

DP Barcode : D228348
PC Code No : 088002
EEB Out : / /

MAY 23 1997

MAY 23 1997

To: Marion Johnson
Product Manager 31
Registration Division (7505C)

From: *Dr. Rueda*
~~Anthony F. Maciorowski~~, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 001258-REEG
Chemical Name : Zinc 2-pyridinethiol-1-oxide
Type Product : antifoulant
Product Name : Zinc Omadine
Company Name : Olin
Purpose : Review data for antifoulant use.

Action Code: 176
Reviewer: Joanne Edwards

Date Due: 11/22/96

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1 (A)			72-2 (A)			72-7 (A)		
71-1 (B)			72-2 (B)			72-7 (B)		
71-2 (A)			72-3 (A)			122-1 (A)		
71-2 (B)			72-3 (B)			122-1 (B)		
71-3			72-3 (C)			122-2		
71-4 (A)			72-3 (D)			123-1 (A)		
71-4 (B)			72-3 (E)			123-1 (B)		
71-5 (A)			72-3 (F)			123-2		
71-5 (B)			72-4 (A)			124-1		
72-1 (A)			72-4 (B)			124-2		
72-1 (B)			72-5			141-1		
72-1 (C)			72-6			141-2		
72-1 (D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur

P=Partial (Study partially fulfilled Guideline but additional information is needed)

S=Supplemental (Study provided useful information but Guideline was not satisfied)

N=Unacceptable (Study was rejected)/Nonconcur



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 23 1997

MEMORANDUM

SUBJECT: Data Evaluations for Zinc 2-pyridinethiol-1-oxide (088002); D228348,
Sponsor: Olin Corporation

TO: Marion Johnson, PM 31 (7505C)
Registration Division

FROM: Dan Rieder, Acting Chief *Dan Rieder* 5/23/97
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

A summary of the findings for studies performed using zinc omadine is provided below:

GUIDELINE	MRID	TOXICITY	ACCEPTABILITY/ CLASSIFICATION
122-2	438646-09	EC50 = 28 ppb NOEC = 7.8 ppb	Core
72-3(b)	438646-08	EC50 = 22 ppb NOEC = 7.1 ppb	Core Very highly toxic
72-3(a)	438646-05	LC50 = 0.40 ppm NOEC = 0.20 ppm	Core Highly toxic
72-2	438646-04	EC50 = 8.25 ppb NOEC = <1.1 ppb	Core Very highly toxic

GUIDELINE	MRID	TOXICITY	ACCEPTABILITY/ CLASSIFICATION
71-2(a)	438646-10	LC50 = 1110 ppm	Core Slightly toxic
71-2(b)	438646-12	LC50 > 5000 ppm	Core Practically non-toxic
71-1(a)	438646-11	LD50 = 82.4 mg/kg NOEC < 31.2 mg/kg	Core Moderately toxic
72-3(c)	438646-07	LC50 = 4.7 ppb NOEC = 1.6 ppb	Core Very highly toxic
72-1 (rainbow trout)	438646-13	LC50 = 3.6 ppb NOEC = 1.6 ppb	Core Very highly toxic
72-1 (fathead minnow)	438646-06	LC50 = 2.68 ppb NOEC = 1.1 ppb	Core Very highly toxic

A summary of the findings for studies performed using pyridine sulfonic acid is provided below:

GUIDELINE	MRID	TOXICITY	ACCEPTABILITY/ CLASSIFICATION
122-2	438646-25	EC50 = 28.9 ppm NOEC = 5.46 ppm	Core
72-3 Mollusc Shell Deposition	438646-24	EC50 = 85.6 ppm NOEC = 51.1 PPM	Core Slightly toxic
72-3(b) Estuarine Shrimp	438646-26	LC50 = 71.0 ppm NOEC = 51.9 ppm	Core Slightly Toxic
72-2	438646-22	LC50 = > 122 ppm NOEC = 122.0	Core Practically non-toxic
72-3 Sheepshead Minnow	438646-23	LC50 > 127.0 ppm NOEC = 127.0 ppm	Core Practically non-toxic
72-1	438646-21	LC50 = 68.5 ppm NOEC = 55.2 ppm	Core Slightly toxic
72-1	438646-27	LC50 = 57.1 ppm NOEC = 46.9 ppm	Core Slightly toxic

These data were submitted to support an antifoulant use. In order for us to review such the use, the proposed labeling must be submitted.

If you have any questions concerning this review please, contact Joanne Edwards (305-6736) or Les Touart (305-6134).

DATA EVALUATION RECORD
§ 72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

1. CHEMICAL: Zinc Omadine

PC Code No.: 001258

2. TEST MATERIAL: Zinc bis-1-oxide-2(1H)-pyridenethionate, off-white powder,
CAS No.13463-41-7. Purity: 97.8%

3. CITATION

Authors: R. L. Boeri, J. P. Magazu, and T. J. Ward

Title: Acute toxicity of Zinc Omadine to the Daphnid,
Daphnia magna.

Study Completion Date: 8 July 1994

Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA

Sponsor: Olin Corporation, New Haven, CT

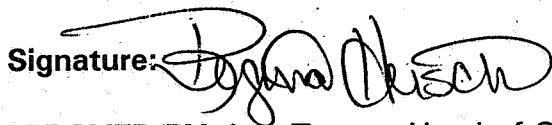
Laboratory Report ID: 21-OL

MRID No.: 438646-04

DP Barcode: D228348

4. REVIEWED BY: Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature:

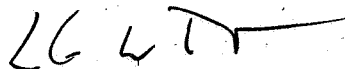


Date:

1/10/97

5. APPROVED BY: Les Touart, Head of Section (1), EEB, EFED

Signature:



Date:

5/19/97

6. STUDY PARAMETERS

Scientific Name of Test Organism:

Daphnia magna

Age of Test Organism:

< 24 hours old/0.08 mg wet
weight

Definitive Test Duration:

48 hours

Study Method:

Flow-through

Type of Concentrations:

Mean measured

7. CONCLUSIONS:

Results Synopsis

LC₅₀: 8.25 µg/L ai

95% C.I.: 5.24-25.82 µg/L ai

NOEL: < 1.1 µg/L ai

Probit Slope: 1.4

8. ADEQUACY OF THE STUDY**A. Classification:** Core.**B. Rationale:** N/A**C. Repairability:** N/A**9. Guideline Deviations**

1. Dechlorinated tapwater was used as the dilution water.
2. The pH was 8.1 instead of 7.2-7.6.
3. Water hardness exceeded (160 mg/L as CaCO₃) the recommended limits (40 to 48 mg/L as CaCO₃).

10. SUBMISSION PURPOSE: Registration**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 th instar.	1 st instar
<u>Supplier</u>	Produced from an in-house culture
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	7 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
<u>Feeding</u> No feeding during the study.	Daphnids were not fed during the study.
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	0% mortality prior to testing

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.	Dechlorinated tapwater collected at T. R. Wilbury Laboratories.
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	19.2-19.8 °C

Guideline Criteria	Reported Information
pH Prefer 7.2 to 7.6.	8.1
Dissolved Oxygen Static: $\geq 60\%$ during 1 st 48 h and $\geq 40\%$ during 2 nd 48 h, flow-through: $\geq 60\%$.	8.7 mg/L at 48 hours
Total Hardness Prefer 40 to 48 mg/L as CaCO_3 .	160 mg/L as CaCO_3
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 ml (daphnids and midges) or 2-3 L.	Glass 20L 15L, Daphnids were exposed in cages suspended in the aquaria that consisted of a Nitex screen cylinder with a glass bottom.
Type of Dilution System Must provide reproducible supply of toxicant.	Intermittent flow proportional diluter. The diluter, constructed at T. R. Wilbury allowed test media to contact only glass, stainless steel, or Teflon surfaces.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	6.6 vol/24 hours
Biomass Loading Rate 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.	0.00005 g/L (0.000008 g/L/24 hours)
Photoperiod 16 hours light, 8 hours dark.	16 hours light, 8 hours dark

Guideline Criteria	Reported Information
<u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	None used

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L, then no definitive test is required.	Screening tests conducted, but none had $LC_{50} > 100$ mg/L.
<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.	Control, 2.4, 4.1, 6.7, 9.8, and 17.0 μ g/L
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.	20 per test level in 2 replicates of 10 daphnids each.
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 $> 1^{\circ}\text{C}$. 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.	The temperature in one test vessel was recorded continuously during the test. DO, pH, and temperature were measured and recorded daily in each test chamber that contained live daphnids.

<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	Analytical determination of test material concentration from the definitive test was performed on pooled samples collected midway between the top, bottom, and sides of the 2 replicates of each concentration at the beginning and end of the test.
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12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> Static: $\leq 10\%$ Flow-through: $\leq 5\%$	0%
<u>Percent Recovery of Chemical</u>	46-67% of nominal
Raw data included?	No

Mortality

Concentration (µg/L)		Number of Organisms	Cumulative Number Dead and Number Affected in ()	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	--	20	0	0
2.4	1.1	20	0	1
4.1	2.4	20	0	5
6.7	4.2	20	2	9
9.8	6.6	20	1	5 (12)
17.0	11.0	20	3	13 (7)

Other Significant Results: Affected daphnids were lethargic.

B. Statistical Results

Method: Probit

48-hr LC₅₀: 8.2 µg/L ai

95% C.I.: 5.2 - >11.0 µg/L ai

Probit Slope: 1.4

NOEC: 1.1 µg/L ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	9.11 (2.4-infinity) µg/L ai
Moving Average Angle LC ₅₀ (95% C.I.)	9.11 (7.17-14.22) µg/L ai
Probit LC ₅₀ (95% C.I.)	8.26 (5.24-25.83) µg/L ai
Probit Slope	1.4
NOEC	<1.1 µg/L ai

14. REVIEWER'S COMMENTS:

Regina Hirsch Zinc Omadine Acute Toxicity to Daphnids

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
11	20	13	65	13.1588
6.6	20	5	25	2.069473
4.2	20	9	45	41.19014
2.4	20	5	25	2.069473
1.1	20	2	10	2.012253E-02

THE BINOMIAL TEST SHOWS THAT 2.4 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 9.112436

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.6047557	9.112436	7.170254	14.22287

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	.349881	1
GOODNESS OF FIT PROBABILITY		
.1887432		

SLOPE = 1.37821
95 PERCENT CONFIDENCE LIMITS = .5629886 AND 2.193432

LC50 = 8.259187
95 PERCENT CONFIDENCE LIMITS = 5.242572 AND 25.82752

LC10 = .9895832
95 PERCENT CONFIDENCE LIMITS = .1035908 AND 1.911994

DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE FISH
§ 72-3(A)

1. **CHEMICAL:** Zinc Omadine

PC Code No.: 001258

2. **TEST MATERIAL:** Zinc bis-1-oxide-2(1H)-pyridenethionate, off-white powder,
CAS No.13463-41-7.

Purity: 97.8%

3. **CITATION**

Authors: R. L. Boeri, J. P. Magazu, and T. J. Ward

Title: Acute Toxicity of Zinc Omadine to the Sheepshead Minnow, *Cyprinodon variegatus*.

Study Completion Date: 13 July 1994

Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA

Sponsor: Olin Corporation, New Haven, CT

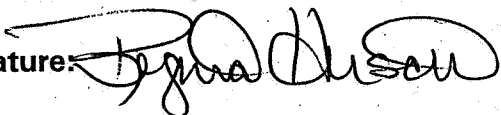
Laboratory Report ID: 22-OL

MRID No.: 438646-05

DP Barcode: D228348

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature:

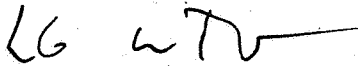


Date:

1/10/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature:



Date:

5/15/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:

Cyprinodon variegatus

Age or Size of Test Organism:

Juvenile/0.26 g, wet weight

Definitive Test Duration:

96 hours

Study Method:

Static renewal

Type of Concentrations:

Mean measured

7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: 0.40 ppm ai

95% C.I.: 0.20-0.59 ppm ai

NOEL: 0.20 ppm ai

8. ADEQUACY OF THE STUDY**A. Classification:** Core.**B. Rationale:** N/A**C. Repairability:** N/A**9. Guideline Deviations**

1. Fish were smaller (0.26 g) than what is recommended (0.5-5 g).
2. Range of fish weights and lengths were not included in report.
3. Water adjusted to appropriate salinity with dechlorinated tapwater.
4. Temperature range varied more than 1 degree.

10. SUBMISSION PURPOSE: Registration**11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are the sheepshead minnow (<i>Cyprinodon variegatus</i>) or the Silverside (<i>Menidia sp.</i>).	<i>Cyprinodon variegatus</i>
<u>Mean Weight</u> 0.5 - 5 g	0.26 g
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 25 mm Range: Not reported.
<u>Supplier</u>	Aquatic Biosystems, Inc., Fort Collins, CO
All fish from same source?	Yes

Guideline Criteria	Reported Information
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 14 days	14 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	48 hours preceding test initiation
Pretest Mortality <3% mortality 48 hours prior to testing	<3% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Natural seawater collected at Marblehead, MA. Water adjusted to appropriate salinity with dechlorinated tapwater.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	16 ppt

Guideline Criteria	Reported Information
<u>Water Temperature</u> 22 $\pm$ 1 °C	21.0-22.4°C
<u>pH</u> 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	7.8-8.0
<u>Dissolved Oxygen</u> Static: $\geq$ 60% during 1 st 48 hrs and $\geq$ 40% during 2 nd 48 hrs, flow-through: $\geq$ 60%	6.8 mg/L at 96 hours
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume:</u> 15-30 L of solution	Glass 20L 15L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Static renewal conditions. Test media were renewed after 48 hours of exposure.
<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	N/A
<u>Biomass Loading Rate</u> Static: $\leq$ 0.8 g/L at $\leq$ 17°C, $\leq$ 0.5 g/L at $>$ 17°C; flow-through: $\leq$ 1 g/L/day	0.17 g/L
<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

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	Screening tests were performed but LC_{50} 's did not exceed 100 mg/L
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Control, 0.13, 0.22, 0.36, 0.60, and 1.0 mg ai/L.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	20 fish per test level (10 in each of 2 replicates)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<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 in one test vessel was recorded continuously throughout the test. DO, pH, and temperature were measured and recorded daily in each test chamber that contained live fish.

