

MRID No.: 436781-62

**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
J.M. Shearing

Title: ICIA5504: Toxicity to the Blue-Green Alga
(*Anabaena flos-aquae*)

Study Completion Date: December 11, 1993

Laboratory: Brixham Environmental Laboratory, Brixham
Devon, UK

Sponsor: Zeneca Ag Products, Wilmington, DE

Laboratory Report ID: BL5054/B

MRID No.: 436781-62

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: 6/21/96

6. **STUDY PARAMETERS**

Definitive Test Duration: 120 hours

Type of Concentrations: Mean measured

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

Results Synopsis

EC₅₀: 13 ppm

NOEC: 9 ppm

95% C.I.: 12 - 14 ppm

Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

①

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

1. Initial cell density (20,000 cells/mL) was greater than recommended (3,000 cells/mL).

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>Anabaena flos-aquae</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	20,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	CCAP 1403/13A

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	Dimethylformamide (DMF)
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	24°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.0 KLux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

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

Signature: *Max Feken*

Date: 12/1/95

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

Signature: *Mark Mossler*

Date: 12/5/95

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS**

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EC₅₀: 13 ppm

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Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

Guideline Criteria	Reported Information
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Initial 7.4 - 7.5 Final 7.2 - 7.6

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses at least 5	6
Controls negative and/or solvent	Negative and solvent control
Replicates per dose 3 or more	3 (6 for solvent control)
Duration of test 120 hours	120 hours
Daily observations were made?	Yes
Method of Observations	Algal cell absorbance
Maximum Labeled Rate	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

Concentration (mg/L)		Algal cell absorbance*	% reduction in area under growth curve**	120-Hour pH
Nominal	Mean Measured			
Control	<0.007	2.337	0(+12)	7.6
Solvent	<0.007	1.947	--	7.5
1.3	1.4	2.133	0(+8)	7.5
2.4	2.4	1.910	7	7.5
4.3	3.9	2.033	2	7.5
7.8	8.5	1.730	12	7.5
14	14	1.303	63	7.5
25	21	<0.016	98	7.3

* Inoculum (Day 0) calculated cell absorbance = 0.005 (2000 cells/mL)

** compared to the solvent control

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

Statistical Results for Areas Under the Growth Curve

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

EC₅₀: 10 ppm

95% C.I.: 6.4 - >21 ppm

Probit Slope: Not reported

NOEC: 8.5 ppm

Statistical Results for Growth Rate

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

EC₅₀: >21 ppm

95% C.I.: N/A

Probit Slope: N/A

NOEC: 8.5 ppm

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Moving average method and Williams' test for solvent control comparisons. Results based on mean measured concentrations.

EC₅₀: 13 ppm
Probit Slope: N/A

95% C.I.: 12 - 14 ppm
NOEC: 9 ppm

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC₅₀ and NOEC for *A. flos-aquae* exposed to ICIA5504 were 13 and 9 ppm, respectively. This study can be categorized as Core.

FEKEN ~~SUBSTITUTION~~ ANABAENA 11-28-95

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
21	100	99	99	0
14	100	33	33	0
8.5	100	11	11	0
3.9	100	4	4	0
2.4	100	2	2	0
1.4	100	0	0	0

THE BINOMIAL TEST SHOWS THAT 14 AND 21 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 15.21465

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	1.155227E-02	13.3062	12.50765 14.20901

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	1.677712	25.68942	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 = 4.259726
95 PERCENT CONFIDENCE LIMITS = -1.257749 AND 9.7772

LC50 = 13.40221
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 6.745826
95 PERCENT CONFIDENCE LIMITS = 0 AND 12.11624

from Absorbance (# of cells)
mean measured
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~~SULFENTRAZONE~~

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Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	6	1.947	1.947	2.009
2	1.4	3	2.133	2.133	2.009
3	2.4	3	1.910	1.910	1.972
4	3.9	3	2.033	2.033	1.972
5	8.5	3	1.730	1.730	1.730
6	14	3	1.303	1.303	1.303

SULFENTRAZONE

File: 43678162

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	2.009				
1.4	2.009	0.431		1.75	k= 1, v=15
2.4	1.972	0.173		1.84	k= 2, v=15
3.9	1.972	0.173		1.87	k= 3, v=15
8.5	1.730	1.500		1.88	k= 4, v=15
14	1.303	4.454	*	1.89	k= 5, v=15

s = 0.204

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

NOEC - 8.5 ppm

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