

US EPA ARCHIVE DOCUMENT

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

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

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

3. **CITATION:**

Authors: S.A. Sankey, S.J. Kent, J.E. Caunter, and A.J. Grinell

Title: ICIA5504: Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*).

Study Completion Date: July 31, 1992

Laboratory: Brixham Environmental Laboratory, Zeneca Ltd., Brixham Devon, UK

Sponsor: Zeneca Inc., Wilmington, DE

Laboratory Report ID: BL4850/B

MRID No.: 436781-17

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: *W. Erickson*

Date: *4/01/96*

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: *H.T. Craven*

Date: *6/21/96*

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: 0.57-1.37 g
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for an acute toxicity test using an estuarine fish. The 96-hour LC₅₀ of 0.67 ppm ai classifies azoxystrobin as highly toxic to the sheepshead minnow.

Results Synopsis

LC₅₀: 0.67 ppm ai
NOEC: 0.18 ppm ai

95% C.I.: 0.56-0.80 ppm ai
Probit Slope: N/A

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

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DP Barcode: D217072/D217078

MRID No.: 436781-17

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

1. **CHEMICAL:** ^{Azoxytolin} Sulfentrazone **PC Code No.:** 129081 1288:0
2. **TEST MATERIAL:** ICIA5504 **Purity:** 96.2%
3. **CITATION:**
Authors: S.A. Sankey, S.J. Kent, J.E. Caunter, and A.J. Grinell
Title: ICIA5504: Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*).
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4. **REVIEWED BY:** Barbara Herbert, B.S., Associate Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: *Barbara Herbert* **Date:** 10-26-95

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

Signature: *P. Kosalwat* **Date:** 10/26/95

5. **APPROVED BY:** (Name), Head of Section (#), EEB, EFED

Signature: **Date:**

6. **STUDY PARAMETERS:**

Age or Size of Test Organism: 0.57-1.37 g
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine fish. The 96-hour LC₅₀ of 0.67 ppm ai classifies Sulfentrazone as highly toxic to the sheepshead minnow. The NOEC was 0.18 ppm ai.

Results Synopsis

LC₅₀: 0.67 ppm ai **95% C.I.:** 0.56-0.80 ppm ai

9. **GUIDELINE DEVIATIONS:** None.
10. **SUBMISSION PURPOSE:** New Chemical.
11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are the sheepshead minnow (<i>Cyprinodon variegatus</i>) or the silverside (<i>Menidia spp.</i>).	<i>Cyprinodon variegatus</i>
<u>Mean Weight</u> 0.5-5 g	0.93 g
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 33 mm Range: 28-36 mm
<u>Supplier</u>	Sea Plantations Inc., Salem, MA, USA.
All fish from same source?	Yes
All fish from the same year class?	Not reported.

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	15 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Reported in "good condition."
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study	Not fed during the 48 hours prior to or during testing.

Guideline Criteria	Reported Information
<u>Pretest Mortality</u> < 3% mortality 48 hours prior to testing	<1% mortality in the 48 hours prior to testing.

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Reconstituted seawater or seawater from a natural source.	Filtered seawater.
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 ‰ Salinity Weekly range should not deviate by more than 6%.	Salinity measured during the test was 35.0-35.2‰.
<u>Water Temperature</u> 22°C	21.8-21.9°C
<u>pH</u> Monthly range must not deviate by more than 0.8 unit. Euryhaline: 7.7-8.0 Stenohaline: 8.0-8.3	7.82-8.07
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	79-90% of saturation during the test.
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume:</u> 15-30 L of solution	Glass 61 x 30.5 x 31 cm 45 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Continuous flow diluter with mixing chambers.

Guideline Criteria	Reported Information
<p><u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period</p>	8 volume replacements/day
<p><u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^\circ\text{C}$, ≤ 0.5 g/L at $> 17^\circ\text{C}$; flow-through: ≤ 1 g/L/day</p>	Instantaneous loading rate of 0.41 g/L.
<p><u>Photoperiod</u> 16 hours light, 8 hours dark</p>	16 hours light, 8 hours dark
<p><u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests</p>	DMF, 0.1 mL/L.

D. Test Design

Guideline Criteria	Reported Information
<p><u>Range Finding Test</u> If $\text{LC}_{50} > 100$ mg/L with 30 fish, then no definitive test is required.</p>	None reported.
<p><u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	Each test concentration was approximately 56-57% of the next highest concentration (0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg ai/L).
<p><u>Number of Test Organisms</u> Minimum 10/level for static test, 20/level for flow-through, may be divided among containers</p>	20 per level
<p>Test organisms randomly or impartially assigned to test vessels?</p>	Yes

Guideline Criteria	Reported Information
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Temperature was monitored continuously in the dilution water and measured daily in all chambers. 2. DO and pH were measured daily in all chambers.
<u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Yes, solutions collected and analyzed at 0, 24, 48, 72, and 96 hours.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	Mean recovery of 93-110%.
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0% mortality in both the dilution water control and in the solvent control.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes, signs observed above 0.33 mg/L level.

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<0.0051	20	0	0	0	0
Sol. Cont.	<0.0051	20	0	0	0	0
0.18	0.18	20	0	0	0	0
0.32	0.33	20	0	1	1	1
0.56	0.60	20	0	0	0	2
1.0	1.1	20	0	6	14	20
1.8	1.8	20	4	20	20	20
3.2	3.0	20	20	20	20	20

Other Significant Results: Fish exposed at all but the two lowest treatment levels demonstrated signs of toxicity which included quiescence, surfacing, sounding, loss of balance, and irregular respiration.

B. Statistical Results

Method: Moving Average Angle (based on mean measured concentrations)

96-hr LC₅₀: 0.66 ppm ai

95% C.I.: 0.56-0.78 ppm ai

Probit Slope: N/A

NOEC: 0.33 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	0.76 (0.6 - 1.1) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	0.67 (0.56 - 0.80) ppm ai
Probit LC ₅₀ (95% C.I.)	0.72 (not determined) ppm ai
Probit Slope	8.10
NOEC	0.18 ppm ai

results based on mean measured concentrations.

14. **REVIEWER'S COMMENTS:** Fish used in the test were treated with 200 ppm formalin ten days before testing. This treatment did not impact the results of the study since mortality was <1% during the 48 hrs prior to testing, the fish were reported as in good health at the initiation of the test, and no fish in the dilution water control group died or showed signs of toxic symptoms.

This study is scientifically sound, fulfills the guideline requirements, and is classified as **CORE**. Based on mean measured concentrations, the 96-hour LC_{50} was determined to be 0.67 ppm ai, which classifies azoxystrobin as highly toxic to the sheepshead minnow. The NOEC was determined to be 0.18 ppm ai based on lack of toxic symptoms and mortality at this test level.

Barbara Herbert Sulfentrazone Cyprinodon Variegatus 10-24-95

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3	20	20	100	9.536742E-05
1.8	20	20	100	9.536742E-05
1.1	20	20	100	9.536742E-05
.6	20	2	10	2.012253E-02
.33	20	1	5	2.002716E-03
.18	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .6 AND 1.1 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 .7607427

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	5.135013E-02	.667974	.5618553	.8043816

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
9	1.542603	4.478536	1.282573E-03

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

SLOPE = 8.099941
95 PERCENT CONFIDENCE LIMITS = -1.960314 AND 18.1602

LC50 = .7159981
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .4990231
95 PERCENT CONFIDENCE LIMITS = 0 AND .7501177
