

MRID No. 438870-08

DATA EVALUATION RECORD
S 72-1 -- ACUTE LC₅₀ TEST WITH A COLDWATER FISH

1. **CHEMICAL:** Pirate (AC 303,630) PC Code No.: 129093
2. **TEST MATERIAL:** CL 357,806 Purity: 97%
(A photolytic degradate of AC 303,630)
3. **CITATION:**
Authors: J.W. Davis, M.R. Dunham, and J.D. Wisk
Title: Acute Toxicity of CL 357,806 to the
Rainbow Trout (*Oncorhynchus mykiss*) Under
Static Test Conditions
Study Completion Date: December 14, 1995
Laboratory: Toxikon Environmental Sciences, Jupiter,
FL
Sponsor: American Cyanamid Company, Princeton, NJ
Laboratory Report ID: J9504005a
MRID No.: 438870-08
DP Barcode: D210808 and D222690
4. **REVIEWED BY:** William Evans, Biologist added
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: *William Evans* Date: 10/9/96
5. **APPROVED BY:** Ann Stavola, Section Chief, Review Section 5
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: *Ann Stavola* Date: 11/5/96
6. **STUDY PARAMETERS:**
Age or Size of Test Organism: 0.20-0.53 g
Definitive Test Duration: 96 hours
Study Method: Static
Type of Concentrations: Nominal
7. **CONCLUSIONS:** This study is not scientifically sound and does
not meet the guideline requirements for an acute freshwater
fish toxicity test. Results of toxicity values are not
recorded for invalid studies. added

Results Synopsis

LC₅₀:
NOEC:

95% C.I.:
Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

- A. Classification: Invalid.
- B. Rationale: The exposure concentrations are unknown.
- C. Repairability: No

9. GUIDELINE DEVIATIONS:

1. The test solutions were aerated during the test and exposure concentrations were not measured.
2. The dissolved oxygen concentration in the test solutions ($\geq 44\%$) was lower than recommended ($\geq 60\%$).
3. The test temperature ranged from 11.3 to 14.5°C. The test temperature should not vary $> 1^\circ\text{C}$.

10. SUBMISSION PURPOSE:11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the rainbow trout (<i>Oncorhynchus mykiss</i>)	<i>Oncorhynchus mykiss</i>
<u>Mean Weight</u> 0.5-5 g	0.36 (0.20-0.53) g
<u>Mean Standard Length</u> Longest not > 2x shortest	3.13 (2.7-3.7) cm
<u>Supplier</u>	Mount 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
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<u>Acclimation Period</u> Minimum 14 days	14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study	The fish were not fed during the test period.
<u>Pretest Mortality</u> < 3% mortality 48 hours prior to testing	No mortality in the 48 hours prior to testing.

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Town of Jupiter water which was carbon-treated, aerated, and filtered prior to use.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 12°C	11.3-14.5°C
<u>pH</u> Prefer 7.2 to 7.6	6.5-7.4
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	≥44% saturation during the first 24 hours. Aeration was initiated when the DO fell below 40% of saturation at 31 hours. Except for the highest test concentration, DO was ≥87% of saturation during the 2nd half the test.
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃	70 mg/L as CaCO ₃

<u>Test Aquaria</u> 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume</u> : 15-30 L of solution	1. Glass 2. 10 liters (22-cm diameter, 30-cm height) 3. 9 liters
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Static system
<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	N/A
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^{\circ}\text{C}$, ≤ 0.5 g/L at $> 17^{\circ}\text{C}$; flow- through: ≤ 1 g/L/day	0.4 g/L
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Solvent: DMF Maximum conc.: 0.1 mL/L

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A range-finding test showed 0% mortality at 1.0 μ g ai/L and 100% mortality at concentrations ≥ 10 μ g ai/L.
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Five nominal test concentrations (0.63, 1.3, 2.5, 5, and 10 μ g/L), a dilution water control and a solvent control were used.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	10 fish/replicate, 2 replicates/treatment
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary $> 1^{\circ}\text{C}$ 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Temperature was measured hourly in the dilution water control and continuously in the water bath. 2. DO and pH were measured daily in each test vessel.
<u>Chemical Analysis</u> 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 chemical analysis of test solutions was conducted.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	N/A
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	5% mortality in each control
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes; signs of toxicity were observed in fish exposed to test concentrations ≥ 1.3 ppb, which included dark coloration and loss of equilibrium.

Mortality

Concentration (ppb)		Number of Fish	Cumulative Number Dead			
Nominal (ppb)	Mean Measured (ppb)		Hour of Study			
			24	48	72	96
Control	N/A	20	0	1	1	1
Solvent Control	N/A	20	0	1	1	1
0.63	N/A	20	0	0	1	1
1.3	N/A	20	0	1	1	1
2.5	N/A	20	1	4	4	4
5.0	N/A	20	17	20	20	20
10.0	N/A	20	20	20	20	20

Other Significant Results: Dissolved oxygen concentrations decreased from 100-102% of saturation at test initiation to 44-64% of saturation at 24 hours. Aeration of the test solutions began at 31 hours and continued until test termination. DO levels were $\geq 87\%$ of saturation for the remainder of the test in all test solutions, except the highest test concentration.

B. Statistical Results - based on nominal concentrations.

Method: Moving Average

96-hr LC_{50} : 2.6 ppb

95% C.I.: 2.1-3.4 ppb

Probit Slope: N/A

NOEC: 0.63 ppb

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC_{50} (C.I.)	3.2 (2.5-5.0) ppb
Moving Average Angle LC_{50} (95% C.I.)	N/A
Probit LC_{50} (95% C.I.)	N/A
Probit Slope	N/A
NOEC	0.63 ppb

- 14. REVIEWER'S COMMENTS:** Although the test solutions were aerated at approximately 31 hours after test initiation and continued until test termination, chemical analysis of the test solutions was not performed. Therefore, the actual concentrations to which the fish were exposed are unknown. This study is not scientifically sound and is classified as **Invalid**. The toxicity values are not recorded for studies classified as invalid.

added

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

RGM O.mykiss CL 357-806

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10	19	19	100	1.907348E-04
5	19	19	100	1.907348E-04
2.5	19	3	15.7895	.2212524
1.3	19	0	0	1.907348E-04
.63	19	0	0	1.907348E-04

THE BINOMIAL TEST SHOWS THAT 2.5 AND 5 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.175985

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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