

MRID No.: 436781-65

DATA EVALUATION RECORD
EC₅₀ TEST WITH *LEMNA GIBBA*
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
R.D. Stanley

Title: ICIA5504: Toxicity to the Duckweed (*Lemna gibba*)

Study Completion Date: October 27, 1993

Laboratory: Brixham Environmental Laboratory, Brixham
Devon, UK

Sponsor: Zeneca Ag Products, Wilmington, DE

Laboratory Report ID: BL5000/B

MRID No.: 436781-65

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. Craven
6/21/96

6. **STUDY PARAMETERS**

Definitive Test Duration: 14 days

Type of Concentrations: Nominal

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute aquatic plant study.

Results Synopsis

EC₅₀: 3.4 ppm
NOEC: 0.8 ppm

95% C.I.: 3.0-3.9 ppm
Probit Slope: 2.8

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core

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B. Rationale: N/A

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:** The number of plants (3) used initially during the study was less than recommended (5 plants).

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Lemna gibba</i>	<i>Lemna gibba</i>
<u>Number of Plants/Fronds</u> 5 plants, 3 fronds per plant.	3 plants, 4 fronds each
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Hoagland's medium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	Dimethylformamide (0.1 mL/L)
<u>Temperature</u> 25°C	25°C
<u>Light Intensity</u> 5.0 KLux ($\pm 15\%$)	5.05 - 5.08 KLux
<u>Photoperiod</u> Continuous	Continuous
<u>pH</u> Approximately 5.0	Fresh solutions (4.5 - 4.8) and old solutions (5.1 - 6.0)
<u>Test System</u> Static or renewal	Renewal after 7 days

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DATA EVALUATION RECORD
EC₅₀ TEST WITH LEMNA GIBBA
GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** ~~Sulfontrazone~~ *Azoxytolin* **PC Code No.:** ¹²⁸⁸¹⁰ ~~129001~~

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

3. **CITATION**

Authors: D.V. Smyth, S.J. Kent, S.A. Sankey and R.D. Stanley

Title: ICIA5504: Toxicity to the Duckweed (*Lemna gibba*)

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DP Barcode: ~~D317072, D317073~~

MRID No.: 436781-65

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

Signature: *[Signature]*

Date: 12/1/95

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

Signature: *[Signature]*

Date: 12/5/95

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS**

Definitive Test Duration: 14 days

Type of Concentrations: Nominal

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute aquatic plant study.

Results Synopsis

EC₅₀: 3232 ppb

NOEC: 800 ppb

95% C.I.: 2705 - 3868 ppb

Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

3

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C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	7
<u>Controls</u> negative and/or solvent	Negative and solvent control
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 14 days	14 days
<u>Daily observations were made?</u>	Yes, every 2 to 3 days
<u>Method of Observations</u>	Frond counts, dry weights
<u>Maximum Labeled Rate</u>	Not reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 14 day frond count?	Yes
Control frond count at 14 day $\geq$ 2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response

Concentration ($\mu\text{g/l}$)		Avg. Frond Density	% Inhibition*	14-Day pH
Nominal	Mean Measured			
Control	<4.8	306	0	5.7
Solvent Control	<4.8	305	-	5.8
100	110	338	0	5.9
200	220	332	0	5.8
400	430	329	0	5.8
800	780	335	0	5.9
1600	1600	232	25	5.8
3200	3200	156	51	5.7
6400	6400	93	72	5.6

Concentration ($\mu\text{g/L}$)		Avg. Dry Weight (mg)	% Inhibition*	14-Day pH
Nominal	Mean Measured			
Control	<4.8	39.9	0	5.7
Solvent Control	<4.8	39.4	-	5.8
100	110	49.2	0	5.9
200	220	43.2	0	5.9
400	430	39.9	0	5.8
800	780	42.9	0	5.9
1600	1600	33.2	16	5.8
3200	3200	27.5	31	5.7
6400	6400	21.5	47	5.6

*compared to the solvent control

Other Significant Results: Reduced root and frond growth was observed in plants at treatment concentrations ≥ 1600 ppb after day 7. Reduced root growth was also observed by day 14 in nominal 800 ppb test solutions.

