

DATA EVALUATION RECORD
S 72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

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

2. **TEST MATERIALS:** ICIA5504

3. **CITATION**

Authors: J.H. Rapley, E. Farrelly, and M.J. Hamer
Title: ICIA5504: Acute Toxicity of the Technical Material to First Instar *Daphnia magna*
Study Completion Date: May 23, 1994
Laboratory: Zeneca Agrochemicals (Zeneca Limited), Bracknell, Berkshire, UK
Sponsor: Zeneca Agricultural Products, Zeneca Inc., Wilmington, Delaware
Laboratory Report ID: 92JH095
MRID No.: 436781-16

4. **REVIEWED BY:**

William Erickson
 Biologist
 EEB/EFED/EPA

Signature:

Date:

W. Erickson
 4/6/96

5. **APPROVED BY:**

Harry Craven
 Section Head 4
 EEB/EFED/EPA

Signature:

Date:

H.T. Craven
 6/21/96

6. **STUDY PARAMETERS**

Age of Test Organism: <24 hours
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for a freshwater invertebrate acute toxicity test. An EC₅₀ of 284 ppb classifies azoxystrobin as highly toxic to *Daphnia magna*.

Results Synopsis

EC₅₀: 259 ppb 95% CI: 126-644 ppb
 Slope: N/A NOEC: 126 ppb

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

(1)

DP Barcode: D217072/D217078

MRID No.: 436781-16

DATA EVALUATION RECORD
§ 72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** *Azoxystrobin*
Sulfentrazone

PC Code No.: 129081

2. **TEST MATERIALS:** ICIA5504

96.2%

3. **CITATION**

Authors: J.H. Rapley, E. Farrelly, and M.J. Hamer
Title: ICIA5504: Acute Toxicity of the Technical Material to First Instar *Daphnia magna*

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DP Barcode: D217072/D217078

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

Signature: *Barbara Herbert*

Date: 10-26-95

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: *P. Kosalwat*

Date: 10/26/95

5. **APPROVED BY:** (Name), Head of Section (#), EEB, EFED

Signature:

Date:

6. **STUDY PARAMETERS**

Age of Test Organism: <24 hours
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate acute test. An EC₅₀ of 284 ppb classifies Sulfentrazone as highly toxic to *Daphnia magna*. The NOEC is 126 ppb.

8. **ADEQUACY OF THE STUDY**

A. **Classification:** Core.

2

7.5

9. Guideline Deviations:

1. Dechlorinated water was used as the dilution water.
2. The pH was slightly higher than recommended.
3. The dilution water used had much higher hardness than recommended (160-180 mg/L as CaCO₃ vs. 40-48 mg/L as CaCO₃).

10. SUBMISSION PURPOSE: New Chemical.**11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Not Reported
<u>Life Stage</u> Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 rd instar.	1 st instar (<24 h)
<u>Supplier</u>	In-house cultures.
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	N/A
Wild caught organisms were quarantined for 7 days?	N/A

Guideline Criteria	Reported Information
Were there signs of disease or injury?	Not reported.
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study.	No feeding during test.
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	Not reported.

C. Test System:

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	A mixture of dechlorinated water and reverse osmosis dechlorinated water.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20.2-20.6°C
<u>pH</u> Prefer 7.2 to 7.6.	7.8-8.0
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 h and ≥ 40% during 2 nd 48 h, flow-through: ≥ 60%.	≥96% of saturation during the test.
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃ .	160-180 mg/L as CaCO ₃ .

Guideline Criteria	Reported Information
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel. 2. <u>Size:</u> 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. <u>Fill volume:</u> 200 mL (daphnids and midges) or 2-3 L.	Glass 250 mL 200 mL
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant.	N/A
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^{\circ}\text{C}$, ≤ 0.5 g/L at $> 17^{\circ}\text{C}$; flow-through: ≤ 1 g/L/day.	Not reported
<u>Photoperiod</u> 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.	No solvent was used.

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L, then no definitive test is required.	A range finding test was conducted using ICIA5504 concentrations ranging from 0.064 to 1,000 μ g/L with and without solvents (methanol, DMSO). The solvents appeared to slightly reduce the toxic effects of ICIA5504.
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Control, 1.3, 2.2, 3.6, 6.1, 10.1, 16.8, 28.0, 46.7, 77.8, 130, 216, 360, 600, and 1000 μ g ai/L
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.	30 per level, 10 per replicate
<u>Test organisms randomly or impartially assigned to test vessels?</u>	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary $> 1^{\circ}\text{C}$. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	1. Temperature was monitored continuously in the water bath. 2. DO and pH were performed on test solutions at 0 and 48 hours.
<u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Chemical analysis was performed on test solutions at 0 and 48 hours.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> Static: $\leq 10\%$ Flow-through: $\leq 5\%$	0%
<u>Percent Recovery of Chemical</u>	53-71% of nominal
Raw data included?	Yes

Mortality

Concentration (ppb)		Number of Organ- isms	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			3	9	24	48
Control	<0.4	30	0	0	0	0
1.3	0.84	30	0	0	0	0
2.2	1.42	30	0	0	0	0
3.6	2.48	30	0	0	0	0
6.1	3.48	30	0	0	0	0
10.1	6.86	30	0	0	0	0
16.8	11.6	30	0	0	0	0
28.0	17.3	30	0	0	0	0
46.7	32.0	30	0	0	0	0
77.8	47.1	30	0	0	0	0
130	71.1	30	0	0	0	0
216	126	30	0	0	0	0
360	209	30	0	0	2	10
600	320	30	0	0	5	20
1000	644	30	0	0	19	27

Other Significant Results: None.

Statistical Results

Method: Iteratively reweighted linear regression on the logit transformation of percent response on $\log_{10}$ (concentration).

48-hr EC_{50} : 280 ppb ai 95% C.I.: 220-380 ppb ai

Probit Slope: N/A NOEC: 126 ppb ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test EC_{50} (C.I.)	259 (126-644) ppb ai
Moving Average Angle EC_{50} (95% C.I.)	284 (248-327) ppb ai
Probit EC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	126 ppb ai

14. REVIEWER'S COMMENTS: Dechlorinated water was used as the dilution water. However, it is probably acceptable in this test since no signs of toxicity or mortality were observed in any control daphnids. This study is scientifically sound and fulfills the guideline requirement for a freshwater invertebrate acute test. An EC_{50} of 259 ppb classifies azoxystrobin as highly toxic to *Daphnia magna*.

Barbara Herbert ~~Sulfentrazone~~ Daphnia magna 10-23-95

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
644	30	27	90	4.215167E-04
320	30	20	66.66667	4.936858
209	30	10	33.33334	4.936858
126	30	0	0	9.313227E-08
71.1	30	0	0	9.313227E-08
47.1	30	0	0	9.313227E-08
32	30	0	0	9.313227E-08
17.3	30	0	0	9.313227E-08
11.6	30	0	0	9.313227E-08
6.86	30	0	0	9.313227E-08
3.48	30	0	0	9.313227E-08
2.48	30	0	0	9.313227E-08
1.42	30	0	0	9.313227E-08
.84	30	0	0	9.313227E-08

THE BINOMIAL TEST SHOWS THAT 126 AND 644 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 258.6115

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	4.857591E-02	283.6174	247.7975 326.6133

NO CONVERGENCE IN 25 ITERATIONS. THE PROBIT METHOD
PROBABLY CANNOT BE USED WITH THIS SET OF DATA.
