DATA EVALUATION RECORD
§ 72-3(B) - ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK EMBRYO/LARVAL STUDY

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

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

3. **CITATION**

Authors: S.J. Kent, S.A. Sankey, J.E. Caunter, and A.J. Grinell
Title: ICIA5504: Acute Toxicity to Larvae of the Pacific Oyster (*Crassostrea gigas*)
Study Completion Date: February 11, 1993
Laboratory: Brixham Environmental Laboratory, Zeneca Ltd., Brixham Devon, UK
Sponsor: Zeneca Ag Products, Zeneca Inc., Wilmington, DE
Laboratory Report ID: BL4842/B
MRID No.: 436781-19

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: 6/2/96

6. **STUDY PARAMETERS**

Age of Test Organism: 2.5 hours post-fertilization
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** The study was categorized as core. The EC_{50} of 1.3 ppm ai classifies azoxystrobin as moderately toxic to Pacific oyster larvae.

Results Synopsis

EC_{50}: 1.3 ppm ai 95% C.I.: 1.1-1.4 ppm ai
NOEC: 0.56 ppm ai Probit Slope: N/A

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

(1)

DP Barcode: ~~D217072/D217078~~ MRID No. 436781-19

DATA EVALUATION RECORD
§ 72-3(B)- ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK EMBRYO/LARVAL STUDY

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

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

3. **CITATION**

Authors: S.J. Kent, S.A. Sankey, J.E. Caunter, and A.J. Grinell
Title: ICIA5504: Acute Toxicity to Larvae of the Pacific Oyster (*Crassostrea gigas*)
Study Completion Date: February 11, 1993
Laboratory: Brixham Environmental Laboratory, Zeneca Ltd., Brixham Devon, UK
Sponsor: Zeneca Ag Products, Zeneca Inc., Wilmington, DE
Laboratory Report ID: BL4842/B
MRID No.: 436781-19
DP Barcode: ~~D217072/D217078~~

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

Signature: Barbara Herbert **Date:** 10-30-95

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

Signature: **Date:** 10/30/95

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

Signature: **Date:**

6. **STUDY PARAMETERS**

Age of Test Organism:	2.5 hours post-fertilization
Definitive Test Duration:	48 hours
Study Method:	Static
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** The study was categorized as core. The EC_{50} was calculated to be 1.3 ppm ai, which classifies sulfentrazone as moderately toxic to Pacific oyster larvae. The NOEC was 0.56 ppm ai.

9. **GUIDELINE DEVIATIONS:**

1. The pH of the seawater used (8.2-8.3) was slightly higher than recommended (7.7-8.0).

2. The salinity of the seawater used (32‰) was greater than recommended (10-17‰).

3. Test vessels (250-mL borosilicate beakers) were smaller than recommended (1-L glass beakers).

4. There were only two replicates of the control groups. For control groups, four replicates are recommended.

11. **SUBMISSION PURPOSE:** New Chemical.

12. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster, the Eastern oyster, the mussel, or the Quahog.	*Crassostrea gigas*
Age of embryos Eggs should be tested within 3 hours of fertilization.	Embryos introduced into test solutions 2.5 hours post-fertilization.
Supplier	Guernsey Sea Farms, Channel Islands, UK.
Are all oysters from same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural seawater from an uncontaminated source or reconstituted water.	Aerated, filtered seawater from Tor Bay, Devon, UK.

Guideline Criteria	Reported Information
Does water support test animals without observable signs of stress? Not more than 10% abnormal embryos and not more than 30% mortality in 48 hours.	Yes
Salinity 10-17 ‰ salinity, weekly range < 6 ‰	32 ±2‰
Water Temperature 20°-25° C, ±2°C	19.6 - 21.0°C
pH 7.7-8.0	8.19 - 8.31
Dissolved Oxygen ≥ 60% throughout	≥96% of saturation during the test.
Total Organic Carbon	Not reported.
Test Vessels Glass 1-liter beakers preferred.	250-mL borosilicate beakers with loose-fitting covers.
Type of Dilution System Must provide reproducible supply of toxicant.	Static test.
Flow rate Consistent flow rate.	N/A
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Aeration Not recommended.	No aeration during the test.
Solvents Not to exceed 0.5 mL/L.	Solvent: DMF Maximum conc.: 0.1 mL/L

C. **Test Design**

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L, then no definitive test is required.	No range finding tests reported.

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.	Treatment levels are approximately 55-56% of the next highest concentration (0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg ai/L), dilution water control and solvent control. Treatments replicated two times.
Number of Controls Four replicates of each control or 10% of the total number of treatment replicates.	Dilution water control and solvent control replicated twice each.
Number of Test Organisms 20,000 to 30,000 embryos/L per treatment level and in each control.	36,000 embryos/L.
Biological observations made? Occurrences of misshapen or malformed shells should be reported.	Normal and abnormal larvae were counted.
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber. 2. DO and pH Measured at beginning of test and at 48 h in the high, medium, and low doses and in the control.	Temperature measured hourly in surrogate vessel and daily in one treatment and control replicate(s). DO and pH measured at 0 and 48 hours in one replicate of controls and treatments.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes, one replicate of control or treatment solution was sampled and analyzed at 0 and 48 hours.

5

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% abnormal embryos and not more than 30% mortality in 48 hours.	The pooled control had 119% embryos developing normally yielding 0% mortality.
Recovery of Chemical	97-106% of nominal.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Only reported as abnormal development.

Larval mortality

Concentration (ppm)		Number of Normal Larvae/mL	Number of Abnormal Larvae/mL*	Mean Percent Decrease in Normal Development**
Nominal	Mean Measured			
Dilution Water Control	<0.0076	41.19	2.06	0%
Solvent Control	<0.0076	39.69	1.00	0%
0.56	0.57	38.94	0.81	0%
1.0	1.0	42.31	5.31	10.3%
1.8	1.9	0.06	28.31	100%
3.2	3.1	0	a	100%

Concentration (ppm)		Number of Normal Larvae/mL	Number of Abnormal Larvae/mL*	Mean Percent Decrease in Normal Development**
Nominal	Mean Measured			
5.6	5.9	0	a	100%
10	10	0	a	100%

*Abnormal larvae include dead and abnormally developed larvae

**In comparison to the solvent control.

a - Subsamples contained a high density of crystaline particles (assumed to be undissolved ICIA5504 technical). This interferred with the counting of the abnormal larvae.

B. Statistical Results

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

48-hr EC_{50}: 1.3 ppm ai 95% C.I.: 1.2 - 1.4 ppm ai
Probit Slope: N/A NOEC: 0.56 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result*
Statistical Method for EC_{50}	Moving average angle
EC_{50} (95% C.I.)	1.3 ppm ai (1.1 - 1.4 ppm ai)
Probit Slope	N/A
Statistical Method for NOEC	None
NOEC	0.56 ppm ai

*based on mean measured concentrations

15. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirement. The EC_{50} of 1.3 ppm ai classifies azoxystrobin as moderately toxic to *Crassostrea gigas*.

7

Barbara Herbert ~~Sulfenbrazone~~ Crassostrea gigas 10-26-95

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10	34	34	100	0
5.9	34	34	100	0
3.1	34	34	100	0
1.9	28	28	100	0
1	47	5	10.6383	0
.57	40	1	2.5	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.283538

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.0314665	1.251664	1.116691	1.397679

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
9	1.854197	8.570041	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 = 9.265556

95 PERCENT CONFIDENCE LIMITS =-3.351252 AND 21.88236

LC50 = 1.253859

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .9144963

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****