US ERA ARCHIVE DOCUMENT

: D228348 DP Barcode PC Code No : 088002 EEB Out

MAY 23 :007

MAY 23 1997

Marion Johnson To:

Product Manager 31

Registration Division (7505C)

Dan Rieda

From: Anthony F. Maciorowski, Chief

Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

: 001258-REEG Req./File #

Chemical Name: Zinc 2-pyridinethiol-1-oxide

: antifoulant Type Product : Zinc Omadine Product Name

Company Name : Olin

: Review data for antifoulant use. Purpose

Action Code: 176

Date Due: 11/22/96

Joanne Edwards Reviewer:

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

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lowing:			<u> </u>					T
GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)		1	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)	Contract Con		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 1007

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

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
		EC50 = 28.9 ppm NOEC = 5.46 ppm	Core
72-3 Mollusc Shell 438646-24 Deposition		EC50 = 85.6 ppm NOEC = 51.1 PPM	Core Slightly toxic
72-3(b) Estuarine 438646-26 Shrimp		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
1		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. <u>TEST MATERIAL</u>: Zinc bis-1-oxide-2(1H)-pryidenethionate, off-white powder, CAS No.13463-41-7. <u>Purity</u>: 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

mature: Do Date: 1/10/97

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

Signature: 2647 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

 LC_{50} : 8.25 μ g/L ai 95% C.I.: 5.24-25.82 μ g/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 $CaCO_3$) the recommended limits (40 to 48 mg/L as $CaCO_3$).

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>	Daphnia magna
All organisms are approximately the same size and weight?	Not Reported
Life Stage Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 th instar.	1 st instar
Supplier	Produced from an in-house culture
All organisms from the same source?	Yes

B. Source/Acclimation

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

C. <u>Test System</u>:

Guideline Criteria	Reported Information
Source of dilution water 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
Water Temperature 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
<u>pH</u> Prefer 7.2 to 7.6.	8.1
Dissolved Oxygen Static: ≥ 60% during 1 st 48 h and ≥ 40% during 2 nd 48 h, flow-through: ≥ 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:	Glass
250 ml (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 ml (daphnids and midges) or 2-3 L.	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 ≤ 17°C, ≤ 0.5 g/L at > 17°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
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	None used

D. <u>Test Design</u>:

Guideline Criteria	Reported Information
Range Finding Test If $LC_{50} > 100 \text{ mg/L}$, then no definitive test is required.	Screening tests conducted, but none had LC50 > 100 mg/L.
Nominal Concentrations of Definitive Test 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 μg/L
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control. 	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.

Chemical Analysis

Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used 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
Control Mortality Static: ≤10% Flow-through: ≤5%	0%
Percent Recovery of Chemical	46-67% of nominal
Raw data included?	No

Mortality

Concentration (μg/L)		Number	Cumulative Number Dead and Number Affected in ()		
		of Organisms	Hour of Study		
Nominal	Mean Measured		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 $\mu g/L$ ai

Probit Slope: 1.4

NOEC: $1.1 \mu g/L$ ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter 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
GOODNESS OF FIT PROBABILITY
3 .349881 1

.1887432

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

LC50 = 8.259187 95 PERCENT CONFIDENCE LIMITS = 5.242572 AND 25.82752

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

1. CHEMICAL: Zinc Omadine PC Code No.: 001258

2. <u>TEST MATERIAL</u>: Zinc bis-1-oxide-2(1H)-pryidenethionate, off-white powder, CAS No.13463-41-7. <u>Purity</u>: 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: Jana Ouson

Date: 1/10/97

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

Signature:

66 w7v

Date: 5 /(5/5)

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
Species Preferred species are the sheepshead minnow (Cyprinodon variegatus) or the Silverside (Menidia sp.).	Cyprinodon variegatus
Mean Weight 0.5 - 5 g	0.26 g
Mean Standard Length Longest not > 2x shortest	Mean: 25 mm Range: Not reported.
Supplier	Aquatic Biosystems, Inc., Fort Collins,
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

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

DP Barcode: D228348

Reported Information Guideline Criteria 21.0-22.4°C Water Temperature 22 ± 1 °C 7.8-8.0 рH 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8 6.8 mg/L at 96 hours Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60% Test Aquaria 1. Material: Glass Glass or stainless steel 2. Size: 20L Volume of 19 L (5 gal) or 30 x 60 x 30 cm 15L 3. Fill volume: 15-30 L of solution Static renewal conditions. Test media Type of Dilution System were renewed after 48 hours of Must provide reproducible supply of exposure. toxicant N/A Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period 0.17 g/L **Biomass Loading Rate** 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 16 h light, 8 h dark. **Photoperiod** 16 hours light, 8 hours dark Solvent: None **Solvents** Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests

MRID No.: 438646-05

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	Screening tests were performed but LC ₅₀ 's did not exceed 100 mg/L
Nominal Concentrations of Definitive Test 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.
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control 	Temperature 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
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	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
Recovery of Chemical	75-100 % of nominal
Control Mortality 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

Concentra	ation (ppm)	Number				
	The second secon	of ⊿⊸ Fish		Hour of	Study	
Nominal	Mean Measured		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 LC₅₀: 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.

