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

1. **CHEMICAL:** S-Dimethenamid **PC Code No.:** 120051

2. **TEST MATERIAL:** SAN 1289H Technical **Purity:** 91.1% as S-Dimethenamid; 96.3% as total dimethenamid

3. **CITATION:**

Authors: W.C. Graves and J.P. Swigert
Title: SAN 1289H Technical: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*)
Study Completion Date: September 3, 1996
Laboratory: Wildlife International Ltd., Easton, MD
Sponsor: Sandoz Agro, Inc., Des Plaines, IL
Laboratory Report ID: 131A-174
MRID No.: 443322-30
DP Barcode: D238350, D238356

4. **REVIEWED BY:** Pim Kosalwat, Ph.D., Senior Scientist
Golder Associates Inc.

Signature: P. Kosalwat **Date:** 10/23/97

APPROVED BY: Max A. Feken, M.S., Environmental Toxicologist
Golder Associates Inc.

Signature: [signature] **Date:** 10/23/97

5. **APPROVED BY:**

Signature: [signature] **Date:** 11/18/97
11/4/98

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: 26 mm
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Mean measured (as total dimethenamid)

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements. The 96-hour LC_{50} for sheepshead minnow was determined to be 12 ppm, which classifies SAN 1289H technical as slightly toxic to the sheepshead minnow.

Results Synopsis: Results are based on mean measured concentrations as total dimethenamid.
LC_{50}: 12 ppm 95% C.I.: 9.2-16 ppm
NOEC: 5.3 ppm Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

   A. **Classification:** Core

   B. **Rationale:** N/A

   C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS:** No significant deviations.

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	0.31 g
Mean Standard Length Longest not > 2x shortest	Mean: 21 mm Range: 18-26 mm
Supplier	In-house cultures
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	Cultures maintained under conditions similar to testing; test fish acclimated to test conditions for 3 days.
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	No feeding during acclimation and test periods.
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Seawater collected from Indian River Inlet, DE, was adjusted to a salinity of 20 ‰ with well water. The water was aerated and filtered.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range <6 ‰	20 ‰
Water Temperature 22 ± 1 °C	22.0-22.9°C

Guideline Criteria	Reported Information
pH 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	8.3-8.4
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥64% saturation during the test
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	 Teflon®-lined polyethylene 25-L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow diluter
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	6 volume replacements/day, meter systems calibrated before the test and checked twice daily during the test.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.034 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.: 0.1 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.	Range-finding test was conducted but results were not reported.
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	Negative control, solvent control; and 3.2, 5.4, 9.0, 15, and 25 mg/L, not corrected for percentage active ingredient
Number of Test Organisms Minimum 10/level, may be divided among containers	20 fish per treatment level and control, 10 per replicate.
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	Temperature measured in each test chamber at test initiation and termination, and monitored continuously in one negative control chamber. DO and pH measured daily in alternate replicate 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	Solutions from alternate replicates were collected and analyzed at 0, 48, and 96 hours using GC.

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	98-108% of nominal
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured*		24	48	72	96
Control	<0.25	20	0	0	0	0
Solv. Cont.	<0.25	20	0	0	0	0
3.2	3.4	20	0	0	0	0
5.4	5.3	20	0	0	0	0
9.0	9.2	20	0	0	0	0
15	16	20	0	9	19	20
25	27	20	5	20	20	20

* Measured as total dimethenamid

Other Significant Results: Signs of toxicity observed at the three highest test levels included erratic swimming, discoloration, lying on bottom with little motion, and lethargy.

B. Statistical Results

Method: Binomial method

96-hr LC_{50}: 12 ppm
Probit Slope: N/A
95% C.I.: 9.2-16 ppm
NOEC: 5.3 ppm

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC_{50} (95% C.I.)	12 ppm (9.2-16 ppm)
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	5.3 ppm

14. REVIEWER'S COMMENTS: This study is scientifically sound and can be classified as **Core.** Based on mean measured concentrations as total dimethenamid, the 96-hour LC_{50} for sheepshead minnow was determined to be 12 ppm, which classifies the test material as slightly toxic to the sheepshead minnow. The NOEC was 5.3 ppm.

KOSALWAT SAN 1289H TECHNICAL CYPRINODON VARIEGATUS 10-23-97

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
27	20	20	100	9.536742E-05
16	20	20	100	9.536742E-05
9.2	20	0	0	9.536742E-05
5.3	20	0	0	9.536742E-05
3.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 9.2 AND 16 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 12.1326

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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