

**DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)**

- 1. **CHEMICAL:** Oryzalin PC Code No.: 104201
- 2. **TEST MATERIAL:** Oryzalin technical herbicide Purity: 96.9%
- 3. **CITATION:**

Authors: H.D. Kirk, J.M. Hugo, J.A. Miller, and K.H. Birk

Title: Evaluation of the Chronic Toxicity of Oryzalin Herbicide (3,5-Dinitro-N4,N4-di(N-propyl)sulfanilamide) to the Daphnid, *Daphnia magna* Straus

Study Completion Date: 16 April 1996

Laboratory: The Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI

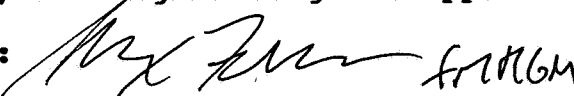
Sponsor: DowElanco, Indianapolis, IN

Laboratory Report ID: DECO-ES-2995

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- 4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Environmental Scientist, KBN Engineering and Applied Sciences, Inc.

Signature:  Date: 5/23/97

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

Signature: P. Kosalwat Date: 5/23/97

- 5. **APPROVED BY:**

Signature:  Date: 6/26/97

- 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 requirements for a freshwater invertebrate life-cycle test using *Daphnia magna*.

Results Synopsis:

NOEC: 0.358 ppm **LOEC:** 0.608 ppm **MATC:** 0.467 ppm

LOEC's for specific effects:

Neonates Produced: 1.01 ppm
 Daphnid Survival: 1.01 ppm
 Growth (weight): 0.608 ppm
 Growth (length): 1.01 ppm

8. ADEQUACY OF THE STUDY:

- A. **Classification:** Core.
- B. **Rationale:** Fulfills requirement.
- C. **Repairability:** N/A

9. GUIDELINE DEVIATIONS: There were no significant deviations from the guidelines.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms/Acclimation:

Guideline Criteria	Reported Information
<u>Species</u> Daphnia magna	Daphnia magna
<u>Source</u>	In-house cultures
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Parental cultures were maintained in dilution water under conditions similar to test conditions.
<u>Parental Acclimation Period</u> At least 21 days.	Continuous acclimation
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	>14 days old
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	3 ml of algal diet (Ankistrodesmus falcatus) per replicate daily

Guideline Criteria	Reported Information
Food Concentration 5 mg/l (dry wt.) of synthetic food or 10^8 cells/l of algae is recommended.	Approximately 20-60 x 10^6 cells of algae per vessel
Were daphnids in good health during acclimation period?	Yes

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).	Raw Lake Huron water which was adjusted to a hardness of 170 mg/L as CaCO_3 , autoclaved, and aerated prior to use. Dilution water was analyzed for organic and inorganic contaminants three times/year.
Water Temperature $20^\circ\text{C} \pm 2^\circ\text{C}$. Must not deviate from 20°C by more than 5°C for more than 48 hours.	Range: $18.9-20.8^\circ\text{C}$
pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.	7.4-8.0; with one measurement at 7.0 on day 14 in the 0.358 ppm group
Total Hardness 160 to 180 mg/l as CaCO_3 is recommended.	154-176 mg/l as CaCO_3
Dissolved Oxygen Renewal: must not drop below 50% for more than 48 hours. Flow-through: $\geq 60\%$ throughout test.	$\geq 74\%$ of saturation throughout the test

Guideline Criteria	Reported Information
<p><u>Test Vessels or Compartments</u> 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.</p>	<p>1. Glass 2. 250 ml borosilicate vessels filled with 150 ml of test solution.</p>
<p><u>Covers</u> 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.</p>	<p>Test vessels were covered with watch glasses.</p>
<p><u>Type of Dilution System</u> Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>N/A</p>
<p><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.</p>	<p>N/A</p>
<p><u>Aeration</u> Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	<p>Dilution water was aerated prior to use in the study.</p>
<p><u>Photoperiod</u> 16 hours light, 8 hours dark.</p>	<p>16 hours light, 8 hours dark</p>
<p><u>Solvents</u> Not to exceed 0.5 ml/l for static tests or 0.1 ml/l for flow-through tests. Acceptable solvents are dimethyl formamide, triethylene glycol, methanol, acetone and ethanol.</p>	<p>Solvent: DMF Maximum conc.: 0.1 ml/L</p>