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

****	*****	A A A A A A A A A A A A A A A A A A A		
CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
. 99	20	20	100	9.536742E-05
.59	20	20	100	9.536742E-05
		6	30	5.765915
.36	20	0	0	9.536742E-05
. 2	20	0	0	9.536742E-05
.098	20	0 :	U	9.5507420 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
.2	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)-pryidenethionate, off-white powder,

CAS No.13463-41-7

Purity: 97.8%

3. CITATION

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

Acute toxicity of Zinc Omadine to the fathead Title:

minnow, Pimephales promelas

Study Completion Date:

12 July 1994

T. R. Wilbury Laboratories, Marblehead, MA Laboratory: Olin Corporation, New Haven, CT

Sponsor:

19-OL,

Laboratory Report ID: 438646-06 MRID No.:

D228348 DP Barcode:

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

Signature:

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

Signature:

4667

Date: 5/19/97

6. STUDY PARAMETERS

Scientific Name of Test Organism:

Pimephales promelas

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

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

NOEC: 1.1μg/L

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, Pimephales promelas
<u>Mean Weight</u> 0.5-5 g	0.28 g
Mean Standard Length Longest not > 2x shortest	32 mm
Supplier	Aquatic Biosystems, Fort Collins, CO
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criterla	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	24 hour period prior to test initiation
Pretest Mortality No more than 3% mortality 48 hours prior to testing	<5% 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 collected at T.R. Wilbury Laboratories.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 17°C or 22°C	21.1-22.9 °C
pH Prefer 7.2 to 7.6	7.3-7.6

'Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	8.5 mg/L at 72 hour
Total Hardness Prefer 40 to 48 mg/L as CaCO ₃	40 mg/L as CaCO ₃
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	glass 20 L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent flow proportional diluter constructed at T.R. Wilbury Laboratories
Flow Rate 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.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.19 g/L (0.03 g/L/day)
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	None

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	3 screening tests and 2 definitive test were performed prior to this definitive test.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Control, 3.0, 4.0, 7.0, 10.0, and 17.0 μg ai/L.
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and 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.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	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		
Recovery of Chemical	37 -88% of Nominal		
Control Mortality 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	Cumulative Number Dead and Number Affected in ()			
	Mean Measured	of Fish	Hour of Study			
Nominal			24	48	72	96
Control	0.0	20	0	1	1	1
3.0	1.1	20	0	О	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.: $2.0 - 3.1 \mu g/L$ ai

Probit Slope: 4.3

NOEC: 1.1 μg/L ai

13. <u>VERIFICATION OF STATISTICAL RESULTS</u>

Parameter	en verde en Euse 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	PROB. (PERCENT)
15 7.9 4.8 2.6	19 19 19 19	19 19 17 7 1	100 100 89.4737 36.8421 5.2632	1.907348E-04 1.907348E-04 3.643036E-02 17.96417 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
GOODNESS OF FIT PROBABILITY
5 .1377375 1

.7144211

SLOPE = 4.886639 95 PÉRCENT CONFIDENCE LIMITS = 3.073061 AND 6.700217

LC50 = 2.762645 95 PERCENT CONFIDENCE LIMITS = 2.2004 AND 3.349051

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 3.814697E-03
1.1	19	1	5.2632	3.01409/15-05

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

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)-pryidenethionate, off-white powder,

CAS No.13463-41-7 **Purity: 97.8%**

3. CITATION

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

Acute toxicity of Zinc Omadine to the Mysid, Title:

Mysidopsis bahia.

11 July 1993 Study Completion Date:

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

Olin Corp., New Haven, CT Sponsor:

23-OL Laboratory Report ID:

> 438646-07 MRID No.: DP Barcode: D228348

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

Date: 1/8/97 , EFED

Date: 5/19/97 5. APPROVED BY: Les Touart, Head of Section (1), EEB, EFED

Signature:

6. STUDY PARAMETERS

Signature:

Scientific Name of Test Organism:

16 67,-

Mysidopsis bahia

Age or Size of Test Organism: 1.5 mg

Definitive Test Duration: 96 hours

> Study Method: Flow-through

Mean measured

Type of Concentrations:

7. **CONCLUSIONS**:

Results Synopsis

LC₅₀: 4.7 μg/L ai 95% C.I.: 4.04 - 5.53 μg/L ai

NOEL: 1.6 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
Species Preferred species are Mysidopsis bahia, Penaeus setiferus, P. duorarun, P. aztecus and Palaemonetes sp.	Mysidopsis bahia
Age 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
Acclimation Period 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
Feeding 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 Artmia salina daily during acclimation and testing.
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	dilution as natural filtered seawater adjusted with dechlorinated tapwater to the correct salinity.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 for marine (stenohaline) shrimp and 10-17 for estuarine (euryhaline) shrimp, weekly range < 6	11 to 19 ppt

Guideline Criteria Water Temperature Approx. 22 ± 1 °C	Reported Information 21.3 to 22.9°C
pH 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
Dissolved Oxygen Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, Flow-through: ≥ 60%	7.9 mg/L at 24 hour
Total Organic Carbon	Not reported
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 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. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	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 Laboratories, 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	5.3 vol/24 hours

Guideline Criteria	Reported Information
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.001 g/L (0.002 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	None

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If 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 concnetrations for the definitive test.
Nominal Concentrations of Definitive Test 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 μg ai/L.
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control 	Temperature 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.
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Analytical determineation of test material concnetration 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. <u>REPORTED RESULTS</u>

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	67-106 % of Nominal
Control Mortality 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

Concentr	Concentration (ppm)					
		Number of		Hour of	Study	
*Nominal	Mean Measured	Shrimp	24	48	72	96
Control	0.0	20	0	0 -	1	1
2.4	1.6	20	О '	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 LC_{50} : 6.3 $\mu g/L$ ai

95% C.I.: 1.6 to 9.4 μ g/L ai

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

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result - 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
9.39999	r .	, * 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	NUMBER	PERCENT	BINOMIAL	
•	EXPOSED	DEAD	DEAD	PROB. (PERCENT)	
17	19	19	100	1.907348E-04	
9.3999	999	19	19	100	3

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

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)-pryidenethionate, 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 Shaw MSM