Guideline Criteria	Reported Information
<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	Analytical determination of test material concentration from the definitive test was performed on pooled samples collected midway between the top, bottom, and sides of the 2 replicates of each concentration at the beginning and end of the test, and before and after media renewal at 48 hours.

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

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	75-100 % of nominal
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0 %
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead and Number Affected in ()			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	--	20	0	0	0	0
0.13	0.098	20	0	0	0	0
0.22	0.20	20	0	0	0	0
0.36	0.36	20	0 (2)	2	5	6
0.60	0.59	20	20	20	20	20
1.0	0.99	20	20	20	20	20

Other Significant Results: Affected fish exhibited a loss of equilibrium.

B. Statistical Results

Method: Binomial/nonlinear interpolation

96-hr LC_{50} : 0.40 ppm ai 95% C.I.: 0.20-0.59 ppm ai

Probit Slope: Not reported NOEC: 0.20 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	0.40 (0.2-0.59) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	When there are less than 2 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.

DP Barcode: D228348

MRID No.: 438646-05

Probit LC ₅₀ (95% C.I.)	see above
Probit Slope	
NOEC	0.20 ppm ai

14. REVIEWER'S COMMENTS:

Regina Hirsch Zinc Omadine Acute Toxicity to the Sheepshead Minnow

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.99	20	20	100	9.536742E-05
.59	20	20	100	9.536742E-05
.36	20	6	30	5.765915
.2	20	0	0	9.536742E-05
.098	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .2 AND .59 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 .4022538

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.

Regina Hirsch Zinc Omadine Acute Toxicity to the Sheepshead Minnow

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.99	20	20	100	9.536742E-05
.59	20	20	100	9.536742E-05
.36	20	6	30	5.765915
.2	20	0	0	9.536742E-05
.098	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .2 AND .59 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 .4022538

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.

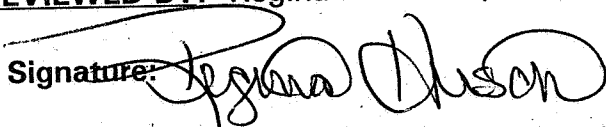
DATA EVALUATION RECORD
§ 72-1(A) -- ACUTE LC₅₀ TEST WITH A WARMWATER FISH

1. **CHEMICAL:** Zinc Omadine PC Code No.: 001258
2. **TEST MATERIAL:** Zinc bis-1-oxide-2(1H)-pyridenethionate, off-white powder,
CAS No.13463-41-7 Purity: 97.8%

3. **CITATION**

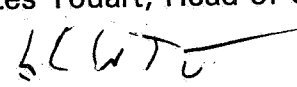
Authors: R. L. Boeri, J. P. Magazu, and T. J. Ward
Title: Acute toxicity of Zinc Omadine to the fathead minnow, *Pimephales promelas*
Study Completion Date: 12 July 1994
Laboratory: T. R. Wilbury Laboratories, Marblehead, MA
Sponsor: Olin Corporation, New Haven, CT
Laboratory Report ID: 19-OL,
MRID No.: 438646-06
DP Barcode: D228348

4. **REVIEWED BY:** Regina M. Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 1/8/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5/19/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Pimephales promelas</i>
Age or Size of Test Organism:	Juvenile/average 0.28 g
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: 2.68 µg/L ai

NOEC: 1.1 µg/L

95% C.I.: 2.10-3.27 g/L ai

Probit Slope: 4.88

8. ADEQUACY OF THE STUDY**A. Classification:** Core.**B. Rationale:** N/A**C. Repairability:** N/A**9. GUIDELINE DEVIATIONS**

1. Average weight of test organisms were smaller (0.28 g) than recommended (0.5-5.0 g).
2. Mortality prior to test initiation should be $\leq 3\%$, study reported mortality as $\leq 5\%$, unsure if meets criteria.
3. Dilution water was dechlorinated tap water.

10. SUBMISSION PURPOSE: Registration**11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the bluegill sunfish (<i>Lepomis macrochirus</i>)	Fathead minnow, <i>Pimephales promelas</i>
<u>Mean Weight</u> 0.5-5 g	0.28 g
<u>Mean Standard Length</u> Longest not > 2x shortest	32 mm
<u>Supplier</u>	Aquatic Biosystems, Fort Collins, CO
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	14 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
<u>Feeding</u> No feeding during the study	24 hour period prior to test initiation
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing	< 5% mortality prior to testing.

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	dechlorinated tap water collected at T.R. Wilbury Laboratories.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 17°C or 22°C	21.1-22.9 °C
<u>pH</u> Prefer 7.2 to 7.6	7.3-7.6

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: $\geq 60\%$	8.5 mg/L at 72 hour
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO_3	40 mg/L as CaCO_3
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume:</u> 15-30 L of solution	glass 20 L 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter constructed at T.R. Wilbury Laboratories
<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	5.8 vol/24 hours. The diluter was calibrated before and after the test.
<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	0.19 g/L (0.03 g/L/day)
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<u>Solvents</u> 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
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	3 screening tests and 2 definitive test were performed prior to this definitive test.
<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	Control, 3.0, 4.0, 7.0, 10.0, and 17.0 μ g ai/L.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	20/level (10 in each replicate - 2 replicates/level)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary $> 1^{\circ}\text{C}$ 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	Temperature in one test vessel was recorded continuously during the test. DO, pH, and temperature were measured and recorded daily in each test chamber that contained live fish.
<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	Test substance is photo-instable, therefore chemical analysis were performed.

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

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	37 -88% of Nominal
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0.05%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead and Number Affected in ()			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	0.0	20	0	1	1	1
3.0	1.1	20	0	0	1	2
4.0	2.6	20	2(1)	4(9)	8	8
7.0	4.8	20	16(4)	18(2)	18(2)	18(2)
10.0	7.9	20	19(1)	19(1)	19(1)	20
17.0	15.0	20	20	20	20	20

Other Significant Results:

After 24 and 48 hours of exposure all affected fish exhibited erratic swimming and lethargy. After 72 and 96 hours of exposure affected fish exhibited lethargy.

B. Statistical Results

Method: Probit Analysis

96-hr LC₅₀: 2.6 µg/L ai 95% C.I.: 2.0 - 3.1 µg/L ai

Probit Slope: 4.3 NOEC: 1.1 µg/L ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	2.99 (1.1-4.8) µg/L ai
Moving Average Angle LC ₅₀ (95% C.I.)	2.68 (2.1-3.3) µg/L ai
Probit LC ₅₀ (95% C.I.)	2.76 (2.2-3.3) µg/L ai
Probit Slope	4.88
NOEC	1.1 µg/L ai

14. REVIEWER'S COMMENTS:

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Regina Hirsch Zinc Omadine Acute Toxicity to Fathead Minnow

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
15	19	19	100	1.907348E-04
7.9	19	19	100	1.907348E-04
4.8	19	17	89.4737	3.643036E-02
2.6	19	7	36.8421	17.96417
1.1	19	1	5.2632	3.814697E-03

THE BINOMIAL TEST SHOWS THAT 1.1 AND 4.8 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 2.992117

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	7.652411E-02	2.684948	2.105504

3.268667

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	.1377375	1

GOODNESS OF FIT PROBABILITY

.7144211

SLOPE = 4.886639
95 PERCENT CONFIDENCE LIMITS = 3.073061 AND 6.700217

LC50 = 2.762645
95 PERCENT CONFIDENCE LIMITS = 2.2004 AND 3.349051

LC10 = 1.518563
95 PERCENT CONFIDENCE LIMITS = .9394259 AND 1.9577

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Regina Hirsch Zinc Omadine Acute Toxicity to the Fathead Minnow

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
15	19	19	100	1.907348E-04
7.9	19	19	100	1.907348E-04
4.8	19	17	89.4737	3.643036E-02
2.6	19	7	36.8421	17.96417
1.1	19	1	5.2632	3.814697E-03

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 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
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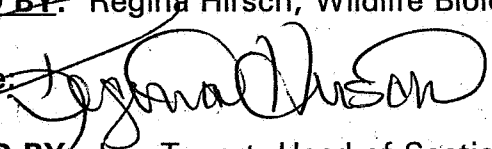
DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE SHRIMP
§ 72-3(C)

1. **CHEMICAL**: Zinc Omadine PC Code No.: 001258
2. **TEST MATERIAL**: Zinc bis-1-oxide-2(1H)-pyridenethionate, off-white powder,
CAS No. 13463-41-7 Purity: 97.8%

3. **CITATION**

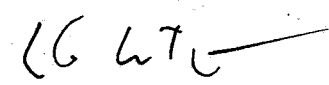
Authors: R. L. Boeri, J. P. Magazu, and T. J. Ward
Title: Acute toxicity of Zinc Omadine to the Mysid,
Mysidopsis bahia.
Study Completion Date: 11 July 1993
Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor: Olin Corp., New Haven, CT
Laboratory Report ID: 23-OL
MRID No.: 438646-07
DP Barcode: D228348

4. **REVIEWED BY**: Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 1/8/97

5. **APPROVED BY**: Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5/19/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Mysidopsis bahia</i>
Age or Size of Test Organism:	1.5 mg
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS**:

Results Synopsis

LC₅₀: 4.7 µg/L ai

NOEL: 1.6 µg/L ai

95% C.I.: 4.04 - 5.53 µg/L ai

8. ADEQUACY OF THE STUDY**A. Classification:** Core.**B. Rationale:** N/A**C. Repairability:** N/A**9. BACKGROUND****10. GUIDELINE DEVIATIONS**

1. Dilution water contained dechlorinated tap water.
2. Total organic carbon was not reported.

11. SUBMISSION PURPOSE: Registration**12. 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>	Aquatic Indicators, St. Augustine, FL
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	14 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
<u>Feeding</u> No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each.	Mysids were fed live <i>Artemia salina</i> daily during acclimation and testing.
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	<3% mortality prior to testing.

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	dilution as natural filtered seawater adjusted with dechlorinated tapwater to the correct salinity.
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 for marine (stenohaline) shrimp and 10-17 for estuarine (euryhaline) shrimp, weekly range < 6	11 to 19 ppt

Guideline Criteria	Reported Information
<u>Water Temperature</u> Approx. 22 ± 1 °C	21.3 to 22.9°C
<u>pH</u> 8.0-8.3 for marine (stenohaline) shrimp; 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.1 to 8.0
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 hrs and $\geq 40\%$ during 2 nd 48 hrs, Flow-through: $\geq 60\%$	7.9 mg/L at 24 hour.
<u>Total Organic Carbon</u>	Not reported
<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 20 L 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter. The diluter was constructed at T. R. Wilbury Laboratories, allowed test media to contact only glass, stainless steel, or Teflon surfaces.
<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	5.3 vol/24 hours

Guideline Criteria	Reported Information
<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	0.001 g/L (0.002 g/L/day)
<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	None

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $\text{LC}_{50} > 100$ mg/L with 30 shrimp, then no definitive test is required.	A screening test was not performed and historic data were used to determine the range of concentrations for the definitive 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, 2.4, 4.0, 6.4, 9.6, 16 $\mu\text{g ai/L}$.
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers	20/Level (2 replicates with 10 organisms per replicate)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
<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	Temperature in one test vessel was recorded continuously during the test. DO, pH, and temperature were measured and recorded daily in each test chamber that contained live shrimp.
<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	Analytical determination of test material concentration from the test was performed on pooled samples collected midway between the top, bottom, and sides of the 2 replicates of each concentration at the beginning and end of the test.

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	67-106 % of Nominal
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0.05%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	0.0	20	0	0	1	1
2.4	1.6	20	0	0	1	1
4.0	3.2	20	1	1	4	8
6.4	5.9	20	0	0	5	8
9.6	9.4	20	3	3	20	20
16.0	17.0	20	12	20	20	20

Other Significant Results:

No sublethal effects were observed.

B. Statistical Results

Method: Binomial/nonlinear interpolation

96-hr LC₅₀: 6.3 µg/L ai

95% C.I.: 1.6 to 9.4 µg/L ai

Probit Slope: Could not be calculated NOEC: 1.6 µg/L ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	6.35 (1.6-9.39) µg/L ai
Moving Average Angle LC ₅₀ (95% C.I.)	4.70 (4.04-5.53) µg/L ai
Probit LC ₅₀ (95% C.I.)	Probability less than 0.05 Probit should not be used.
NOEC	1.6 µg/L ai

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Regina Hirsch Zinc Omadine Acute Toxicity to Mysid Shrimp

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
17	19	19	100	1.907348E-04
9.399999		19	19	100
1.907348E-04				
5.9	19	7	36.8421	17.96417
3.2	19	7	36.8421	17.96417
1.6	19	0	0	1.907348E-04

THE BINOMIAL TEST SHOWS THAT 1.6 AND 9.399999 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 6.351465

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.359227E-02		4.705913	4.042063
5.531044				

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
GOODNESS OF FIT PROBABILITY		
4	1.198996	3.96021
7.803023E-03		

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

SLOPE = 4.443864
95 PERCENT CONFIDENCE LIMITS = -.4221077 AND 9.309834

LC50 = 4.856263
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 2.514866
95 PERCENT CONFIDENCE LIMITS = 0 AND 4.610631

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Regina Hirsch Zinc Omadine Acute Toxicity to Mysids

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
17	19	19	100	1.907348E-04
9.399999		19	19	100

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- ☐ Identity of product inert ingredients.
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DATA EVALUATION RECORD
§ 72-3(B) -- ACUTE EC₅₀ TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY

1. **CHEMICAL:** Zinc Omadine

PC Code No.: 001258

2. **TEST MATERIAL:** Zinc bis-1-oxide-2(1H)-pyridenethionate, off-white powder,
CAS No.13463-41-7. Purity: 97.8%

3. **CITATION**

Authors: R. L. Boeri, J. P. Magazu, and T. J. Ward
Title: Acute flow-through mollusc shell deposition test
with Zinc Omadine.

