

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD  
AQUATIC INVERTEBRATE LIFE CYCLE TEST  
GUIDELINE 72-4(B)

1. CHEMICAL: Mepiquat chloride PC Code No.: 109101

2. TEST MATERIAL: Mepiquat-chloride Purity: 99%

3. CITATION:

Authors: Dr. Elendt-Schneider  
Title: Determination of the Chronic Toxicity of Mepiquat Chloride to the Water Flea *Daphnia magna* Straus

Study Completion Date: April 6, 1993

Laboratory: BASF Aktiengesellschaft, Ludwigshafen, Germany

Sponsor: BASF Corporation, Agricultural Products Group, Research Triangle Park, NC

Laboratory Report ID: 93/10316

MRID No.: 431559-02

DP Barcode: Not available.

4. REVIEWED BY: Rosemary Graham Mora, M.S., Staff Scientist  
KBN Engineering and Applied Sciences, Inc.

Signature: *R. Mora* Date: 9/12/96

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,  
KBN Engineering and Applied Sciences, Inc.

Signature: *P. Kosalwat* Date: 9/12/96

5. APPROVED BY: Already checked out of PRAT. Do secondary if we need to use data. KJC 12/21/99

Signature: Date:

6. STUDY PARAMETERS:

Age of Test Organism: 2-24 hours  
Definitive Test Duration: 21 days  
Study Method: Static-Renewal  
Type of Concentrations: Nominal

7. CONCLUSIONS: This study is scientifically sound but does not fulfill the guideline requirements for a freshwater invertebrate life-cycle test using *Daphnia magna*. Chemical analysis was conducted on only three test concentrations, and the growth of first generation daphnids was not measured at test termination.

**Results Synopsis:** Results were based on nominal concentrations.

NOEC: 12.5 ppm

LOEC: 25 ppm

MATC: 17.7 ppm

LOEC's for specific effects:

Neonates Produced: 50 ppm  
Daphnid Survival: 25 ppm  
Growth (weight): Not measured.  
Growth (length): Not measured.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental.

B. Rationale: Only solutions from three test concentrations were analyzed and the growth of first generation daphnids was not measured at test termination.

C. Repairability: No.

9. GUIDELINE DEVIATIONS: This study followed the OECD guidelines which are somewhat different from the EPA's SEP. Significant deviations from the SEP are noted as follows:

1. While the study consisted of eight test concentrations, chemical analysis was conducted on only three concentrations. Therefore, the results were reported as nominal concentrations.
2. The growth (length and dry weight) of first generation daphnids was not measured at test termination. The measurement of these growth parameters is required by the guidelines.
3. Ten replicates were used for each treatment and the control. Each replicate contained one daphnid. This design differs from that suggested in the EPA's SEP. However, this study design is acceptable.
3. The pH (7.6-8.4) was at times slightly higher than recommended (7.6-8.0).
4. The hardness, alkalinity, and conductivity of the test solutions were not measured weekly in one concentration and one control as recommended.
5. The fill volume of the test beakers (50 ml) was less than recommended (80 ml).

10 SUBMISSION PURPOSE:11. MATERIALS AND METHODS:

## A. Test Organisms/Acclimation:

Guideline Criteria	Reported Information
<u>Species</u> <i>Daphnia magna</i>	<i>Daphnia magna</i> Straus
<u>Source</u>	In-house cultures (originally obtained from Institut National de Recherche Chimique Appliquée, France).
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Parental cultures were maintained in the dilution water under "standard conditions in the laboratory."
<u>Parental Acclimation Period</u> At least 21 days.	Not reported.
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	Parental stock was 2-4 weeks old.
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Daphnids were fed daily with a concentrate of <i>Scenedesmus subspicatus</i> .
<u>Food Concentration</u> 5 mg/L (dry wt.) of synthetic food or $10^6$ cells/L of algae is recommended.	Approx. $\leq 0.3$ mL/50 mL of test solution were added to the test vessels up to three times daily.
<u>Were daphnids in good health during acclimation period?</u>	Not reported.