Date: 1/9/9-7

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

Signature:

LG WIV

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

95% C.I.: 18.9 - 27.3 μg/L ai

EC₅₀: 22.0 μ g/L ai NOEL: 7.1 μ g/L ai

Probit Slope: 4.24

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>)	Crassostrea virginica
Mean valve height 25 - 50 mm along the long axis	28-50 mm
Supplier	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
Acclimation Period 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
Amount of peripheral shell growth removed prior to testing	3-5 mm
Feeding during the acclimation 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.
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 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
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	33-34 ‰

Guideline Criteria	Reported Information
Water Temperature 1530 C, consistent in all test vessels	19.1-21.4C
рН	7.7-8.1
Dissolved Oxygen ☐ 60% throughout	Range: 7.1-7.6 mg/L
Total Organic Carbon	Not reported.
Test Aquaria Should be constructed of glass or stainless steel.	Glass
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent flow proportional diluter, constructed at T. R. Wilbury Laboratories, Inc.
Flow rate 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
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	Solvent: None

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test 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 µg/L

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test 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
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH 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
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	O %
Control Shell Deposition Must be at least 2 mm.	2.4 mm
Recovery of Chemical	79 - 113 % of Nominal
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (ppm)		Number -		Mean Shell	Mean	
Nominal	Mean Measured	∷Per Level	Number Dead	Deposition (mm)	Percent Reduction	
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.

96-hr EC₅₀: 22 μg/L ai 95% C.I.: 17-25 μg/L ai

NOEC: 10 μ g/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

GOODNESS OF FIT PROBABILITY

5 .1331501 1

.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

•	and the second s	•					
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	e v 🌉 meniji	33	20		0.522	21.9	1.710

Regina Hirsch Zinc Omadine Shell Deposition for the Mollusc

				,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
CONC.	NUMBER	NUMBER	PERCENT	BINOMIAL ·
	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
3.3	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

GOODNESS OF FIT PROBABILITY

5 .1331501 1

.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	NUMBER	PERCENT	BINOMIAL
	EXPOSED	DEAD	DEAD	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

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 OBSERVED	8.040	29.040 35	45.840 47	29.040 23	8.040 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.

nc 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.

'TE: 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		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
hin (Error)	114	59.878	0.525	·
Total	119	118.024		

Critical F value = 2.37 (0.05,5,60)

Since F > Critical F REJECT Ho: All groups equal

Zinc Omadine for Mollusc shell deposition

File: molldat Transform: NO TRANSFORMATION

UDOUT	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 Ho:	Control <t< th=""><th>reatment</th></t<>	reatment
GROUP	IDENTIFICATION		Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1 2	Control 7.1	L 20 L 20	0.522	21.9	-0.005 50

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)-pryidenethionate, 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:

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

Signature:

7,-

Date: 1/9/97Date: 5/(9/37)

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. <u>SUBMISSION PURPOSE</u>: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Skeletonema costatum Anabaena flos-aquae Selenastrum capricornutum Navicula pelliculosa	Selenastrum capricornutum
Initial Number of Cells 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
Solvent	None
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	23.8-24.3°C
Light Intensity Anabaena: 2.0 Lux (±15%) Others: 4.0-5.0 Lux (±15%)	31 - 33 footcandles

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

C. Test Design

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

12. <u>REPORTED RESULTS</u>

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

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

Dose Response

Mean Measured Concentration (μg ai/L)	Cell Density (x 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 μg/L

95% C.I.: 24 - 33 μg/L

NOEC: 7.8 μg/L

13. <u>Verification of Statistical Results</u>

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

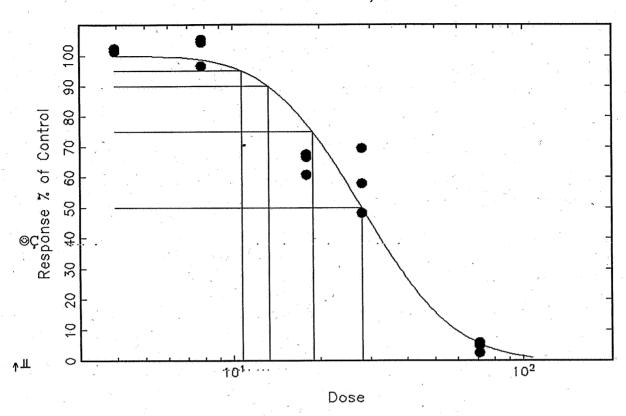
95% C.I.: 24-33 μg/L

NOEC: 7.8 μg/L

14. REVIEWER'S COMMENTS:

Program: Nuthatch _ ______ 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 7.8 ·)

JE.DAT : Selenastrum / zinc omadine



JE:DAT : Selenastrum / zinc omadine _______ Williams Test [One-Sided Test for Decrease, alpha = 0.050000] . . . Isotone T-bar P-value Significance Dose Means 211 211 -0.03569 7.8 N.S. 135 8.137 < 0.005 18 9.564 < 0.005 28 121 9 21.59 < 0.005 71 "*"=Significant; "N.S."=Not Significant. Estimates of EC%
 Parameter
 Estimate
 95% Bounds
 Std.Err.
 Lower Bound

 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 Obs. Pred. Obs. Pred. %Change Mean Mean -Pred. %Control Dose #Reps.

 0.00
 3.00
 211.
 207.
 3.51
 100.

