

DATA EVALUATION RECORD
§ 72-1 - ACUTE LC₅₀ TEST WITH A COLDWATER FISH

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 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)

Study Completion Date: August 23, 1996

Laboratory: Wildlife International Ltd., Easton, MD

Sponsor: ISP Sutton Laboratories, Chatham, NJ

Laboratory Report ID: 300A-107

MRID No.: 441058-02

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 or Size of Test Organism: 24-27 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. A 96-hour LC₅₀ of 106 ppm ai classifies Suttocide A as practically non-toxic to the trout.

Results Synopsis:

LC₅₀: 106 ppm ai
NOEC: 35 ppm ai

95% C.I.: 90 - 130 ppm ai
Probit Slope: 7.1

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 9.4) 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 the rainbow trout (<i>Oncorhynchus mykiss</i>)	<i>Oncorhynchus mykiss</i>
<u>Mean Weight</u> 0.5-5 g	0.25 g
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 26 mm Range: 24-27 mm
<u>Supplier</u>	Troutlodge, Inc., Sumner, WA
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 14 days	Held under similar conditions to testing for 35 days and under testing conditions for 51 hours prior to testing
Wild caught organisms were quarantined for 7 days?	N/A

Guideline Criteria	Reported Information
Were there signs of disease or injury?	No signs of disease or stress
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	Last fed 48 hours prior to testing
<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	Moderately-hard well water, filtered and aerated
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 12°C	11.5-12.5°C
<u>pH</u> Prefer 7.2 to 7.6	8.3-9.4
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	≥88% of saturation during the test
<u>Total Hardness</u> Prefer 40 to 200 mg/L as CaCO ₃	132 mg/L as CaCO ₃

Guideline Criteria	Reported Information
<p><u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume:</u> 15-30 L of solution</p>	<p>Teflon®-lined polyethylene 25-L 15 L</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>6 volume replacements every 24 hours</p>
<p><u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^\circ\text{C}$, ≤ 0.5 g/L at $> 17^\circ\text{C}$; flow-through: ≤ 1 g/L/day</p>	<p>0.028 g/L/day</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 $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.</p>	<p>Yes, but results were not reported</p>

Guideline Criteria	Reported Information
<p><u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	<p>Control, 16, 26, 43, 72, and 120 mg active ingredient (ai)/L</p>
<p><u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers</p>	<p>20, 10 per replicate</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>
<p>Biological observations made every 24 hours?</p>	<p>Yes</p>
<p><u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, 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</p>	<p>Temperature measured constantly in one negative control chamber and at test initiation and termination in each test chamber DO and pH measured daily in alternating chambers that contained live fish</p>
<p><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</p>	<p>Samples were collected from one replicate chamber at 0, 48, and 96 hours after test initiation and analyzed using HPLC coupled with pulsed electrochemical detection.</p>

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes</p>

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Guideline Criteria	Reported Information
<u>Recovery of Chemical</u>	71-100%
<u>Control Mortality</u> 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

Mortality

Concentration (ppm ai)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<0.26	20	0	0	0	0
16	11	20	0	0	0	0
26	21	20	0	0	0	0
43	35	20	0	0	0	0
72	62	20	0	1	1	1
120	120	20	4	6	6	13

Other Significant Results: Lethargy, surfacing, and discoloration were signs noted among fish exposed at the two highest-concentration exposure levels.

B. Statistical Results

Method: Probit

96-hr LC₅₀: 106 ppm ai
Probit Slope: Not reported

95% C.I.: 90 - 130 ppm ai
NOEC: 35 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	104 (0 - ∞) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	104 (89 - 134) ppm ai
Probit LC ₅₀ (95% C.I.)	106 (90 - 130) ppm ai
Probit Slope	7.1
NOEC	35 ppm ai

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound, fulfills the guideline requirements, and can be classified as **Core for a formulated product**. The 96-hour LC₅₀ for rainbow trout exposed to Suttocide A was determined to be 106 ppm ai (214 ppm of whole product), which classifies this material as practically non-toxic to the rainbow trout.

Mossler Suttocide A Oncorhynchus mykiss 6-17-97

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
120	20	13	65	13.1588
62	20	1	5	2.002716E-03
35	20	0	0	9.536742E-05
21	20	0	0	9.536742E-05
11	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 103.87

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.2127723	103.87	89.12939	133.8395

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
11	.2672274	1	.9998336

SLOPE = 7.117801
 95 PERCENT CONFIDENCE LIMITS = 3.438322 AND 10.79728

LC50 = 105.8812
 95 PERCENT CONFIDENCE LIMITS = 89.72939 AND 130.2839

LC10 = 70.2091
 95 PERCENT CONFIDENCE LIMITS = 45.32696 AND 84.03901
