MRID No.: 436781-14

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

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

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

3. **CITATION**

Authors: S.A. Sankey, S.J. Kent, B.G. Maddock, and S.K. Cornish
Title: ICIA5504: Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*)
Study Completion Date: August 21, 1992
Laboratory: Brixham Environmental Laboratory, Zeneca Limited, Brixham, U.K.
Sponsor: Zeneca Ag Products, Zeneca Inc., Wilmington, DE
Laboratory Report ID: BL4602/B
MRID No.: 436781-14

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
Date: 6/20/96

6. **STUDY PARAMETERS**

Age or Size of Test Organism: 0.82 g, 35 mm
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 freshwater fish toxicity test. An LC_{50} of 1.1 ppm mean measured concentration classifies azoxystrobin as moderately toxic to bluegill sunfish.

Results Synopsis
LC_{50}: 1.1 ppm ai — 95% C.I.: 0.9-1.7 ppm ai
NOEC: 0.5 ppm ai — Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:** Core. (1)

DP Barcode: ~~D217072/D217078~~ MRID No.: 436781-14

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

1. **CHEMICAL:** ~~Sulfentrazone~~ Azoxystrobin **PC Code No.:** ~~129081~~ 129810

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

3. **CITATION**

Authors: S.A. Sankey, S.J. Kent, B.G. Maddock, and S.K. Cornish
Title: ICIA5504: Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*)
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Laboratory Report ID: BL4602/B
MRID No.: 436781-14
DP Barcode: ~~D217072/D217078~~

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

Signature: Barbara H. Herbert **Date:** 10-25-95

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

Signature: P. Kosalwat **Date:** 10/25/95

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

Signature: **Date:**

6. **STUDY PARAMETERS**

Age or Size of Test Organism: mean weight of 0.82 g, mean length of 35 mm
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 freshwater fish toxicity test. An LC_{50} of 1.1 ppm mean measured concentration classifies ~~Sulfentrazone~~ as moderately toxic to bluegill sunfish. The NOEC was 0.5 ppm since no mortality

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9. **GUIDELINE DEVIATIONS:**

1. Dechlorinated water was used as the dilution water.

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

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is the bluegill sunfish (*Lepomis macrochirus*)	*Lepomis macrochirus*
Mean Weight 0.5-5 g	0.82 g
Mean Standard Length Longest not > 2x shortest	Mean: 35 mm Range: 30-41 mm
Supplier	Sea Plantations Inc., Salem Massachusetts
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 14 days	48 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?	A five-day medicated diet of tetracycline in Promin ended 8 days prior to test initiation. No sign of disease.

Guideline Criteria	Reported Information
Feeding No feeding during the study	The fish were not fed 48 hours prior to test initiation or during the test period.
Pretest Mortality < 3% mortality 48 hours prior to testing	<1% mortality 48 hours 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 (passed through activated carbon, filtered, dechlorinated with sodium thiosulphate, and then UV sterilized).
Does water support test animals without observable signs of stress?	Yes, no mortality or signs of toxicity in the control fish.
Water Temperature 17°C or 22°C	21.8-21.9°C
pH Prefer 7.2 to 7.6	7.11-7.65
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	78-101% saturation during the 96-hour exposure.
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	Dilution water had a total hardness of 23.3-48.4 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	1. Glass 2. 54 liters (61 x 30.5 x 31 cm) 3. 45 liters

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant	Continuous flow diluter.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	250 ml/minute (approximately 8 volume replacements per day).
Biomass Loading Rate Static: ≤0.8g/L static test at ≤17°C; ≤0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	Instantaneous loading of 0.36 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	Solvent: DMF Maximum conc.: 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 concentration was 56-57% of the next highest concentration (0.10, 0.18, 0.32, 0.56, 1.0, and 1.8 mg/L). A solvent control and a dilution water control were also included.
Number of Test Organisms Minimum 10/level, may be divided among containers	20 fish per test container; one container per treatment level.

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Guideline Criteria	Reported Information
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	1. Temperature was monitored continuously in the dilution water control and daily in all containers. 2. DO and pH were measured daily in all test containers.
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 of test solutions was conducted at 0, 24, 48, 72, and 96 hours. The mean measured concentrations based on percentage active ingredient were 0.09, 0.16, 0.30, 0.50, 0.90, and 1.7 mg ai/L.

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 89-94%
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes.

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal (mg ai/L)	Mean Measured (mg ai/L)		24	48	72	96
Control	<0.009	20	0	0	0	0
Solvent Control	<0.009	20	0	0	0	0
0.10	0.09	20	1	1	1	1
0.18	0.16	20	0	0	0	0
0.32	0.30	20	0	0	0	0
0.56	0.50	20	0	0	0	0
1.0	0.90	20	3	3	3	4
1.8	1.7	20	20	20	20	20

Other Significant Results: Signs of toxicity were noted at concentrations ≥0.90 mg ai/L and included sounding and dark discoloration.

B. Statistical Results

Method: Moving average angle

96-hr LC_{50}: 1.1 ppm ai 95% C.I.: 0.95-1.2 ppm ai .

Probit Slope: N/A NOEC: 0.5 ppm ai

13. **VERIFICATION OF STATISTICAL RESULTS:**

Parameter	Result
Binomial Test LC_{50} (C.I.)	1.1 (0.9-1.7) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	1.1 (could not be determined)
Probit Slope	3.56
NOEC	0.5 ppm ai

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14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirement for a freshwater fish acute toxicity testing. Although dechlorinated water was used as the dilution water, no mortality or signs of toxicity occurred in the control fish. The residual chlorine measured in the dilution water was below the detection limit (<4 µg/L). An LC_{50} value of 1.1 ppm classifies azoxystrobin as moderately toxic to bluegill sunfish.

HERBERT [redacted] LEPOMIS MACROCHIRUS 10-19-95

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.7	20	20	100	9.536742E-05
.9	20	4	20	.5908966
.5	20	0	0	9.536742E-05
.3	20	0	0	9.536742E-05
.16	20	0	0	9.536742E-05
9.000001E-02	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT .9 AND 1.7 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 1.096764

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	54.28853	190.9013	0

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

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

SLOPE = 3.555127

95 PERCENT CONFIDENCE LIMITS =-22.63932 AND 29.74957

LC50 = 1.069278

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .4697429

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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