MRID No. 436781-18

DATA EVALUATION RECORD
§ 72-3(C) - ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP

1. **CHEMICAL:** Azoxystrobin PC Code No.: 128810

2. **TEST MATERIAL:** ICIA5504 Purity: 96.2%

3. **CITATION**

Authors: S.J. Kent, S.A. Sankey, and A.J. Grinell
Title: ICIA5504: Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*)
Study Completion Date: March 19, 1993
Laboratory: Brixham Environmental Laboratory, Zeneca Ltd., Brixham Devon, UK
Sponsor: Zeneca Ag Products, Zeneca Inc., Wilmington, DE
Laboratory Report ID: BL4785/B
MRID No.: 436781-18

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: W. Erickson
Date: 4/04/96

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: H-T Craven 6/21/96
Date:

6. **STUDY PARAMETERS**

Age or Size of Test Organism: <24 hours old
Definitive Test Duration: 96 hours
Study Method: Static, no aeration
Type of Concentrations: Nominal (confirmed by analysis)

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for an estuarine shrimp toxicity test. Based on nominal concentrations (confirmed by analysis) of ICIA5504, the 96-hour LC_{50} for mysid shrimp was 56 ppb ai, which classifies azoxystrobin as very highly toxic to *Mysidopsis bahia*.

(1)

DP Barcode: ~~D217072/D217078~~ MRID No. 436781-18

DATA EVALUATION RECORD
§ 72-3(C) - ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP

1. **CHEMICAL:** ~~Sulfentrazone~~ Azoxystrobin **PC Code No.:** ~~129081~~ 128810

2. **TEST MATERIAL:** ICIA5504 **Purity:** 96.2%

3. **CITATION**

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DP Barcode: ~~D217072/D217078~~

4. **REVIEWED BY:** Barbara Herbert, B.S., Associate Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: Barbara Herbert **Date:** 10-31-95

APPROVED BY: Mark Mossler, M.S., Toxicologist, KBN Engineering and Applied Sciences, Inc.

Signature: Mark Mossler **Date:** 10/31/95

5. **APPROVED BY:** ~~William Erickson~~, Section 4, EEB, EFED

Signature: **Date:**

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Age or Size of Test Organism: <24 hours old
Definitive Test Duration: 96 hours
Study Method: Static, no aeration
Type of Concentrations: Nominal (confirmed by analysis)

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an estuarine shrimp test. Based on nominal concentrations (confirmed by analysis) of ICIA5504, the 96-hour LC_{50} for mysid shrimp was 55 ppb ai, which classifies sulfentrazone as very highly toxic to *Mysidopsis bahia*. The NOEC was 32 ppb ai.

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Results Synopsis

LC_{50}: 56 ppb ai 95% C.I.: 35-110 ppb ai
NOEC: 35 ppb ai Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:** Core.

9. **GUIDELINE DEVIATIONS**

1. The pH (8.0-8.3) of the test solutions during this study were higher than the recommended values (7.7-8.0) for a euryhaline species.

2. The salinity of the dilution water (20‰) was higher than the recommended 10-17‰.

10. **SUBMISSION PURPOSE:** New Chemical.

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarum*, *P. aztecus* and *Palaemonetes sp.*	*Mysidopsis bahia*
Age Juvenile, mysids should be ≤ 24 hours old	<24 hours old.
Supplier	In house cultures originally obtained from Sea Plantations Inc., Salem, MA, USA.
All shrimp are from same source?	Yes
All shrimp are from the same year class?	N/A

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B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	Adult mysids were held under test conditions.
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 hours before the beginning of the test if organisms are over 0.5 g each.	Mysids (<24 hours old at test initiation) were fed 10-20 *Artemia salina* nauplii per mysid daily during the study.
Pretest Mortality <3% mortality 48 hours prior to testing	Mortality prior to testing not reported.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Aged, aerated, filtered natural seawater (collected from Tor Bay, Devon, UK) diluted with distilled water.
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 ‰	20.2-20.5‰
Water Temperature Approx. 27 ± 1 °C	25-26°C

Guideline Criteria	Reported Information
pH 8.0-8.3 for marine (steno-haline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	8.0-8.3
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	≥71% of saturation during the test.
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.	1. Glass 2. 1000 mL beakers with loose fitting glass lids. 3. Fill volume of test chambers was 800 ml.
Type of Dilution System Must provide reproducible supply of 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	Static
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	An instantaneous rate of 10 mysids per test container (800 ml), otherwise not reported.
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h 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	Solvent: DMF Maximum conc.: 0.10 mL/L.

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.	None reported.
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.	A dilution water control and a solvent control (0.1 mL/L DMF) plus seven exposure concentrations were used: 180, 100, 56, 32, 18, 10, and 5.6 µg/L. Each being 56-57% of the next highest concentration.
Number of Test Organisms Minimum 20/level, may be divided among containers	10 mysids per test chamber, one chamber used per level.
Test organisms randomly or impartially assigned to test vessels?	Mysids randomly allocated to vessels and positions of controls and treatments randomly assigned.
Biological observations made every 24 hours?	Yes; biological observations were made at 24, 48, 72, and 96 hours.
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	1. Temperature measured continuously throughout the test period in a dilution water surrogate vessel. Temperature was also measured daily in each test vessel. 2. DO was measured every 48 h in all test vessels. pH was measured at 0 h and 96 h in all test vessels.

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	ICIA5504 concentration was measured in all solutions at 0, 48, and 96 h.

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	106-116%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% mortality in both the dilution water and solvent control.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes. Signs of toxicity reported were quiescence and reduced feeding.

Mortality

Concentration (ppb)		Number of Shrimp	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	<0.59	10	0	0	0	0
Solvent Control	<0.59	10	0	0	0	0
5.6	6.5	10	0	0	0	0
10	11	10	0	0	0	0

Concentration (ppb)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
18	20	10	0	0	0	0
32	35	10	0	0	0	0
56	60	10	0	3	6	6
100	110	10	1	9	10	10
180	190	10	4	10	10	10

Other Significant Results: Mysids present in the 56 ppb ai solution (and mysids present at the two higher concentrations before total death) were noted as quiescent and failed to clear the introduced *Artemia* (i.e., reduced feeding).

B. Statistical Results

Method: Moving average angle (based on nominal conc.)

96-hr LC_{50}: 55 ppb ai 95% C.I.: 41-79 ppb ai

Probit Slope: N/A NOEC: 32 ppb ai

14. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result*
Binomial Test LC_{50} (C.I.)	56 (35-110) ppb ai
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	35 ppb ai

*based on mean measured concentrations

15. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an estuarine test using the mysid shrimp. An LC_{50} of 56 ppb ai places azoxystrobin in the very highly toxic category for mysid shrimp.

Barbara Herbert [redacted] Mysid shrimp 10-30-95

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
190	10	10	100	9.765625E-02
110	10	10	100	9.765625E-02
60	10	6	60.00001	37.69531
35	10	0	0	9.765625E-02
20	10	0	0	9.765625E-02
11	10	0	0	9.765625E-02
6.5	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 35 AND 110 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 56.03616

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

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