Study Completion Date: 11 July 1994

Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead MA

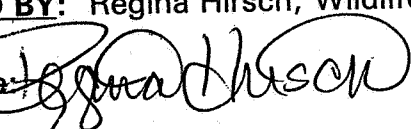
Sponsor: Olin Corporation, New Haven, CT

Laboratory Report ID: 24-OL

MRID No.: 438646-08

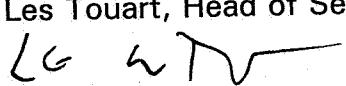
DP Barcode: D228348

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 1/9/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5/19/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:

Crassostrea virginica

Age or Size of Test Organism:

Juvenile/28-50 mm height

Definitive Test Duration:

96 hours

Study Method:

Flow-through

Type of Concentrations:

Mean measured

7. **CONCLUSIONS:**

Results Synopsis

EC₅₀: 22.0 µg/L ai

NOEL: 7.1 µg/L ai

95% C.I.: 18.9 - 27.3 µg/L ai

Probit Slope: 4.24

DP Barcode: D228348

MRID No.: 438646-08

8. ADEQUACY OF THE STUDY

A. Classification: CORE

B. Rationale: N/A

C. Repairability: N/A

9. BACKGROUND

10. GUIDELINE DEVIATIONS

1. Total organic carbon was not reported.

11. SUBMISSION PURPOSE: Registration

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are the Pacific oyster (<i>Crassostrea gigas</i>) and the Eastern oyster (<i>Crassostrea virginica</i>)	<i>Crassostrea virginica</i>
<u>Mean valve height</u> 25 - 50 mm along the long axis	28-50 mm
<u>Supplier</u>	P. Cummins Oyster Company, Pasadena MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 10 days	11 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
<u>Amount of peripheral shell growth removed prior to testing</u>	3-5 mm
<u>Feeding during the acclimation</u> Must be fed to avoid stress.	Continuously supplied with live marine phytoplankton to supplement the available food in the unfiltered natural seawater that as used as dilution water.
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	<3% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater from the Atlantic Ocean at T. R. Wilbury Laboratories in Marblehead, MA
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 ‰ salinity, weekly range < 6 ‰	33-34 ‰

Guideline Criteria	Reported Information
<u>Water Temperature</u> 15...-30... C, consistent in all test vessels	19.1-21.4...C
<u>pH</u>	7.7-8.1
<u>Dissolved Oxygen</u> ☐ 60% throughout	Range: 7.1-7.6 mg/L
<u>Total Organic Carbon</u>	Not reported.
<u>Test Aquaria</u> Should be constructed of glass or stainless steel.	Glass
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter, constructed at T. R. Wilbury Laboratories, Inc.
<u>Flow rate</u> Consistent flow rate	8.6 vol/24 hours
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Not reported
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<u>Solvents</u> Not to exceed 0.5 ml/L	Solvent: None

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $EC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	Range finding test found reduction in shell deposition at 5.0 μ g/L

Guideline Criteria	Reported Information
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Control, 6.3, 11.0, 17.0, 25.0, 42.0 µg ai/L
<u>Number of Test Organisms</u> Minimum 20 individual per test level and in each control	20
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured hourly in at least one chamber 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	The temperature in one test vessel was recorded continuously during the test. were measured and recorded daily in each test chamber that contained live animals.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

13. REPORTED RESULTS

A. General Results

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

Guideline Criteria	Reported Information
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0 %
<u>Control Shell Deposition</u> Must be at least 2 mm.	2.4 mm
<u>Recovery of Chemical</u>	79 - 113 % of Nominal
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Control	--	20	0	2.4	--
6.3	7.1	20	0	2.4	0%
11.0	10.0	20	0	2.3	4%
17.0	17.0	20	0	1.5	38%
25.0	25.0	20	0	1.0	58%
42.0	33.0	20	0	0.7	71%

B. Statistical Results

Method: Chi-square test was used to determine that data were normally distributed and Hartley's test was used to determine that variances were homogeneous. Because data were homogeneous a parametric one-way ANOVA was performed, followed by the Dunnett's test to compare treatment means to the control.

DP Barcode: D228348

MRID No.: 438646-08

96-hr EC₅₀: 22 µg/L ai

95% C.I.: 17-25 µg/L ai

NOEC: 10 µg/L ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC ₅₀	Probit
EC ₅₀ (95% C.I.)	22.0 (18.9-27.3)µg/L ai
Probit Slope	4.24
Statistical Method for NOEC	Dunnett's Test NOEC
NOEC	7.1 µg/L ai

15. REVIEWER'S COMMENTS:

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 20.61553

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.2209053	21.70102	17.51696	27.68258

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	.1331501	1

GOODNESS OF FIT PROBABILITY
.6241866

SLOPE = 4.24223
95 PERCENT CONFIDENCE LIMITS = 2.694251 AND 5.790208

LC50 = 22.40276
95 PERCENT CONFIDENCE LIMITS = 18.99795 AND 27.31605

LC10 = 11.24425
95 PERCENT CONFIDENCE LIMITS = 7.612051 AND 13.89673

3	10.0	20	0.522	21.9	0.050
4	17	20	0.522	21.9	0.930
5	25	20	0.522	21.9	1.355
6	33	20	0.522	21.9	1.710

Regina Hirsch Zinc Omadine Shell Deposition for the Mollusc

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
33	20	14	70	5.765915
25	20	12	60.00001	25.17223
17	20	8	40	25.17223
10	20	1	5	2.002716E-03
7.1	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10 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 20.61553

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	.2209053	21.70102	17.51696 27.68258

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	.1331501	1

GOODNESS OF FIT PROBABILITY
.6241866

SLOPE = 4.24223
95 PERCENT CONFIDENCE LIMITS = 2.694251 AND 5.790208

LC50 = 22.40276
95 PERCENT CONFIDENCE LIMITS = 18.99795 AND 27.31605

LC10 = 11.24425
95 PERCENT CONFIDENCE LIMITS = 7.612051 AND 13.89673

Regina Hirsch Zinc Omadine Shell deposition to the mollusc

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
33	20	14	70	5.765915
25	20	12	60.00001	25.17223
17	20	8	40	25.17223
10	20	1	5	2.002716E-03
7.1	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10 AND +INFINITY CAN BE

Zinc Omadine for Mollusc shell deposition
 File: molldat Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	8.040	29.040	45.840	29.040	8.040
OBSERVED	4	35	47	23	11

Calculated Chi-Square goodness of fit test statistic = 5.6286
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Zinc Omadine for Mollusc shell deposition
 File: molldat Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 4.05
 Closest, conservative, Table H statistic = 6.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 15
 Actual values ==> R (# groups) = 6, df (# avg reps-1) = 19.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Zinc Omadine for Mollusc shell deposition
 File: molldat Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	8.040	29.040	45.840	29.040	8.040
OBSERVED	4	35	47	23	11

Calculated Chi-Square goodness of fit test statistic = 5.6286
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Zinc Omadine for Mollusc shell deposition
File: molldat Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statist
Zinc Omadine for Mollusc shell deposition
File: molldat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	58.146	11.629	22.150
Within (Error)	114	59.878	0.525	
Total	119	118.024		

Critical F value = 2.37 (0.05,5,60)
Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

Zinc Omadine for Mollusc shell deposition
File: molldat Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	2.385	2.385		
2	7.1	2.390	2.390	-0.022	
3	10.0	2.335	2.335	0.218	
4	17	1.455	1.455	4.059	*
5	25	1.030	1.030	5.914	*
6	33	0.675	0.675	7.463	*

Dunnett table value = 2.28 (1 Tailed Value, $P=0.05$, $df=60,5$)

Zinc Omadine for Mollusc shell deposition
File: molldat Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	20			
2	7.1	20	0.522	21.9	-0.005 SD

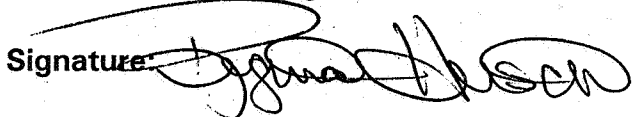
**DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 122-2 OR 123-2 (TIER I OR II)**

1. **CHEMICAL:** Zinc Omadine **PC Code No.:** 001258
2. **TEST MATERIAL:** Zinc bis-1-oxide-2(1H)-pyridenethionate, off-white powder,
CAS No.13463-41-7 **Purity:** 97.8%

3. **CITATION**

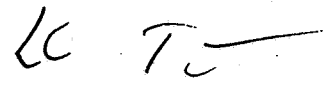
Authors: T. J. Ward, J. P. Magazu, and R. L. Boeri
Title: Growth and Reproduction Test with Zinc Omadine
and the Freshwater Alga, *Selenastrum capricornutum*.
Study Completion Date: 13 July 1994
Laboratory: T. R. Wilbury Laboratories, Inc.
Sponsor: Olin Corporation
Laboratory Report ID: 25-OL
DP Barcode: D228348
MRID No.: 438646-09

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 1/9/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5/12/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Selenastrum capricornutum*
Definitive Test Duration: 120 hours
Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

Results Synopsis

EC₅₀: 28 µg/L ai

95% C.I.: 24-33 µg/L ai

NOEL: 7.8 µg/L ai

8. **ADEQUACY OF THE STUDY**

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS**

1. Standard nutrients were not reported.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Selenastrum capricornutum</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/ml	10,000 cells/ml
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Not Reported

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	23.8-24.3°C
<u>Light Intensity</u> Anabaena: 2.0 Lux ($\pm 15\%$) Others: 4.0-5.0 Lux ($\pm 15\%$)	31 - 33 footcandles

Guideline Criteria	Reported Information
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous light
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	7.2-7.7

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	Control, 9.8, 20.0, 39.0, 78.0, 160.0
<u>Doses</u> at least 5	5
<u>Controls</u> negative and/or solvent	Control (no solvent)
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Cellular counts
<u>Maximum Labeled Rate</u>	Not reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes

Guideline Criteria	Reported Information
Control cell count at 120 hr $\geq 2X$ initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	No

Dose Response

Mean Measured Concentration ($\mu\text{g ai/L}$)	Cell Density ($\times 10^3$ cells/ml)	% Inhibition	120-Hour pH
Control	211		7.7
7.8	211	100%	7.7
18.0	135	64%	7.7
28.0	121	58%	7.7
71.0	< 11	< 5%	7.7
170.0	< 10	< 5%	7.6

Other Significant Results:**Statistical Results**

Statistical Method: Shapiro-Wilk's test was used to determine that the data were normally distributed, and Bartlett's test was used to determine that variances were homogeneous. Because the of homogeneity was met a parametric one-way ANOVA was performed followed by Dunnett's test to compare treatment to control means.

EC₅₀: 28 $\mu\text{g/L}$ 95% C.I.: 24 - 33 $\mu\text{g/L}$ NOEC: 7.8 $\mu\text{g/L}$

13. Verification of Statistical Results

Statistical Method: Shapiro-Wilk's test was used to determine that the data were normally distributed, and Bartlett's test was used to determine that variances were homogeneous. Because the of homogeneity was met a parametric one-way ANOVA was performed followed by Dunnett's test to compare treatment to control means.

EC₅₀: 28 µg/L

95% C.I.: 24-33 µg/L

NOEC: 7.8 µg/L

14. REVIEWER'S COMMENTS:

Program: Nuthatch

Date: 3/10/97

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

Input file: JE.DAT

Raw data:

Selenastrum / zinc omadine

5
3
3
3
3
3

0

212
210
210

7.8

200
216
218

18

140
126
138

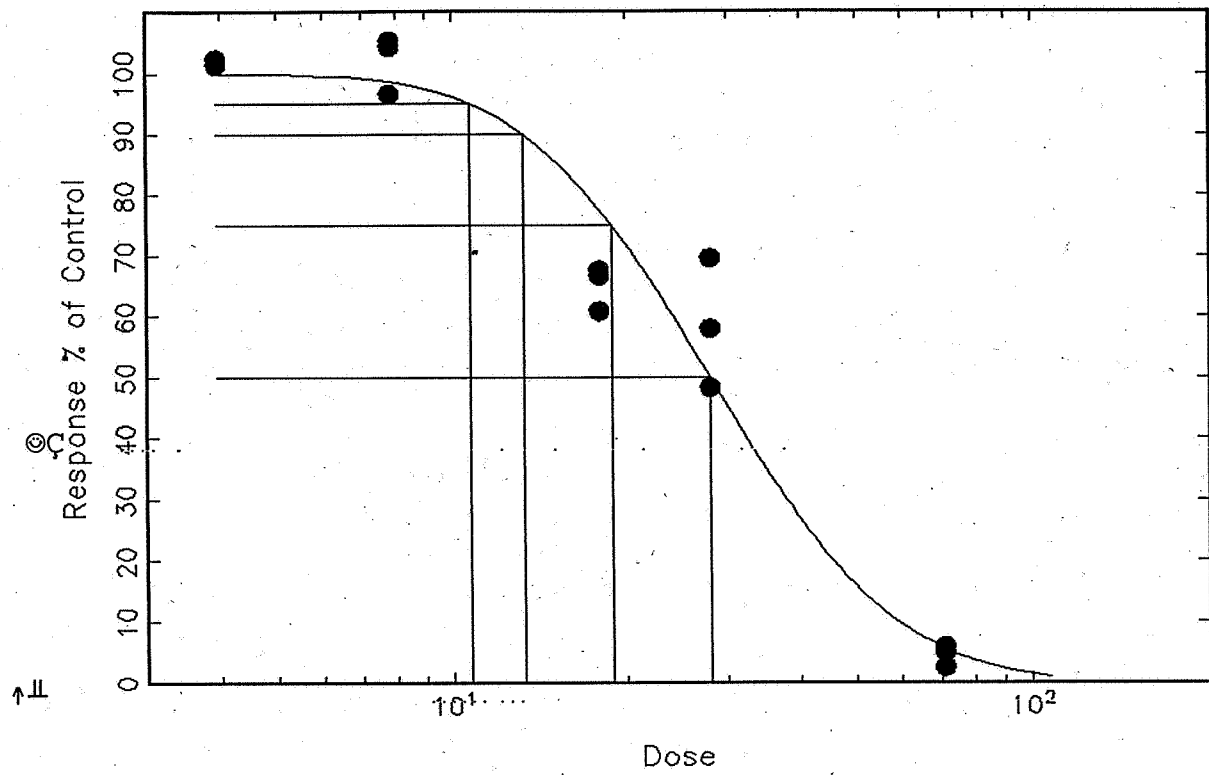
28

144
120
7

71

10
12
5

JE.DAT : Selenastrum / zinc omadine



JE.DAT : Selenastrum / zinc omadine

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	211	.		
7.8	211	-0.03569	N.S.	
18	135	8.137	<0.005	*
28	121	9.564	<0.005	*
71	9	21.59	<0.005	*

"*"=Significant; "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
5	11.	7.7	15.	0.068	0.71
EC10	13.	9.9	18.	0.059	0.74
EC25	19.	15.	24.	0.045	0.80
EC50	28.	24.	33.	0.033	0.85

Slope = 3.97 Std.Err. = 0.426

!!!Poor fit: p = 0.0057 based on DF= 2.0 10.

