10-8-96

MRID No. 438870-09

DATA EVALUATION RECORD
§ 72-2 -- ACUTE LC_{50} 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: For RGM **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:** 10/8/96

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Daphnia magna*
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_{50} of 18 ppb classifies CL 357,806 as very highly toxic to *Daphnia magna*. The NOEC was 13 ppb.

Results Synopsis

EC_{50}: 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
Species Preferred species is *Daphnia magna*	*Daphnia magna*
All organisms are approximately the same size and weight?	Not reported.
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3rd instar.	<24 hour neonates
Supplier	In-house cultures.
All organisms from the same source?	Yes.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period 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.
Feeding No feeding during the study.	Daphnids were not fed during the test.
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	N/A.

C. Test System:

Guideline Criteria	Reported Information
Source of dilution water 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-µm filters) prior to use.
Does water support test animals without observable signs of stress?	Yes.
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20.2-22.4°C
pH Prefer 7.2 to 7.6.	7.2-7.5

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%.	≥97% of saturation throughout the test.
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$.	84 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 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
Type of Dilution System Must provide reproducible supply of toxicant.	Static system.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.	Not reported.
Photoperiod 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
Solvents 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
Range Finding Test If LC_{50} >100 mg/L, then no definitive test is required.	The range-finding test showed ≤15% mortality at concentrations ≤10.0 ppb and 100% mortality at concentrations ≥50 ppb.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Dilution water control, solvent control, and six nominal test concentrations (1.6, 3.1, 6.3, 13, 25, and 50 ppb).
Number of Test Organisms Minimum 20/level, may be divided among containers.	10 daphnids per dish, 2 dishes per treatment and control.
Test organisms randomly or impartially assigned to test vessels?	Yes.
Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, 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.	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.
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	None.

12. **REPORTED RESULTS:**

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

Mortality

Concentration (ppb)		Number of Organisms	Cumulative Number Immobile	
			Hour of Study	
Nominal	Mean Measured		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_{50}: 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_{50} (C.I.)	18 (13-25) ppb
Moving Average Angle EC_{50} (95% C.I.)	N/A
Probit EC_{50} (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_{50} 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 CL357,806

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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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