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

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

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

3. **CITATION:**

Authors:	T.J. Ward, P.L. Kowalski, and R.L. Boeri
Title:	Oryzalin: Acute Toxicity to the Sheepshead Minnow, *Cyprinodon variegatus*
Study Completion Date:	December 11, 1995
Laboratory:	T.R. Wilbury Laboratories, Inc., Mablehead, MA
Sponsor:	DowElanco, Midland, MI
Laboratory Report ID:	654-DO
MRID No.:	438877-01
DP Barcode:	D223419

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

Signature: for RGM **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:**

Signature: Robert K. **Date:** May 22 97

In) 6/11/97

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	0.33 g
Definitive Test Duration:	96 hours
Study Method:	Flow-Through
Type of Concentrations:	Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using sheepshead minnows. The study was conducted with exposure concentrations up to the maximum obtainable water solubility of this material for the conditions of this study (3.04 ppm). No mortality or sublethal effects were observed, therefore, the LC_{50} is determined to be >3.04 ppm which, at worst, classifies Oryzalin as moderately toxic to the sheepshead minnow. The NOEC was 3.04 ppm, the highest concentration tested.

Results Synopsis

LC_{50}: >3.04 ppm
NOEC: 3.04 ppm
95% C.I.: N/A
Probit Slope: N/A

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: 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 (3.04 ppm).

C. Repairability: N/A.

9. Guideline Deviations:

1. The salinity (15-17‰) of test solutions during this study was lower than recommended (salinity of 30‰).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the Silverside (*Menidia sp.*).	*Cyprinodon variegatus*
Mean Weight 0.5 - 5 g	0.33 g
Mean Standard Length Longest not > 2x shortest	27 mm
Supplier	Aquatic BioSystems, Fort Collins, CO
All fish from same source?	Yes.
All fish from the same year class?	Not reported.

2

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 14 days	14 days
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	The fish were not fed during the 48 hours prior to test initiation or during the test period.
Pretest Mortality <3% mortality 48 hours prior to testing	<3%

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 collected at T.R. Wilbury in Mablehead, MA. The water was analyzed and found to be free of pesticides and PCBs.
Does water support test animals without observable signs of stress?	Yes.
Salinity 30-34‰ salinity, weekly range < 6 ‰	15-17‰
Water Temperature 22 ± 1 °C	21.8-22.6°C

Guideline Criteria	Reported Information
pH 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	7.7-7.9
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥80% of saturation
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	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	6.8 volume additions/24 hours
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.22 g/L instantaneous loading or 0.03 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 fish, then no definitive test is required.	During a range-finding test, no mortality or sublethal effects occurred at concentrations ≤1 mg/L and total mortality at 10 mg/L.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Dilution water control, solvent control and five nominal concentrations (1.5, 2.5, 4.0, 6.0, and 10 mg/L).
Number of Test Organisms Minimum 10/level, may be divided among containers	10 fish 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.
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.

12. **REPORTED RESULTS:**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Recovery of Chemical	30-39%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No mortality occurred in the dilution water control or the solvent control.
Raw data included?	Yes.
Signs of toxicity (if any) were described?	No signs of toxicity were observed.

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	ND	20	0	0	0	0
Solvent Control	ND	20	0	0	0	0
1.5	0.59	20	0	0	0	0
2.5	0.94	20	0	0	0	0
4.0	1.40	20	0	0	0	0
6.0	1.96	20	0	0	0	0
10.0	3.04	20	0	0	0	0

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-hr LC_{50}: >3.04 ppm 95% C.I.: N/A

Probit Slope: N/A NOEC: 3.04 ppm

13. 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	3.04 ppm

14. REVIEWER'S COMMENTS: This study is scientifically sound, meets the guideline requirements for an acute toxicity test using sheepshead minnow, 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 (3.04 ppm). The LC_{50} is determined to be >3.04 ppm which, at worst, classifies Oryzalin as moderately toxic to the sheepshead minnow. The NOEC was 3.04 ppm, the highest concentration tested.