DATA EVALUATION RECORD
§ 72-3(A) - ACUTE LC_{50} 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 LC_{50} of 0.67 ppm ai classifies azoxystrobin as highly toxic to the sheepshead minnow.

Results Synopsis

LC_{50}: 0.67 ppm ai 95% C.I.: 0.56-0.80 ppm ai
NOEC: 0.18 ppm ai Probit Slope: N/A

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

(1)

DP Barcode: ~~D217072/D217078~~ MRID No.: 436781-17

DATA EVALUATION RECORD
§ 72-3(A) - ACUTE LC_{50} TEST WITH A ESTUARINE/MARINE FISH

1. **CHEMICAL:** ~~Sulfentrazone~~ Azomethrin PC Code No.: ~~129081~~ 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*).
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   Laboratory Report ID: BL4850/B
   MRID No.: 436781-17
   DP Barcode: ~~D217072/D217078~~

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 LC_{50} of 0.67 ppm ai classifies ~~Sulfentrazone~~ as highly toxic to the sheepshead minnow. The NOEC was 0.18 ppm ai.

   Results Synopsis
   LC_{50}: 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
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the silverside (*Menidia* spp.).	*Cyprinodon variegatus*
Mean Weight 0.5-5 g	0.93 g
Mean Standard Length Longest not > 2x shortest	Mean: 33 mm Range: 28-36 mm
Supplier	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
Acclimation Period 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
Feeding No feeding during the study	Not fed during the 48 hours prior to or during testing.

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

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Reconstituted seawater or seawater from a natural source.	Filtered seawater.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ Salinity Weekly range should not deviate by more than 6%.	Salinity measured during the test was 35.0-35.2‰.
Water Temperature 22°C	21.8-21.9°C
pH 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
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	79-90% of saturation during the test.
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 61 x 30.5 x 31 cm 45 L
Type of Dilution System Must provide reproducible supply of toxicant	Continuous flow diluter with mixing chambers.

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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	8 volume replacements/day
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	Instantaneous loading rate of 0.41 g/L.
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	DMF, 0.1 mL/L.

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.	None reported.
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	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).
Number of Test Organisms Minimum 10/level for static test, 20/level for flow-through, may be divided among containers	20 per level
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	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.
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	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
Recovery of Chemical	Mean recovery of 93-110%.
Control Mortality 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			
			Hour of Study			
Nominal	Mean Measured		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 LC_{50}: 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

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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.

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Barbara Herbert Sulfentrazone Cyprinodon Variegatus 10-24-95

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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

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