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MRID No. 421423-05

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

1. CHEMICAL: Oxyfluorfen PC Code No.: 111601

2. TEST MATERIAL: RH-2915 Purity: 71.8%

3. CITATION:

Author: A.D. Forbis

Title: Chronic Toxicity of RH-2915 to *Daphnia magna* Under Flow-Through Test Conditions

Study Completion Date: September 18, 1986

Laboratory: ABC Laboratories, Inc., Columbia, MO

Sponsor: Rohm and Haas Company, Spring House, PA

Laboratory Report ID: 33943

MRID No.: 421423-05

DP Barcode: Not available

4. REVIEWED BY: Karl Bullock, M.S., Environmental Scientist,
Golder Associates Inc.

Signature:

Date:

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
Golder Associates Inc.

Signature:

Date:

5. APPROVED BY:

Signature:

Date:

6. STUDY PARAMETERS:

Age of Test Organism: <24 hours

Definitive Test Duration: 21 days

Study Method: Flow-Through

Type of Concentrations: Mean Measured

7. CONCLUSIONS: This study is scientifically sound but does not fulfill the guideline requirements for a freshwater invertebrate life-cycle test.

Results Synopsis: Most sensitive endpoints: Growth and reproduction

NOEC: 13 ppb ai LOEC: 28 ppb ai MATC: 19 ppb ai

LOEC's for specific endpoints:

Young / Adult
Reproductive Day: 28 ppb ai
Daphnid Survival: >28 ppb ai
Growth (weight): Not measured
Growth (length): 28 ppb ai

8. ADEQUACY OF THE STUDY:

- A. **Classification:** Supplemental
- B. **Rationale:** Statistical analyses could not be verified due to the lack of raw data.
- C. **Repairability:** Yes, raw data for survival, reproduction, and growth must be submitted for statistical verification.

9. GUIDELINE DEVIATIONS:

- 1. Daphnid weight was not measured as required by the guideline.
- 2. Raw data were not reported; survival, reproduction, and growth data were only reported as treatment means. Statistical analyses cannot be verified without raw data.
- 3. Dilution water hardness (225-275 mg/L as CaCO₃) was higher than recommended (160-180 mg/L CaCO₃).

10 SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms/Acclimation:

Guideline Criteria	Reported Information
<u>Species</u> <i>Daphnia magna</i>	<i>Daphnia magna</i>
<u>Source</u> Laboratory, commercial, or wild stock.	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.	Held under test conditions at $20 \pm 2^{\circ}\text{C}$ in dilution water
<u>Parental Acclimation Period</u> At least 21 days.	Continuous
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	Not reported
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Green algae (<i>Selenastrum capricornutum</i>), supplemented with Tetramin [®] -Cerophyl suspension.
<u>Food Concentration</u> 5 mg/L (dry wt.) of synthetic food or 10^8 cells/L of algae is recommended.	Fed three times daily with a 20-30 mL of an algal suspension, supplemented once daily with 2 mL of Tetramin [®] -Cerophyl suspension. Food concentration in test solutions was not reported.
Were daphnids in good health during acclimation period?	Not reported

B. Test System:

Guideline Criteria	Reported Information
<p><u>Test Water</u> Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</p>	Aerated, aged ABC well water, periodically screened for contaminants.
<p><u>Water Temperature</u> 20°C ±2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.</p>	20 - 21°C
<p><u>pH</u> 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.</p>	8.1 - 8.4
<p><u>Total Hardness</u> 160 to 180 mg/L as CaCO₃ is recommended.</p>	225-275 mg/L as CaCO ₃
<p><u>Dissolved Oxygen</u> <u>Renewal</u>: must not drop below 50% for more than 48 hours. <u>Flow-through</u>: >60% throughout test.</p>	≥93% of saturation during the test
<p><u>Test Vessels or Compartments</u> 1. <u>Material</u>: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <u>Size</u>: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.</p>	1. Glass 2. 1-L test chambers; fill volume appeared to be 1 L.
<p><u>Covers</u> <u>Renewal</u>: Test vessels should be covered with a glass plate. <u>Flow-through</u>: openings in test compartments should be covered with mesh nylon or stainless steel screen.</p>	Test chambers had notched drains covered with 50-mesh stainless steel screens.

