DATA EVALUATION RECORD
§ 72-1(C) -- ACUTE LC_{50} TEST WITH A COLDWATER FISH

6/12/1995

1. **CHEMICAL:** RPA 201772 (Isoxaflutole) PC Code No.: 123000

2. **TEST MATERIAL:** Batch No. 21 ADM 93 Purity: 98.7%

3. **CITATION**

Authors:	Bettencourt, M.
Title:	RPA 201772 - Acute Toxicity to Rainbow Trout (*Onchorhynchus mykiss*) Under Flow-Through Conditions
Study Completion Date:	December 8, 1993
Laboratory:	Springborn Laboratories, Inc.
Sponsor:	Rhone-Poulenc Ag Company
Laboratory Report ID:	SLI Report No. 93-8-4860; SLI Study # 10566.0493.6284.108
MRID No.:	435732-36
DP Barcode:	D213874

4. **REVIEWED BY:** Michael Davy, Agronomist, ERCB, EFED

Signature: Michael Davy **Date:** 6-8-95

5. **PEER REVIEWER:** Andrew Bryceland, Fishery Biologist, ERCB/EFED

Signature: **Date:** 6-12-95

6. **STUDY PARAMETERS**

Age or Size of Test Organism:	1.3 gm, 48 mm long
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for 72-1 freshwater acute toxicity study. It is not possible to classify , precisely, the toxicity of RPA 201772 based on this study, since no LC_{50} was derived. However, based on this study, it is possible to state that RPA 201772 is probably no more than moderately toxic to fish.

Results Synopsis: 96-hr LC_{50}: >1.7 ppm ai; NOEC: 1.7 ppm ai

8. **ADEQUACY OF THE STUDY**

A. **Classification:** Core.
B. **Rationale:** meets guidelines
C. **Repairability:** n/a

9. **BACKGROUND**

10. GUIDELINE DEVIATIONS

1. Solvent amount in flow-through test exceeded 0.1 ml/L.
2. Total hardness is below 40 to 48 mg/L as $CaCO_3$
3. LC_{50} was not calculated due to solubility.

11. SUBMISSION PURPOSE: EUP

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species: Preferred species is the rainbow trout (*Onchorhynchus mykiss*)	*Onchorhynchus mykiss*
Mean Weight : 0.5-5 g	0.5 g
Mean Standard Length Longest not > 2x shortest	Mean: 43 mm Range: 35-50 mm
Supplier	Wellington River Aquafarm
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 14 days	14 days
Wild caught organisms were quarantined for 7 days?	N/A
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
Feeding No feeding during the study	48 hours prior to test
Pretest Mortality No more than 3% mortality 48 hours prior to testing	0% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	well water
Does water support test animals without observable signs of stress?	Yes
Water Temperature : 12°C	13 °C
pH : Prefer 7.2 to 7.6	7.3-7.4
Dissolved Oxygen flow-through: ≥ 60%	lowest is 89% at 72 hour
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	28 - 30 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	Glass aquarium measured 39 x 20 x 25 cm maintained with 15 L volume
Type of Dilution System Must provide reproducible supply of toxicant	Harvard peristaltic pump
Flow Rate: Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	diluter system calibrated before test and checked twice daily during test to deliver 6.3 vol/24 hours
Biomass Loading Rate : Flow-through: ≤ 1 g/L/day	0.053 g/L/day
Photoperiod 16 hours light, 8 hours dark	16 light, 8 dark
Solvents : Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: Acetone Maximum conc.: 0.5 ml/L.

In order to optimize the solubility of the test material, ultrasonication and mechanical stirring were utilized.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	No mortalities observed in 10 and 100 ppm range, however sublethal effects observed at these levels (erratic swimming and loss of equilibrium).
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	0.32, 0.54, 0.90, 1.5, and 2.5 mg ai/L.
Number of Test Organisms Minimum 10/level, may be divided among containers	10/vessel and 2 vessel/level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	temperature, DO, and pH were measured once daily in each replicate of each level throughout test
Chemical Analysis 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	No aeration done. No precipitate observed, but precipitate observed at 2.5 and higher concentration levels during the range finding. Measured concentrations are 0.36, 0.57, 0.85, 1.1 and 1.7 mg ai/L during definitive test.

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	112-16%
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

The recovery of the chemical at 1.5 nominal level is 73% and at 2.5 is 65%. This may indicate that the limit of solubility may reached.

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	0	20	0	0	0	0
Solvent Control	0	20	0	0	0	0
0.32	0.36	20	0	0	0	0
0.54	0.57	20	0	0	0	0
0.90	0.85	20	0	0	0	0
1.5	1.1	20	0	0	0	0
2.5	1.7	20	0	0	0	0

Other Significant Results: No toxic symptoms observed

B. Statistical Results Method: Observational
96-hr LC_{50}: >1.7 ppm ai NOEC: 1.7 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Method: Observational

96-hr LC_{50}: >1.7 ppm ai NOEC: 1.7 ppm ai

15. **REVIEWER'S COMMENTS:** According to the registrant, the solubility of this chemical is between 2 and 3 mg ai/L. The author increased the percentage of acetone to 0.5 ml/L to make the chemical more soluble. This is within the maximum for static test but the flow-through maximum is 0.1 ml/L. In the range finding test, the fish showed sublethal effects at (erratic swimming and loss of equilibrium)at 10 and 100 ppm level. Undissolved material was observed at concentrations as low as 2.5 ppm during range finding tests. No mortality were observed at the higher concentrations during the 96-hour range finding tests. This would indicate that the LC_{50} for fish may be greater than the 100 ppm level from the range finding test.

The recovery of the chemical at 1.5 nominal level is 73% and at 2.5 is 65%. This may indicate that the limit of solubility may reached. Although no undissolved chemical was noted in the definitive test at the highest level of 1.7 ppm ai, it could be safely assumed that the measured concentration reached the limit of solubility at temperature of 13°C. Due to no significant mortality in the range finding test and the solubility limit of the chemical at 1.7 ppm ai and no mortality and symptoms during the definitive test, the reviewer believes that the chemical is practically not toxic to the rainbow trout. However, the EPA's criteria define chemicals with LC_{50} values of $\geq$1.0 and $\leq$10.0 ppm to be moderately toxic to aquatic organisms.

This study is scientifically sound and meets the guideline requirements for 72-1 freshwater acute toxicity study.

Isoxaflutole Review 123000

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