 7.80
 3.00
 211.
 204.
 7.03
 98.6

 18.0
 3.00
 135.
 161.
 -26.2
 77.7

 28.0
 3.00
 121.
 103.
 17.9
 49.9

 71.0
 3.00
 9.00
 11.2
 -2.19
 5.40

 0.00 1.38 22.3 50.1

94.6

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- TABLE 1 OF 2

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 <tre< th=""><th colspan="2">eatment</th></tre<>				eatment	
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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control 7.8	210.667 211.333	210.667 211.333	-0.079	
3	18.0	134.667	134.667	8.987	*
4	28.0	121.333	121.333	10.563	*
5 6	71.0 170	10.333 9.000	10.333 9.000	23.689 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 - T	ABLE 2 OF	2 Ho:	Control <t< th=""><th>reatment</th></t<>	reatment
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1 2 3 4 5	Control 7.8 18.0 28.0 71.0 170	3 3 3 3 3 3	21.142 21.142 21.142 21.142 21.142	10.0 10.0 10.0 10.0 10.0	-0.667 76.000 89.333 200.333 201.667

Zinc Omadine and Alga

File: 122dat Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1287.333

0.864

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

rtley test for homogeneity of variance Lirtletts 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.

7 nc Omadine and Alga

e: 122dat Transform: NO TRANSFORMATION

ANOVA TABLE

9	164		**	
SOURCE	DF	SS	MS	F
Between	5	123123.778	24624.756	229.542
Within (Error)	12	1287.333	107.278	· ·
Total	17	124411.111		3

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

. .

TABLE 1 CUMULATIVE MORTALITY OF NORTHERN BOBWHITE DOSED WITH ZINC OMADINE

		Total	0/10	0/10	2/10	9/10	10/10	10/10	10/10
		14	0/5 0/5	0/5	0/5 2/5	5/5 4/5	5/5 5/5	5/5 5/5	5/5
		13	0/5 0/5	0/5 0/5	0/5 2/5	5/5 4/5	5/5 5/5	5/5 5/5	5/5 5/5
		12	0/5	0/5 0/5	0/5	5/5 4/5	5/5 5/5	5/5 5/5	5/5 5/5
		귀	0/5	0/5	0/5 2/5	5/5 4/5	5/5 5/5	5/5 5/5	5/5 5/5
		의	0/5 0/5	0/5	0/5	5/5	5/5 5/5	5/5	5/5
b		6	0/5 0/5	0/5	0/5 2/5	5/5 4/5	5/5 5/5	5/5	5/5 5/5
Expose	,	8	0/5	0/5	0/5 2/5	5/5 4/5	5/5 5/5	5/5	5/5
lumber	F Study	7	0/5 0/5	0/5 0/5	0/5 1/5	5/5 4/5	5/5 5/5	5/5	5/5 5/5
Number Dead/Number Exposed	Day of	9	0/5	0/5 0/5	0/5 1/5	5/5 4/5	5/5 5/5	5/5 5/5	5/5 5/5
Number		2	0/5	0/5 0/5	0/5 1/5	5/5 4/5	5/5 5/5	5/5 5/5	5/5 5/5
		4	0/5	0/5 0/5	0/5 1/5	5/5 4/5	5/5 5/5	5/5 5/5	5/5 5/5
		က	0/5 0/5	0/5	0/5 0/5	3/5 1/5	5/5 5/5	5/5	5/5
		2	0/5	0/5 0/5	0/5 0/5	0/5 0/5	1/5 2/5	5/5	4/5 5/5
			0/5	0/5	0/5 0/5	0/5	1/5	3/5	2/5 4/5
		0	0/5 0/5	0/5 0/5	0/5	0/5 0/5	0/5 0/5	0/5	0/5 0/5
		g Sex	ΣLL	Σι⊾	Σι∟	Σ∟	£⊯	Σι∟	Σι⊥
	Docade	mg a.i./kg	Control	31.2	62.5	125	250	200	1000

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 LC $_{50}$ 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 pyrithione); 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: James () US CM

Date: 12/12/96

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

Signature:

W Tu

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. <u>CONCLUSIONS</u>:

Results Synopsis

LC₅₀: 1063 ppm ai

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

NOEL: < 253 ppm ai Probit Slope: 4.55

(LCso = 1110 ppm based on nominal) TSE

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS

1. Brooder temperature (39°C \pm 2°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>).	Colinus virginianus
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:	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

MRID No.: 438646-10

DP Barcode: D228348

Mortalit	v
TIVE CHARA	

<u> 10rtanty</u>										
Conc. (ppm)		Cumulative Number of Dead								
1	All P	No. of	Day of Study							
Nominal	Mean Measured	Birds	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	01	01	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	34	84	10	10	10	10	10

¹ Signs of toxicity: wing droop, ruffled appearance 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

² Signs of toxicity: depression, reduced reaction to external stimuli (sound and movement), wing droop, prostate 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.

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

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

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

MRID No.: 438646-11 DP Barcode: D228348

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. **Purity: 96%**

(Poisonous solid contains zinc pyrithione); white powder.

3. CITATION

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

Zinc Omadine: An acute oral toxicity study with the Title:

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

Date: 5/19/97

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

Signature:

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>).	Colinus virginianus
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		

DP Barcode: D228348

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

viorcanty									
	Cumulative Number of Dead								
.	No. of				Da	y of Stu	dy .		
Dosage (mg/kg)	Birds	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 these 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 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. <u>Verification of Statistical Results:</u>

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 WI's statistics in error?