Guideline Criteria	Reported Information
<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>	Intermittent-flow proportional diluter equipped with a mixing chamber.
<p><u>Renewal Rate</u> Three times weekly.</p>	3.3 volume replacements/day
<p><u>Aeration</u> Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	Dilution water was aerated before delivery to the exposure chambers. Exposure chambers were not aerated.
<p><u>Photoperiod</u> 16 hours light, 8 hours dark</p>	16 hours light, 8 hours dark
<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 dimethylformamide, triethylene glycol, methanol, acetone and ethanol.</p>	Solvent: triethylene glycol Maximum conc.: Not reported

C. Test Design:

Guideline Criteria	Reported Information
<p><u>Duration</u> 21 days</p>	21 days
<p><u>Nominal Concentrations</u> Control(s) and at least 5 test concentrations; dilution factor not less than 50%.</p>	Dilution water control, solvent control, and five treatment concentrations: 3.7, 6.0, 14, 24, and 50 $\mu\text{g ai/L}$
<p><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.</p>	40 daphnids/level; 4 test chambers with 10 daphnids each

Guideline Criteria	Reported Information
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).	N/A
<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.	1. DO and pH were measured on days 0, 4, 7, 14, and 21 in the dilution water control and the low (3.7 $\mu\text{g ai/L}$), middle (14 $\mu\text{g ai/L}$), and high (50 $\mu\text{g ai/L}$) treatments. 3. Temperature was measured daily and monitored continuously in the water bath.
<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.	Samples collected and analyzed on days 0, 4, 7, 14, and 21 by GLC resulted in mean recoveries ranging from 48.6 to 71.7% of nominal. The limit of detection was not reported. Quality control mean recoveries were 90-98%.

12. REPORTED RESULTS:

A. General Results:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> ≤ 30%	8% in both the dilution water control and 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
<u>Data Endpoints</u> - 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.	-Mean survival of parental daphnids, -Mean number of young/adult reproductive days, -Average length of surviving first-generation daphnids.
Raw data included?	No

Effects Data

Toxicant Concentration ($\mu\text{g ai/L}$)		Percent Dead or Immobile (21 Days)	Young/Adult Reproductive Days	Mean Length (mm)
Nominal	Measured			
Control	-	8%	4.7	3.4
Solvent Control	-	8%	4.9	3.4
3.7	1.8	8%	5.3	3.5
6.0	4.3	2%	5.0	3.4
14	7.4	10%	5.0	3.4

Toxicant Concentration ($\mu\text{g ai/L}$)		Percent Dead or Immobile (21 Days)	Young/Adult Reproductive Days	Mean Length (mm)
Nominal	Measured			
24	13	5%	4.4	3.4
50	28	25%	1.2*	2.7*

* Significantly reduced compared to the pooled controls ($p \leq 0.05$).

Toxicity Observations: None reported.

B. Statistical Results: Comparisons made between treatments and the pooled controls. Percentage data (survival) were arcsin transformed prior to analysis.

Endpoint	Method	NOEC ($\mu\text{g ai/L}$)	LOEC ($\mu\text{g ai/L}$)
Survival	One-way ANOVA and Fisher's Least Significant Difference test	28	>28
Reproduction	One-way ANOVA and Fisher's Least Significant Difference test	13	28
Length	One-way ANOVA and Fisher's Least Significant Difference test	13	28

13. **VERIFICATION OF STATISTICAL RESULTS:** Verification of statistical results were not possible due to the lack of raw data. Only treatment means were reported.
14. **REVIEWER'S COMMENTS:** This study is scientifically sound, but does not fulfill the guideline requirements for a daphnid

life-cycle test. As no data were reported other than treatment means, verification of statistical analyses was not possible. Based on mean measured concentrations and the most sensitive endpoints (growth and reproduction) reported by the author, the LOEC and NOEC were determined to be 28 and 13 ppb ai, respectively. The geometric mean MATC is 19 ppb ai. This study is classified as **Supplemental**.