

DATA EVALUATION RECORD
§ 72-2 - ACUTE EC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

- 1. **CHEMICAL:** Suttocide A PC Code No.: 128972
- 2. **TEST MATERIAL:** Sodium Hydroxymethylglycinate Purity: 49.53%
- 3. **CITATION:**

Authors: W.C. Graves and J.P. Swigert
Title: Suttocide A (Integra 44): A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

Study Completion Date: August 23, 1996
Laboratory: Wildlife International Ltd., Easton, MD
Sponsor: ISP Sutton Laboratories, Chatham, NJ
Laboratory Report ID: 300A-109
MRID No.: 441058-01
DP Barcode: D230022 & D230023

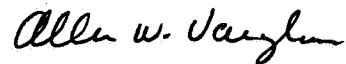
- 4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist,
KBN Engineering and Applied Sciences, Inc.,

Signature:  Date: 6/18/97

- APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist,
KBN Engineering and Applied Sciences, Inc.,

Signature: P. Kosalwat Date: 6/18/97

- 5. **APPROVED BY:** Allen W. Vaughan 6/27/97

Signature: Date:  01/06/98

- 6. **STUDY PARAMETERS:**

Age of Test Organism: <24 hours
Definitive Test Duration: 48 hours
Study Method: Flow-through
Type of Concentrations: Mean measured

- 7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements. The 48-hour EC₅₀ value of 39 ppm ai classifies Suttocide A as slightly toxic to *Daphnia magna*.

Results Synopsis:
 EC₅₀: 39 ppm ai 95% C.I.: 29 - 53 ppm ai
 NOEC: 15 ppm ai Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

- A. **Classification:** Core for a formulated product.
- B. **Rationale:** N/A.
- C. **Repairability:** N/A.

9. GUIDELINE DEVIATIONS: The pH of the test solutions (up to 9.3) was greater than recommended (7.2-7.6).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Not reported
<u>Life Stage</u> Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 rd instar.	1 st instar (<24 h)
<u>Supplier</u>	In-house cultures
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	Cultures maintained for 16-30 days under conditions similar to testing prior to neonate collection
Wild caught organisms were quarantined for 7 days?	N/A

Guideline Criteria	Reported Information
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
<u>Feeding</u> No feeding during the study.	No feeding during the study
<u>Pretest Mortality</u> No more than 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.	Moderately-hard well water, filtered and aerated
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	19.5-20.5°C
<u>pH</u> Prefer 7.2 to 7.6.	8.3-9.3
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 h and ≥ 40% during 2 nd 48 h, flow-through: ≥ 60%.	≥95% of saturation during the test
<u>Total Hardness</u> Prefer 40 to 200 mg/L as CaCO ₃ .	125 mg/L as CaCO ₃

Guideline Criteria	Reported Information
<p><u>Test Aquaria</u></p> <p>1. <u>Material:</u> Glass or stainless steel.</p> <p>2. <u>Size:</u> 250 mL (daphnids and midges) or 3.9 L (1 gal).</p> <p>3. <u>Fill volume:</u> 200 mL (daphnids and midges) or 2-3 L.</p>	<p>Glass</p> <p>300 mL</p> <p>≈270 mL</p>
<p><u>Type of Dilution System</u> Must provide reproducible supply of toxicant.</p>	<p>Continuous-flow diluter</p>
<p><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.</p>	<p>14 volume replacements every 24 hours</p>
<p><u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.</p>	<p>N/A</p>
<p><u>Photoperiod</u> 16 hours light, 8 hours dark.</p>	<p>16 hours light, 8 hours dark</p>
<p><u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.</p>	<p>Solvent: none Maximum conc.: N/A</p>

D. Test Design

Guideline Criteria	Reported Information
<p><u>Range Finding Test</u> If EC₅₀ >100 mg/L, then no definitive test is required.</p>	<p>Yes, but results were not reported</p>
<p><u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.</p>	<p>Control, 16, 26, 43, 72, and 120 mg active ingredient (ai)/L</p>

Guideline Criteria	Reported Information
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.	20 per level, 10 per replicate
Test organisms randomly or impartially assigned to test vessels?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary > 1°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 measured continuously in one negative control chamber and in each chamber at initiation and termination DO and pH measured daily in alternating replicates
<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 alternating replicates at 0 and 48 hours after test initiation and analyzed using HPLC coupled with pulsed electrochemical detection.

12. REPORTED RESULTS:**A. General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> Static: ≤10% Flow-through: ≤5%	0% mortality in the dilution water control group
Percent Recovery of Chemical	46-97%
Raw data included?	Yes

Mortality

Concentration (ppm ai)		Number of Organisms	Cumulative Number Dead/Immobile	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	<0.26	20	0	0
16	7.3	20	0	0
26	15	20	0	0
43	29	20	0	0
72	53	20	2	20
120	116	20	20	20

Other Significant Results: Three daphnids exposed at the 29 ppm level were noted as lethargic at test termination.

B. Statistical Results

Method: Binomial method

48-hr EC₅₀: 39 ppm ai

Probit Slope: N/A

95% C.I.: 29 - 53 ppm ai

NOEC: 15 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test EC ₅₀ (C.I.)	39 (29 - 53) ppm ai
Moving Average Angle EC ₅₀ (95% C.I.)	N/A
Probit EC ₅₀ (95% C.I.)	N/A
Probit Slope	N/A
NOEC	15 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound, fulfills the guideline requirements for an acute toxicity study using freshwater invertebrates, and can be classified as Core for a formulated product. The 48-hour EC₅₀ value of 39 ppm ai (79 ppm as whole product) classifies Suttocide A as slightly toxic to *Daphnia magna*.

Mossler Suttocide A Daphnia magna 6-17-97

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
116	20	20	100	9.536742E-05
53	20	20	100	9.536742E-05
29	20	0	0	9.536742E-05
15	20	0	0	9.536742E-05
7.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 29 AND 53 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 39.2046

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.
