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

1. **CHEMICAL:** CGA-77102 PC Code No.: 108800

2. **TEST MATERIAL:** CGA-77102 Technical Purity: Not reported.

3. **CITATION:**

Authors: W.C. Spare
Title: The Acute Toxicity of CGA-77102 Technical to *Mysidopsis bahia* (Bay Shrimp)
Study Completion Date: September 26, 1983
Laboratory: Biospherics Incorporated, Rockville, MD
Sponsor: Ciba-Geigy Corporation, Greensboro, NC
Laboratory Report ID: 83-E-168M
MRID No.: 439289-13
DP Barcode: D223753 and D223769

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: Allen W. Vaughan 04.07.97 **Date:** 3/13/97

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	1-5 days old
Definitive Test Duration:	96 hours
Study Method:	Static
Type of Concentrations:	Mean Measured

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements for an acute toxicity test using estuarine invertebrates. The age of the test organisms (1-5 days at test initiation) was ≥24 hours old and was variable among the test population. A 96-hour LC_{50} of 1.41 ppm ai classifies CGA-77102 as moderately toxic to *Mysidopsis bahia*. The NOEC was not determined.

Results Synopsis

96-Hour LC_{50}: 1.41 ppm ai 95% C.I.: 1.17-1.68 ppm ai
NOEC: Not determined. Probit Slope: 4.95

8. **ADEQUACY OF THE STUDY:**

   A. **Classification:** Invalid.

   B. **Rationale:** The age of the test organisms (1-5 days old at test initiation) was ≥24 hours at test initiation and was variable among the test population.

   C. **Repairability:** No.

9. **BACKGROUND:**

10. **GUIDELINE DEVIATIONS:** This study was conducted in 1983 before the EPA SEP guidance was available (1985); therefore, many deviations from the current guidelines were noted and include the following:

   1. The age of the test organisms (1-5 days old at test initiation) was ≥24 hours and was variable among the test population. The current guidelines require mysids ≤24 hours old at test initiation.

   2. Pretest mortality of the test population was not reported.

   3. The concentration of solvent used in the solvent control and test solutions was not reported; the guidelines limit the solvent concentration to ≤0.5 ml/L for a static test.

   4. The purity of the test material was not reported.

   5. The salinity (30‰) of test solutions during this study was higher than recommended (salinity of 10-17‰) for a euryhaline species.

   6. The pH of the test solutions (7.2-7.4) was lower than recommended (7.7-8.0) for a euryhaline species.

   7. The construction material of the test vessels was not reported. Glass or stainless steel is recommended.

   8. The system used to control the temperature was not reported. The reviewer assumes that it was control by

ambient air. The test temperature was recorded daily, not continuously as recommended. In addition, it is not clear from which vessel the temperature was recorded.

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	1-5 days old at test initiation
Supplier	Sea Plantation Inc., Salem, MA
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Mysids were 1-5 days old at test initiation.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	Test organisms were acclimated to the dilution water for one day prior to test initiation.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported.
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A

Guideline Criteria	Reported Information
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 *Artemia* at test initiation.
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural or reconstituted seawater	Reconstituted seawater, rigorously aerated before use.
Does water support test animals without observable signs of stress?	Not reported.
Salinity 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6%	30‰
Water Temperature Approx. 22 ± 1 °C	21°C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.2-7.4
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	≥65% saturation during 1st 48 hrs and ≥55% during 2nd 48 hrs
Total Organic Carbon	Not reported

Guideline Criteria	Reported Information
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. Not reported 2. 250-mL beakers 3. 200 mL test solution
Type of Dilution System Must provide reproducible supply of toxicant	Static system
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
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: Acetone Maximum conc.: Not reported

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 the results of preliminary testing.
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 0.66, 1.1, 1.8, 3.0, 5.0 mg/L
Number of Test Organisms Minimum 20/level, may be divided among containers	5 mysids per test chamber; 4 replicate test chambers 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. Temperature was recorded daily. 2. DO and pH were measured daily in each treatment and control.
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 using gas chromatography at test initiation and termination.

13. **REPORTED RESULTS:**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	The GLP statement indicated that this study was conducted prior to the implementation of GLP standards.
Recovery of Chemical	77-104%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% in both controls
Raw data included?	Mean % survival was reported.
Signs of toxicity (if any) were described?	None reported.

Mortality

Concentration		Number of Shrimp	Cumulative Number Dead			
Nominal (ppm)	Mean Measured (ppm ai)		Hour of Study			
			24	48	72	96
Control	<0.01	20	0	0	0	0
Solvent Control	<0.01	20	0	0	0	0
0.66	0.51	20	1	1	1	1
1.1	0.96	20	2	2	2	2
1.8	1.7	20	2	8	14	14
3.0	3.1	20	2	8	15	19
5.0	4.6	20	9	15	15	20

Other Significant Results: None reported.

B. Statistical Results

Method: Moving Average Method

96-Hour LC_{50}: 1.4 ppm ai 95% C.I.: 1.16-1.67 ppm ai
Probit Slope: Not reported. NOEC: <0.51 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC_{50} (C.I.)	1.41 (0.96-3.13) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	1.40 (1.15-1.67) ppm ai
Probit LC_{50} (95% C.I.)	1.41 (1.17-1.68) ppm ai
Probit Slope	4.95
NOEC	Not determined.

15. REVIEWER'S COMMENTS: The reviewer questions whether the reported value for the hardness of the dilution water was a typographical error. The value (6,000 mg/L as $CaCO_3$) seems unusually high.

This study is not scientifically sound and does not fulfill the guideline requirements for an acute toxicity test using estuarine invertebrates. This study was conducted in 1983 before the EPA SEP was available (1985), therefore, many deviations from the current guidelines were noted; most importantly, the age of the test organisms (1-5 days old at test initiation) was $\geq$24 hours at test initiation and was variable among the test population. Current guidelines require mysids which are $\leq$24 hours old at test initiation. This study is classified as **Invalid.**

A 96-hour LC_{50} value of 1.41 ppm ai classifies CGA-77102 as moderately toxic to mysids. The NOEC could not be determined since mortality was noted at all concentrations tested.

RGM Bay Shrimp CGA-77102

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
4.61	20	20	100	9.536742E-05
3.13	20	19	95	2.002716E-03
1.67	20	14	70	5.765915
.96	20	2	10	2.012253E-02
.51	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT .96 AND 3.13 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 1.406309

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.0517176	1.403039	1.152042	1.668302

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1094463	1	.3183674

SLOPE = 4.945184

95 PERCENT CONFIDENCE LIMITS = 3.309185 AND 6.581183

LC50 = 1.407836

95 PERCENT CONFIDENCE LIMITS = 1.172586 AND 1.680962

LC10 = .7793569

95 PERCENT CONFIDENCE LIMITS = .5405514 AND .9662971

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