JE.DAT : Selenastrum / zinc omadine

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	211.	207.	3.51	100.	0.00
7.80	3.00	211.	204.	7.03	98.6	1.38
18.0	3.00	135.	161.	-26.2	77.7	22.3
28.0	3.00	121.	103.	17.9	49.9	50.1
71.0	3.00	9.00	11.2	-2.19	5.40	94.6

Page 59 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
 - ☐ Identity of product impurities.
 - ☐ Description of the product manufacturing process.
 - ☐ Description of quality control procedures.
 - ☐ Identity of the source of product ingredients.
 - ☐ Sales or other commercial/financial information.
 - ☐ A draft product label.
 - ☐ The product confidential statement of formula.
 - ☐ Information about a pending registration action.
 - ☒ FIFRA registration data.
 - ☐ The document is a duplicate of page(s) _____.
 - ☐ The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	210.667	210.667		
2	7.8	211.333	211.333	-0.079	
3	18.0	134.667	134.667	8.987	*
4	28.0	121.333	121.333	10.563	*
5	71.0	10.333	10.333	23.689	*
6	170	9.000	9.000	23.846	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Zinc Omadine and Alga

File: 122dat Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	3			
2	7.8	3	21.142	10.0	-0.667
3	18.0	3	21.142	10.0	76.000
4	28.0	3	21.142	10.0	89.333
5	71.0	3	21.142	10.0	200.333
6	170	3	21.142	10.0	201.667

Zinc Omadine and Alga

File: 122dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	123123.778	24624.756	229.542
Within (Error)	12	1287.333	107.278	
Total	17	124411.111		

Critical F value = 3.11 (0.05,5,12)

Since F > Critical F REJECT Ho:All groups equal

Zinc Omadine and Alga

File: 122dat Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

60

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	210.667	210.667		
2	7.8	211.333	211.333	-0.079	
3	18.0	134.667	134.667	8.987	*
4	28.0	121.333	121.333	10.563	*
5	71.0	10.333	10.333	23.689	*
6	170	9.000	9.000	23.846	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Zinc Omadine and Alga

File: 122dat Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	3			
2	7.8	3	21.142	10.0	-0.667
3	18.0	3	21.142	10.0	76.000
4	28.0	3	21.142	10.0	89.333
5	71.0	3	21.142	10.0	200.333
6	170	3	21.142	10.0	201.667

Zinc Omadine and Alga
File: 122dat Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1287.333

W = 0.864

Critical W (P = 0.05) (n = 18) = 0.897

Critical W (P = 0.01) (n = 18) = 0.858

Data PASS normality test at P=0.01 level. Continue analysis.

Zinc Omadine and Alga
File: 122dat Transform: NO TRANSFORMATION

Bartlett test for homogeneity of variance
Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Zinc Omadine and Alga
File: 122dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	123123.778	24624.756	229.542
Within (Error)	12	1287.333	107.278	
Total	17	124411.111		

Critical F value = 3.11 (0.05,5,12)

Since F > Critical F REJECT Ho: All groups equal

Zinc Omadine and Alga
File: 122dat Transform: NO TRANSFORMATION

- 13 -

TABLE 1
CUMULATIVE MORTALITY OF NORTHERN BOBWHITE
DOSED WITH ZINC OMADINE

Dosage mg a.i./kg		Sex	Number Dead/Number Exposed																
			Day of Study																
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total	
Control	M		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/10
	F		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
31.2	M		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/10	
	F		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		0/5
62.5	M		0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/10	
	F		0/5	0/5	0/5	0/5	1/5	1/5	1/5	1/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5		2/5
125	M		0/5	0/5	0/5	3/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	9/10	
	F		0/5	0/5	0/5	1/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5		4/5
250	M		0/5	1/5	1/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10	
	F		0/5	0/5	2/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5		5/5
500	M		0/5	3/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10	
	F		0/5	1/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5		5/5
1000	M		0/5	2/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10	
	F		0/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5		5/5

The LD50 value was determined to be 60 mg a.i./kg, with a 95% confidence interval of 44 mg a.i./kg to 81 mg a.i./kg.

DP Barcode: D228348

MRID No.: 438646-10

DATA EVALUATION RECORD
§ 71-2(A) -- UPLAND GAME BIRD DIETARY LC₅₀ TEST

1. **CHEMICAL:** Zinc Omadine

PC Code No.: 001258

2. **TEST MATERIAL:** Zinc Omadine Powder, white powder; CAS No. 13463-41-7; UN 2811; D.O.T. (poisonous solid contains zinc pyrethione); Wildlife International Ltd. ID. No. 2503. Purity: 96 %

3. **CITATION**

Authors: S.M. Campbell and J.B. Beavers

Title: Zinc Omadine: a dietary LC₅₀ study with the Northern Bobwhite.

Study Completion Date: 24 May 1994

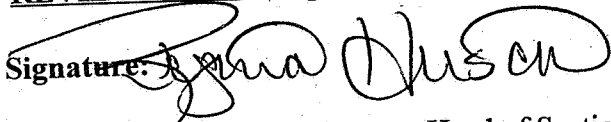
Laboratory: Wildlife International, Ltd., Easton, Maryland

Sponsor: Olin Corp., New Haven, Connecticut

Laboratory Report ID: 133-109

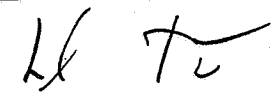
MRID No.: 438646-10

4. **REVIEWED BY:** Regina M. Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 12/12/96

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5-19-97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Colinus virginianus*

Age of Test Organisms at Test Initiation: 10 days

Definitive Study Duration: 8 days

7. **CONCLUSIONS:**

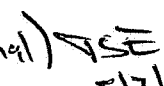
Results Synopsis

LC₅₀: 1063 ppm ai

NOEL: < 253 ppm ai

95% C.I.: 789 - 1412 ppm ai

Probit Slope: 4.55

(LC₅₀ = 1110 ppm based on nominal) 
5/7/97

8. ADEQUACY OF THE STUDY**A. Classification:** Core.**B. Rationale:****C. Repairability:****9. GUIDELINE DEVIATIONS**

1. Brooder temperature ($39^{\circ}\text{C} \pm 2^{\circ}\text{C}$) was higher than what is recommended (35°C).

10. SUBMISSION PURPOSE: Registration of an Antifoulant

11. MATERIALS AND METHODS**A. Test Organisms**

Guideline Criteria	Reported Information
Species: An upland game bird species, preferably the bobwhite (<i>Colinus virginianus</i>).	<i>Colinus virginianus</i>
Age at beginning of test: 10-14 days old.	10 days
Supplier	Wildlife International Ltd. Production Flock, Easton, Maryland
Chicks appeared healthy and did not have excessive mortality before the test?	Not reported
Acclimation period: As long as possible.	10 days

B. Test System

Guideline Criteria	Reported Information
Pen size: about 35 x 100 x 24 cm	72 x 90 x 23 cm

Guideline Criteria	Reported Information
Brooder temperature: about 35°C (95°F)	39°C ± 2°C
Room temperature: 22-27°C (71-81°F)	23.6°C ± 1.8°C
Relative humidity: 30-80%	44% ± 10% (SD)
Adequate ventilation?	Yes
Photoperiod Minimum of 14 h of light.	16 hours of light
Diet: A commercial diet for game birds.	Game bird ration formulated to Wildlife International Ltd. specifications.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	Not reported
Definitive Test Nominal concentrations: Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC ₅₀ > 5000 ppm.	275, 492, 1568, 2800, 5000 ppm (analytical support values: 253, 468, 920, 1640, 2990, 5420 ppm)
Controls: Control group tested with diet containing the maximum amount of vehicle used in treated diets?	Yes
Number of birds per group: 10 (strongly recommended)	10 birds per group
Vehicle: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic.	None reported
Vehicle amount (% of diet by weight): Not more than 2%	N/A

Guideline Criteria	Reported Information
Test durations: 5 days with treated feed and at least 3 days observation with "clean" feed.	5 days with treated feed and 3 days observation with "clean" feed.
No mortality during last 72 hr of observations?	No

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Body weights measured at beginning and end of study?	Yes, body weights measured on test initiation, Day 5 of test and at test termination -- body weights were measured by group not individually.
Estimated consumption per pen reported for pretreatment, treatment, and observation periods?	Partially done, feed consumption was measured during the exposure period and post treatment only on Days 0-1, 1-2, 2-3, 3-4, 4-5, and 6-8.
Control Mortality: Not more than 10%	0%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Mortality

Conc. (ppm)		No. of Birds	Cumulative Number of Dead							
Nominal	Mean Measured		Day of Study							
			1	2	3	4	5	6	7	8
Control		30	0	0	0	0	0	0	0	0
275	253	10	0	0	0	0	0	0	0	0
492	468	10	0	0	0	0	1	1	1	1
878	920	10	0	0	0 ¹	0 ¹	3 ¹	3 ¹	3 ¹	3
1568	1640	10	0	0 ²	1 ²	8 ²	8 ²	8 ²	8	8
2800	2990	10	0	0 ³	8 ³	10	10	10	10	10
5000	5420	10	0	3 ⁴	8 ⁴	10	10	10	10	10

¹ Signs of toxicity: wing droop, ruffled appearance and lethargy.

² Signs of toxicity: depression, reduced reaction to external stimuli (sound and movement), wing droop, prostrate posture, loss of righting reflex, shallow and rapid respiration, loss of coordination, a ruffled appearance, and lethargy.

³ Signs of toxicity: wing droop, loss of coordination, a ruffled appearance, and lethargy.

⁴ Signs of toxicity: depression, reduced reaction, wing droop, a ruffled appearance, lower limb weakness, and lethargy.

Other Significant Results: When compared to the controls, there was reduction in body weight gain noted among birds at the 275, 492, 878, and 1568 ppm a.i. test concentrations during the exposure period (Days 0-5). Body weight for birds at 2800 and 5000 ppm a.i. test concentrations could not be measured due to total mortality by Day 5. There was a reduction in feed consumption at the 878, 1568, 2800, and 5000 ppm a.i. test concentrations during exposure period, while birds at the 275 and 492 ppm a.i. concentrations also exhibited a reduction in feed consumption on the 4th day of the exposure period.

Statistical Results:

Statistical Method: Probit Method

LC₅₀: 1110 ppm a.i. 95% C.I.: 866 - 1423 ppm a.i.

NOEL: < 275 ppm a.i. Probit Slope: 6

DP Barcode: D228348

MRID No.: 438646-10

13. Verification of Statistical Results

Statistical Method: Probit Method

LC₅₀: 1063 ppm a.i. 95% C.I.: 789 - 1412 ppm a.i.

NOEL: < 253 ppm a.i. Probit Slope: 4.55

14. REVIEWER'S COMMENTS: The difference in the results are due using the analytical support values instead of the nominal values.

Regina Hirsch Zinc Oxamide Acute Dietary for the Bobwhite Quail

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5420	10	10	100	9.765625E-02
2990	10	10	100	9.765625E-02
1640	10	8	80	5.46875
920	10	3	30	17.1875
468	10	1	10	1.074219
253	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 468 AND 2990 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 1153.805

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
 SPAN G LC50 95 PERCENT CONFIDENCE LIMITS
 5 .1169362 1019.918 709.1691 1399.374

RESULTS CALCULATED USING THE PROBIT METHOD
 ITERATIONS G H
 GOODNESS OF FIT PROBABILITY
 5 .2156766 1
 .9074671

SLOPE = 4.546629
 95 PERCENT CONFIDENCE LIMITS = 2.435129 AND 6.658128

LC50 = 1063.313
 95 PERCENT CONFIDENCE LIMITS = 789.2681 AND 1412.535

LC10 = 558.8991
 95 PERCENT CONFIDENCE LIMITS = 284.553 AND 759.9463

DP Barcode: D228348

MRID No.: 438646-11

DATA EVALUATION RECORD
§ 71-1(A) - AVIAN SINGLE-DOSE LD₅₀ TEST

1. **CHEMICAL:** Zinc Omadine

PC Code No.: 001258

2. **TEST MATERIAL:** Zinc Omadine Powder; CAS No. 13463-41-7; UN2811 D.O.T.
(Poisonous solid contains zinc pyrithione); white powder. Purity: 96 %

3. **CITATION**

Authors: S.M. Campbell, J.B. Beavers, M. Jaber.

