

MRID No.: 436781-63

**DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 123-2 (TIER II)**

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

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

3. **CITATION**

Authors: D.V. Smyth, S.J. Kent, S.A. Sankey and
P.A. Johnson

Title: ICIA5504: Toxicity to the Marine Alga
(*Skeletonema costatum*)

Study Completion Date: December 4, 1993

Laboratory: Brixham Environmental Laboratory, Brixham
Devon, UK

Sponsor: Zeneca Ag Products, Wilmington, DE

Laboratory Report ID: BL5053/B

MRID No.: 436781-63

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature:

Date:

W. Erickson
4/04/96

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature:

Date:

H. T. Craven
6/21/96

6. **STUDY PARAMETERS:**

Definitive Test Duration: 120 hours

Type of Concentrations: Nominal

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test.

Results Synopsis

EC₅₀: 453 ppb
NOEC: 100 ppb

95% C.I.: 386 - 532 ppb
Probit Slope: 2.3

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core

①

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS: None noted.10. SUBMISSION PURPOSE:11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Skeletonema costatum</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	10,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	CCAP 1077/1C

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	Acetone (0.1 mL/L)
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	20°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.5 KLux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	16 hours light, 8 hours dark
<ph </ph <i>Skeletonema</i> : approx. 8.0 Others: approx. 7.5	Initial 8.1 Final 8.2 - 8.9

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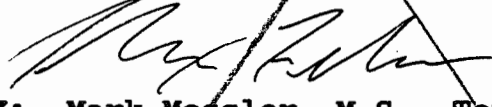
Sponsor: Zeneca Ag Products, Wilmington, DE

Laboratory Report ID: BL5053/B

~~PP-Barcode: B217072, B217073~~

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4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,
KBN Engineering and Applied Sciences, Inc.

Signature: 

Date: 12/1/95

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

Signature: 

Date: 12/5/95

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS**

Definitive Test Duration: 120 hours

Type of Concentrations: Nominal

7. **CONCLUSIONS:** This study is scientifically sound and fulfills
the guideline requirements for an algal toxicity test.

Results Synopsis

EC₅₀: 453 ppb

NOEC: 100 ppb

95% C.I.: 386 - 532 ppb

Probit Slope: 2.3

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	3X
<u>Doses</u> at least 5	7
<u>Controls</u> negative and/or solvent	Negative and solvent controls
<u>Replicates per dose</u> 3 or more	3 (6 for solvent control)
<u>Duration of test</u> 120 hours	120 hours
<u>Daily observations were made?</u>	Yes
<u>Method of Observations</u>	Cellular counts
<u>Maximum Labeled Rate</u>	Not reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes
Control cell count at 120 hr $\geq 2X$ initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response

Nominal Dose ($\mu\text{g/L}$)	Avg. Particle Density ($\times 10^6$ particles/mL)	% Reduction in Area under Growth Curve*	120-Hour pH
Control	59.0	0(+1)	8.7
Solvent	56.8	--	8.6

Nominal Dose ($\mu\text{g/L}$)	Avg. Particle Density ($\times 10^6$ particles/mL)	% Reduction in Area under Growth Curve*	120-Hour pH
3.2	57.8	0(+2)	8.6
10	62.2	0(+7)	8.6
32	57.5	12	8.7
100	55.3	55	8.9
320	33.4	77	8.5
1000	10.8	92	8.3
3200	2.89	98	8.2

* Compared to the solvent control

Other Significant Results: Area under growth curve was the most sensitive end-point measure in the study.

Statistical Results for Areas under the Growth Curve

Statistical Method: Probit analysis and Dunnett's test for mean comparisons. Results based on nominal concentrations.

EC₅₀: 200 ppb
Probit Slope: Not reported

95% C.I.: 88 - 550 ppb
NOEC: 10 ppb

Statistical Results for Growth Rate

Statistical Method: Probit analysis and Dunnett's test for mean comparisons. Results based on nominal concentrations.

EC₅₀: 1800 ppb
Probit Slope: Not reported

95% C.I.: 240 - >3200 ppb
NOEC: 100 ppb

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Probit analysis and Williams' test for mean separation.

EC₅₀: 453 ppb
Probit Slope: 2.3

95% C.I.: 386 - 532 ppb
NOEC: 100 ppb

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. Based on nominal concentrations, the 120-hour EC₅₀ and NOEC for *S. costatum* exposed to ICIA5504 were 453 and 100 ppb, respectively. This study is categorized as Core.

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

FEKEN ~~3012211301~~ SKELETONEMA 11-29-95

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3200	100	95	95	0
1000	100	81	81	0
320	100	41	41	0
100	100	3	3	0
32	100	1	1	0
10	100	0	0	0
3.2	100	0	0	0

THE BINOMIAL TEST SHOWS THAT 320 AND 1000 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 408.0235

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.294525E-02	471.4168	395.7124	567.4299

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.102904E-02	1	.2963777

SLOPE = 2.250735
95 PERCENT CONFIDENCE LIMITS = 1.924347 AND 2.577123

LC50 = 452.6716
95 PERCENT CONFIDENCE LIMITS = 385.6939 AND 531.896

LC10 = 123.459
95 PERCENT CONFIDENCE LIMITS = 93.77839 AND 153.888

Cell Density

~~XXXXXXXXXXXX~~ (SKELETONEMA)

File: 43678163

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	6	56.833	56.833	58.400
2	3.2	3	57.767	57.767	58.400
3	10	3	62.167	62.167	58.400
4	32	3	57.467	57.467	57.467
5	100	3	55.333	55.333	55.333
6	320	3	33.400	33.400	33.400
7	1000	3	10.800	10.800	10.800
8	3200	3	2.890	2.890	2.890

SULFENTRAZONE (SKELETONEMA)

File: 43678163

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT	58.400				
3.2	58.400	0.660		1.73	k= 1, v=19
10	58.400	0.660		1.81	k= 2, v=19
32	57.467	0.267		1.84	k= 3, v=19
100	55.333	0.632		1.85	k= 4, v=19
320	33.400	9.876	*	1.86	k= 5, v=19
1000	10.800	19.400	*	1.87	k= 6, v=19
3200	2.890	22.734	*	1.87	k= 7, v=19

s = 3.356

Note: df used for table values are approximate when v > 20.

NOEC = 100 ppb