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MRID No. 436781-21

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4

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

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

3. **CITATION:**

Authors: J.H. Rapley, E. Farrelly and M.J. Hamer
Title: ICIA5504 : Chronic Toxicity to *Daphnia magna*
Study Completion Date: May 23, 1994
Laboratory: Jealotts Hill Research Station, Berkshire, UK
Sponsor: Zeneca Inc., Wilmington, Delaware
Laboratory Report ID: RJ1493B
MRID No.: 436781-21

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: W. Erickson
Date: 4/01/96

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: H. J. Craven 6/21/96
Date:

6. **STUDY PARAMETERS:**

Age of Test Organism: ≤24 hours
Definitive Test Duration: 21 days
Study Method: Static Renewal
Type of Concentrations: Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for a freshwater invertebrate life-cycle test using *Daphnia magna*.

Results Synopsis: Most sensitive endpoint: Reproduction

NOEC: 44 ppb ai LOEC: 84 ppb ai MATC: 61 ppb ai

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DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4

1. **CHEMICAL:** ~~Sulfentrazone~~ Azoxystrobin **PC Code No.:** ~~129081~~ 128810

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

3. **CITATION:**

Authors: J.H. Rapley, E. Farrelly and M.J. Hamer
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MRID No.: 436781-21
DP Barcode: ~~D217072, D217078~~

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

Signature: [signature] **Date:** 1/19/96

APPROVED BY: Rosemary Mora, M.S., Associate Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat for RGM **Date:** 1/22/96

5. **APPROVED BY:**

Signature: **Date:**

6. **STUDY PARAMETERS:**

Age of Test Organism: ≤24 hours
Definitive Test Duration: 21 days
Study Method: Static Renewal
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7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate life-cycle test using *Daphnia magna*.

Results Synopsis: Most sensitive endpoint: Reproduction

NOEC: 44 ppb ai LOEC: 84 ppb ai MATC: 61 ppb ai

LOEC's for specific endpoints:

Neonates Produced:	84 ppb ai
Daphnid Survival:	169 ppb ai
Growth (length):	169 ppb ai

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core

B. **Rationale:** Although the water temperature of the test solutions was not recorded, the temperature of the constant temperature room (18.8°C - 22.3°C) was monitored continuously and was within acceptable limits. The test conditions appeared to be adequate considering only one mortality was observed in both the negative and solvent control combined.

C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS:**

1. The temperature of the test solutions was not measured. However, the temperature of the test room was measured continuously.

2. Dry weight of daphnids was not measured as recommended. However, mean growth (length) appeared to be a much less sensitive endpoint than reproduction or survival.

3. The percentage of methanol in the test solutions was changed from 0.01% to 0.003% on day 9. The study states that "the amount of methanol used to introduce the test chemical was reduced to 0.003% on day 9". It is not clear whether the amount of methanol in the solvent control was decreased on day 9.

4. The acclimation period of the parental stock was not reported.

5. The dilution water was not tested for contaminants.

6. Hardness, alkalinity, and conductivity were not measured in one test concentration and in one control as recommended. These parameters were measured on the blended water used to prepare test solutions.

7. The pH of the dilution water (8.0-8.3) was slightly higher than recommended (7.6-8.0).

8. Dechlorinated water was used. However, survival, growth, reproduction, and survival of the control daphnids was acceptable.

9. Dissolved oxygen in some 48- and 72-hour test solutions (≥39% of saturation) was lower than recommended (≥50% saturation recommended during test).

10 **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms/Acclimation:

Guideline Criteria	Reported Information
Species *Daphnia magna*	*Daphnia magna*
Source Laboratory, commercial, or wild stock.	In-house cultures.
Parental Acclimation Conditions Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Held under test conditions at 21°C in blended (dechlorinated and deionized) water.
Parental Acclimation Period At least 21 days.	Not specified.
Age of Parental Stock At least 10-12 days old at the beginning of the acclimation period.	5 weeks old.
Food Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Diet of yeast and *Chlorella vulgaris*.
Food Concentration 5 mg/L (dry wt.) of synthetic food or 10^8 cells/L of algae is recommended.	Algae (50 - 125 µg total carbon per vessel) and dried yeast (0.1-0.2 mg per vessel).
Were daphnids in good health during acclimation period?	Not reported.

