

US EPA ARCHIVE DOCUMENT

MRID No. 434928-15

**DATA EVALUATION RECORD**  
**S 72-1(A) -- ACUTE LC<sub>50</sub> TEST WITH A WARMWATER FISH**

1. **CHEMICAL:** Pirate (AC 303,630) PC Code No.: 129093
2. **TEST MATERIAL:** CL 312,094 Purity: 93.6%  
(A metabolite of AC 303,630)
3. **CITATION:**

Authors: J.W. Davis, D.L. Youngerman, and J.D. Wisk

Title: Acute Toxicity of CL 312,094 to the Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Test Conditions

Study Completion Date: October 28, 1994

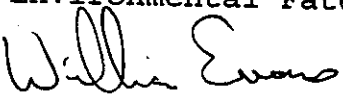
Laboratory: Toxikon Environmental Sciences, Jupiter, FL

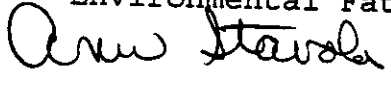
Sponsor: American Cyanamid Company, Princeton, NJ

Laboratory Report ID: J9304007

MRID No.: 434928-15

DP Barcode: D210808 and D222690
4. **REVIEWED BY:** William Evans, Biologist  
Ecological Effects Branch  
Environmental Fate and Effects Division
 

Signature:  Date: 7/30/96 added
5. **APPROVED BY:** Ann Stavola, Section Chief, Review Section 5  
Ecological Effects Branch  
Environmental Fate and Effects Division
 

Signature:  Date: 8/8/96
6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	0.29 ±0.10 g
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 an acute freshwater fish toxicity test. The study was conducted with exposure concentrations up to the maximum obtainable water solubility of this material for the conditions of this study (928 ppb). Only 35% mortality occurred at the highest concentration tested, therefore, the LC<sub>50</sub> was determined to be >928 ppb which, at worst, classifies CL 312,094 as highly toxic to the bluegill. The NOEC was 242 ppb.

**Results Synopsis**

LC<sub>50</sub>: >928 ppb  
 NOEC: 242 ppb

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

**8. ADEQUACY OF THE STUDY:**

A. **Classification:** Core

B. **Rationale:** Due to the low solubility of the test material the LC<sub>50</sub> could not be achieved. Further, the LC<sub>50</sub> of the parent compound of this major degradate is more toxic.

C. **Repairability:** No. Since the LC<sub>50</sub> greatly exceeds the LC<sub>50</sub> of the parent compound, and higher test concentrations could not be tested due to the bracket of the low solubility (< 1300 µg/L) of the test material, the test will not need to be repeated.

*added*

9. **GUIDELINE DEVIATIONS:** Test fish were slightly smaller than recommended.

10. **SUBMISSION PURPOSE:** Test major metabolite of AC 303,630 (Pirate).

**11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species is the bluegill sunfish ( <i>Lepomis macrochirus</i> )	<i>Lepomis macrochirus</i>
<b><u>Mean Weight</u></b> 0.5-5 g	0.29 (0.11-0.43) g
<b><u>Mean Standard Length</u></b> Longest not > 2x shortest	23 (18-27) mm
<b><u>Supplier</u></b>	Northeastern Biologists, Inc., Rhinebeck, NY
All fish from same source?	Yes
All fish from the same year class?	Yes

## B. Source/Acclimation

Guideline Criteria	Reported Information
<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	No feeding during the test period.
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing	0%

## 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 filtered (carbon and 20- $\mu$ m) and aerated prior to use.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 17°C or 22°C	21.5-23.4°C
<u>pH</u> Prefer 7.2 to 7.6	6.7-6.9
<u>Dissolved Oxygen</u> Static: $\geq$ 60% during 1 <sup>st</sup> 48 hrs and $\geq$ 40% during 2 <sup>nd</sup> 48 hrs, flow-through: $\geq$ 60%	$\geq$ 70% saturation throughout the test

