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

1. **CHEMICAL**: Cloquintocet-mexyl PC Code No.: 125203

2. **TEST MATERIAL**: CGA-185072 Purity: 96.3%

3. **CITATION**

Authors:	S.J. Palmer and H.O. Krueger
Title:	CGA-185072: A 96-hour Flow-Through Acute Toxicity Test with the Rainbow Trout
Study Completion Date:	April 30, 1998
Laboratory:	Wildlife International Ltd., Easton, MD
Sponsor:	Novartis Crop Protection, Inc.
Laboratory Report ID:	108A-196
MRID No.:	445651-02
DP Barcode:	D246816

4. **REVIEWED BY**: Stephen Carey, Biologist, EFED, ERBIII

Signature: Stephen Carey **Date**: 10/18/99

5. **APPROVED BY**: Harry Craven, EFED, ERBIII

Signature: Henry Craven **Date**: 12/99

6. **STUDY PARAMETERS**

Age or Size of Test Organism:	33-38 mm
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean-measured

7. **CONCLUSIONS**: This study is scientifically sound but does not fulfill the guideline requirements for an acute toxicity test with the rainbow trout.

Results Synopsis

LC_{50}: >0.97 ppm ai 95% C.I.: N/A
NOEL: >0.97 ppm ai Probit Slope: N/A

8. **ADEQUACY OF THE STUDY**

A. Classification: Supplemental

B. Rationale: Despite the presence of precipitates in all test solutions except the lowest concentration, samples were

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A. Classification: Supplemental.

B. Rationale: Despite the presence of precipitates in all test solutions except the lowest concentration, samples were not filtered before chemical analysis.

C. Repairability: No.

9. **GUIDELINE DEVIATIONS:**

1. While precipitates were present in all test concentrations with the exception of the lowest one (0.063 mg ai/L nominal), they were not removed before the chemical analysis.

2. pH of the dilution water (8.1 - 8.3) was higher than recommended (7.2 - 7.6).

3. Water hardness (136 mg/L as $CaCO_3$) was higher than recommended (40 - 48 mg/L as $CaCO_3$).

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is the rainbow trout (*Oncorhynchus mykiss*)	*Oncorhynchus mykiss*
Mean Weight 0.1-5 g	0.45 g
Mean Standard Length Longest not > 2x shortest	Mean: 36 mm Range: 33-38 mm
Supplier	Mt. Lassen Trout Farm, Red Bluff, CA
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	21-day holding period, 51-hour acclimation period
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No sickness or injury within the 7 days prior to testing
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	Last fed ≥48 hours prior to testing
Pretest Mortality < 3% mortality 48 hours prior to testing	Pretest mortality not reported

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, filtered, aerated, and screened for contaminants before use.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 12°C	11.3-12.5°C
pH Prefer 7.2 to 7.6	8.1-8.3

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥81% of saturation during the test
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$	Mean: 136 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	 Stainless steel 25-L 15 L
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow diluter.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	Approximately 6 vol/24 hours, meter systems checked twice daily
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.05 g/L/day
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Solvent: dimethylformamide Maximum conc.: 0.1 mL/L

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.	Nominal concentrations based upon results of one exploratory range finding toxicity test.
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	Negative control, solvent control, 0.063, 0.13, 0.25, 0.50, and 1.0 mg ai/L.
Number of Test Organisms Minimum 10/level, may be divided among containers	20 fish per treatment level or control
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 ever 48 h in the high, medium, and low doses and in the control	Temperature measured in each chamber at test initiation and termination, and also monitored continuously in one negative control replicate. DO and pH measured every 24 hours in alternate replicates from each control and treatment group.
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	Solutions were collected daily from each replicate of the controls and treatments and analyzed by HPLC. Samples were not filtered or centrifuged before the analyses.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	Mean recoveries for each concentration ranged from 71 to 97% of the nominal values.
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0% mortality in both negative control and solvent control groups
Raw data included?	Yes
Signs of toxicity (if any) were described?	No signs of test material toxicity were observed

Mortality

Concentration (ppm ai)				Number of Fish	Cumulative Number Dead
Nominal	0-hour	96-hour	Mean Measured		Hour of Study
					96
Negative Control	ND	ND	ND	20	0
Solvent Control	ND	ND	ND	20	0
0.063	0.045	0.043	0.045	20	0
0.13	0.10	0.098	0.097	20	0
0.25	0.20	0.19	0.19	20	0
0.50	0.37	0.39	0.38	20	0
1.0	0.92	0.98	0.97	20	0

Other Significant Results: No signs of test material toxicity wre observed. All treatment solutions with the exception of the 0.063 ppm ai treatment contained a white precipitate in the mixing chambers. The test solutions in the test chambers appeared clear and colorless.

B. Statistical Results

Method: Visual estimation

96-hr LC_{50}: >0.97 ppm ai 95% C.I.: N/A

Probit Slope: N/A NOEC: 0.97 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	>0.97 ppm ai

14. REVIEWER'S COMMENTS: In the mixing chambers, all test solutions, with the exception of the control and lowest test concentration, contained white precipitates. Solubility trials revealed that CGA-185072 has a propensity to adhere to Teflon and glass, therefore insoluble CGA-185072 was present in all test solutions greater than 0.063 mg ai/L nominal. Since the test solutions were not filtered or centrifuged to remove the insoluble portion of CGA-185072 before chemical analysis, the actual concentrations (soluble portion) to which the fish were exposed are unknown. A different solvent system should be employed.

This study is scientifically sound but does not fulfill the guideline requirements for an acute toxicity test with the rainbow trout. Based on measured concentrations of both soluble and insoluble CGA-185072, the 96-hour LC50 was determined to be >0.97 ppm ai, greater than the solubility limit of the test compound (0.59 mg ai/L). CGA-185072 was classified as no more than highly toxic to the rainbow trout. The NOEC was determined to be >0.97 ppm ai. This study is classified as **supplemental**.

The study protocol deviated from recommended guidelines of pH and water hardness of the dilution water.