Regina Hirsch Zinc Oxadine BWQ Acute Oral ************************ BINOMIAL . PERCENT NUMBER NUMBER PROB. (PERCENT) CONC. DEAD DEAD · EXPOSED 9.765625E-02 100 10 10 1000 9.765625E-02 100. 10 500 10 9.765625E-02 100

10 10 250 1.074219 90 9 10 125 5.46875 20 2 10 62.5 9.765625E-02 0 0

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

RESULTS CALCULATED USING THE PROBIT METHOD . G TTERATIONS GOODNESS OF FIT PROBABILITY .3508794 1

10

31.2

.999959

7.175594 SLOPE AND 11.42606 95 PERCENT CONFIDENCE LIMITS = 2.925126

82.37645 95 PERCENT CONFIDENCE LIMITS = 63.00147 AND 108.0656

54.80426 LC10 =69.56859 27.88084 AND 95 PERCENT CONFIDENCE LIMITS = *************************

Regina Hirsch Zinc Oxadine Acute Oral BWQ

Regina	Hirsch Zinc	coxadine Acut	+*********	****************
CONC.	NUMBER EXPOSED	NUMBER DEAD	DEAD DEAD	BINOMIAL PROB. (PERCENT) 9.765625E-02
1000 500 250 125 62.5	10 10 10 10 10	10 10 10 9 2	100 100 100 90 20	9.765625E-02 9.765625E-02 9.765625E-02 1.074219 5.46875 9.765625E-02
31.2	10	Ü	0	-

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.

MRID No.: 438646-12 DP Barcode: D228348

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 pyrithione), white powder. Purity: 96 %

3. CITATION

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

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

24 May 1994 Study Completion Date:

> Laboratory: Wildlife International Ltd., Easton Maryland

Olin Corporation, New Haven, Connecticut Sponsor:

Laboratory Report ID: 133-111

438646-12 MRID No.:

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

Date: 11/1/910

Date: 5/19/97

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

Signature:

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

 LC_{50} : > 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 $(31^{\circ}\text{C} \pm 3^{\circ}\text{C})$ 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 (Anas platyrhynchos).	Anas platyrhynchos
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

DP Barcode: D228348

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

Mortanty										
Conc. (ppm)				Cı	ımulati	vė Num	ber of	Dead		
		No. of	Day of Study							
Nominal	Mean Measured	Birds	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	01	01	01	0	0	0
2800	2990	10	0	0	- 02	0^2	1 ²	1 ²	1 ²	1
5000	5420	10	0	0	0^3	0^3	13	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.

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

 LC_{50} : > 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.

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

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)-pryidenethionate, off-white powder,

CAS No.13463-41-7.

Purity: 97.8%

3. CITATION

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

Acute toxicity of Zinc Omadine to the rainbow trout, Title:

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:

20-0L

MRID No.:

438646-13

DP Barcode:

D228348

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

Date: 1/10/97

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

Signature:

16 WT

Date: 5/19/97

6. STUDY PARAMETERS

Scientific Name of Test Organism:

Oncorhynchus mykiss

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

95% C.I.: 3.07-4.33 μg/L ai

NOEL: 1.6 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
Species Preferred species is the rainbow trout (Onchorhynchus mykiss)	Onchorhynchus mykiss
Mean Weight 0.5-5 g	0.52 g
Mean Standard Length Longest not > 2x shortest	Mean: 36 mm Range: Not reported
Supplier	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
<u>pH</u> Prefer 7.2 to 7.6	7.1-7.4

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	9.4 mg/L at 0 and 24 hours
Total Hardness Prefer 40 to 48 mg/L as CaCO ₃	44 mg/L CaCO ₃
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	Glass aquaria 20 L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent flow proportional diluter, constructed at T. R. Wilbury Laboratories.
Flow Rate 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.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.35 g/L (0.05 g/L/day)
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: None

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC ₅₀ > 100 mg/L with 30 fish, then no definitive test is required.	A screening test was not performed and historic data were used to determine the range of concentrations.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Control, 2.9, 3.9, 6.8, 9.8, and 17.0 μg ai/L.
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and 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.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Chemical analysis performed.

MRID No.: 438646-13 DP Barcode: D228348

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				and the same of th	7. M. S.	
Concentration (μg/L)		Number	Cumulative Number Dead and Number Affected in ()			
Property of the control of the contr		of Fish	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.

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 toxicty to rainbow trout

****** CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
15 8.7	18 18	18 18 15	100 100 83.3333	3.814697E-04 3.814697E-04 .3768921
5.5 3.2 1.6	18 18 18	7 0	38.8889 0	24.03412 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
GOODNESS OF FIT PROBABILITY
6 .1652566 1
.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

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DATA EVALUATION RECORD § 72-1(A) -- ACUTE LC $_{50}$ TEST WITH A WARMWATER FISH

1. <u>CHEMICAL</u>: Pyridine Sulfonic Acid <u>PC Code No.</u>:

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 promeles.

Date: 1/15/97

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

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

Signature: $(C \sim)$ Date: $\sqrt[5]{\sqrt{\gamma}}$

6. STUDY PARAMETERS

Scientific Name of Test Organism: Pimephales promeles

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.

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
Species Preferred species is the bluegill sunfish (Lepomis macrochirus)	Pimephales promeles
<u>Mean Weight</u> 0.5-5 g	0.32 g
Mean Standard Length Longest not > 2x shortest	Mean: 35.7 mm Range: Not reported
Supplier	Aquatic Biosystems, Fort Collins, CO
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Critéria	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 prior to test initiation
Pretest Mortality No more than 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	Dilution water was dechlorinated tap water collected from Marblehead, MA.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 17°C or 22°C	22.0-22.9 °C
pH Prefer 7.2 to 7.6	3.6-7.9

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	8.4 at 48 hours.
Total Hardness Prefer 40 to 48 mg/L as CaCO ₃	48 mg/L as CaCO ₃
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	Glass 20 L 15 L
Type of Dilution System 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.
Flow Rate 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.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.21 g/L (0.04 g/L/day)
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: None

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A screening test was not performed and historic data was used to determine the range of concentrations for the definitive test.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Control, 18.8, 31.2, 50.0, 75.1, and 125.0 mg ai/L.
Number of Test Organisms 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
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may	The temperature in one test vessel was recorded continuously during the test.
not vary > 1°C 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	DO, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live fish.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	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
Recovery of Chemical	108-113% of nominal
Control Mortality 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

Concentr	ation (ppm)		C	umulative N	Number De	ead
	1	Number of	P. 10	Hour o	f Study	
Nominal	Mean Measured	Fish	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	О	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

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 , 2.2.	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	4
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

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

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

Acute toxicity of Pyridine Sulfonic Acid to the Title:

Daphnid, Daphnia magna.

