US EPA ARCHIVE DOCUMENT

8.26-92

MRID No. 417778-01

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Calcium oxytetracycline; 60.4% purity; a tan powder.

3. **STUDY TYPE:** Avian Single Dose Oral LD_{50} Test.
Species Tested: Bobwhite quail (*Colinus virginianus*).

4. **CITATION:** Campbell, S., K.A. Hoxter, and G.J. Smith. 1991. Oxytetracycline Calcium Complex: An Acute Oral Toxicity Study with the Northern Bobwhite. Project No. 260-103A. Performed by Wildlife International Ltd., Easton, MD. Submitted by Pfizer Incorporated, New York, NY. EPA MRID No. 417778-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark Mossler

Date: 4/27/92

6. **APPROVED BY:**

Michael Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
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Signature: Michael L. Whitten

Date: 4/27/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Ann Stavola

Date: 8/26/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an acute oral toxicity test using bobwhite quail. The LD_{50} value of >2000 mg ai/kg classifies oxytetracycline calcium complex as practically non-toxic to bobwhite quail. The NOEL was 250 mg ai/kg.

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Animals: The birds used in the study were 18-week-old bobwhite quail (*Colinus virginianus*) obtained from a supplier in Phillipsburg, NJ. The birds were from the same hatch, pen-reared and phenotypically indistinguishable from wild birds. They were acclimated to the laboratory for 7 weeks prior to testing and ranged in weight from 170 to 206 g at test initiation. Except for a 15-hour fasting period immediately prior to dosing, water and a game bird ration were offered *ad libitum* during acclimation and testing. No antibiotics were administered during the test.

Fritts Quail Farm

B. Test System: All birds were housed indoors in pens constructed of galvanized wire and galvanized sheeting (side walls). The floors (78 x 51 cm) of the pens were sloped giving a ceiling height which ranged from 20 to 25 cm. Fluorescent lights provided 8 hours of 130 lux illumination per day. The average temperature was 25 ±1°C and the average relative humidity was 28 ±2%.

C. Dosage: Fourteen-day single dose oral LD_{50} test. Five nominal dosages [125, 250, 500, 1000, and 2000 mg active ingredient (ai)/kg of body weight] and a diluent (water) control were used in the test.

D. Design: Groups of ten birds (five males and five females) were assigned to each treatment and control group by random draw. Each dosage group was assigned two pens in which the birds were segregated by sex.

The test substance was dispersed in water and intubated directly into the crop or proventriculus of each bird using a stainless steel cannula. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight. The control birds received a corresponding volume of water only. Each bird received a constant dosage volume of 6 ml per kilogram of body weight.

All birds were observed once a day during acclimation and at least twice daily during testing for mortality, signs of toxicity, and abnormal behavior. The birds were individually weighed at test initiation and by group on days 3, 7, and 14. Group food consumption was determined for days 0-3, 4-7, and 8-14 by measuring the change in feed presented to the birds over a period of

time. However, this is an estimate due to wastage by the birds.

Samples of each dosing solution were taken to determine the homogeneity and concentration of the test material. The samples were sent to Hazleton Laboratories America, Inc., for analysis by microbial zone inhibition.

E. **Statistics:** The LD_{50} was estimated by visual inspection due to the pattern of mortality in this study.

12. **REPORTED RESULTS:** There were no mortalities in the control or treatment groups. All birds in these groups were normal in appearance and behavior.

There were no reductions in body weight gain or feed consumption in the treatment groups in comparison to the controls (Table 2, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The acute oral LD_{50} value for bobwhite quail exposed to oxytetracycline calcium complex was determined to be greater than 2000 mg ai/kg. The no mortality dosage and no-observed-effect dosage was 2~~000~~ 50 mg ai/kg.

Quality Assurance and Good Laboratory Practice statements were included in the biological and analytical reports which indicated compliance with the regulations set forth in 40 CFR Part 160.

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

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, ASTM, and SEP guidelines with the following exceptions:

Necropsies were not performed. These are recommended, but not required by the guidelines.

Group body weights, rather than individual body weights, were taken at the end of the test.

B. **Statistical Analysis:** Upon review of the mortality data, the reviewer concurs that the LD_{50} was greater than 2000 mg ai/kg.

C. **Discussion/Results:** A review of the analytical portion of the report (included separately) indicated that the

concentrations of test material were at or above the nominal concentrations.

Upon review of the body weight gain data, it appears that there was a treatment related reduction during the test period for the three highest treatment levels (500, 1000, and 2000 mg ai/kg). The no-observed-effect level (NOEL) was therefore determined to be 250 mg ai/kg.

This study is scientifically sound and meets the requirements for an acute oral toxicity test using bobwhite quail. The LD_{50} value of >2000 mg ai/kg classifies oxytetracycline calcium complex as practically non-toxic to bobwhite quail. The NOEL was determined to be 250 mg ai/kg based on a reduction in body weight gain in the three highest treatment groups.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 4-14-92.

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Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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Shaughnessey # 006304 Chemical Name Oxytetracycline calcium complex Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Oral LD_{50}	60.4	LD_{50} = >2000 mg/kg * 95% C.L. (N/A) Control Mortality (%) = 0		
Species: Colinus virginianus		Slope = N/A # Animals/Level = 10 (5♀ 5♂) Age (Days) = 126 Sex = 5♀ 5♂		
Lab: Wildlife International		14-Day Dose Level mg/kg*/(% Mortality): 125 (0), 250 (0), 500 (0), 1000 (0), 2000 (0)	M. Hosler 4/14/92	Core
MRID #: 417778-01		Comments: based on active ingredient. NOEL = 250 mg ai/kg		

8-Day Dietary LC_{50}		LC_{50} = pp 95% C.L. ( ) Control Mortality (%) =		
Species		Slope = # Animals/Level = Age (Days) = Sex =		
Lab		8-Day Dose Level pp /(% Mortality): ( ), ( ), ( ), ( ), ( )		
MRID #		Comments:		

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 KBH ~~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:		
8.				
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)	K.R. 4/14/92	Suppl.
		10.9 (0), 19.5 (0), 33.7 (0), 44.1 (0), 94.9 (0)		
MRID # 417832-01		Comments: NOEC > 94.9 mg a.i./l *		
		* Based on mean measured concentrations.		