8-26-92

MRID No. 417832-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Oxytetracycline.
Shaughnessey No. 006304.

2. **TEST MATERIAL:** Oxytetracycline HCl; RO2202-51010; 90.9% active ingredient; a yellow powder.

3. **STUDY TYPE:** Freshwater Fish Static Acute Toxicity Test.
Species Tested: Bluegill (*Lepomis macrochirus*).

4. **CITATION:** Murphy, D. and G.T. Peters. 1991. Oxytetracycline Hydrochloride: A 96-Hour Static Acute Toxicity Test With the Bluegill (*Lepomis macrochirus*). Project No. 260A-102. Performed by Wildlife International Ltd., Easton, Maryland. Submitted by Pfizer, Inc., New York, New York. EPA MRID No. 417832-01.

5. **REVIEWED BY:**

Sara R. Ager, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Sara R. Ager 8/20/92

originally KBN ~~study~~ reviewed

6. **APPROVED BY:**

Ann Stavola, Section Head
Ecological Effects Branch
Environmental Fate and Effects Division

Ann Stavola 8/26/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using freshwater fish. The dosage levels were less than 100 mg/l but not high enough to produce a precise LC_{50} and NOEC. However, since no mortality or sublethal effects occurred in any test concentration (up to 94.9 mg a.i./l mean measured concentration), the LC_{50} was probably >100 mg/l which would classify Oxytetracycline hydrochloride as practically non-toxic to bluegill. The NOEC was 94.9 mg a.i./l.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile bluegill (*Lepomis macrochirus*) were obtained from Fattig Fish Hatchery, Brady, Nebraska 66 days prior to test initiation. The fish were maintained at the testing facility in well water and fed frozen brine shrimp and flake food. Water temperatures during the holding period ranged from 12.7°C to 21.5°C. Changes in water temperature did not exceed 3°C in any 72-hour period during the holding period. The well water had a hardness of 144 mg/l as $CaCO_3$, an alkalinity of 170 to 190 mg/l as $CaCO_3$, and a pH of 7.7 to 8.6. The organisms did not exhibit signs of disease or stress during the holding period.

The fish were acclimated to test conditions for approximately 9 days prior to test initiation. The fish were not fed 48 hours prior to the test or during the test. No mortality occurred during the 48-hour period immediately preceding the test.

All fish used in the test were from the same source and year class. The average length of 10 control organisms was 30 mm with a range of 25 to 35 mm. The average weight of 10 control organisms was 0.69 g with a range of 0.48 to 1.11 g.

B. **Test System:** The static test was conducted in Teflon®-lined, 25-l polyethylene aquaria filled with 15 l of test solution. The depth of the test solution in each test chamber was approximately 18 cm. The test chambers were positioned in a temperature-controlled water bath designed to maintain a temperature of 22 ±1°C. The photoperiod was 16 hours of light at an intensity of 42 footcandles and 8 hours of darkness with 30-minute dawn and dusk simulation periods.

The dilution water was soft, reconstituted freshwater prepared according to ASTM (1988) recommendations. The water quality of the dilution water was measured in the control at test initiation and had a hardness of 40 mg/l as $CaCO_3$, an alkalinity of 40 mg/l as $CaCO_3$, and a pH of 7.4. The dilution water was aerated using spray nozzles and filtered (0.2 µm) to remove microorganisms and particles prior to delivery to the test chamber.

The stock solution (3,000 mg/l) was prepared by dissolving 3 g of Oxytetracycline hydrochloride in 1 l of reconstituted water. The appropriate amount of stock was added to the dilution water in each test chamber and the solution was gently mixed.

C. **Dosage:** Ninety-six-hour static test. The nominal concentrations were 11.8, 19.6, 32.7, 54.5, and 90.9 mg a.i./l. A dilution water control was also included in the test.

D. **Design:** Bluegill were impartially distributed, in groups of two, to each test chamber for a total of 10 fish per concentration. The biomass loading was 0.46 g/l of test solution.

Observations of mortality and sublethal effects were made every 24 hours after test initiation. The dissolved oxygen concentration and pH were measured every 24 hours in each concentration and the control. The temperature was measured at test initiation and test termination in all concentrations and the control. The temperature was also measured continuously in the dilution water control.