Title: Zinc Omadine: An acute oral toxicity study with the
Northern Bobwhite.

Study Completion Date: 17 August 1993

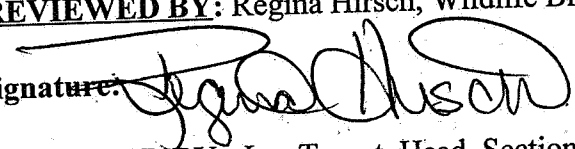
Laboratory: Wildlife International Ltd., Easton, Maryland

Sponsor: Olin Corp., New Haven, Connecticut

Laboratory Report ID: 133-110

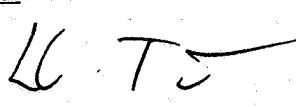
MRID No.: 438646-11

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 11/8/96

5. **APPROVED BY:** Les Touart, Head, Section (1), EEB, EFED

Signature: 

Date: 5/17/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Colinus virginianus*

Test Organisms Age/Size: 19 weeks of age

Definitive Study Duration: 14 days

7. **CONCLUSIONS:**

Results Synopsis

LD₅₀: 82.4 mg ai/kg

NOEL: < 31.2 mg ai/kg

95% C.I.: 63-108 mg ai/kg

Probit Slope: 7.17

8. **ADEQUACY OF THE STUDY**

A. Classification: Core.

B. Rationale: N/A/**C. Repairability:** N/A**9. GUIDELINE DEVIATIONS**

1. Test animals were older than (19 weeks) what is recommended in guidelines (16 weeks).
2. Individual body weights were only taken prior to test initiation, and group weights taken 3 times during test. Individual weights should have been taken upon test termination as well as test initiation.

10. SUBMISSION PURPOSE: Registration of an Antifoulant**11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
Species: A wild waterfowl species, preferably the mallard (<i>Anas platyrhynchos</i>), or an upland game bird species, preferably the bobwhite (<i>Colinus virginianus</i>).	<i>Colinus virginianus</i>
Age at beginning of test: At least 16 weeks old.	19 weeks of age.
Supplier	Top Flight Quail Farm, Belvidere, NJ 07823
Acclimation period: At least 15 days.	5 weeks

B. Test System

Guideline Criteria	Reported Information
Pen facilities adequate?	Yes

Guideline Criteria	Reported Information
Photoperiod: 10-h light, 14-h dark is recommended.	8 hours of light per day.
Diet was nutritious and appropriate for species?	Yes
Feed withheld at least 15 hours prior to dosing?	Yes

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	Not Reported
Definitive Test Nominal concentrations: At least five, in a geometric scale, unless $LD_{50} > 2000$ mg ai / kg.	31.2, 62.5, 125, 250, 500, 1000 mg a.i. pre kg body weight
Controls: Water control or vehicle control (if vehicle is used)	Substance was administered by gelatin capsule, Control group was given blank gelatin capsules.
Number of birds per group: 10 (strongly recommended)	5 males and 5 females in each group.
Vehicle: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic.	No vehicle was used.
Amount of vehicle per body weight: Constant volume/weight % of body weight, not to exceed 1% (1ml/100g).	N/A
Observations period: At least 14 days.	14 days

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Individual body weights measured at beginning of test, on day 14 and at end of test if extended beyond 14 days?	Yes, individual body weights taken one day prior to test initiation, and by the group on days 3, 7, and 14.
Mean feed consumption measured at beginning of test, on day 14, and at end of test if extended beyond 14 days?	Yes, average feed consumption was determined for each dosage and control group for days 0-3, 4-7, and 8-14.
Control Mortality: Not more than 10%	0 %
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Dosage (mg/kg)	No. of Birds	Cumulative Number of Dead							
		Day of Study							
		1	2	3	4	5	6-8	9-12	13-14
Control	10	0	0	0	0	0	0	0	0
31.2	10	0	0	0	0	0	0	0	0
62.5	10	0	0*	0*	1*	1*	2*	2*	2
125	10	0*	0*	4*	9*	9*	9*	9*	9
250	10	0*	1*	3*	10	10	10	10	10
500	10	0*	4*	9*	10	10	10	10	10
1000	10	0*	6*	9*	10	10	10	10	10

* Signs of toxicity in remaining test animals noticed: reduced reaction to external stimuli, ruffled appearance, lethargy, wing droop, loss of coordination, depression, prostrate posture, loss of righting reflex and lower limb weakness.

Other Significant Results: When compared to the control group there was a marked loss in body weight among surviving birds at the 31.2, 62.5, 125 mg a.i./kg dosage groups from the period of 0-3 days. The single surviving hen at the 125 mg/kg dosage group continued to exhibit a loss in body weight through day 7. A compensatory gain in body weight by surviving birds at the 62.5 and 125 mg/kg dosage level was seen from days 7-14. Changes in body weight could not be determined for males in 125 mg/kg group nor the 250, 500, and 1000 mg/kg groups due to total mortality by day 3. There was a marked dose response reduction in feed consumption during the period from day 0-3 in all dosage groups with surviving birds.

Reported Statistical Results:

Statistical Method: Probit Method

LD₅₀: 60 mg a.i./kg 95% C.I.: 44- 81 mg a.i./kg

NOEL: < 31.2 mg a.i./kg Probit Slope: 5

13. Verification of Statistical Results:

Statistical Method: Probit Method

LD₅₀: 82.4 mg/kg 95% C.I.: 63 - 108 mg/kg

NOEL: < 31.2 mg/kg Probit Slope: 7.17

- 15. REVIEWER'S COMMENTS:** There seems to be a discrepancy with the stats used in this study. If there was only 20% mortality at the 62.5 dosage level and 0% at the 31.2 mg/kg dosage level, how can the LC50 be 60 mg/kg? Was the mortality data reported in the table in error, or was WT's statistics in error?

Regina Hirsch Zinc Oxadine BWQ Acute Oral

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1000	10	10	100	9.765625E-02
500	10	10	100	9.765625E-02
250	10	10	100	9.765625E-02
125	10	9	90	1.074219
62.5	10	2	20	5.46875
31.2	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 31.2 AND 125 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 83.24136

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	.1144904	82.65652	61.92978 108.4238

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
6	.3508794	1

GOODNESS OF FIT PROBABILITY
.999959

SLOPE = 7.175594
95 PERCENT CONFIDENCE LIMITS = 2.925126 AND 11.42606

LC50 = 82.37645
95 PERCENT CONFIDENCE LIMITS = 63.00147 AND 108.0656

LC10 = 54.80426
95 PERCENT CONFIDENCE LIMITS = 27.88084 AND 69.56859

Regina Hirsch Zinc Oxadine Acute Oral BWQ

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1000	10	10	100	9.765625E-02
500	10	10	100	9.765625E-02
250	10	10	100	9.765625E-02
125	10	9	90	1.074219
62.5	10	2	20	5.46875
31.2	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 31.2 AND 125 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 83.24136

DP Barcode: D228348

MRID No.: 438646-12

DATA EVALUATION RECORD
§ 71-2(B) -- WATERFOWL DIETARY LC₅₀ TEST

1. **CHEMICAL:** Zinc Omadine

PC Code No.: 001258

2. **TEST MATERIAL:** Zinc Omadine Powder; CAS No. 13463-41-7; UN 2811; D.O.T. poisonous solid (contains zinc pyrrhione), white powder. **Purity:** 96 %

3. **CITATION**

Authors: Campbell, S.M., J.B. Beavers, and M. Jaber

Title: Zinc Omadine: A dietary LC₅₀ study with the mallard.

Study Completion Date: 24 May 1994

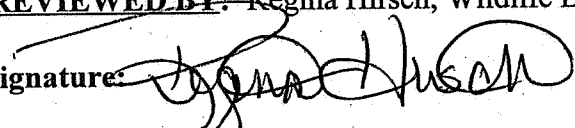
Laboratory: Wildlife International Ltd., Easton Maryland

Sponsor: Olin Corporation, New Haven, Connecticut

Laboratory Report ID: 133-111

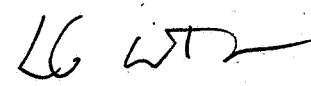
MRID No.: 438646-12

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 11/6/90

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5/9/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Anas platyrhynchos*

Age of Test Organisms at Test Initiation: 10 days

Definitive Study Duration: 16 days

7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: > 5000 ppm ai

NOEL: < 275 ppm ai

8. **ADEQUACY OF THE STUDY**

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS**

1. Brooder temperature was lower (31°C ± 3°C) than what is recommended (35°C).

10. SUBMISSION PURPOSE: Registration of an Antifoulant**11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
Species: A wild waterfowl species, preferably the mallard (<i>Anas platyrhynchos</i>).	<i>Anas platyrhynchos</i>
Age at beginning of test: 5-10 days old (preferably 5).	10 days old
Supplier	Whistling Wings, 113 Washington Street, Hanover, Illinois 61041
Chicks appeared healthy and did not have excessive mortality before the test?	Yes
Acclimation period: As long as possible.	8 days

B. Test System

Guideline Criteria	Reported Information
Pen size: about 70 x 100 x 24 cm	72 X 90 X 25.5 cm
Brooder temperature: about 35°C (95°F)	31°C ± 3°C
Room temperature: 22-27°C (71-81°F)	21.4°C ± 1.2°C
Relative humidity: 30-80%	53% ± 11% (SD)
Adequate ventilation?	Yes
Photoperiod Minimum of 14 h of light.	16 hours of light per day

Guideline Criteria	Reported Information
Diet: A commercial waterfowl feed.	game bird ration formulated to Wildlife International's specifications.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	Not Reported
Definitive Test Nominal concentrations: Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless $LC_{50} > 5000$ ppm.	275, 492, 878, 1568, 2800, and 5000 ppm a.i. (analytical values 253, 468, 920, 1640, 2990, and 5420 ppm a.i.)
Controls: Control group tested with diet containing the maximum amount of vehicle used in treated diets?	Yes, no vehicle was used to prepare test or control diets.
Number of birds per group: 10 (strongly recommended)	10
Vehicle: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic.	None used
Vehicle amount (% of diet by weight): Not more than 2%.	N/A
Test durations: 5 days with treated feed and at least 3 days observation with "clean" feed.	5 days exposure and 3 days post-exposure observation
No mortality during last 72 hr of observations?	No

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Body weights measured at beginning and end?	Yes, body weights were measured at the initiation of the test, on Day 5, and at test termination of the test on Day 8.
Estimated consumption per pen reported for pretreatment, treatment, and observation periods?	Yes, average feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time (Days 0-1, 1-2, 2-3, 3-4, and 4-5).
Control Mortality: Not more than 10%	0%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Conc. (ppm)		No. of Birds	Cumulative Number of Dead							
Nominal	Mean Measured		Day of Study							
			1	2	3	4	5	6	7	8
Control		30	0	0	0	0	0	0	0	0
275	253	10	0	0	0	0	0	0	0	0
492	468	10	0	0	0	0	0	0	0	0
878	920	10	0	0	0	0	0	0	0	0
1568	1640	10	0	0	0 ¹	0 ¹	0 ¹	0	0	0
2800	2990	10	0	0	0 ²	0 ²	1 ²	1 ²	1 ²	1
5000	5420	10	0	0	0 ³	0 ³	1 ³	1 ³	1	1

¹ Signs of toxicity: ruffled appearance and lethargy.

² Signs of toxicity: ruffled appearance, loss of coordination, impaired walking, and lethargy.

³ Signs of toxicity: ruffled appearance, lethargy, depression, loss of coordination, and a reduced reaction to external stimuli (sound and movement).

Other Significant Results: When compared to the controls, during the exposure period (Days 0-5), there was a concentration dependent reduction in body weight gain noted among birds at the 275, 492, and 878 ppm a.i. test concentrations, and a loss in body weight among birds at the 1568, 2800, and 5000 ppm a.i. test concentrations. A concentration related reduction in feed consumption was also noted among birds at all treatment groups during the exposure period.

Statistical Results

Statistical Method: Visual inspection of mortality data

LC₅₀: > 5000 ppm

NOEL: < 275 ppm (based on the effects of body weight gain and feed consumption)

13. Verification of Statistical Results

Statistical Method: None needed as an LC50 could not be determined from the data given.

LC₅₀: > 5000ppm

NOEL: < 275 ppm

14. REVIEWER'S COMMENTS: Study appears scientifically sound.

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

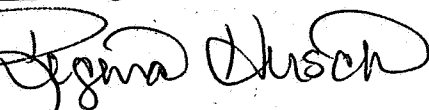
1. **CHEMICAL:** Zinc Omadine PC Code No.: 001258
2. **TEST MATERIAL:** Zinc bis-1-oxide-2(1H)-pyridenethionate, off-white powder,
CAS No.13463-41-7. Purity: 97.8%

3. **CITATION**

Authors: R. L. Boeri, J. P. Magazu, and T. J. Ward
Title: Acute toxicity of Zinc Omadine to the rainbow trout,
Oncorhynchus mykiss.
Study Completion Date: 7 July 1994
Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor: Olin Corporation, New Haven, CT
Laboratory Report ID: 20-OL
MRID No.: 438646-13
DP Barcode: D228348

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature:



Date:

1/10/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature:



Date:

5/19/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Oncorhynchus mykiss</i>
Age or Size of Test Organism:	Juvenile/average 0.52 g wet weight
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: 3.6 µg/L ai

NOEL: 1.6 µg/L ai

95% C.I.: 3.07-4.33 µg/L ai

Probit Slope: 6.52

8. ADEQUACY OF THE STUDY**A. Classification:** Core.**B. Rationale:** N/A**C. Repairability:** N/A**9. GUIDELINE DEVIATIONS**

1. Range of fish weights and lengths were not included in report.
2. The fish were fed the 48 hours prior to the initiation of the study.
3. Dechlorinated tap water was used as dilution water.
4. Temperature range varied more than 1 degree.