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B. Test System:

Guideline Criteria	Reported Information
Test Water Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).	The dilution water was a blend of dechlorinated water and deionized water.
Water Temperature 20°C ±2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.	Temperature of the test solutions was not measured. The room temperature ranged from 18.8 to 22.3°C.
pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.	The pH was 8.0 to 8.3 for fresh solutions and 7.7 to 8.6 for old solutions.
Total Hardness 160 to 180 mg/L as $CaCO_3$ is recommended.	172 mg/L as $CaCO_3$.
Dissolved Oxygen Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥60% throughout test.	The DO was ≥94% of saturation in fresh solutions and ≥39% of saturation in old solutions.
Test Vessels or Compartments 1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.	1. Glass. 2. 250 ml beakers containing 200-mL of test solution.
Covers Renewal: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	A watch glass cover was used to cover each test vessel.

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.	N/A.
Renewal Rate Three times weekly.	Three times weekly (Monday, Wednesday, and Friday).
Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.	Not reported.
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark.
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.	Solvent: Methanol. Maximum conc.: 0.1 mL/L.

C. **Test Design:**

Guideline Criteria	Reported Information
Duration 21 days	21 days.
Nominal Concentrations Control(s) and at least 5 test concentrations; dilution factor not less than 50%.	Dilution water control; solvent control (0.1 mL methanol/l), and seven test concentrations: 6.25, 12.5, 25, 50, 100, 200 and 400 µg/l.

Guideline Criteria	Reported Information
Number of Test Organisms 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.	22 daphnids/level; 7 test chambers containing 1 daphnid each (for measuring survival only), and 3 test chambers containing 5 daphnids each (for measuring survival, growth, and reproduction).
Test organisms randomly or impartially assigned to test vessels?	Not stated.
Renewal Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).	Yes.
Water Parameter Measurements 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	1. D.O. was measured at test initiation and in the new and old test solutions on days 2, 5, 7, 9, 12, 14, 16, 19, and 21. 2. pH was measured at test initiation and in the new and old test solutions on days 2, 5, 7, 9, 12, 14, 16, 19, and 21. The blended water used to prepare test solutions was sampled on days 0, 9, and 15 to analyze for alkalinity, hardness, and conductivity. 3. Temperature of the test room was measured continuously.

Guideline Criteria	Reported Information
Chemical Analysis Needed 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.	Mean measured concentrations are the average of concentrations determined in the new and 48- and 72-hour solutions sampled on days 0, 2, 5, 7, 9, 12, 14, 16, 19, and 21.

12. REPORTED RESULTS:

A. General Results:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Control Mortality ≤ 30%	No mortality was observed in the negative control and 4.5% (1 out of 22) was observed in the solvent control.
Did daphnids in each control produce at least 40 young after 21 days?	Yes
Were no ephippia produced in any of the controls?	Not reported.
Data Endpoints - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs.	- Survival of parental daphnids, - Number of live young produced per adult, - Length of surviving first-generation daphnids.
Raw data included?	Yes.

Effects Data:

Toxicant Concentration (µg/L)		No. (%) Dead or Immobile (21 Days)	Avg. No. Live Young per Female	Mean Total Length (mm)
Nominal	Measured			
Control	<0.4	0/22 (0%)	107	4.26
Solvent Control	<0.4	1/22 (4.5%)	111	4.23
6.25	5.18	0/22 (0%)	120	4.28
12.5	10.4	2/22 (9%)	114	4.27
25	21.4	1/22 (4.5%)	118	4.26
50	43.9	1/22 (4.5%)	108	4.24
100	84.1	3/22 (14%)	32	4.27
200	169	9/22 (41%)	41	4.26
400	372	22/22 (100%)	0	--

Toxicity Observations: Many broods were wholly or partially aborted or dead at the 84 and 169 µg/L concentration levels. Only a few aborted or partially aborted broods were reported at the 44 µg/L concentration. However, there was no significant difference in the total young produced at this treatment level.

B. Statistical Results:

Endpoint	Method	NOEC (µg/L)	LOEC (µg/L)
Survival (21-day)		Not determined (ND)	ND
Reproduction (14- and 21-day)	Least significant difference (LSD)	44	84
Wet Weight	N/A	N/A	N/A
Length	LSD	169	372

13. **VERIFICATION OF STATISTICAL RESULTS:**

Endpoint	Method	NOEC (µg/L)	LOEC (µg/L)
Survival (21-day)	Williams' test	84	169
Reproduction (14- and 21-day)	Williams' test	44	84
Weight	N/A	N/A	N/A
Length	Williams' test	169	ND

14. **REVIEWER'S COMMENTS:** Although the water temperature of the test solutions was not recorded, the temperature of the test room (18.8°C - 22.3°C) was monitored continuously and was within the recommended water quality limits (20°C ±2°C). The test conditions appeared to be adequate considering only one mortality was observed in both the negative and solvent control combined.