Guideline Criteria	Reported Information
<b>Total Hardness</b> Prefer 40 to 48 mg/L as CaCO <sub>3</sub>	60-72 mg/L as CaCO <sub>3</sub>
<b>Test Aquaria</b> 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume</u> : 15-30 L of solution	1. Glass 2. 23.6 L (40 X 29.5 X 20 cm) 3. 15.3 L (13 cm maximum solution depth)
<b>Type of Dilution System</b> Must provide reproducible supply of toxicant	Modified proportional vacuum- siphon diluter
<b>Flow Rate</b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	9.2 volume additions/24 hours
<b>Biomass Loading Rate</b> Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow- through: ≤ 1 g/L/day	0.04 g/L/day; 0.38 g/L instantaneous loading
<b>Photoperiod</b> 16 hours light, 8 hours dark	16 hours of light/day
<b>Solvents</b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Solvent: DMF Maximum conc.: 0.092 ml/L

## D. Test Design

Guideline Criteria	Reported Information
<b>Range Finding Test</b> If LC <sub>50</sub> >100 mg/L with 30 fish, then no definitive test is required.	The results of two range finding tests were variable and showed 0% mortality at 10 and 100 µg/L, 40-60% mortality at 1,000 and 5,000 µg/L and 20% mortality at 10,000 µg/L.

Guideline Criteria	Reported Information
<p><b><u>Nominal Concentrations of Definitive Test</u></b> Control &amp; 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	A dilution water control, solvent control and five nominal concentrations (130, 216, 360, 600, and 1000 µg/L).
<p><b><u>Number of Test Organisms</u></b> Minimum 10/level, may be divided among containers</p>	20 fish/vessel; one vessel/treatment level and control
<p>Test organisms randomly or impartially assigned to test vessels?</p>	Yes
<p>Biological observations made every 24 hours?</p>	Yes
<p><b><u>Water Parameter Measurements</u></b> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary &gt; 1°C 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control</p>	<p>1. Temperature was measured daily in each test vessel and continuously in the dilution water control. 2. D.O. and pH were measured daily in each test vessel.</p>
<p><b><u>Chemical Analysis</u></b> 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</p>	Chemical analysis of test solutions was conducted at 0, 48, and 96 hours in each treatment and control.

## 12. REPORTED RESULTS

### A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes

Guideline Criteria	Reported Information
<b>Recovery of Chemical</b>	93-112% of nominal
<b>Control Mortality</b> Not more than 10% control organisms may die or show abnormal behavior.	0%
<b>Raw data included?</b>	Yes
<b>Signs of toxicity (if any) were described?</b>	Signs of toxicity were exhibited in the three highest test concentrations and included partial loss of equilibrium, dark coloration, and lethargy.

### Mortality

Concentration (ppb)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<30	20	0	0	0	0
Solvent Control	<30	20	0	0	0	0
130	140	20	0	0	0	0
216	242	20	0	0	0	0
360	363	20	0	0	1	1
600	632	20	0	0	0	1
1000	928	20	1	1	5	7

**Other Significant Results:** Undissolved test material was observed as a white precipitate in the high concentration test chamber after 48-hours of exposure, "which indicates that the water solubility of the test substance was exceeded in the high concentration. The water solubility of CL 312,094 in freshwater was determined to be 369 µg/L..."

A diluter malfunction occurred during the test. "As a result of this intermittent problem, the recorded cycle number values for day 0 and 1 were higher than the actual number of cycles... In

addition, the volume of toxicant and solvent/cycle on days one and two of the test was approximately 1.5 times higher than the desired rate due to the chemical delivery system double injecting."

### B. Statistical Results

Method: Visual Inspection

96-hr LC<sub>50</sub>: >928 ppb

Probit Slope: N/A

95% C.I.: N/A

NOEC: 242 ppb

### 13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC <sub>50</sub> (C.I.)	N/A
Moving Average Angle LC <sub>50</sub> (95% C.I.)	N/A
Probit LC <sub>50</sub> (95% C.I.)	N/A
Probit Slope	N/A
LC <sub>50</sub> (Visual Inspection)	>928 ppb
NOEC	242 ppb

14. **REVIEWER'S COMMENTS:** Although there was a diluter malfunction, the test solutions which analyzed at 0, 48, and 96 hours and the ratio of the lowest to highest measured concentration within a treatment was not >1.5.

This study is scientifically sound, meets the guideline requirements for an acute freshwater fish toxicity test, and is classified as Core. The study was conducted with exposure concentrations up to the maximum obtainable water solubility of this material for the conditions of this study (928 ppb). Only 35% mortality occurred at the highest concentration tested, therefore, the LC<sub>50</sub> was determined to be >928 ppb which, at worst, classifies CL 312,094 as highly toxic to the bluegill. The NOEC was 242 ppb.