10. SUBMISSION PURPOSE: Registration**11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the rainbow trout (<i>Onchorhynchus mykiss</i>)	<i>Onchorhynchus mykiss</i>
<u>Mean Weight</u> 0.5-5 g	0.52 g
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 36 mm Range: Not reported
<u>Supplier</u>	Aquatic Research Organisms, Hampton, New Hampshire
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	14 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	Fish were not fed throughout the study, however they were fed the 48 hours prior to the initiation of the study.
Pretest Mortality < 3% mortality 48 hours prior to testing	< 3 % mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Dechlorinated tap water was used as dilution water.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 12°C	11.5-12.8 °C
pH Prefer 7.2 to 7.6	7.1-7.4

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: $\geq 60\%$	9.4 mg/L at 0 and 24 hours
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO_3	44 mg/L CaCO_3
<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	Glass aquaria 20 L 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter, constructed at T. R. Wilbury Laboratories.
<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	6.9 vol/24 hours, Calibrated before and after the test, not anytime during the test.
<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	0.35 g/L (0.05 g/L/day)
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours 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

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A screening test was not performed and historic data were used to determine the range of concentrations.
<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	Control, 2.9, 3.9, 6.8, 9.8, and 17.0 μ g ai/L.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	20 fish per treatment (10 per replicate, 2 replicates)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary $> 1^{\circ}\text{C}$ 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	Temperature in one test vessel was measured continuously throughout the test. DO, pH, and temperature were measured daily in each test chamber that contained live fish.
<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	Chemical analysis performed.

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	55-89% of nominal
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	10 %
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Mortality		Number of Fish	Cumulative Number Dead and Number Affected in ()			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	--	20	1	2	2	2
2.9	1.6	20	1	2	2	2
3.9	3.2	20	1	8	8	9
6.8	5.5	20	5	14	16	17
9.8	8.7	20	13 (7)	19(1)	20	20
17.0	15.0	20	12 (8)	20	20	20

Other Significant Results: Affected fish exhibited discoloration after 24 hours of exposure, and discoloration and lethargy after 48 hours.

DP Barcode: D228348

MRID No.: 438646-13

B. Statistical Results

Method: Probit

96-hr LC₅₀: 3.2 µg/L ai

95% C.I.: 2.6-3.8 µg/L ai

Probit Slope: 4.8

NOEC: 1.6 µg/L ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	3.64 (1.6-5.5) µg/L ai
Moving Average Angle LC ₅₀ (95% C.I.)	3.65 (3.07-4.25) µg/L ai
Probit LC ₅₀ (95% C.I.)	3.69 (3.07-4.33) µg/L ai
Probit Slope	6.52
NOEC	1.6 µg/L ai

14. REVIEWER'S COMMENTS:

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Regina Hirsch Zinc Omadine Acute toxicity to rainbow trout

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
15	18	18	100	3.814697E-04
8.7	18	18	100	3.814697E-04
5.5	18	15	83.3333	.3768921
3.2	18	7	38.8889	24.03412
1.6	18	0	0	3.814697E-04

THE BINOMIAL TEST SHOWS THAT 1.6 AND 5.5 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 3.637446

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	6.185815E-02		3.64698 3.069758

4.252189

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
6	.1652566	1

GOODNESS OF FIT PROBABILITY
.8750214

SLOPE = 6.521434
95 PERCENT CONFIDENCE LIMITS = 3.870356 AND 9.172511

LC50 = 3.688994
95 PERCENT CONFIDENCE LIMITS = 3.071397 AND 4.326476

LC10 = 2.355947
95 PERCENT CONFIDENCE LIMITS = 1.578602 AND 2.874784

Page 90 is not included in this copy.

Pages _____ through _____ are not included in this copy.

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- _____ Identity of product inert ingredients.
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-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD
§ 72-1(A) -- ACUTE LC₅₀ TEST WITH A WARMWATER FISH

1. **CHEMICAL:** Pyridine Sulfonic Acid

PC Code No.:

2. **TEST MATERIAL:** Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook# D104334-15, 1-10-92, a white crystal.

Purity: 98%

3. **CITATION:**

Authors: R. L. Boeri, R. L. Kowalski, and T. J. Ward
Title: Acute toxicity of Pyridine Sulfonic Acid to the fathead minnow, *Pimephales promelas*.

Study Completion Date: 22 March 1994

Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA

Sponsor: Olin Corporation, New Haven, CT

Laboratory Report ID: 40-OL

MRID No.: 438646-21

DP Barcode:

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature:

Date: 1/15/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature:

Date: 5/15/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Pimephales promelas</i>
Age or Size of Test Organism:	Juvenile/35.7 mm, 0.32 g
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: 68.5 ppm ai

95% C.I.: 55.2-85.0 ppm ai

NOEC: 55.2 ppm ai

8. **ADEQUACY OF THE STUDY**

A. Classification: Core.

DP Barcode: D

MRID No.: 438646-21

B. Rationale: N/A

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS**

1. Range of weight and length of fish used in study was not reported.
2. Dilution water was dechlorinated tap water collected from Marblehead, MA.
3. The pH of the test water ranged from 3.6-7.9.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the bluegill sunfish (<i>Lepomis macrochirus</i>)	<i>Pimephales promeles</i>
<u>Mean Weight</u> 0.5-5 g	0.32 g
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 35.7 mm Range: Not reported
<u>Supplier</u>	Aquatic Biosystems, Fort Collins, CO
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	14 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
<u>Feeding</u> No feeding during the study	48 hours prior to test initiation
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing	<3 % mortality prior to testing.

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	Dilution water was dechlorinated tap water collected from Marblehead, MA.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 17°C or 22°C	22.0-22.9 °C
<u>pH</u> Prefer 7.2 to 7.6	3.6-7.9

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: $\geq 60\%$	8.4 at 48 hours.
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO_3	48 mg/L as CaCO_3
<u>Test Aquaria</u> 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume</u> : 15-30 L of solution	Glass 20 L 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter. The diluter, which was constructed at T. R. Wilbury, allowed test media to contact only glass, stainless steel, or Teflon-coated surfaces.
<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	5.5 vol/24 hours Calibrated before and after the test.
<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	0.21 g/L (0.04 g/L/day)
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours 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

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A screening test was not performed and historic data was used to determine the range of concentrations for the definitive test.
<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	Control, 18.8, 31.2, 50.0, 75.1, and 125.0 mg ai/L.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	2 replicates per test level with 10 fish in each replicate (total 20 fish/test level).
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<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 every 48 h in the high, medium, and low doses and in the control	The temperature in one test vessel was recorded continuously during the test. DO, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live fish.
<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	Analytical determination of test material concentrations from the definitive test was performed on pooled samples collected beneath the surface of the 2 replicates of each concentration at the beginning and end of the test.

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

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	108-113% of nominal
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	--	20	0	0	0	0
18.8	21.0	20	0	0	0	0
31.2	33.8	20	0	0	0	0
50.0	55.2	20	0	0	0	0
75.1	85.0	20	20	20	20	20
125.0	135.0	20	20	20	20	20

Other Significant Results/Protocol Deviations: Fish were not fed on day 0 of the 14 day acclimation period prior to the initiation of the test. Food used in feeding the test organisms during acclimation contained measurable

DP Barcode: D

MRID No.: 438646-21

concentrations of 2 pesticides (names of which were not reported). The slope of the 96 hour dose response curve could not be calculated.

B. Statistical Results

Method: Binomial/nonlinear interpolation

96-hr LC₅₀: 68.5 ppm ai 95% C.I.: 55.2-85.0 ppm ai

Probit Slope: could not be calculated NOEC: 55.2 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	68.5 (55.2-85.0) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	
Probit LC ₅₀ (95% C.I.)	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.
Probit Slope	
NOEC	55.2 ppm ai

14. REVIEWER'S COMMENTS:

• RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G H
GOODNESS OF FIT PROBABILITY
5 .1377375 1
.7144211

SLOPE = 4.886639
95 PERCENT CONFIDENCE LIMITS = 3.073061 AND 6.700217

LC50 = 2.762645
95 PERCENT CONFIDENCE LIMITS = 2.2004 AND 3.349051

LC10 = 1.518563
95 PERCENT CONFIDENCE LIMITS = .9394259 AND 1.9577

DATA EVALUATION RECORD
§ 72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** Pyridine Sulfonic Acid

PC Code No.:

2. **TEST MATERIAL:** Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook# D104334-15, 1-10-92, a white crystal.

Purity: 98%

3. **CITATION**

Authors: T. J. Ward, P. L. Kowalski, R. L. Boeri

Title: Acute toxicity of Pyridine Sulfonic Acid to the Daphnid, *Daphnia magna*.

Study Completion Date: 4 May 1994

Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA

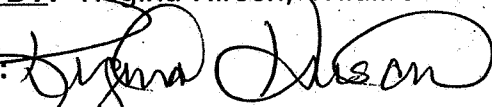
Sponsor: Olin Corporation, New Haven, CT

Laboratory Report ID: 42-OL

MRID No.: 438646-22

DP Barcode:

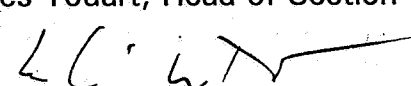
4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date:

1/17/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date:

5/19/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Daphnia magna*

Age of Test Organism: > 24 hours

Definitive Test Duration: 48 hours

Study Method: Flow-through

Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: > 122.0 ppm ai

NOEL: 122.0 ppm ai

8. **ADEQUACY OF THE STUDY**

A. Classification: Core.

DP Barcode: Dxxxxxx

MRID No.438646-22

B. Rationale: N/A

C. Repairability: N/A

9. Guideline Deviations

1. Study's dilution water was dechlorinated tap water collected at Marblehead, MA.
2. The pH range for the dilution water was higher (7.1-8.7) than what is recommended (7.2-7.6).
3. Total water hardness was 4X higher (164 mg/L as CaCO₃) than what is recommended (42-48 mg/L as CaCO₃).

10. SUBMISSION PURPOSE: Registration

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?	Yes
<u>Life Stage</u> Daphnids: 1 st instar (< 24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 th instar.	1 st instar
<u>Supplier</u>	T. R. Wilbury Laboratories, Inc
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	7 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
<u>Feeding</u> No feeding during the study.	Daphnids not fed during the test
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	0% mortality prior to testing

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.	Dechlorinated tap water collected at Marblehead, MA.
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	19.5-20.0°C
<u>pH</u> Prefer 7.2 to 7.6.	7.1-8.7

Guideline Criteria	Reported Information
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 h and $\geq 40\%$ during 2 nd 48 h, flow-through: $\geq 60\%$.	8.4-9.0 mg/L
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO_3 .	164 mg/L as CaCO_3
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel. 2. <u>Size:</u> 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. <u>Fill volume:</u> 200 ml (daphnids and midges) or 2-3 L.	Glass 20 L 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant.	Intermittent flow proportional diluter. The diluter was constructed at T. R. Wilbury, allowed test media to contact only glass, stainless steel, or Teflon-coated surfaces.
<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.	5.9 vol/24 hours Diluter was calibrated before and after the test.
<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.	0.0001 g/L (0.0002 g/L/24 hours)
<u>Photoperiod</u> 16 hours light, 8 hours dark.	16 hours light, 8 hours dark.
<u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	No solvent used.

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L, then no definitive test is required.	A screening test was not conducted and historic data were used to select the range of concentrations for the definitive test.
<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.	Control, 19.0, 31.0, 50.0, 75.0, 125.0 mg/L.
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.	20 daphnids (10 per replicate, 2 replicates per treatment level and control).
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 $> 1^{\circ}\text{C}$. 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.	The temperature in one test vessel was recorded continuously during the test. DO, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live animals.
<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	Analytical determination of test material concentration from the definitive test was performed on pooled samples collected midway between the top, bottom, and sides of the 2 replicates of each concentration at the beginning and end of the test.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> Static: $\leq 10\%$ Flow-through: $\leq 5\%$	0%
<u>Percent Recovery of Chemical</u>	87-100% of nominal
Raw data included?	No

Mortality

Concentration (ppm)		Number of Organ- isms	Cumulative Number Dead	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	--	20	0	0
19.0	16.6	20	0	0
31.0	30.4	20	0	0
50.0	49.1	20	0	0
75.0	75.0	20	0	0
125.0	122.0	20	0	0

Other Significant Results:**B. Statistical Results**

Method: None, as no daphnids died during study.

48-hr LC_{50} : > 122.0 ppm ai

DP Barcode: Dxxxxxx

MRID No.438646-22

NOEC: 122.0 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	____ (____ - ____) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	____ (____ - ____) ppm ai
Probit LC ₅₀ (95% C.I.)	____ (____ - ____) ppm ai
Probit Slope	
NOEC	122.0 ppm ai

14. REVIEWER'S COMMENTS:

DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE FISH
§ 72-3(A)

1. **CHEMICAL:** Pyridine Sulfonic Acid PC Code No.:
2. **TEST MATERIAL:** Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook# D104334-15, 1-10-92, a white crystal. Purity: 98%
3. **CITATION**

Authors: R. L. Boeri, R. L. Kowalski, and T. J. Ward

Title: Acute toxicity of Pyridine Sulfonic Acid to the sheepshead minnow, *Cyprinodon variegatus*.

