10-8-96

MRID No.: 434928-18

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

1. **CHEMICAL:** Pirate (AC 303,630) PC Code No.: 129093

2. **TEST MATERIAL:** ^{14}C-AC 303,630 Purity: 96.8%
Radiopurity: 97%

3. **CITATION:**

Authors: J.W. Davis, J.D. Wisk, and G.S. Ward
Title: Acute Toxicity of AC 303,630 to the Mysid (*Mysidopsis bahia*) Under Flow-Through Test Conditions
Study Completion Date: March 7, 1994
Laboratory: Toxikon Environmental Sciences, Jupiter, FL
Sponsor: American Cyanamid Company, Princeton, NJ
Laboratory Report ID: J9304005
MRID No.: 434928-18
DP Barcode: D210808 and D222690

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

Signature: for RGM **Date:** 5/8/96

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

Signature: P. Kosalwat **Date:** 5/8/96

5. **APPROVED BY:**

Signature: **Date:** 10/8/96

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	<24 hours old
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 an acute toxicity test using estuarine invertebrates. A 96-hour LC_{50} value of 2.03 ppb classifies ^{14}C-AC 303,630 as very highly toxic to *Mysidopsis bahia*. The NOEC was 0.32 ppb.

7/30/96

1

Results Synopsis
96-Hour LC_{50}: 2.03 ppb 95% C.I.: 1.69-2.51 ppb
NOEC: 0.32 ppb Probit Slope: 3.97

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core

B. **Rationale:** Fulfills requirement.

C. **Repairability:** N/A

9. **BACKGROUND:**

10. **GUIDELINE DEVIATIONS:**

1. The temperature (26.1-28.2°C), pH (8.3-8.6), and salinity (20‰) of test solutions during this study were higher than the recommended values (temperature of 22 ±1°C; pH of 7.7-8.0; salinity of 10-17‰) for a euryhaline species.

2. The dissolved oxygen concentrations in the solvent control and treatment solutions ranged from 45 to 61% of saturation; the guidelines recommend D.O. concentrations of ≥60% for flow-through studies.

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.*	*Mysidopsis bahia*
Age Juvenile, mysids should be ≤ 24 hours old	<24 hours old
Supplier	In-house cultures
All shrimp are from same source?	Yes

Guideline Criteria	Reported Information
All shrimp are from the same year class?	Not reported.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	Mysid cultures were maintained under conditions similar to test conditions.
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.	Mysids were fed live brine shrimp nauplii daily during the study to reduce cannibalism.
Pretest Mortality <3% mortality 48 hours prior to testing	N/A

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural or reconstituted seawater	Natural seawater (collected from a shallow well) which was filtered and diluted with Town of Jupiter freshwater to adjust the salinity to 20%.
Does water support test animals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
Salinity 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6‰	21-23‰
Water Temperature Approx. 22 ± 1 °C	26.1-28.2°C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	8.3 to 8.6
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	45 to >100% saturation
Total Organic Carbon	1.44 mg/L
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. 300-mL crystallizing dishes (10-cm diameter X 5-cm height) with Nitex screen collars set inside 11.3-L glass tanks (42 X 21.5 X 12.5 cm) 3. Crystallizing dishes and glass tanks had a fill volume of 300 mL and 5.4 L, respectively.
Type of Dilution System Must provide reproducible supply of toxicant	Proportional diluter system
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	36 volume additions to crystallizing dishes every 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	Not reported.
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.: 0.037 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.	Test concentrations for the definitive study were based upon previous mysid acute toxicity results.
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 0.86, 1.43, 2.38, 3.96, 6.60, and 11.0 µg/L of AC 303,630 as whole material.
Number of Test Organisms Minimum 20/level, may be divided among containers	10 mysids per test chamber; 2 replicate test chambers 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 n in the high, medium, and low doses and in the control	1. Temperature measured continuously in the water bath and hourly in the dilution water control. 2. DO and pH were measured daily in all test solutions.
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	Test solutions were analyzed on Days 0, 2, and 4 using liquid scintillation counter. The highest test concentration was also measured using HPLC.

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	37-51%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No mortality in either control.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes; signs of toxicity were observed in concentrations ≥0.73 ppb mean measured concentration and included lethargy.

Mortality

Concentration (ppb as whole material)		Number of Shrimp	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	<0.05	20	0	0	0	0
Solvent Control	<0.05	20	0	0	0	0
0.86	0.32	20	0	0	0	0
1.43	0.73	20	0	1	1	1
2.38	0.89	20	0	0	0	1
3.96	1.52	20	0	6	8	9
6.60	2.52	20	5	7	9	9
11.0	5.08	20	19	20	20	20

Other Significant Results: The single mortality at 0.73 and 0.89 ppb represents one mysid not found.

The measured concentrations in the highest test level averaged 46% of nominal by radioassay and 45% of nominal using HPLC.

Dissolved oxygen concentrations in the treatment and solvent control solutions ranged from 79 to >100% saturation on Day 0, from 45 to 61% of saturation on Day 1, and from 64 to 77% of saturation on Day 2. Aeration was initiated in the test tanks on Day 1 and in the test dishes on Day 2.

B. Statistical Results

Method: Probit

96-hr LC_{50}: 2.03 ppb

95% C.I.: 1.69-2.51 ppb

Probit Slope: Not reported

NOEC: 0.32 ppb

14. **VERIFICATION OF STATISTICAL RESULTS:**

Parameter	Result
Binomial Test LC_{50} (C.I.)	2.64 (0.89-5.08) ppb
Moving Average Angle LC_{50} (95% C.I.)	2.03 (1.71-2.41) ppb
Probit LC_{50} (95% C.I.)	2.03 (1.69-2.51) ppb
Probit Slope	3.97
NOEC	0.32 ppb

15. **REVIEWER'S COMMENTS:** By 24 hours the dissolved oxygen concentrations in the treatment and solvent control solutions dropped below the recommended limit of 60%; test solutions were aerated for the remainder of the study. No mortality in the solvent control occurred during the test. Therefore, it is not likely that this decrease in dissolved oxygen affected the results of the study.

A 96-hour LC_{50} value of 2.03 ppb classifies AC 303,630 as very highly toxic to mysids. The NOEC was 0.32 ppb based on the lack of mortality and sublethal effects at this test concentration. The study is classified as **Core.**

RGM M.bahia AC 303-630

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5.08	20	20	100	9.536742E-05
2.52	20	9	45	41.19014
1.52	20	9	45	41.19014
.89	20	1	5	2.002716E-03
.73	20	1	5	2.002716E-03
.32	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .89 AND 5.08 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.639385

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	6.572953E-02	2.028742	1.708819	2.411373

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	9.600473E-02	1	.1523618

SLOPE = 3.972571

95 PERCENT CONFIDENCE LIMITS = 2.741685 AND 5.203458

LC50 = 2.028143

95 PERCENT CONFIDENCE LIMITS = 1.687716 AND 2.510154

LC10 = .9714183

95 PERCENT CONFIDENCE LIMITS = .6882573 AND 1.207707

****