquaria were designated for each temperature and each aquarium contained 20 fish. Final methiocarb concentration of each aquarium was adjusted as 5.43 mg L^{-1}. During the 24 h acute toxicity experiment, water characteristics were: dissolved oxygen 7.6 ± 0.7 mg L^{-1}, pH 7.6 ± 0.2, alkalinity 23.1 ± 1.7 mg L^{-1} as $CaCO_3$, hardness 34.5 ± 1.5 mg L^{-1} as $CaCO_3$, unionized ammonia 9.2 ± 2 ng L^{-1} and nitrite 0.13 ± 0.05 μg L^{-1}.

2.8. Histopathology

A separate experiment was conducted to evaluate histopathology of fish exposed to methiocarb. Twenty fish were exposed to 3.75 and 7.5 mg L^{-1} methiocarb for 96 h. Moribund (lethargic and loosing balance) fish were removed from aquaria, the second gill arch, liver, spleen, kidneys, and head were removed and preserved in 10% neutral buffered formalin (NBF). Before exposure to methiocarb, 10 rainbow trout were fixed in 10% NBF as controls. Different organs were embedded in paraffin, 5-μm tissue sections placed on microscope slides and stained with hematoxylin and eosin (Luna, 1968).

2.9. Statistical analyses

Statistical test analysis was described by Altinok (2004). Briefly, the estimated concentration of methiocarb that kill 50% of rainbow trout within 24, 48, 72, and 96 h (24-h LC_{50}, 48-h LC_{50}, 72-h LC_{50}, 96-h LC_{50}) was calculated by probit analysis (SPSS 2002, SPSS Inc., Chicago, IL, USA). After exposing fish to different concentrations of methiocarb, survival of fish were analyzed by Kaplan–Meier survival and failure time analysis tests (KMSFTAT). After analyzing survival data with KMSFTAT, when significant differences were found