C. Test Design:

Guideline Criteria	Reported Information
<u>Duration</u> 21 days	21 days
<u>Nominal Concentrations</u> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.	Dilution water control, solvent control (0.1 ml DMF/L), and five exposure concentrations: 0.130, 0.216, 0.360, 0.600, 1.00 ppm.
<u>Number of Test Organisms</u> 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.	25 daphnids/level; 10 test vessels with 1 daphnid each (growth/reproduction group) and 3 test compartments with 5 daphnids each (mortality/sublethal effects group).
Test organisms randomly or impartially assigned to test vessels?	Yes
<u>Renewal</u> 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).	Renewal every Monday, Wednesday, and Friday during the study.
<u>Water Parameter Measurements</u> 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.	D.O., pH, and temperature were measured in all test chambers every Monday, Wednesday, and Friday. Alkalinity, hardness, and conductivity were measured weekly for all concentration levels. Temperature was also monitored continuously in one representative test vessel.

Guideline Criteria	Reported Information
<p><u>Chemical Analysis</u> 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.</p>	<p>Concentrations of test material were measured for each treatment and control in samples collected from the bulk dose solutions on days 0, 2, 5, 7, 9, 12, 14, 16, and 19, and in pre-renewal solutions collected on days 2 (11 out of 13 replicates) and 21 (13 out of 13 replicates).</p>

12. REPORTED RESULTS:

A. General Results:

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes</p>
<p><u>Control Mortality</u> ≤ 30%</p>	<p><u>Solvent Control:</u> 7% in the the mortality/sublethal effect group and 0% in the growth/reproduction group</p> <p><u>Dilution Water Control:</u> 0% in both groups</p>
<p>Did daphnids in each control produce at least 40 young after 21 days?</p>	<p>Yes</p>
<p>Were no ehippia produced in any of the controls?</p>	<p>None.</p>

Guideline Criteria	Reported Information
<p>Data Endpoints</p> <ul style="list-style-type: none"> - 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. 	<ul style="list-style-type: none"> - Number of live/dead and immobile first-generation daphnids, - Number of live/dead and immobile second-generation daphnids produced in the growth/reproduction group, - Number of young produced per female in the growth/reproduction group, - Dry weight and length of surviving first-generation daphnids in the growth/reproduction group, - Clinical observations.
Raw data included?	Yes.

Effects Data:

Toxicant Concentration (ppm)		# Dead Daphnids/total	Mean Number Young per Female ^a	# Young/female/reprod. day ^a	Mean Total Length (mm) ^a	Mean Dry Weight (µg) ^a
Nominal	Measured					
Control	--	0/10 ^a 0/15 ^b	264.0	20.9	4.18	730.2
Solvent Control	--	0/10 1/15	281.7	21.7	4.17	557.2
0.130	0.130	0/10 0/15	252.9	20.1	4.08	475.8
0.216	0.217	0/10 1/15	275.2	21.2	4.08	450.7
0.360	0.358	1/10 2/15	256.1	20.0	4.21	502.0
0.600	0.608	0/10 1/15	256.1	19.7	4.01	406.8

Toxicant Concentration (ppm)		# Dead Daphnids/total	Mean Number Young per Female ^a	# Young/female/reprod. day ^a	Mean Total Length (mm) ^a	Mean Dry Weight (µg) ^a
Nominal	Measured					
1.00	1.01	5/10 7/15	150.9	12.0	3.42	281.7

^a Represents results for the growth/reproduction group.

^b Represents results for the mortality/sublethal effect group.

Toxicity Observations: By test termination, all adults in the highest test concentration of the mortality/sublethal effect group were immobile or pale in appearance. Several progeny in the two highest test concentrations of the growth/reproduction group were found dead.

B. Statistical Results: Statistical differences from the control or solvent control were determined using Dunnett's Test, Steel's Many-One Rank Test, or Kruskal-Wallis Test with the Wilcoxon procedure.

Most sensitive endpoint:

Endpoint	Method	NOEC (ppm)	LOEC (ppm)
Dry Weight	Dunnett's test	0.358	0.608

13. VERIFICATION OF STATISTICAL RESULTS: All treatment responses were compared to the pooled control (length) or the solvent control (weight and reproduction).

Most sensitive endpoint:

Endpoint	Method	NOEC (ppm)	LOEC (ppm)
Dry Weight	Williams' test	0.358	0.608

14. REVIEWER'S COMMENTS: Based on the most sensitive endpoint (weight), the MATC was between 0.358 and 0.608 ppm. The geometric mean MATC was 0.467 ppm.

MRID No. 439869-01

This study is scientifically sound and fulfills the guideline requirements for a daphnid life-cycle test. The study is classified as **Core**.