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

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

2. **TEST MATERIAL:** AC 303,630 Purity: 94.5%

3. **CITATION:**

Authors: G.S. Ward and J.D. Wisk
Title: Acute Toxicity of AC 303,630 to the Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Test Conditions
Study Completion Date: October 18, 1993
Laboratory: Toxikon Environmental Sciences, Jupiter, FL
Sponsor: American Cyanamid Company, Princeton, NJ
Laboratory Report ID: J9203020c
MRID No.: 434928-16
DP Barcode: D210808 and D222690

4. **REVIEWED BY:** William Evans, Biologist
Ecological Effects Branch
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added

Signature: William Evans **Date:** 10/9/96

5. **APPROVED BY:** Ann Stavola, Section Chief, Review Section 5
Ecological Effects Branch
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added

Signature: Ann Stavola **Date:** 11/8/96

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: 0.31 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. A 96-hour LC_{50} value of 60.2 ppb ai classifies AC 303,630 as very highly toxic to the sheepshead minnow. The NOEC was 30.7 ppb ai.

Results Synopsis
96-Hour LC_{50}: 60.2 ppb ai 95% C.I.: 53.5-68.6 ppb ai
NOEC: 30.7 ppb ai Probit Slope: 11.1

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: Fulfills requirement.

C. Repairability: N/A.

9. Guideline Deviations:

1. The weight and length of the test organisms ranged from 0.07 to 0.63 g (mean of 0.31 ±0.14 g) and from 9 to 28 mm (mean of 21 ±4 mm), respectively; the guidelines recommend a weight of 0.5-5 g and a length range in which the longest fish is not greater than 2 times the shortest fish.

2. The dissolved oxygen concentrations in the three highest treatment levels ranged from 56 to 58% of saturation on Day 2; the guidelines recommend dissolved oxygen concentrations of ≥60% of saturation throughout the study.

3. The salinity of the dilution water (20‰) was lower than recommended (30-34‰).

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	Mean: 0.31 ±0.14 g Range: 0.07-0.63 g
Mean Standard Length Longest not > 2x shortest	Mean: 21 ±4 mm Range: 9-28 mm
Supplier	Aquatic Biosystems, Inc., Fort Collins, CO
All fish from same source?	Yes.

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

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 14 days	Fish were maintained under conditions similar to test conditions for approximately 79 days prior to use.
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	No mortality occurred in the 48 hours prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Natural filtered saltwater collected from a saltwater well, adjusted to 20‰ and aerated prior to use.
Does water support test animals without observable signs of stress?	Yes.

Guideline Criteria	Reported Information
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	20‰
Water Temperature 22 ± 1 °C	Mean: 23.1 ±0.9°C Range: 21.4-24.7°C
pH 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	8.1-8.4
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥56% 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. 24 L (40 X 29.5 X 20 cm) 3. 15 L
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	10 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	0.04 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.: 0.04 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, mortality was 0% at 50 and 100 ppb, 33% at 200 ppb, and 100% at 400 ppb.
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 test concentrations (19.4, 32.4, 54, 90, 150 µg ai/L).
Number of Test Organisms Minimum 10/level, may be divided among containers	20 fish per test vessel; 1 test vessel per treatment and control.
Test organisms randomly or impartially assigned to test vessels?	Yes.
Biological observations made every 24 hours?	Yes; biological observations were made at 24, 48, 72, and 96 hours.
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 hourly in the dilution water control and continuously in the water bath. 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	Test solutions were analyzed at 0, 24, and 96 hours using 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	84-103%
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 (ppb ai)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	<0.5	20	0	0	0	0
Solvent Control	<0.5	20	0	0	0	0
19.4	16.2	20	0	0	0	0
32.4	30.7	20	0	0	0	0
54	48.4	20	0	0	0	3
90	84.9	20	5	11	18	19
150	155	20	11	12	16	20

Other Significant Results: On Day 2 the dissolved oxygen concentrations in the three highest treatment levels ranged from 56-58% of saturation. Gentle aeration was initiated and continued for the remainder of the study. This did not appear to affect the recovery of the test substance at these

added

levels when measurements were made at 96 h. added

B. Statistical Results

Method: Probit

96-hr LC_{50}: 60.2 ppb ai 95% C.I.: 53.5-68.6 ppb ai

Probit Slope: 11.1 NOEC: 30.7 ppb ai

13. **VERIFICATION OF STATISTICAL RESULTS:**

Parameter	Result
Binomial Test LC_{50} (C.I.)	61.1 (48.4-84.9) ppb ai
Moving Average Angle LC_{50} (95% C.I.)	62.4 (52.7-75.7) ppb ai
Probit LC_{50} (95% C.I.)	60.2 (53.5-68.6) ppb ai
Probit Slope	11.1
NOEC	30.7 ppb ai

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 the range for length and weight of the test organisms was greater than recommended, there was a clear dose-response for mortality established by this study and no control mortality occurred. Therefore, it is not likely that the size of the test organisms affected the results of this study.

A 96-hour LC_{50} of 60.2 ppb ai classifies AC 330,630 as very highly toxic to the sheepshead minnow. The NOEC was 30.7 ppb ai.

RGM Sheepshead AC 303-630

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
155	20	20	100	9.536742E-05
84.9	20	19	95	2.002716E-03
48.4	20	3	15	.1288414
30.7	20	0	0	9.536742E-05
16.2	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 48.4 AND 84.9 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 61.06565

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	5.135007E-02	62.38448	52.65196	75.68496

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
11	.169592	1	.9996002

SLOPE = 11.05648
95 PERCENT CONFIDENCE LIMITS = 6.503252 AND 15.60971

LC50 = 60.17536
95 PERCENT CONFIDENCE LIMITS = 53.45256 AND 68.63686

LC10 = 46.19063
95 PERCENT CONFIDENCE LIMITS = 37.18905 AND 52.17522

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