

DATA EVALUATION RECORD

§ 72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE1. CHEMICAL: Methyl Bromide PC Code No.: 0532012. TEST MATERIAL: Methyl Bromide Purity: 99.87%3. CITATION

Authors: Drottar, K.R. and J.P. Swigert
Title: Methyl Bromide: A 48-hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*).

Study Completion Date: July 31, 1993Laboratory: Wildlife International Ltd., Easton, MD

Sponsor: Methyl Bromide Industry Panel
 Chemical Manufacturers Association

Laboratory Report ID: 264A-102BMRID No.: 429329-01DP Barcode: D1971124. REVIEWED BY: (Tom A. Bailey), (Fishery Biologist), EEB, EFEDSignature: *Tom A. Bailey*Date: *April 18, 1995*5. APPROVED BY: (Henry T. Craven), Head of Section (4), EEB, EFEDSignature: *Henry T. Craven*Date: *4/18/95*6. STUDY PARAMETERS

Scientific Name of Test Organism: (*Daphnia magna*)
Age of Test Organism: 1st instar <24 hours old
Definitive Test Duration: 48 hours
Study Method: Static (sealed serum bottoms with septa)
Type of Concentrations: Mean measured

7. CONCLUSIONS: The study was scientifically sound and meets guideline requirements a 72-2 acute freshwater invertebrate toxicity study. On the basis of mean measured concentrations, the 48-hour EC50 as determined by the binomial method was 2.6 mg a.i./L, which classifies methyl bromide as moderately toxic to *Daphnia magna*. The No Observable Effects Concentration was 1.2 mg a.i./L.

Results SynopsisLC₅₀: 2.6 ppm ai

95% C.I.: 2.2 - 3.5 ppm ai

NOEL: 1.2 ppm ai

Probit Slope: _____

8. ADEQUACY OF THE STUDY

2010700

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

9. BACKGROUND

Because of the volatile nature of methyl bromide, special precautions and procedures were necessary. Subsamples of the purchased test material were removed and stored at -14 C. The remaining test substance was maintained at 4 C. Test concentrations were prepared directly into test containers on a weight basis. Evacuated Tedlar bags were filled with liquid methyl bromide, the methyl bromide was allowed to vaporize, methyl bromide was removed from the Tedlar bags with gas-tight syringes, and known quantities of methyl bromide were transferred to 100 mL air-tight serum bottles (covered with septa) filled with known weights of serum water.

10. GUIDELINE DEVIATIONS

1. The Brood number of test daphnids were not reported.

11. SUBMISSION PURPOSE: This study was submitted pursuant to an Agency Data-Call-In. The objective of the test is to determine the acute toxicity of methyl bromide to a cladoceran, *Daphnia magna* under static conditions.

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Not Reported
<u>Life Stage</u> Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 rd instar.	1st instar

Guideline Criteria	Reported Information
<u>Supplier</u>	Cultures maintained by Wildlife International, Easton Maryland
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 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.	(Adults fed prior to test initiation)
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	0% mortality 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.	Well water (45 meters deep) Medium-hard water source
Does water support test animals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
<p><u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C</p>	20°C
<p><u>pH</u> 7.6 to 8.0 is recommended.</p>	8.1
<p><u>Dissolved Oxygen</u> Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%.</p>	(lowest=89% DO & 48-hour)
<p><u>Total Hardness</u> 160 to 180 mg/L as CaCO₃ is recommended.</p>	144 mg/L as CaCO ₃
<p><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.</p>	1. glass 2. 100 mL 3. 125 mL
<p><u>Type of Dilution System</u> Must provide reproducible supply of toxicant.</p>	(N/A)
<p><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.</p>	— vol/24 hours (N/A)
<p><u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.</p>	5 daphnids per test chamber
<p><u>Photoperiod</u> 16 hours light, 8 hours dark.</p>	16 hours light, 8 hours dark. 30 minute transition period.

Guideline Criteria	Reported Information
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	(N/A)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If $LC_{50} > 100$ mg/L, then no definitive test is required.	Results less than 100 mg/L
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	1.3, 2.2, 3.6, 6.0, 10 mg/L
Number of Test Organisms Minimum 20/level, may be divided among containers.	20 organisms/level
Test organisms randomly or impartially assigned to test vessels?	Yes
Water Parameter Measurements <ol style="list-style-type: none"> Temperature Measured continuously or, if water baths are used, every 6 h, may not vary $> 1^{\circ}C$. 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 continuously in surrogate negative control; ranged from 19.5 to 20 C. pH measured in surrogate of each treatment at test initiation, in all actual test vessels at test termination.

Guideline Criteria	Reported Information
<p>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</p>	<p>3 uL were removed from each test vessel to confirm presence of methyl bromide. At 0 and 48 hours, 2 mL were removed from each test chamber (gas-tight syringe) and transferred to autosampler.</p>

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	Yes
<p>Control Mortality Static: ≤10% Flow-through: ≤5%</p>	0 %
<p>Recovery of Chemical</p>	93-99%
<p>Raw data included?</p>	Yes

Mortality

Concentration (ppm)		Number of Organisms	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			2	24	48	96
Control	No Detect	20	0	0	0	
Solvent Control						
1.3	1.2	20	0	0	0	
2.2	2.2	20	0	0	1	
3.6	3.5	20	0	0	20	
6.0	5.8	20	0	13	20	
10	9.8	20	0	20	20	

DP Barcode: D197112

MRID No.: 429329-01

Other Significant Results:

B. Statistical Results

Method: Binomial

48-hr LC₅₀: 2.6 ppm ai 95% C.I.: _____ - _____ ppm ai

Probit Slope: _____ NOEC: 1.2 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	2.6 (2.2 - 3.5) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	_____ (_____ - _____) ppm ai
Probit LC ₅₀ (95% C.I.)	_____ (_____ - _____) ppm ai
Probit Slope	
NOEC	1.2 ppm ai (observed)

15. REVIEWER'S COMMENTS:

This study is scientifically sound. The modified test design was acceptable and provided good results. The analytical procedure appeared to be adequate, however a qualified chemist should review the procedure to confirm its suitability. Methyl Bromide is moderately toxic to *Daphnia magna* with an LC50 of 2.6 mg a.i./L and an NOEC of 1.2 mg a.i./L.