Study Completion Date: 4 May 1994

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

Olin Corporation, New Haven, CT Sponsor:

Laboratory Report ID: 42-OL

> 438646-22 MRID No.:

DP Barcode:

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

Signature:

Date: 1/17/97
EFED
Date: 5/19/97

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

Signature:

46.67

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

 LC_{50} : > 122.0 ppm ai

NOEL: 122.0 ppm ai

8. ADEQUACY OF THE STUDY

A. Classification: Core.

DP Barcode: Dxxxxxx

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 $CaCO_3$) than what is recommended (42-48 mg/L as $CaCO_3$).

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

DP Barcode: Dxxxxxx

B. Source/Acclimation

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

C. <u>Test System</u>:

Guideline Criteria	Reported Information
Source of dilution water 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
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	19.5-20.0°C
pH Prefer 7.2 to 7.6.	7.1-8.7

DP Barcode: Dxxxxxx

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1 st 48 h and ≥ 40% during 2 nd 48 h, flow-through: ≥ 60%.	8.4-9.0 mg/L
Total Hardness Prefer 40 to 48 mg/L as CaCO ₃ .	164 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 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.	5.9 vol/24 hours Diluter was calibrated before and after the test.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.	0.0001 g/L (0.0002 g/L/24 hours)
Photoperiod 16 hours light, 8 hours dark.	16 hours light, 8 hours dark.
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	No solvent used.

DP Barcode: Dxxxxxx

D. <u>Test Design</u>:

Guideline Criteria	Reported Information
Range Finding Test 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.
Nominal Concentrations of Definitive Test 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.
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature	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.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	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.

DP Barcode: Dxxxxxx

12. REPORTED RESULTS:

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

Mortality

Concentration (ppm)		Number of Organ-	Cumulative Number Dead Hour of Study	
Nominal	Mean Measured	isms	24	48
Control	<u>-</u>	20	0	0
19.0	16.6	20	0	0
31.0	30.4	20	o	O
50.0	49.1	20	o	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

NOEC: 122.0 ppm ai

13. <u>VERIFICATION OF STATISTICAL RESULTS</u>

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)

PC Code No.: 1. CHEMICAL: Pyridine Sulfonic Acid

2. TEST MATERIAL: Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook#

D104334-15, 1-10-92, a white crystal. Purity: 98%

3. CITATION

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

Acute toxicity of Pyridine Sulfonic Acid to the Title:

sheepshead minnow, Cyrinodon variegatus.

28 April 1994 Study Completion Date:

> T. R. Wilbury Laboratories, Inc., Marblehead, MA <u>Laboratory</u>:

Olin Corporation, New Haven, CT Sponsor:

Laboratory Report ID: 43-OL

> MRID No .: 438646-23

DP Barcode:

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

Date: 1/13/97

1, EFED

Date: 5/15/97 5. APPROVED BY: Les Touart, Head of Section (1), EEB, EFED

6. STUDY PARAMETERS

Signature:

Signature:

Scientific Name of Test Organism: Cyprinodon variegatus

Age or Size of Test Organism: Juvenile/35.6 mm, 0.73 g

Definitive Test Duration: 96 hours

> Flow-through Study Method:

Type of Concentrations: Mean measured

7. CONCLUSIONS:

Results Synopsis

 LC_{50} : >127.0 ppm ai NOEL: 127.0 ppm ai

8. ADEQUACY OF THE STUDY

A. Classification: Core.

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
Species Preferred species are the sheepshead minnow (Cyprinodon variegatus) or the Silverside (Menidia sp.).	Cyprinodon variegatus
<u>Mean Weight</u> 0.5 - 5 g	0.73 g
Mean Standard Length Longest not > 2x shortest	Mean: 35.6 mm Range: Not reported
Supplier	Aquatic Biosystems, Inc., Fort Collins,
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	48 hours prior to 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.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	11 to 17 ‰
Water Temperature 22 <u>+</u> 1 °C	21.9-22.6°C

Guideline Criteria	Reported Information
<u>pH</u>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: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	6.9 mg/L at 48 hours.
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	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 ≤ 17°C, ≤ 0.5 g/L at > 17°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
Range Finding Test 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.
Nominal Concentrations of Definitive Test 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.
Number of Test Organisms 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
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C	The temperature in one test vessel was recorded continuously during the test.
DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	DO, pH, salinity, and temperature were measured and recorded daily in each test chamber that contained live animals.
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	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
Recovery of Chemical	95-104% of Nominal
Control Mortality 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

iviortanty					2	
Concentra	Concentration (ppm)			mulative N	lumber De	ad .
The second second second		Number of Fish		Hour of	Study	
Nominal	Mean Measured		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	o

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 LC₅₀: >127.0 ppm ai

NOEC: 127.0 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter 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

P.C Code No.: 1. CHEMICAL: Pyridine Sulfonic Acid

2. TEST MATERIAL: Pyridine Sulfonic Acid, Cas# 15103-48-7, G95087-24, a

Purity: 99.1% white crystal.