The concentration of Oxytetracycline hydrochloride present in each test chamber was determined at test initiation and test termination by using microbial zone inhibition method (Association of Official Analytical Chemists, 1990).

E. **Statistics:** The LC_{50} value could not be determined since no mortality occurred in any of the treatment levels.

12. **REPORTED RESULTS:** Mean measured concentrations were 10.9, 19.5, 33.7, 44.1, and 94.9 mg a.i./l and ranged from 81 to 104% of nominal concentrations (Table 1, attached). No mortality or signs of toxicity were observed in any of the Oxytetracycline hydrochloride test concentrations of up to 94.9 mg a.i./l, the highest concentration tested (Table 3, attached). The 24-, 48-, 72-, and 96-hour LC_{50} values for Oxytetracycline hydrochloride were >94.9 mg a.i./l. The no observed effect concentration (NOEC) for the study was 94.9 mg a.i./l.

During the study, the dissolved oxygen concentration ranged from 6.3 to 8.6 mg/l (72% to 99% of saturation at 22°C), the pH ranged from 6.2 to 7.2, and the continuous temperature of the dilution water control ranged from 21.4°C to 22.4°C. The temperature measured at the beginning and end of the test ranged from 21.4°C to 22.8°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author categorized the test material as practically non-toxic to bluegill.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP, but deviated from the guidelines as follows:

The test organisms were acclimated to the test conditions 9 days prior to test initiation. The SEP recommends an acclimation period of two weeks.

The test aquaria were Teflon®-lined polyethylene chambers. The SEP states that test chambers should be constructed of glass or stainless steel, however, the system used in this study is probably adequate.

The SEP states that static tests are initiated either by adding the test material to the test chambers after the fish are added or by adding the fish to the test chambers within 30 minutes after the test material is added to the dilution water. Appendix II (Changes in Protocol) states that the test organisms were added to the test chambers within 2 hours after the test material was added to the dilution water.

The dosage levels tested were less than 100 mg/l but not high enough to produce a precise LC_{50} and NOEC.

The SEP states that it must be determined that a chemical will have an LC_{50} greater than 100 mg/L, by exposing at least 30 individuals to a concentration of 100 mg/l or greater. During this toxicity test, five concentrations were tested and the highest nominal test concentration in this test was 90.9 mg a.i./l with only 10 fish tested.

added The temperature varied 1.4°C which exceeds the guidelines maximum variance of 1°C.

B. Statistical Analysis: Statistical analysis was not needed since no mortality occurred in any of the treatment levels.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a static acute freshwater fish toxicity study. In this test, the dosage levels tested were less than 100 mg/l but not high enough to produce a precise LC_{50} or NOEC. In order to determine that a chemical will have an LC_{50} greater than 100 mg/l, the test should have been conducted by exposing 30 individuals to a concentration of 100 mg/L or greater. However, since no mortality or sublethal effects occurred in any test concentration (up to 94.9 mg a.i./l mean measured concentration), the LC_{50} was probably >100 mg/l which would classify Oxytetracycline hydrochloride as practically non-toxic to bluegill. The NOEC was 94.9 mg a.i./l.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: See Section 14.C.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, April 14, 1992.

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Shaughnessey # 006304 Chemical Name Oxytetracycline hydrochloride Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} = pp (95% C.L.) Control Mortality (%) = Solvent Control Mortality (%) =		
Species:		Slope = # Animals/Level = Temperature =		
Lab:		48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( )	_____	_____
MRID #		Comments:		

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96-Hour LC_{50}	90.9 %	LC_{50} = >94.9 ppm (95% C.L. N/A) Control Mortality (%) = 0 Solvent Control Mortality (%) = N/A		
Species: Lepomis macrochirus		Slope = N/A # Animals/Level = 10 Temperature = 21.4 - 22.4 °C (continuous temperature reading from the control)		
Lab: Wildlife International, Ltd.		mg a.i./l * 96-Hour Dose Level ppm /(% Mortality) 10.9 (0), 19.5 (0), 33.7 (0), 44.1 (0), 94.9 (0)	K.R. 4/14/92	Suppl.
MRID # 417832-01		Comments: NOEC > 94.9 mg a.i./l * * Based on mean measured concentrations.		

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