

MRID No. 448047-03

DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE SHRIMP
§ 72-3

1. **CHEMICAL:** Hydrogen cyanamide **PC Code No.:** 014002

2. **TEST MATERIAL:** Aqueous hydrogen cyanamide **Purity:** 50.8%

3. **CITATION:**

Authors: Gettmann, W., K.R. Drottter, and H.O. Krueger

Title: Hydrogen Cyanamide: A 96-Hour Flow-Through Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)

Study Completion Date: November 24, 1998

Laboratory: Wildlife International Ltd., Easton, MD

Sponsor: SKW Trostberg AG, Trostberg, Germany

Laboratory Report ID: 248A-102

MRID No.: 448047-03

DP Barcode: D255592

4. **REVIEWED BY:** Mark Mossler, M.S., Environmental Scientist,
Golder Associates Inc.

Signature: 

Date: 9/20/99

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
Golder Associates Inc.

Signature: 

Date: 9/20/99

5. **APPROVED BY:** 

Signature: 

Date: 1/27/00

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. The 96-hour LC₅₀ for mysids exposed to hydrogen cyanamide was 6.3 ppm ai, which classifies this compound as moderately toxic to *Mysidopsis bahia*. The NOEC was 1.4 ppm ai.



8. ADEQUACY OF THE STUDY:**A. Classification:** Core.**B. Rationale:** N/A.**C. Repairability:** N/A.**9. GUIDELINE DEVIATIONS:** No deviations of consequence were noted.**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are <i>Mysidopsis bahia</i> , <i>Penaeus setiferus</i> , <i>P. duorarum</i> , <i>P. aztecus</i> and <i>Palaemonetes sp.</i>	<i>Mysidopsis bahia</i>
<u>Age</u> Juvenile, mysids should be ≤ 24 hours old	<24 hours old
<u>Supplier</u>	In-house cultures
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> minimum 10 days	Adult mysids were cultured under the same temperature, salinity, and pH as those used during the study.
Wild caught organisms were quarantined for 7 days?	N/A

Guideline Criteria	Reported Information
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
<u>Feeding</u> 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.
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Natural filtered seawater collected from Indian River Inlet, DE - adjusted to 20 % with well water and aerated
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 % for marine (steno-haline) shrimp and 10-17 % for estuarine (euryhaline) shrimp, weekly range < 6 %	20
<u>Water Temperature</u> Approx. 22 ± 1 °C	24.5-25.1 °C
<u>pH</u> 8.0-8.3 for marine (steno-haline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	8.2-8.3

Guideline Criteria	Reported Information
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 hrs and $\geq 40\%$ during 2 nd 48 hrs, Flow-through: $\geq 60\%$	$\geq 80\%$ of saturation
<u>Total Organic Carbon</u>	<1.0 mg/L
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> 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. <u>Fill volume:</u> 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	Glass 300-mL beakers were suspended in 8-L stainless steel aquaria. Openings in the beakers were covered with nylon mesh to allow solution flow ≈ 220 mL
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Continuous-flow proportional diluter
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	14 volume additions per day
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^\circ\text{C}$, \leq 0.5 g/L at $> 17^\circ\text{C}$; flow- through: ≤ 1 g/L/day	Not reported
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 h light, 8 h dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Solvent: none Maximum conc.: N/A

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 shrimp, then no definitive test is required.	Concentrations selected based on consultation with the sponsor and a range finding test
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	Control, 1.3, 2.5, 5.1, 10, and 20 ppm of whole material, or 0.7, 1.3, 2.5, 5.0, and 10 ppm active ingredient (ai)
<u>Number of Test Organisms</u> 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?	Observations were made daily
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary $> 1^{\circ}C$ 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature was measured continuously throughout the test in one control vessel. Temperature was also measured at 0 and 96 hours in each test vessel. DO and pH were measured daily in alternating replicates of each test group.
<u>Chemical Analysis</u> 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	Samples were collected from each vessel at 0, 24, 48, 72, and 96 hours and analyzed by HPLC.

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 Percent of nominal, procedural recovery, limit of quantitation (LOQ)	108-112% of nominal, procedural recovery of 106%, LOQ = 0.30 ppm ai,
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No mortality in the control group
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Analytical results

Nominal concentration (ppm ai)	Measured concentration (ppm ai)				
	Hour of Study				
	0	24	48	72	96
Negative control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.7	0.6	0.7	0.7	0.7	0.7
1.3	1.5	1.4	1.4	1.4	1.5
2.5	2.7	2.8	2.7	2.8	2.9
5.1	5.3	5.1	5.3	5.5	5.7
10	11	11	11	12	N/A

Mortality

Concentration (ppm ai)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured*		Hour of Study			
			24	48	72	96
Control	<0.30	20	0	0	0	0
0.7	0.7	20	0	0	0	0
1.3	1.4	20	0	0	0	0
2.5	2.8	20	1	1	1	1
5.1	5.6	20	0	0	3	4
10	11	20	0	19	20	20

*Measured concentrations were not corrected for a procedural recovery of 106%.

Other Significant Results: Signs of test material toxicity noted at the two highest-concentration treatment levels included erratic swimming and lethargy.

B. Statistical Results

Method: probit method

96-hr LC₅₀: 6.6 ppm ai
Probit Slope: 6.7

95% C.I.: 5.6-7.6 ppm ai
NOEC: 1.4 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Method: moving average method

96-hr LC₅₀: 6.3 ppm ai
Probit Slope: N/A

95% C.I.: 5.5-7.5 ppm ai
NOEC: 1.4 ppm ai

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine shrimp. Based on mean measured concentrations, the 96-hour LC₅₀ of 6.3 ppm ai classifies hydrogen cyanamide as moderately toxic to the mysid shrimp. The NOEC was determined to be 1.4 ppm ai. This study is classified as **Core**.

Mossler Hydrogen cyanamide Mysidopsis bahia 9-15-99

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
11	20	20	100	9.536742E-05
5.6	20	4	20	.5908966
2.8	20	1	5	2.002716E-03
1.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 5.6 AND 11 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 6.907859

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	6.572952E-02	6.346616	5.45163 7.524563

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	3.465233	3.921085	1.981968E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 6.74757
95 PERCENT CONFIDENCE LIMITS = -5.813123 AND 19.30826

LC50 = 6.408731
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.154858
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