3. CITATION:

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

Acute flow-through mollusc shell deposition test Title:

with Pyridine Sulfonic Acid

Study Completion Date: 2 May 1994

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

Olin Corporation, New Haven, CT Sponsor:

Laboratory Report ID: 45-OL

> MRID No .: 438646-24

DP Barcode:

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

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

Signature:

6. STUDY PARAMETERS

Signature:

Crassostrea virginica Scientific Name of Test Organism:

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 95% C.I.: 73.3-102.5 ppm ai

NOEL: 51.1 ppm ai Probit Slope: 5.4 5/10/-

Date:

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
Species Preferred species are the Pacific oyster (Crassostrea gigas) and the Eastern oyster (Crassostrea virginica)	Crassostrea virginica
Mean valve height 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
Acclimation Period 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
Amount of peripheral shell growth removed prior to testing	3-5 mm
Feeding during the acclimation Must be fed to avoid stress.	Oysters were continuously supplied with live marine phytoplankton
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 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
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	34 ‰
Water Temperature 15°-30° C, consistent in all test vessels	21.6-22.2°C

Guideline Criteria	Reported Information
На	6.8-8.0
Dissolved Oxygen ≥ 60% throughout	6.4 mg/L at 24 hours
Total Organic Carbon	Not reported
Test Aquaria Should be constructed of glass or stainless steel.	Glass
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	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
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	Solvent: None

D. Test Design

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

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test 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
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH 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	Yes
included in the report?	

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

Shell Growth

Concentr	Concentration (ppm) Mean Nominal Measured		Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Control	1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 -	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 EC_{50} : 85.6 ppm ai

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

Probit Slope: Not reported

NOEC: 51.1 ppm ai

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

		en e	· · · · · · · · · · · · · · · · · · ·	
SOURCE	DF	SS	MS	F
Between	5	101.957	20.391	23.989
Within (Error)	114	96.869	0.850	
r \tal	119	198.826		

Critical F value = 2.37 (0.05,5,60) Since F > Critical F REJECT Ho:All groups equal

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

	BONFERRONI T-TEST -	TABLE 1 OF 2	Ho:Contro	l <treatm< th=""><th>ent</th></treatm<>	ent
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	*
					120

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	29.040	50	29.040	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

nartley 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
	0.040	20.010	45.040	0.0.010	
EXPECTED OBSERVED	8.040 6	29.040 29	45.840 50	29.040 29	8.040 6

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

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:Contr	ol <treatment< th=""></treatment<>
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	20		٠	
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 Conti	rols Ho:GRP1 MEAN = GRP2 MEAN
GRP1 (SOLVENT CRTL) MEAN = 2.8650 GRP2 (BLANK CRTL) MEAN = 3.1600 DIFFERENCE IN MEANS = -0.2950	CALCULATED t VALUE = -0.9269 DEGREES OF FREEDOM = 38
TABLE t VALUE (0.05 (2),40) = 2.021 TABLE t VALUE (0.01 (2),40) = 2.704	NO significant difference at alpha=0.05 NO significant difference at alpha=0.01

Regina Hirsch Pyridine Sulfonic Acid Shell depositon in the Mollusc

131 80.2	NUMBER EXPOSED .20 20	**************************************	PERCENT DEAD 85 40 15	BINOMIAL PROB. (PERCENT) .1288414 25.17223 .1288414 9.536742E-05
51.1 31.5	20 20	0	0	9.536742E-05 9.536742E-05
18.1	20	υ		• • • • • •

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
GOODNESS OF FIT PROBABILITY
4 .14262 1
.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

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

1. <u>CHEMICAL</u>: Pyridine Sulfonic Acid <u>PC Code No.</u>:

2. TEST MATERIAL: Pyridine Sulfonic Acid, Cas# 15103-48-7, Notebook#

D104334-15, 1-10-92, a white crystal. Pur

Purity: 98%

3. CITATION

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

<u>Title</u>: 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: De Julian Mism

Date: 1/17/97

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

Signature:

LIT

Date: 5.19-97

6. STUDY PARAMETERS

Scientific Name of Test Organism:

Selenastrum capriconuturm

Definitive Test Duration:

120 hours

Type of Concentrations:

Mean measured

7. CONCLUSIONS:

Results Synopsis EC₅₀: 21.7 ppm ai

23-41,2 JSE 6113/97

23.9 ppm 91 95% C.I.: 17.9-26.1 ppm ai

NOEL: 5.46 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
Species Skeletonema costatum Anabaena flos-aquae Selenastrum capricornutum Navicula pelliculosa	Selenastrum capricornutum
Initial Number of Cells 3,000 - 10,000 cells/ml	10,000 cells/ml
Nutrients Standard formula, e.g. 20XAAP	Not reported

B. Test System

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

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

C. Test Design

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

12. REPORTED RESULTS

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

Dose Response

Mean Measured Dose (mg ai/L)	Cell Density (x 10° 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

13. Verification of Statistical Results

Statistical Method: Moving Average

EC₅₀: 21.7 ppm

95% C.I.:17.9-26.1ppm

Probit Slope: 3.85

NOEC: 5.46 ppm

14. REVIEWER'S COMMENTS:

Verified using Northatch and obtained SImilar results to that of 196 113/97

J.DAT : pyridine sulfonic acid				
Williams Test				
[One-Sided Test for Decrease, alpha				
Dose Isotone T-bar P-va Means	lue Significance			
0 2.41E+03 . 5.46 2.39E+03 0.1001 N 11.6 1.96E+03 3.181 0.0 23 1.96E+03 3.181 0.0 46.2 5 17.17 <0.	056 * 058 * 005 *			
"*"=Significant; "N.S."=Not Signif				
Estimates of EC%				
Lower U Lc5 21. 18. EC10 23. 20. EC25 25. 22.	Std.Err. Lower Bound pper /Estimate 25. 0.031 0.86 26. 0.028 0.87 29. 0.024 0.88 32. 0.022 0.90			
Slope = 13.5 Std.Err. !!!Poor fit: p = 0.011 based o				
J.DAT : pyridine sulfonic acid				
Observed vs. Predicted Treatment Gr	oup Means			
	red. Obs. Pred. %Change lean -Pred. %Control			
23.0 3.00 2.000+03 2.00	e+03 151. 100. 2.03e-14 e+03 -316. 100. 8.09e-06			

