
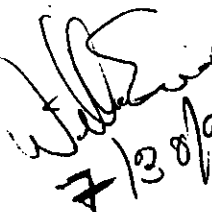
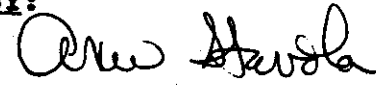


US EPA ARCHIVE DOCUMENT

10-8-96

MRID No. 438870-09

**DATA EVALUATION RECORD**  
**S 72-2 -- ACUTE LC<sub>50</sub> TEST WITH A FRESHWATER INVERTEBRATE**

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 *Daphnia magna* 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:** J9504005b  
**MRID No.:** 438870-09  
**DP Barcode:** D210808 and D222690
4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Environmental Scientist, KBN Engineering and Applied Sciences, Inc.  
**Signature:**  **Date:** 5/20/96   
**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.  
**Signature:** P. Kosalwat **Date:** 5/20/96
5. **APPROVED BY:**  
**Signature:**  **Date:** 10/1/96
6. **STUDY PARAMETERS:**  

Scientific Name of Test Organism:	<i>Daphnia magna</i>
Age of Test Organism:	<24 hours
Definitive Test Duration:	48 hours
Study Method:	Static
Type of Concentrations:	Nominal
7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate acute test using daphnids. An EC<sub>50</sub> of 18 ppb classifies CL 357,806 as very highly toxic to *Daphnia magna*. The NOEC was 13 ppb.

**Results Synopsis**

EC<sub>50</sub>: 18 ppb  
 NOEC: 13 ppb

95% C.I.: 13-25 ppb  
 Probit Slope: N/A

**8. ADEQUACY OF THE STUDY:**

- A. **Classification:** Core.  
 B. **Rationale:** Fulfills requirement.  
 C. **Repairability:** N/A.

9. **Guideline Deviations:** The temperature was monitored continuously only in the environmental chamber, not in one of the test solutions or control as recommended.

**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
<b>Species</b> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Not reported.
<b>Life Stage</b> Daphnids: 1 <sup>st</sup> instar (<24 h). Amphipods, stoneflies, and mayflies: 2 <sup>nd</sup> instar. Midges: 2 <sup>nd</sup> & 3 <sup>rd</sup> instar.	<24 hour neonates
<b>Supplier</b>	In-house cultures.
All organisms from the same source?	Yes.

A

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b>Acclimation Period</b> Minimum 7 days	Parents were isolated and maintained at 25.2°C in the dilution water <24 hours before the test.
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.
<b>Feeding</b> No feeding during the study.	Daphnids were not fed during the test.
<b>Pretest Mortality</b> No more than 3% mortality 48 hours prior to testing.	N/A.

**C. Test System:**

Guideline Criteria	Reported Information
<b>Source of dilution water</b> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	Town of Jupiter water which was aerated and filtered (carbon and 5- $\mu$ m filters) prior to use.
Does water support test animals without observable signs of stress?	Yes.
<b>Water Temperature</b> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20.2-22.4°C
<b>pH</b> Prefer 7.2 to 7.6.	7.2-7.5

Guideline Criteria	Reported Information
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 <sup>st</sup> 48 h and $\geq 40\%$ during 2 <sup>nd</sup> 48 h, flow-through: $\geq 60\%$ .	$\geq 97\%$ of saturation throughout the test.
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO <sub>3</sub> .	84 mg/L as CaCO <sub>3</sub> .
<u>Test Aquaria</u> 1. <u>Material</u> : Glass or stainless steel. 2. <u>Size</u> : 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. <u>Fill volume</u> : 200 ml (daphnids and midges) or 2-3 L.	1. Glass. 2. 300 ml (10-cm diameter, 5- cm height) crystallizing dishes 3. 250 ml
<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: $\leq 0.8$ g/L at $\leq 17^\circ\text{C}$ , $\leq 0.5$ g/L at $> 17^\circ\text{C}$ ; flow- through: $\leq 1$ g/L/day.	Not reported.
<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 Concentration: 0.1 ml/L

**D. Test Design**

Guideline Criteria	Reported Information
<p><b><u>Range Finding Test</u></b> If <math>LC_{50} &gt; 100</math> mg/L, then no definitive test is required.</p>	<p>The range-finding test showed <math>\leq 15\%</math> mortality at concentrations <math>\leq 10.0</math> ppb and 100% mortality at concentrations <math>\geq 50</math> ppb.</p>
<p><b><u>Nominal Concentrations of Definitive Test</u></b> Control &amp; 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.</p>	<p>Dilution water control, solvent control, and six nominal test concentrations (1.6, 3.1, 6.3, 13, 25, and 50 ppb).</p>
<p><b><u>Number of Test Organisms</u></b> Minimum 20/level, may be divided among containers.</p>	<p>10 daphnids per dish, 2 dishes per treatment and control.</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>Yes.</p>
<p><b><u>Water Parameter Measurements</u></b> 1. <b><u>Temperature</u></b> Measured continuously or, if water baths are used, every 6 h, may not vary <math>&gt; 1^\circ\text{C}</math>. 2. <b><u>DO and pH</u></b> 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 a control or blank replicate and continuously in the temperature-controlled chamber. 2. DO and pH were measured in composite solutions at test initiation and in each replicate at test termination.</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>	<p>None.</p>

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
<u>Control Mortality</u> Static: $\leq 10\%$ Flow-through: $\leq 5\%$	No mortality in either control.
<u>Percent Recovery of Chemical</u>	N/A.
Raw data included?	Yes.

Mortality

Concentration (ppb)		Number of Organisms	Cumulative Number Immobile	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	-	20	0	0
Solvent control	-	20	0	0
1.6	-	20	0	0
3.1	-	20	0	0
6.3	-	20	0	0
13	-	20	0	0
25	-	20	7	20
50	-	20	20	20

Other Significant Results: None.

B. Statistical Results - Based on nominal concentrations.

Method: Binomial method

48-hr EC<sub>50</sub>: 18 ppb

95% C.I.: 13-25 ppb

Probit Slope: N/A.

NOEC: 13 ppb

**13. VERIFICATION OF STATISTICAL RESULTS:**

Parameter	Result
Binomial Test EC <sub>50</sub> (C.I.)	18 (13-25) ppb
Moving Average Angle EC <sub>50</sub> (95% C.I.)	N/A
Probit EC <sub>50</sub> (95% C.I.)	N/A
Probit Slope	N/A
NOEC	13 ppb

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate acute toxicity test using daphnids. Based on nominal concentrations, an EC<sub>50</sub> value of 18 ppb classifies CL 357,806 as very highly toxic to *Daphnia magna*. The NOEC was 13 ppb since no mortality or sublethal effects were noted at or below this concentration. The study is classified as Core.



RGM D.magna CL 357 806

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
50	20	20	100	9.536742E-05
25	20	20	100	9.536742E-05
13	20	0	0	9.536742E-05
6.3	20	0	0	9.536742E-05
3.1	20	0	0	9.536742E-05
1.6	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 13 AND 25 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 18.02775

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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