Dry weights of surviving parental daphnids were not measured. However, there was no significant difference in daphnid length between the control and treatment levels. Overall, daphnid growth (length) appeared to be a much less sensitive endpoint than reproduction or survival.

This study is scientifically sound and fulfills the guideline requirements for a daphnid life-cycle test. This study is classified as **Core**. Based on the most sensitive endpoint (reproduction), the NOEC and LOEC are 44 and 84 ppb ai, respectively. The MATC is 61 ppb ai.

[redacted] (reproduction)
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t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	111.4286	CALCULATED t VALUE =	0.5162
GRP2 (BLANK CRTL) MEAN =	107.1429	DEGREES OF FREEDOM =	12
DIFFERENCE IN MEANS =	4.2857		

TABLE t VALUE (0.05 (2),12) = 2.179 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha=0.01

[redacted] (reproduction)
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	14	109.286	109.286	114.000
2	5.18	7	120.000	120.000	114.000
3	10.4	7	113.857	113.857	114.000
4	21.4	7	117.571	117.571	114.000
5	43.9	7	107.714	107.714	107.714
6	84.1	7	32.286	32.286	36.429
7	169	7	40.571	40.571	36.429

[redacted] (reproduction)
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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	114.000				
5.18	114.000	0.434		1.68	k= 1, v=49
10.4	114.000	0.434		1.76	k= 2, v=49
21.4	114.000	0.434		1.79	k= 3, v=49
43.9	107.714	0.145		1.80	k= 4, v=49
84.1	36.429	6.706	*	1.80	k= 5, v=49
169	36.429	6.706	*	1.81	k= 6, v=49

s = 23.470
Note: df used for table values are approximate when v > 20.

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t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	4.2314	CALCULATED t VALUE =	-0.5222
GRP2 (BLANK CRTL) MEAN =	4.2571	DEGREES OF FREEDOM =	12
DIFFERENCE IN MEANS =	-0.0257		

TABLE t VALUE (0.05 (2),12) = 2.179 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha=0.01

[redacted] (LENGHT)
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	14	4.244	4.244	4.244
2	5.2	7	4.283	4.283	4.263
3	10	6	4.270	4.270	4.263
4	21	7	4.257	4.257	4.263
5	44	7	4.244	4.244	4.263
6	84	5	4.270	4.270	4.263
7	169	6	4.255	4.255	4.263

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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	4.244				
5.2	4.263	0.458		1.68	k= 1, v=45
10	4.263	0.434		1.76	k= 2, v=45
21	4.263	0.458		1.79	k= 3, v=45
44	4.263	0.458		1.80	k= 4, v=45
84	4.263	0.407		1.80	k= 5, v=45
169	4.263	0.434		1.81	k= 6, v=45

s = 0.088
Note: df used for table values are approximate when v > 20.

12

[redacted] (SURVIVAL)
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t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.0000	CALCULATED t VALUE =	-1.0000
GRP2 (BLANK CRTL) MEAN =	0.3333	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	-0.3333		

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

[redacted] (SURVIVAL)
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	0.167	0.167	0.111
2	5.18	3	0.000	0.000	0.111
3	10.4	3	0.333	0.333	0.333
4	21.4	3	0.333	0.333	0.333
5	43.9	3	0.333	0.333	0.333
6	84.1	3	0.333	0.333	0.333
7	169	3	2.667	2.667	2.667
8	372	3	5.000	5.000	5.000

[redacted] (SURVIVAL)
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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	0.111				
5.18	0.111	0.138		1.73	k= 1, v=19
10.4	0.333	0.414		1.81	k= 2, v=19
21.4	0.333	0.414		1.84	k= 3, v=19
43.9	0.333	0.414		1.85	k= 4, v=19
84.1	0.333	0.414		1.86	k= 5, v=19
169	2.667	6.206	*	1.87	k= 6, v=19
372	5.000	11.998	*	1.87	k= 7, v=19

s = 0.570
Note: df used for table values are approximate when v > 20.