

DATA EVALUATION RECORD
§ 72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** Mepiquat Chloride PC Code No.: 109101
2. **TEST MATERIAL:** Mepiquat Chloride Purity: 54.6 %
3. **CITATION** Authors: Drottar, Kurt R., James P. Swigert, and Catherine M. Holmes.
Title: Mepiquat chloride: A 48-hour static acute toxicity test with the Cladoceran (*Daphnia magna*).
Study Completion Date: November 8, 1994
Laboratory: Wildlife International Ltd.
Sponsor: BASF Corporation
Laboratory Report ID: 147A-118
MRID No.: 434710-01
DP Barcode: D210498

4. **REVIEWED BY:** William S. Rabert, Biologist, EEB, EFED

Signature: *William S. Rabert* Date: 10/10/95

5. **APPROVED BY:** Harry Craven, Head of Section 4, EEB, EFED

Signature: *Harry Craven* Date: 10/12/95

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Daphnia magna*
Age of Test Organism: < 24 hours old
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

Results Synopsis:

LC₅₀: 106 ppm a.i. 95% C.I.: 92 - 151 ppm a.i.
NOEL: 44 ppm a.i. Probit Slope: 6.2

8. **ADEQUACY OF THE STUDY**

A. **Classification:** Core

B. **Rationale:** N/A

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C. Repairability: N/A

9. Guideline Deviations

1. Water hardness was medium-hard, 133 mg/L, instead of soft water 40- 48 mg/L. Since mepiquat chloride is a quaternary ammonium compound, its toxicity may be affected by water hardness. No mortality occurred in acute mysid shrimp test with a water hardness (salinity) of 20,000 mg/L.
2. Test water pH was 8.2 - 8.3 units, instead of the recommended pH of 7.2 -7.6 units. The pH level is likely to have a strong affect on the toxicity of cationic compounds, such as mepiquat chloride.

10. SUBMISSION PURPOSE: Reregistration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Yes
Life Stage Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 th instar.	1st instar
Supplier	Laboratory cultures
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	14 days

Guideline Criteria	Reported Information
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Yes/No/Not reported (if yes, describe)
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	10 hours prior to testing with no sign of disease
Feeding No feeding during the study.	Neonates not fed during the test
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	not reported

C. Test System:

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water.	well water
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	20.0 - 20.2 °C
pH Prefer 7.2 to 7.6.	8.2 - 8.3
Dissolved Oxygen Static: ≥ 60% during 1 st 48 h and ≥ 40% during 2 nd 48 h, flow-through: ≥ 60%.	8.4 - 8.6 (lowest % DO at 48 hours)
Total Hardness Prefer 40 to 48 mg/L as CaCO ₃ .	136 mg/L as CaCO ₃

Guideline Criteria	Reported Information
<p>Test Aquaria</p> <p>1. <u>Material</u>: Glass or stainless steel.</p> <p>2. <u>Size</u>: 250 ml (daphnids and midges) or 3.9 L (1 gal).</p> <p>3. <u>Fill volume</u>: 200 ml (daphnids and midges) or 2-3 L.</p>	<p>glass beakers in larger glass beaker</p> <p>300 ml</p> <p>1 L of test water in 2-L glass beaker</p>
<p>Type of Dilution System</p> <p>Must provide reproducible supply of toxicant.</p>	<p>static test; good replication of test concentrations</p>
<p>Flow Rate</p> <p>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.</p>	<p>N/A vol/24 hours</p>
<p>Biomass Loading Rate</p> <p>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.</p>	<p>Unreported</p>
<p>Photoperiod</p> <p>16 hours light, 8 hours dark.</p>	<p>16 hours of light; 8 hours of dark</p>
<p>Solvents</p> <p>Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.</p>	<p>No solvent</p>

D. Test Design:

Guideline Criteria	Reported Information
<p>Range Finding Test</p> <p>If $LC_{50} > 100$ mg/L, then no definitive test is required.</p>	<p>Yes; $EC_{50} > 60$ ppm (0 deaths) 120 ppm (100 % death) nominal concentrations only</p>

<p><u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.</p>	<p>0, 16, 26, 43, 72, and 120 mg a.i./L</p>
<p><u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.</p>	<p>20; 10 daphnids per replicate</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>
<p><u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary > 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>continuously measured in control; varied < 1.0 °C D.O. and pH measured every 24 hours</p>
<p><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</p>	<p>Measured Concentrations</p>

12. REPORTED RESULTS:

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

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Percent Recovery of Chemical	102 - 109 % at 0 hours 72 - 133 % at 48 hours
Raw data included?	Yes

Mortality

Concentration (ppm a.i.)		Number of Organisms	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	< LOQ	20	0	0		
Solvent Control	N/A	--	--	--		
16	16	20	0	0		
26	28	20	0	0		
43	44	20	0	0		
72	79	20	5	5		
120	109	20	0	10		

Other Significant Results: Some surviving daphnids were affected at 79 and 109 mg/L. Symptoms included lethargic effects.

B. Statistical Results

Method: Probit Method

48-hr LC₅₀: 106 ppm a.i.

95% C.I.: 92 - 151 ppm a.i.

Probit Slope: not reported

NOEC: 44 ppm a.i.

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
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Binomial Test LC_{50} (C.I.)	N/A (79 - infinity) ppm a.i.
Moving Average Angle LC_{50} (95% C.I.)	108 (89 - infinity) ppm a.i.
Probit LC_{50} (95% C.I.)	106 (92 - 151) ppm a.i.
Probit Slope	6.2
NOEC	44 ppm a.i.

