DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP
§ 72-3(c)

1. **CHEMICAL:** Oryzalin **PC Code No.:** 104201

2. **TEST MATERIAL:** Oryzalin **Purity:** 96.9%

3. **CITATION:**

Authors:	R.L. Boeri, P.L. Kowalski, and T.J. Ward
Title:	Oryzalin: Acute Toxicity to the Grass Shrimp, *Palaemonetes pugio*
Study Completion Date:	December 15, 1995
Laboratory:	T.R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor:	DowElanco, Midland, MI
Laboratory Report ID:	652-DO
MRID No.:	438877-03
DP Barcode:	D223419

4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Environmental Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: for RBM **Date:** 5/22/96

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat **Date:** 5/22/96

5. **APPROVED BY:** Robert K. Hill

Signature: **Date:** 5/22/97

RM 6/16/97

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	0.061 g
Definitive Test Duration:	96 hours
Study Method:	Flow Through
Type of Concentrations:	Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a marine shrimp acute toxicity test. The study was conducted with exposure concentrations up to the maximum obtainable water solubility of this material for the conditions of this study (3.11 mg/L). Only 20% mortality occurred at the highest concentration tested, therefore, the LC_{50} was determined to be >3.11 mg/L which, at worst, classifies Oryzalin as moderately toxic to grass shrimp. The NOEC was 1.95 mg/L.

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Results Synopsis

96-Hour LC_{50}: 3.11 mg/L 95% C.I.: N/A
NOEC: 1.95 mg/L Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: Fulfills requirement.

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS: None.

11. SUBMISSION PURPOSE:

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Palaemonetes pugio*
Age Juvenile, mysids should be ≤ 24 hours old	Juveniles; age not reported. (Report only indicated 33 days after collection).
Supplier	Aquatic Research Organisms, Hampton, NH
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	Test organisms were acclimated to test conditions for at least 14 days prior to test initiation.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each.	No feeding during the 48 hours prior to test initiation or during the test.
Pretest Mortality <3% mortality 48 hours prior to testing	0%

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Carbon-filtered, natural seawater, salinity adjusted with deionized water. The water was analyzed and found to be free of pesticides and PCBs.
Does water support test animals without observable signs of stress?	Not reported.

Guideline Criteria	Reported Information
Salinity 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰	17‰
Water Temperature Approx. 22 ± 1 °C	21.7-22.9°C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.8-8.0
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	≥85% of saturation throughout the test
Total Organic Carbon	Not reported.
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	1. Glass 2. 20 L 3. 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent-flow proportional diluter
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	5.3 volume additions/24 hours

Guideline Criteria	Reported Information
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.041 g/L instantaneous loading or 0.008 g/L/day
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Solvent: DMF Maximum conc.: 0.1 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	During a range-finding test, there was 100% survival at concentrations ≤1 mg/L and 60% survival at 10 mg/L.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	Control, solvent control, and five nominal concentrations (1.5, 2.5, 4.0, 6.0, and 10 mg/L).
Number of Test Organisms Minimum 20/level, may be divided among containers	10 shrimp per test vessel; 2 test vessels per treatment and control.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Water temperature was measured daily in each test vessel and continuously in one test vessel. 2. DO and pH were measured daily in each test vessel.
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Centrifuged samples of the test solutions were analyzed at 0, 48, and 96 hours using high performance liquid chromatography.

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	31-38%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% in both controls
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes; one shrimp at the highest test concentration exhibited loss of equilibrium and immobilization at 48 hours.

Mortality

Concentration		Number of Shrimp	Cumulative Number Dead			
Nominal (mg/L)	Mean Measured (mg/L)		Hour of Study			
			24	48	72	96
Control	<0.0526	20	0	0	0	0
Solvent Control	<0.0526	20	0	0	0	0
1.5	0.57	20	0	0	0	0
2.5	0.94	20	0	0	0	0
4.0	1.36	20	0	0	0	0
6.0	1.95	20	0	0	0	0
10	3.11	20	0	2	4	4

Other Significant Results: Insoluble material was noted in all test solutions during the test. The solutions turned orange, and orange particles were observed in the bottom of each tank. The color and concentration of particles increased with increasing toxicant concentration.

B. Statistical Results

Method: Visual Inspection

96-Hour LC_{50}: >3.11 mg/L 95% C.I.: N/A
Probit Slope: N/A NOEC: 1.95 mg/L

14. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC_{50} (C.I.)	N/A
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	1.95 mg/L

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15. **REVIEWER'S COMMENTS:** This study is scientifically sound, meets the guideline requirements for an acute toxicity test using grass shrimp, and is classified as **Core.** Although a more precise LC_{50} was not determined, this study was conducted with concentrations up to the maximum water solubility obtainable under the conditions of this test (3.11 mg/L). The LC_{50} was >3.11 mg/L which, at worst, classifies Oryzalin as moderately toxic to the grass shrimp. The NOEC was 1.95 mg/L since no mortality or sublethal effects occurred at or below this concentration.