

MRID No.: 436781-64

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

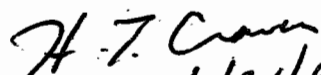
1. **CHEMICAL:** Azoxystrobin **PC Code No.:** 128810
2. **TEST MATERIAL:** ICIA5504 technical **Purity:** 96.2%
3. **CITATION**

Authors: D.V. Smyth, S.A. Sankey, S.J. Kent and R.D. Stanley
Title: ICIA5504: Toxicity to the Freshwater Diatom (*Navicula pelliculosa*)
Study Completion Date: February 5, 1994
Laboratory: Brixham Environmental Laboratory, Brixham Devon, UK
Sponsor: Zeneca Ag Products, Wilmington, DE
Laboratory Report ID: BL5087/B
MRID No.: 436781-64
4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: 
Date: 4/01/96
5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: 
Date: 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 a diatom toxicity test.

Results Synopsis

EC₅₀: 49 ppb
NOEC: 20 ppb

95% C.I.: 43 - 58 ppb
Probit Slope: N/A
8. **ADEQUACY OF THE STUDY:** 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>Navicula pelliculosa</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	3,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	UTEX 667

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	Acetone (0.1 mL/L)
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	24°C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.4 KLux
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial 8.4 - 8.6 Final 7.5 - 8.5

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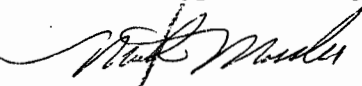
1. **CHEMICAL:** Azoxystrobin ~~Salicylic acid~~ PC Code No.: 128810 ~~128801~~
2. **TEST MATERIAL:** ICIA5504 technical Purity: 96.2%
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DP Barcode: D217072, D217078
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4. **REVIEWED BY:** Max Feken, 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 a diatom toxicity test.

Results Synopsis

EC₅₀: 49 ppb
NOEC: 20 ppb

95% C.I.: 43 - 58 ppb
Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

A. **Classification:** Core

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	8
<u>Controls</u> negative and/or solvent	Negative and solvent controls
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	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. Cell Density ($\times 10^4$ cells/mL)	$\frac{1}{2}$ Reduction of Area under Growth Curve*	120-Hour pH
Control	109	-	8.1
Solvent	119	-	8.0
1.0	154	0(+42)	8.3

Nominal Dose ($\mu\text{g/L}$)	Avg. Cell Density ($\times 10^4$ cells/mL)	% Reduction of Area under Growth Curve*	120-Hour pH
5.0	143	0(+15)	8.0
10	86.0	32	8.0
20	99.6	18	7.9
40	54.8	59	7.8
80	33.9	70	7.8
160	23.8	80	7.7
320	20.7	86	7.6

* Compared to the pooled control

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

Statistical Results for Areas under the Growth Curve

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

EC₅₀: 57 ppb

95% C.I.: 27 - 170 ppb

Probit Slope: Not reported

NOEC: 20 ppb

Statistical Results for Growth Rate

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

EC₅₀: >320 ppb

95% C.I.: N/A

Probit Slope: Not reported

NOEC: 20 ppb

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Moving average method and Williams' test for pooled control comparisons. Results based on nominal concentrations.

EC₅₀: 49 ppb

95% C.I.: 43 - 58 ppb

Probit Slope: N/A

NOEC: 20 ppb

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for a diatom toxicity test. Based on nominal concentrations, the 120-hour EC₅₀ and NOEC for *N. pelliculosa* exposed to ICIA5504 were 49 and 20 ppb, respectively. This study is categorized as Core.

FEKEN ~~SCHEMATIC~~ NAVICULA 11-29-95

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
320	100	82	82	0
160	100	80	80	0
80	100	70	70	0
40	100	52	52	0
20	100	13	13	0
10	100	25	25	0
5	100	0	0	0
1	100	0	0	0

THE BINOMIAL TEST SHOWS THAT 20 AND 40 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 38.74579

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	1.642423E-02	49.28439	42.5248	57.518

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1557426	6.398898	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 = 1.558406
95 PERCENT CONFIDENCE LIMITS = .9433931 AND 2.173419

LC50 = 50.68946
95 PERCENT CONFIDENCE LIMITS = 30.12127 AND 90.24423

LC10 = 7.762157
95 PERCENT CONFIDENCE LIMITS = 2.136227 AND 14.93165

from cellular Density

~~SULFENTRAZONE~~ (NAVICULA)

File: 43678164

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	113.917	113.917	131.208
2	1.0	3	154.333	154.333	131.208
3	5.0	3	142.667	142.667	131.208
4	10	3	85.967	85.967	92.767
5	20	3	99.567	99.567	92.767
6	40	3	54.767	54.767	54.767
7	80	3	33.900	33.900	33.900
8	160	3	23.833	23.833	23.833
9	320	3	20.700	20.700	20.700

SULFENTRAZONE (NAVICULA)

File: 43678164

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
GRPS 1&2 POOLED	131.208				
1.0	131.208	1.065		1.72	k= 1, v=21
5.0	131.208	1.065		1.80	k= 2, v=21
10	92.767	1.302		1.83	k= 3, v=21
20	92.767	1.302		1.84	k= 4, v=21
40	54.767	3.643	*	1.85	k= 5, v=21
80	33.900	4.928	*	1.85	k= 6, v=21
160	23.833	5.548	*	1.85	k= 7, v=21
320	20.700	5.741	*	1.86	k= 8, v=21

s = 22.964

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

NOEC- 20 ppb