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

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

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

3. **CITATION:**

Authors: Gettmann, W., K.R. Drottar, and H.O. Krueger
Title: Hydrogen Cyanamide: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead minnow (*Cyprinodon variegatus*)
Study Completion Date: November 24, 1998
Laboratory: Wildlife International Ltd., Easton, MD
Sponsor: SKW Trostberg AG, Trostberg, Germany
Laboratory Report ID: 248A-103
MRID No.: 448047-02
DP Barcode: D255592

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

Signature: [signature] **Date:** 9/20/99

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

Signature: P. Kosalwat **Date:** 9/20/99

5. **APPROVED BY:**

Signature: [signature] Andrew Brycelend **Date:** 1/27/00

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	18-23 mm
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 an estuarine fish. Based on mean measured concentrations, the 96-hour LC_{50} was determined to be 58 ppm ai, which classifies hydrogen cyanamide as slightly toxic to the sheepshead minnow. The NOEC was determined to be 26 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
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the silverside (*Menidia* spp.).	*Cyprinodon variegatus*
Mean Weight 0.1-5 g	Mean: 0.32 g Range: 0.19 - 0.47 g
Mean Standard Length Longest not > 2x shortest	Mean: 20 mm Range: 18 - 23 mm
Supplier	Aquatic Bio Systems, Fort Collins, CO
All fish from same source?	Yes
All fish from the same year class?	Yes

   B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Held under similar conditions for 28 days and under test conditions for 51 hours
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
Feeding No feeding during the study	Not fed 51 hours prior to or during testing
Pretest Mortality < 3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Reconstituted seawater or seawater from a natural source.	Natural seawater pumped from Indian River Inlet, DE, diluted to a salinity of approximately 20‰ with well water, filtered, and aerated
Does water support test animals without observable signs of stress?	Yes
Salinity Weekly range should not deviate by more than 6‰.	21‰
Water Temperature 22°C	21.8 - 23.3°C
pH Monthly range must not deviate by more than 0.8 unit. Euryhaline: 7.7-8.0 Stenohaline: 8.0-8.3	8.1 - 8.2
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥82% throughout test

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	 Stainless steel 25-L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-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	Six volume additions per day
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.035 g/L/day
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	None

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.	Concentrations were selected based on an exploratory range finding toxicity test and consultation with the sponsor.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Control, 16, 26, 43, 72, and 120 mg/L of total product (8, 13, 22, 37, and 61 mg active ingredient (ai)/L
Number of Test Organisms Minimum 10/level for static test, 20/level for flow-through, may be divided among containers	10 per replicate, 20 per treatment level
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 measured at test initiation and termination in each test chamber and continuously in one negative control replicate. 2. DO and pH were measured every 24 hours in alternating replicate test chambers.
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	Yes, solutions were collected for analysis from each replicate at test initiation, 48 hours, and termination 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)	121-125% of nominal, 103% procedural recovery, LOQ = 0.30 ppm ai
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0% 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	48	96
Negative control	<LOQ	<LOQ	<LOQ
8	10	9	11
13	17	15	16
22	29	23	27
37	50	39	45
61	84	64	74

Mortality

Concentration (ppm ai)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured*		24	48	72	96
Control	<LOQ	20	0	0	0	0
8	10	20	0	0	0	0
13	16	20	0	0	0	0
22	26	20	0	0	0	0
36	45	20	0	0	0	1
60	74	20	0	0	1	19

*Measured concentrations were not corrected for a procedural recovery of 103%.

Other Significant Results: Signs of test material toxicity noted at the two highest-concentration treatment levels included surfacing, lethargy, loss of equilibrium, and quiescence.

B. Statistical Results

Method: probit analysis

96-hr LC_{50}: 57 ppm ai
Probit Slope: 15.2
95% C.I.: 51-64 ppm ai
NOEC: 26 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Method: probit analysis

96-hr LC_{50}: 58 ppm ai
Probit Slope: 15.0
95% C.I.: 52-64 ppm ai
NOEC: 26 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine fish. Based on mean measured concentrations, the 96-hour LC_{50} of 58 ppm ai classifies hydrogen cyanamide as slightly toxic to the sheepshead minnow. The NOEC was determined to be 26 ppm ai. This study is classified as **Core.**

Sheepshead minnow mortality (96 h) - probit

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
26.0000	20	0	0.0000	0.0000	0.0000
45.0000	20	1	0.0500	0.0500	0.0500
74.0000	20	19	0.9500	0.9500	0.9500

Chi - Square Heterogeneity = -0.000

Mu = 1.761222
Sigma = 0.065665

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-21.821522	5.458572	(-32.520325, -11.122720)
Slope	15.228927	3.093500	(9.165666, 21.292187)

Theoretical Spontaneous Response Rate = 0.0000

Sheepshead minnow mortality (96 h)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	40.5945	31.1562	46.3274
EC 5.00	44.9998	36.6028	50.3748
EC10.00	47.5408	39.7839	52.8105
EC15.00	49.3364	42.0219	54.6034
EC50.00	57.7062	51.8015	64.2839
EC85.00	67.4958	60.9851	79.2447
EC90.00	70.0451	63.0556	83.7026
EC95.00	74.0003	66.1044	90.9769
EC99.00	82.0309	71.8796	106.8812