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

*****	****	****	******	******
CONC.	NUMBER	NUMBER	PERCENT	BINOMIAL
	EXPOSED	DEAD	DEAD	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 GOODNESS OF FIT PROBABILITY

Ó

1.378478 7.237763

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.

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

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

11.0433 95 PERCENT CONFIDENCE LIMITS = 0 AND 23.4581 *************************

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	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.
4	Information about a pending registration action.
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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:

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

Acute toxicity of Pyridine Sulfonic Acid to the Title:

Mysid, Mysidopsis bahia.

28 March 1994 Study Completion Date:

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

Olin Corporation, New Haven, CT Sponsor:

44-OL Laboratory Report ID:

> MRID No.: 438646-26

DP Barcode:

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

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

Date: 1/17/96,
EFED
Date: 5/15/17 WT Signature:

6. STUDY PARAMETERS

Mysidopsis bahia Scientific Name of Test Organism:

Age or Size of Test Organism: < 24 hours old

96 hours **Definitive Test Duration:**

Study Method: Flow-through

Type of Concentrations: Mean measured

7. CONCLUSIONS:

Results Synopsis

95% C.I.: 62.8-81.1 ppm ai LC₅₀: 71.0 ppm ai

NOEL: 51.9 ppm ai

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
Species Preferred species are Mysidopsis bahia, Penaeus setiferus, P. duorarun, P. aztecus and Palaemonetes sp.	Mysidopsis bahia
Age Juvenile, mysids should be ≤ 24 hours old	≤ 24 hours old
Supplier	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
Acclimation Period 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
Feeding 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 Artemia salina daily during acclimation and testing.
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 was adjusted to appropriate salinity using dechlorinated tapwater.
Does water support test animals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
Salinity 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰	11 to 17 ‰
Water Temperature Approx. 22 <u>+</u> 1 °C	21.6 to 22.9 °C
pH 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
Dissolved Oxygen Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, Flow-through: ≥ 60%	7.2 mg/L at test initiation
Total Organic Carbon	Not reported
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 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. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	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.

Guideline Criteria	Reported Information
Flow Rate 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
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	.0006 g/L (0.0001 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
Range Finding Test If $LC_{50} > 100$ 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.
Nominal Concentrations of Definitive Test 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.
Number of Test Organisms 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
 Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control 	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.
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	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)
Recovery of Chemical	96-106%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	No

Guideline Criteria	Re	ported Info	rmation	
Signs of toxicity (if any) were described?	Yes			3

Mortality

Concentration (ppm)		Number of	Cumulative Number Dead and Number Affected in ()			
	Mean	Shrimp		Hour of	Study	
Nominal	Measured		24. ***	48	72	96
Control	()	20	0	0	0	O
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
GOODNESS OF FIT PROBABILITY
7 7.117079 22.0

0

7 7.117079 22.02362

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

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: 1/5/97
, EFED
Date: 5/9/97

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

Signature:

6. STUDY PARAMETERS

Scientific Name of Test Organism:

Oncorhynchus mykiss

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

 LC_{50} : 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.

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
Species Preferred species is the rainbow trout (Onchorhynchus mykiss)	Onchorhynchus mykiss
<u>Mean Weight</u> 0.5-5 g	0.41
Mean Standard Length Longest not > 2x shortest	Mean: 36.6 Range: Not reported
Supplier	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
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	Dilution water was dechlorinated tap water collected from Marblehead, MA.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 12°C	11.2-13.0°C
<u>pH</u> Prefer 7.2 to 7.6	3.7-7.2

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	8.8 mg/L at 72 hours
Total Hardness Prefer 40 to 48 mg/L as CaCO ₃	48 mg/L as CaCO ₃
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	Glass 20 L 15 L
Type of Dilution System 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.
Flow Rate 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.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.27 g/L (0.05 g/L/day)
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: None

D. Test Design

Guideline Criteria	Reported Information			
Range Finding Test If LC ₅₀ > 100 mg/L with 30 fish, then no definitive test is required.	A screening test was not performed and historic data was used to determine the range of concentrations for the definitive test.			
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Control, 19.0, 31.0, 50.0, 75.0, and 125.0 mg ai/L.			
Number of Test Organisms 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			
 Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control 	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.			
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	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			
Recovery of Chemical	88-96% of Nominal			
Control Mortality 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	Cumulative Number Dead and Number Affected in ()				
	Moon	of Fish	Hour of Study				
Nominal	Mean Nominal Measured		24	48	72	96	
Control	4.5	20	0	0	O	0	
18.0	15.8	20	0	0	1	1	
31.0	28.5	20	0	О	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 2		20		

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

<u>Protocol Deviations</u>: 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. <u>REVIEWER'S COMMENTS</u>: Since the probability is less than 0.05, results calculated using the probit method probably should not be used.

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CONC.	NUMBER	NUMBER		PERCENT	BINOMIAL
	-EXPOSED	DEAD	į.	DEAD	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 TLE MOVING AVERAGE METHOD

SPAN G LC 0 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

0

6 28.55956 86.07247

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