Study Completion Date: 28 April 1994

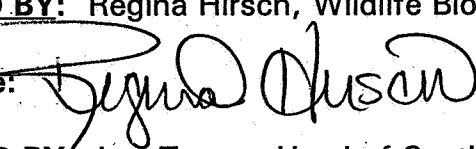
Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA

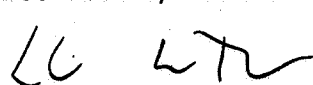
Sponsor: Olin Corporation, New Haven, CT

Laboratory Report ID: 43-OL

MRID No.: 438646-23

DP Barcode:
4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature:  Date: 1/13/97
5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature:  Date: 5/15/97
6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Cyprinodon variegatus</i>
Age or Size of Test Organism:	Juvenile/35.6 mm, 0.73 g
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured
7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: >127.0 ppm ai

NOEL: 127.0 ppm ai
8. **ADEQUACY OF THE STUDY**

A. Classification: Core.

DP Barcode: Dxxxxxx

MRID No.: 438646-23

B. Rationale: N/A

C. Repairability: N/A

9. Guideline Deviations

1. Range of weight and length of fish used in study was not reported.
2. Salinity was less (11 to 17 ‰) than recommended (30-34 ‰).
3. The pH in the higher doses was lower (6.8) than recommended (8.2).

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are the sheepshead minnow (<i>Cyprinodon variegatus</i>) or the Silverside (<i>Menidia sp.</i>).	<i>Cyprinodon variegatus</i>
<u>Mean Weight</u> 0.5 - 5 g	0.73 g
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 35.6 mm Range: Not reported
<u>Supplier</u>	Aquatic Biosystems, Inc., Fort Collins, CO
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	14 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
<u>Feeding</u> No feeding during the study	48 hours prior to test initiation.
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	<3% mortality prior to testing.

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 seawater collected at Marblehead, MA.
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 ‰ salinity, weekly range < 6 ‰	11 to 17 ‰
<u>Water Temperature</u> 22 ± 1 °C	21.9-22.6°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	6.8-8.2
Dissolved Oxygen Static: $\geq 60\%$ during 1 st 48 hrs and $\geq 40\%$ during 2 nd 48 hrs, flow-through: $\geq 60\%$	6.9 mg/L at 48 hours.
Test Aquaria 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume</u> : 15-30 L of solution	Glass 20 L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent flow proportional diluter. The diluter was constructed at T. R. Wilbury, allowed test media to contact only glass, stainless steel, or Teflon-coated surfaces.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	6.8 vol/24 hours
Biomass Loading Rate 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	0.49 g/L (0.07 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: None

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A screening test was not conducted and historic data were used to select the range of concentrations for the definitive test.
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Control, 18.8, 31.2, 50.0, 75.0, and 125.0 mg ai/L.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	20 (10 per replicate, 2 replicates)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<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	The temperature in one test vessel was recorded continuously during the test. DO, pH, salinity, and temperature were measured and recorded daily in each test chamber that contained live animals.
<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	Analytical determination of test material concentration was performed on pooled samples collected beneath the surface of the 2 replicates of each concentration at the beginning and end of the test.

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

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	95-104% of Nominal
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	--	20	0	0	0	0
18.8	17.8	20	0	0	0	0
31.2	31.1	20	0	0	0	0
50.0	50.2	20	0	0	0	0
75.0	78.4	20	0	0	0	0
125.0	127.0	20	0	0	0	0

Other Significant Results: Study deviations include the following.
Food used in feeding the test organisms during acclimation contained measurable concentrations of 2 pesticides. Survival results were not

presented graphically. Dilution water was natural filtered seawater adjusted to a salinity of 11 to 17 parts per thousand with dechlorinated tapwater rather than natural seawater. These deviations did not affect the outcome of the study.

B. Statistical Results

Method: None, as no fish died during study.

96-hr LC_{50} : > 127.0 ppm ai

NOEC: 127.0 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	___ (___ - ___) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	___ (___ - ___) ppm ai
Probit LC_{50} (95% C.I.)	___ (___ - ___) ppm ai
Probit Slope	
NOEC	127.0 ppm ai

14. REVIEWER'S COMMENTS:

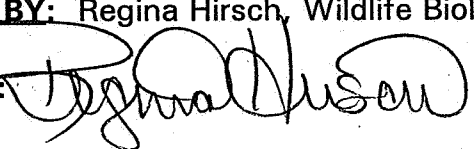
DATA EVALUATION RECORD
§ 72-3(B) -- ACUTE EC₅₀ TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY

1. **CHEMICAL:** Pyridine Sulfonic Acid PC Code No.:
2. **TEST MATERIAL:** Pyridine Sulfonic Acid, Cas# 15103-48-7, G95087-24, a white crystal. Purity: 99.1%

3. **CITATION:**

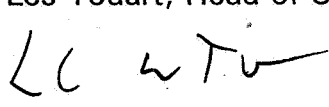
Authors: R. L. Boeri, R. L. Kowalski, and T. J. Ward
Title: Acute flow-through mollusc shell deposition test with Pyridine Sulfonic Acid
Study Completion Date: 2 May 1994
Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor: Olin Corporation, New Haven, CT
Laboratory Report ID: 45-OL
MRID No.: 438646-24
DP Barcode:

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 1/17/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5/19/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Crassostrea virginica</i>
Age or Size of Test Organism:	Juvenile/28-45 mm in height
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**

Results Synopsis

EC₅₀: 85.6 ppm ai
NOEL: 51.1 ppm ai

95% C.I.: 73.3-102.5 ppm ai
Probit Slope: 5.4

DP Barcode: Dxxxxxx

MRID No.: 438646-24

8. ADEQUACY OF THE STUDY

A. Classification: CORE

B. Rationale: N/A

C. Repairability: N/A

9. BACKGROUND

10. GUIDELINE DEVIATIONS

11. SUBMISSION PURPOSE: Registration

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are the Pacific oyster (<i>Crassostrea gigas</i>) and the Eastern oyster (<i>Crassostrea virginica</i>)	<i>Crassostrea virginica</i>
<u>Mean valve height</u> 25 - 50 mm along the long axis	28 to 45 mm
<u>Supplier</u>	P. Cummins Oyster Company, Pasadena, MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 10 days	11 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
<u>Amount of peripheral shell growth removed prior to testing</u>	3-5 mm
<u>Feeding during the acclimation</u> Must be fed to avoid stress.	Oysters were continuously supplied with live marine phytoplankton
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	<3% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Natural unfiltered seawater from an uncontaminated source.	Unfiltered natural seawater collected from the Atlantic Ocean at T. R. Wilbury, Marblehead, MA
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 ‰ salinity, weekly range < 6 ‰	34 ‰
<u>Water Temperature</u> 15°-30° C, consistent in all test vessels	21.6-22.2°C

Guideline Criteria	Reported Information
pH	6.8-8.0
<u>Dissolved Oxygen</u> ≥ 60% throughout	6.4 mg/L at 24 hours
<u>Total Organic Carbon</u>	Not reported
<u>Test Aquaria</u> Should be constructed of glass or stainless steel.	Glass
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter. The diluter was constructed at T. R. Wilbury, allowed test media to contact only glass, stainless steel, or Teflon-coated surfaces.
<u>Flow rate</u> Consistent flow rate	9.2 vol/24 hours
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Not reported
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<u>Solvents</u> Not to exceed 0.5 ml/L	Solvent: None

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $EC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A static test was performed with the highest concentration of 150 mg/L. 100% survival at this level.

Guideline Criteria	Reported Information
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Control, 18.8, 31.2, 50.0, 75.0, and 125.0 mg ai/L
<u>Number of Test Organisms</u> Minimum 20 individual per test level and in each control	20 oysters per treatment level and control (2 replicates of ten oysters each)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured hourly in at least one chamber 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	The temperature in one test vessel was recorded continuously during the test. DO, pH, salinity, and temperature were measured and recorded daily in each test chamber that contained live animals.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

13. REPORTED RESULTS

A. General Results

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

Guideline Criteria	Reported Information
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0 %
<u>Control Shell Deposition</u> Must be at least 2 mm.	2.9 mm
<u>Recovery of Chemical</u>	96-107% of Nominal
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Control	--	20	0	2.9	--
18.8	18.1	20	0	3.2	0%
31.2	31.5	20	0	2.9	0%
50.0	51.1	20	1	2.4	17%
75.0	80.2	20	0	1.7	41%
125.0	131.0	20	0	0.5	82%

B. Statistical Results

Method:

96-hr EC₅₀: 85.6 ppm ai

95% C.I.: 81.4-90.3 ppm ai

Probit Slope: Not reported

NOEC: 51.1 ppm ai

DP Barcode: Dxxxxxx

MRID No.: 438646-24

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC ₅₀	Probit
EC ₅₀ (95% C.I.)	85.6 (73.4-102.5) ppm ai
Probit Slope	5.4
Statistical Method for NOEC	Bonferroni T-test
NOEC	51.1 ppm ai

15. REVIEWER'S COMMENTS:

Data PASS normality test. Continue analysis.

Shell deposition with Pyridine Sulfonic Acid
File: moll Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 4.03
Closest, conservative, Table H statistic = 6.4 (alpha = 0.01)
Used for Table H ==> R (# groups) = 6, df (# reps-1) = 15
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 19.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Shell deposition with Pyridine Sulfonic Acid
File: moll Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	101.957	20.391	23.989
Within (Error)	114	96.869	0.850	
Total	119	198.826		

Critical F value = 2.37 (0.05,5,60)
Since $F > \text{Critical } F$ REJECT H_0 :All groups equal

Shell deposition with Pyridine Sulfonic Acid
File: moll Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	2.865	2.865		
2	18.1	3.160	3.160	-1.012	
3	31.5	2.890	2.890	-0.086	
4	51.1	2.245	2.245	2.127	
5	80.2	1.685	1.685	4.047	*

100

Shell deposition with Pyridine Sulfonic Acid
File: moll Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	8.040	29.040	45.840	29.040	8.040
OBSERVED	6	29	50	29	6

Calculated Chi-Square goodness of fit test statistic = 1.4129
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Shell deposition with Pyridine Sulfonic Acid
File: moll Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 4.03
Closest, conservative, Table H statistic = 6.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 15
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 19.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Shell deposition with Pyridine Sulfonic Acid
File: moll Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	8.040	29.040	45.840	29.040	8.040
OBSERVED	6	29	50	29	6

Calculated Chi-Square goodness of fit test statistic = 1.4129
Table Chi-Square value (alpha = 0.01) = 13.277

6 131.0 0.470 0.470 8.215 *

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=110,5)

Shell deposition with Pyridine Sulfonic Acid
 File: moll Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	20			
2	18.1	20	0.688	24.0	-0.295
3	31.5	20	0.688	24.0	-0.025
4	51.1	20	0.688	24.0	0.620
5	80.2	20	0.688	24.0	1.180
6	131.0	20	0.688	24.0	2.395

Shell deposition with Pyridine Sulfonic Acid
 File: moll Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls		Ho:GRP1 MEAN = GRP2 MEAN	
GRP1 (SOLVENT CRTL) MEAN =	2.8650	CALCULATED t VALUE =	-0.9269
GRP2 (BLANK CRTL) MEAN =	3.1600	DEGREES OF FREEDOM =	38
DIFFERENCE IN MEANS =	-0.2950		
TABLE t VALUE (0.05 (2),40) = 2.021		NO significant difference at alpha=0.05	
TABLE t VALUE (0.01 (2),40) = 2.704		NO significant difference at alpha=0.01	

Regina Hirsch Pyridine Sulfonic Acid Shell depositon in the Mollusc

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
131	20	17	85	.1288414
80.2	20	8	40	25.17223
51.1	20	3	15	.1288414
31.5	20	0	0	9.536742E-05
18.1	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 51.1 AND 131 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 88.80805

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	.1750741	86.16258	72.16457 105.6069

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
4	.14262	1

GOODNESS OF FIT PROBABILITY
.8944887

SLOPE = 5.411857
95 PERCENT CONFIDENCE LIMITS = 3.368066 AND 7.455648

LC50 = 85.63688
95 PERCENT CONFIDENCE LIMITS = 73.35446 AND 102.5476

LC10 = 49.8874
95 PERCENT CONFIDENCE LIMITS = 35.53345 AND 60.02092

**DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 122-2 OR 123-2 (TIER I OR II)**

1. **CHEMICAL:** Pyridine Sulfonic Acid PC Code No.:
2. **TEST MATERIAL:** Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook# D104334-15, 1-10-92, a white crystal. Purity: 98%

3. **CITATION**

Authors: R. L. Boeri, R. L. Kowalski, and T. J. Ward
Title: Growth and reproduction test with Pyridine Sulfonic Acid and the freshwater alga, *Selenastrum capricornutum*.
Study Completion Date: 4 April 1994
Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor: Olin Corporation, New Haven, CT
Laboratory Report ID: 46-OL
MRID No.: 438646-25
DP Barcode:

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature:

Date:

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature:

Date:

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Selenastrum capricornutum*
Definitive Test Duration: 120 hours
Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

Results Synopsis

EC₅₀: ~~21.7~~ ppm ai

NOEL: 5.46 ppm ai

23-46.2 28.9 ppm ai 95% C.I.: ~~17.9-26.1~~ ppm ai
Probit Slope: 3.85

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

1. Amount of nutrients in test solutions was not reported.
2. The pH of the test solutions were either too low or too high (4.0-9.8) of the pH recommended for the test (7.5).
3. Maximum label rate was not reported.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Selenastrum capricornutum</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/ml	10,000 cells/ml
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Not reported

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	23.7-24.0°C

Guideline Criteria	Reported Information
<u>Light Intensity</u> Anabaena: 2.0 Lux ($\pm 15\%$) Others: 4.0-5.0 Lux ($\pm 15\%$)	342-350 footcandles
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	4.0-9.8

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X progression
<u>Doses</u> at least 5	5 doses (6.25, 12.5, 25.0, 50.0, 100.0 mg/L)
<u>Controls</u> negative and/or solvent	Negative
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Cellular counts using hemacytometer
<u>Maximum Labeled Rate</u>	Not Reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes
Control cell count at 120 hr $\geq 2X$ initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	No

Dose Response

Mean Measured Dose (mg ai/L)	Cell Density ($\times 10^3$ cells/ml)	% Inhibition	120-Hour pH
Control	2407	--	9.8
5.46	2393	1%	9.8
11.6	1925	20%	9.7
23.0	1999	17%	9.6
46.2	<10	99%	4.0
91.9	<10	99%	3.4

Other Significant Results: No effects (size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells) were noted.