14. **REVIEWER'S COMMENTS:** Both water hardness and pH levels were higher than recommended for daphnids. Either may affect the toxicity of quaternary compounds like mepiquat chloride. It is uncertain whether the deviations in test conditions increased or decreased the toxicity. I expect that the higher pH level may have increased the toxicity of the test substance. Comparison of Columbia National Fisheries data (Mayer & Ellersieck, 1986) at 3 pH levels (6.5, 7.5 and 8.5) for 2 quaternary compounds show pH effects, but in opposite directions. Fathead minnow LC_{50} values for 2 series of 3 pH levels with 2,4-D diaminyllamine salt were most toxic at pH 6.5 units; for 2 series of 3 pH levels with 2,4-D dodecyl/tetradodecyl amine salt were most toxic at pH 8.5 units. Hence, it is unclear what effect the higher pH and water hardness levels had on the toxicity values for mepiquat chloride.

William Rabert Mepiquat Chloride 54.6 % a.i. Daphnia magna EC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
109	20	10	50	58.80985
79	20	5	25	2.069473
44	20	0	0	9.536742E-05
28	20	0	0	9.536742E-05
16	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 79 AND +INFINITY 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 109

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
1	1.51662	108.9999	88.73033 +INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
7	.3894105	1

GOODNESS OF FIT PROBABILITY
 .9365286

SLOPE = 6.239455
 95 PERCENT CONFIDENCE LIMITS = 2.345863 AND 10.13305

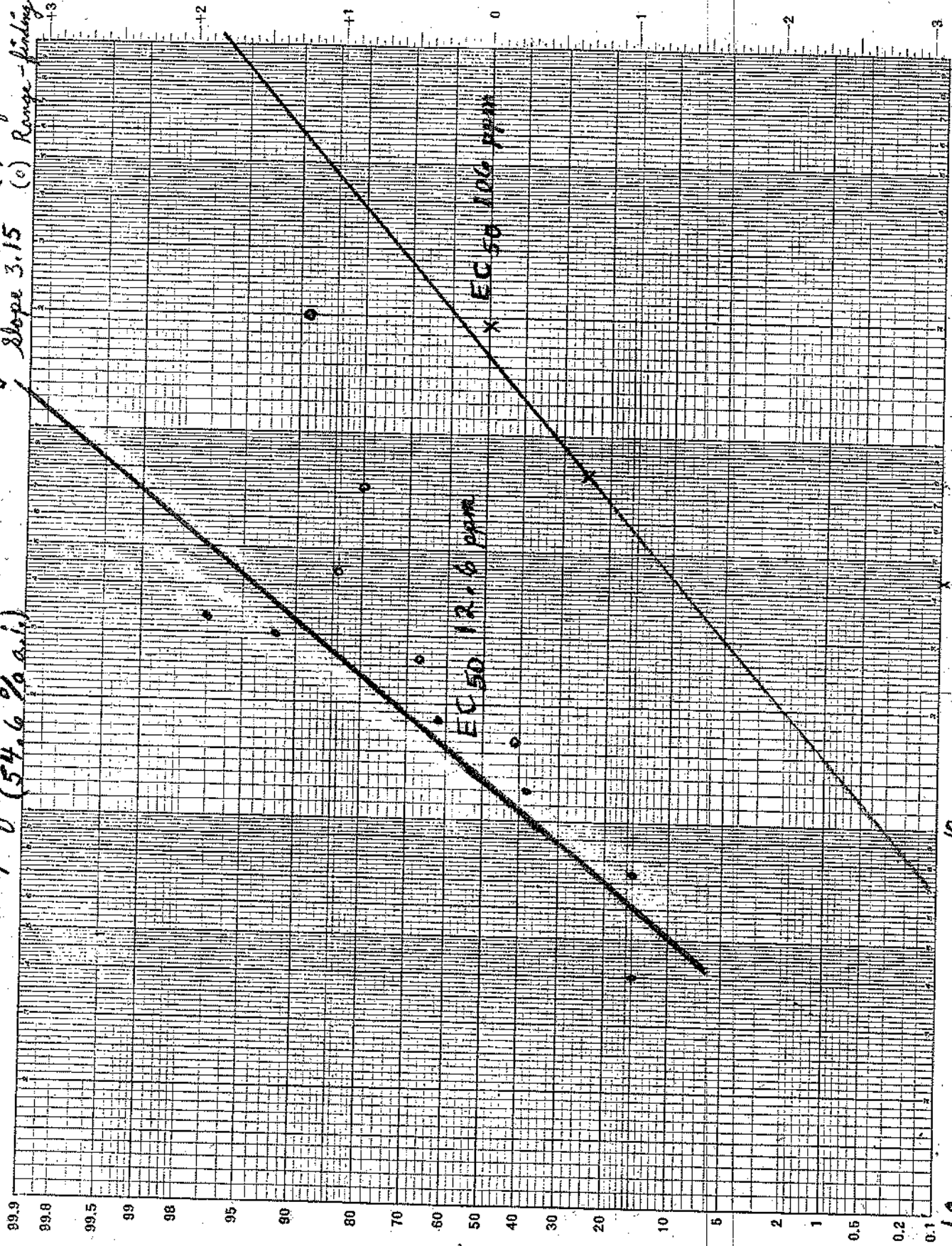
LC50 = 106.2534
 95 PERCENT CONFIDENCE LIMITS = 91.66772 AND 150.8358

LC10 = 66.49662
 95 PERCENT CONFIDENCE LIMITS = 37.7517 AND 78.89927

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Mepiquat Chloride
(54.6% a.i.)

— *Daphnia magna* EC50 (X)
— Eastern Oyster EC50 (•)
Slope 3.15 (o) Range-finding Test



% Effect

Test Concentration