Statistical Results

Number of Fronds:

Statistical Method: Moving average angle method and Dunnett's procedure for mean comparisons.
Comparisons made against the solvent control.

EC ₅₀ : 3200 ppb	95% C.I.: 2700 - 3900 ppb
Probit Slope: N/A	NOEC: 800 ppb

Frond dry weight:

Statistical Method: Moving average angle and Dunnett's procedure for mean comparisons.
Comparisons made against the solvent control.

EC ₅₀ : >6400 ppb	95% C.I.: N/A
Probit Slope: N/A	NOEC: 3200 ppb

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method (frond number): Probit method and Williams' test for comparing means against the solvent control. Results based on nominal concentrations.

EC ₅₀ : 3.4 ppm	95% C.I.: 3.0 - 3.92 ppm
Probit Slope: 2.8	NOEC: 0.8 ppm

Statistical Method (dry weight): Moving average angle and Williams' test for comparing means against the solvent control. Results based on nominal concentrations.

EC ₅₀ : >6.4 ppm	95% C.I.: N/A
Probit Slope: N/A	NOEC: 1.6 ppm

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an acute aquatic plant study. Based on nominal concentrations, the 14-day EC₅₀ and NOEC for *Lemna gibba* exposed to ICIA5504 were 3.4 and 0.8 ppm, respectively. This study is classified as Core.

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
6400	100	72	72	0
3200	100	51	51	0
1600	100	25	25	0
800	100	0	0	0
400	100	0	0	0
200	100	0	0	0
100	100	0	0	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 3119.342

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.0814682	3231.682	2704.931	3867.761

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.661919E-02	1	.0605588

SLOPE = 2.784829
 95 PERCENT CONFIDENCE LIMITS = 2.330473 AND 3.239184

LC50 = 3429.596
 95 PERCENT CONFIDENCE LIMITS = 3039.267 AND 3922.037

LC10 = 1200.053
 95 PERCENT CONFIDENCE LIMITS = 979.3174 AND 1407.112

~~SULFENTRAZONE~~ (FRONDS)

File: 43678165

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	3	293.333	293.333	315.867
2	100	3	326.333	326.333	315.867
3	200	3	319.667	319.667	315.867
4	400	3	317.000	317.000	315.867
5	800	3	323.000	323.000	315.867
6	1600	3	220.000	220.000	220.000
7	3200	3	144.000	144.000	144.000
8	6400	3	81.333	81.333	81.333

SULFENTRAZONE (FRONDS)

File: 43678165

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT	315.867				
100	315.867	0.933		1.75	k= 1, v=16
200	315.867	0.933		1.83	k= 2, v=16
400	315.867	0.933		1.86	k= 3, v=16
800	315.867	0.933		1.87	k= 4, v=16
1600	220.000	3.037	*	1.88	k= 5, v=16
3200	144.000	6.185	*	1.89	k= 6, v=16
6400	81.333	8.781	*	1.89	k= 7, v=16

s = 29.571

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

NOEC - 800 ppb

~~SULFENTRAZONE~~ (DRY WEIGHT)

File: 43678165

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	3	39.400	39.400	41.342
2	200	3	43.167	43.167	41.342
3	400	3	39.867	39.867	41.342
4	800	3	42.933	42.933	41.342
5	1600	3	33.200	33.200	33.200
6	3200	3	27.500	27.500	27.500
7	6400	3	21.533	21.533	21.533

SULFENTRAZONE (DRY WEIGHT)

File: 43678165

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	41.342				
200	41.342	0.502		1.76	k= 1, v=14
400	41.342	0.502		1.85	k= 2, v=14
800	41.342	0.502		1.88	k= 3, v=14
1600	33.200	1.604		1.89	k= 4, v=14
3200	27.500	3.078	*	1.90	k= 5, v=14
6400	21.533	4.621	*	1.91	k= 6, v=14

s = 4.735

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

NOEC - 1600 ppb