Statistical Results

Statistical Method: Binomial method/nonlinear interpolation

EC₅₀: 28.9 ppm

95% C.I.: 23.0 to 46.2 ppm

NOEC: 5.46 ppm

LOEC: 11.6 ppm

DP Barcode: D

MRID No.: 438646-25

13. Verification of Statistical Results

Statistical Method: Moving Average

EC₅₀: 21.7 ppm

95% C.I.: 17.9-26.1 ppm

Probit Slope: 3.85

NOEC: 5.46 ppm

14. REVIEWER'S COMMENTS:

Verified using Notchatch and obtained
Similar results to that of lab ^{SD} 6/12/97

J.DAT : pyridine sulfonic acid

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	2.41E+03	.		
5.46	2.39E+03	0.1001	N.S.	
11.6	1.96E+03	3.181	0.0056	*
23	1.96E+03	3.181	0.0058	*
46.2	5	17.17	<0.005	*

"*"=Significant; "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	21.	18.	25.	0.031	0.86
EC10	23.	20.	26.	0.028	0.87
EC25	25.	22.	29.	0.024	0.88
EC50	28.	25.	32.	0.022	0.90

Slope = 13.5 Std.Err. = 1.88

!!!Poor fit: p = 0.011 based on DF= 2.0 10.

J.DAT : pyridine sulfonic acid

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	2.41e+03	2.24e+03	165.	100.	0.00
5.46	3.00	2.39e+03	2.24e+03	151.	100.	2.03e-14
11.6	3.00	1.93e+03	2.24e+03	-316.	100.	8.09e-06
23.0	3.00	2.00e+03	2.00e+03	0.00132	89.2	10.8
46.2	3.00	5.00	5.00	-5.84e-05	0.223	99.8

Regina Hirsch Pyridine Sulfonic Acid
Growth and Reproduction of the Freshwater Alga

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
91.9	30	30	100	9.313227E-08
46.2	30	30	100	9.313227E-08
23	30	5	16.66667	1.624572E-02
11.6	30	6	20	7.154533E-02
5.46	30	1	3.333334	2.8871E-06

THE BINOMIAL TEST SHOWS THAT 23 AND 46.2 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 29.06455

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	3.972055E-02		21.66839 17.87237

26.136

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
5	1.378478	7.237763

GOODNESS OF FIT PROBABILITY

0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 3.850581
95 PERCENT CONFIDENCE LIMITS = -.6703331 AND 8.371495

LC50 = 23.60039
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 11.0433
95 PERCENT CONFIDENCE LIMITS = 0 AND 23.4581

Page _____ is not included in this copy.

Pages 131 through 132 are not included in this copy.

The material not included contains the following type of information:

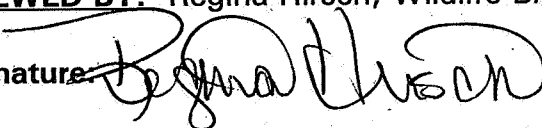
- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
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 - _____ The product confidential statement of formula.
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 - X _____ FIFRA registration data.
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 - _____ The document is not responsive to the request.
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DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE SHRIMP
§ 72-3(C)

1. **CHEMICAL:** Pyridine Sulfonic Acid PC Code No.:
2. **TEST MATERIAL:** Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook# D104334-15, 1-10-92, a white crystal. Purity: 98%
3. **CITATION:**
- Authors: R. L. Boeri, R. L. Kowalski, and T. J. Ward
Title: Acute toxicity of Pyridine Sulfonic Acid to the Mysid, *Mysidopsis bahia*.
Study Completion Date: 28 March 1994
Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor: Olin Corporation, New Haven, CT
Laboratory Report ID: 44-OL
MRID No.: 438646-26
DP Barcode:

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 1/17/96

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature: 

Date: 5/19/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Mysidopsis bahia</i>
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:**

Results Synopsis

LC₅₀: 71.0 ppm ai

NOEL: 51.9 ppm ai

95% C.I.: 62.8-81.1 ppm ai

DP Barcode: Dxxxxxx

MRID No.: 438646-26

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

9. BACKGROUND

10. GUIDELINE DEVIATIONS

1. Water was adjusted to appropriate salinity using dechlorinated tapwater.
2. Total organic was not reported.
3. The pH in the higher doses was lower (6.8) than recommended (7.7-8.3).

11. SUBMISSION PURPOSE: Registration

12. 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>	Adults obtained from Aquatic Biosystems, Inc., Fort Collins, CO. Juveniles produced from in-house culture.
All shrimp are from same source?	Yes

Guideline Criteria	Reported Information
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> minimum 10 days	14 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
<u>Feeding</u> No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each.	Mysids were fed live <i>Artemia salina</i> daily during acclimation and testing.
<u>Pretest Mortality</u> < 3% mortality 48 hours prior to testing	< 3% mortality prior to testing.

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 seawater collected at Marblehead, MA. Water was adjusted to appropriate salinity using dechlorinated tapwater.
Does water support test animals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
<u>Salinity</u> 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰	11 to 17 ‰
<u>Water Temperature</u> Approx. 22 $\pm$ 1 °C	21.6 to 22.9 °C
<u>pH</u> 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	6.8 to 8.5
<u>Dissolved Oxygen</u> Static: $\geq$ 60% during 1 st 48 hrs and $\geq$ 40% during 2 nd 48 hrs, Flow-through: $\geq$ 60%	7.2 mg/L at test initiation
<u>Total Organic Carbon</u>	Not reported
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> 19.6 L is acceptable for organisms $\geq$ 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 $\geq$ 0.5 g, 2-3 L is acceptable for smaller organisms.	Glass 20 L 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter. The diluter was constructed at T. R. Wilbury, allowed test media to contact only glass, stainless steel, or Teflon-coated surfaces.

Guideline Criteria	Reported Information
<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	6.0 vol/24 hours
<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	.0006 g/L (0.0001 g/L/day)
<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

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $\text{LC}_{50} > 100$ mg/L with 30 shrimp, then no definitive test is required.	A screening test was not conducted and historic data were used to select the range of concentrations for the definitive 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, 18.5, 31.2, 50.0, 75.0, and 125.0 mg ai/L.
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers	20 per test level (2 replicates with 10 shrimp in each)
Test organisms randomly or impartially assigned to test vessels?	Yes

Guideline Criteria	Reported Information
Biological observations made every 24 hours?	Yes
<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	The temperature in one test vessel was recorded continuously during the test. DO, pH, salinity, and temperature were measured and recorded daily in each test chamber that contained live animals.
<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	Analytical determination of test material concentration was performed on pooled samples collected beneath the surface of the 2 replicates of each concentration at the beginning and end of the test.

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes)
<u>Recovery of Chemical</u>	96-106%
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	No

Guideline Criteria	Reported Information
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Shrimp	Cumulative Number Dead and Number Affected in ()			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	--	20	0	0	0	0
18.5	17.8	20	0	0	0	1
31.2	31.1	20	0	0	0	0
50.0	51.9	20	0	0	0	2
75.0	79.8	20	0	7	12	13
125.0	128.0	20	19 (1)	20	20	20

Other Significant Results: Affected mysid exhibited lethargy.

B. Statistical Results

Method: Binomial/nonlinear interpolation

96-hr LC₅₀: 71.6 ppm ai 95% C.I.: 51.9-128.0 ppm ai

NOEC: 51.9 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	71.6 (51.9-128.0) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	71.0 (62.9-81.1) ppm ai
Probit LC ₅₀ (95% C.I.)	68.7 (0-infinity) ppm ai
Probit Slope	5.39
NOEC	51.9 ppm ai

15. REVIEWER'S COMMENTS:

regina pyridine sulfonic acid Acute Toxicity to the Mysid

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
128	20	20	100	9.536742E-05
79.8	20	13	65	13.1588
51.9	20	2	10	2.012253E-02
31.1	20	0	0	9.536742E-05
17.8	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 51.9 AND 128 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 71.65694

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.416779E-02		70.99755	62.86088

81.08239

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
7	7.117079	22.02362

GOODNESS OF FIT PROBABILITY

0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 5.388355
95 PERCENT CONFIDENCE LIMITS = -8.986619 AND 19.76333

LC50 = 68.66116
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 39.90411
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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

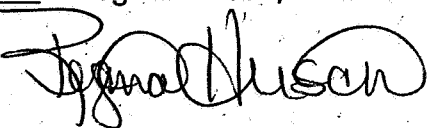
1. **CHEMICAL:** Pyridine Sulfonic Acid PC Code No.:
2. **TEST MATERIAL:** Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook#
D104334-15, 1-10-92, a white crystal. Purity: 98%

3. **CITATION**

Authors: T. J. Ward, P. L. Kowalski, and R. L. Boeri
Title: Acute toxicity of Pyridine Sulfonic Acid to the
rainbow trout, *Oncorhynchus mykiss*.
Study Completion Date: 3 September 1993
Laboratory: T. R. Wilbury Laboratories, Inc., Marblehead, MA
Sponsor: Olin Corporation, New Haven, CT
Laboratory Report ID: 41-OL
MRID No.: 438646-27
DP Barcode:

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature:

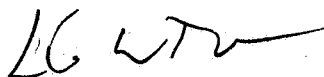


Date:

1/15/97

5. **APPROVED BY:** Les Touart, Head of Section (1), EEB, EFED

Signature:



Date:

5/9/97

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Oncorhynchus mykiss</i>
Age or Size of Test Organism:	Juvenile/36.6 mm, 0.41 g
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**

Results Synopsis

LC₅₀: 57.1 ppm ai

95% C.I.: 48.3-69.8 ppm ai

NOEL: 46.9 ppm ai

8. **ADEQUACY OF THE STUDY**

A. Classification: Core.

DP Barcode: Dxxxxxx

MRID No.: 438646-27

B. Rationale: N/A

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS**

1. Range of weight and length of fish used in study was not reported.
2. Dilution water was dechlorinated tap water collected from Marblehead, MA.
3. The pH of the test water ranged from 3.7-7.8.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the rainbow trout (<i>Onchorhynchus mykiss</i>)	<i>Onchorhynchus mykiss</i>
<u>Mean Weight</u> 0.5-5 g	0.41
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 36.6 Range: Not reported
<u>Supplier</u>	Mount Lassen Trout Farm, Red Bluff, CA
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	14 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
<u>Feeding</u> No feeding during the study	48 hours preceding test initiation
<u>Pretest Mortality</u> < 3% mortality 48 hours prior to testing	< 3% mortality prior to testing.

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	Dilution water was dechlorinated tap water collected from Marblehead, MA.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 12°C	11.2-13.0°C
<u>pH</u> Prefer 7.2 to 7.6	3.7-7.2

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: $\geq 60\%$	8.8 mg/L at 72 hours
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO_3	48 mg/L as CaCO_3
<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	Glass 20 L 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Intermittent flow proportional diluter. The diluter, which was constructed at T. R. Wilbury, allowed test media to contact only glass, stainless steel, or Teflon-coated surfaces.
<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	5.5 vol/24 hours The diluter was calibrated before and after the test.
<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	0.27 g/L (0.05 g/L/day)
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours 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

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A screening test was not performed and historic data was used to determine the range of concentrations for the definitive test.
<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	Control, 19.0, 31.0, 50.0, 75.0, and 125.0 mg ai/L.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	2 replicates per test level with 10 fish in each replicate (total 20 fish/test level).
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<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	The temperature in one test vessel was recorded continuously during the test. DO, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live fish.
<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	Analytical determination of test material concentration from the definitive test was performed on pooled samples collected from the 2 replicates of each concentration at the beginning and end of the test.

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

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	88-96% of Nominal
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead and Number Affected in ()			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	--	20	0	0	0	0
18.0	15.8	20	0	0	1	1
31.0	28.5	20	0	0	0	0
50.0	46.9	20	0	0	1	1
75.0	71.7	20	0	0 (20)	12 (8)	19 (1)
125.0	112.0	20	20	20	20	20

Other Significant Results: Affected fish exhibited a loss of equilibrium, erratic swimming, and lethargy.

DP Barcode: Dxxxxxx

MRID No.: 438646-27

Protocol Deviations: The fish food was not free of measurable concentrations of pesticides (2 of the 47 pesticides that were analyzed for occurred at concentrations at or slightly above the reporting limit). However, the two pesticides were not identified.

B. Statistical Results

Method: Moving Average

96-hr LC₅₀: 57.1 ppm ai 95% C.I.: 48.3-69.8 ppm ai

Probit Slope: Not reported NOEC: 46.9 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	58.0 (46.9-71.7) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	57.1 (48.3-69.8) ppm ai
Probit LC ₅₀ (95% C.I.)	54.7 (0-infinity) ppm ai
Probit Slope	6.56
NOEC	46.9 ppm ai

14. **REVIEWER'S COMMENTS:** Since the probability is less than 0.05, results calculated using the probit method probably should not be used.

Regina Hirsch Pyridine Sulfonic Acid Acute Toxicity to Rainbow Trout

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
112	20	20	100	9.536742E-05
71.7	20	19	95	2.002716E-03
46.9	20	1	5	2.002716E-03
28.5	20	0	0	9.536742E-05
15.8	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 46.9 AND 71.7 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 57.98905

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.0772644	57.13292	48.29792 69.80888

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
GOODNESS OF FIT PROBABILITY		
6	28.55956	86.07247

0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 6.561669
 95 PERCENT CONFIDENCE LIMITS = -28.50464 AND 41.62798

LC50 = 54.69585
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 35.02725
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