## B. Test System:

Guideline Criteria	Reported Information
<p><b><u>Test Water</u></b> Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</p>	<p>Synthetic medium M4 (reconstituted ultrapure, deionized water).</p>
<p><b><u>Water Temperature</u></b> 20°C ±2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.</p>	<p>Range: 19.6-21.0°C</p>
<p><b><u>pH</u></b> 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.</p>	<p>Range: 7.6-8.4</p>
<p><b><u>Total Hardness</u></b> 160 to 180 mg/L as CaCO<sub>3</sub> is recommended.</p>	<p>167-243 mg/L as CaCO<sub>3</sub></p>
<p><b><u>Dissolved Oxygen</u></b> <b>Renewal:</b> must not drop below 50% for more than 48 hours. <b>Flow-through:</b> ≥ 60% throughout test.</p>	<p>≥80% of saturation</p>
<p><b><u>Test Vessels or Compartments</u></b> 1. <b>Material:</b> Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <b>Size:</b> 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.</p>	<p>1. Glass  2. 100-mL beakers containing 50 mL of test solution.</p>
<p><b><u>Covers</u></b> <b>Renewal:</b> Test vessels should be covered with a glass plate. <b>Flow-through:</b> openings in test compartments should be covered with mesh nylon or stainless steel screen.</p>	<p>Each test beaker was covered with a cap.</p>

Guideline Criteria	Reported Information
<p><b><u>Type of Dilution System</u></b> Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>The test was conducted under static renewal conditions.</p>
<p><b><u>Flow Rate</u></b> 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</p>
<p><b><u>Aeration</u></b> Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	<p>The dilution water was aerated to oxygen saturation.</p>
<p><b><u>Photoperiod</u></b> 16-hour light/8-hour dark.</p>	<p>16-hour light/8-hour dark</p>
<p><b><u>Solvents</u></b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.</p>	<p>Solvent: None Maximum conc.: N/A</p>

**C. Test Design:**

Guideline Criteria	Reported Information
<p><b><u>Duration</u></b> 21 days</p>	<p>21 days</p>
<p><b><u>Nominal Concentrations</u></b> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.</p>	<p>Control and 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 mg/L.</p>

Guideline Criteria	Reported Information
<p><b>Number of Test Organisms</b> 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.</p>	<p>10 daphnids/level; 10 test chambers with 1 daphnid each.</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>Not reported.</p>
<p><b>Renewal</b> Parent daphnids in all beakers must be transferred to containers with fresh test solution (&lt;4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).</p>	<p>The test solutions were renewed every Monday, Wednesday and Friday.</p>
<p><b>Water Parameter Measurements</b> 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.</p>	<p>Dissolved oxygen and pH were measured in one replicate of each treatment and the control at the start of the test and at each change of test solution in the 48- or 72-hour-old test solution.</p> <p>Alkalinity, hardness, and conductivity were measured in the dilution water only.</p> <p>Temperature was recorded continuously in an extra vessel located close to the test vessels.</p>
<p><b>Chemical Analysis</b> Needed 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>Samples of the freshly prepared solution and the corresponding 48- or 72-hour-old solution of three test concentrations (3.13, 12.5, and 100 ppm) were analyzed weekly.</p>

**12. REPORTED RESULTS:****A. General Results:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes; however, the GLP statement indicated compliance with OECD principles of good laboratory practice (February 4, 1983).
<u>Control Mortality</u> ≤ 30%	0%
Did daphnids in each control produce at least 40 young after 21 days?	Yes
Were no ephippia produced in any of the controls?	Not reported.
<u>Data Endpoints</u> - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs.	- Survival of first-generation daphnids; - Live young/parent daphnid - Dead young/parent daphnid - Aborted eggs/parent daphnid
Raw data included?	Yes



Effects Data:

Toxicant Concentration (ppm)		Percent Survival of Parent Daphnids (surviving daphnids/total)	Mean # of Young per Live Female	Mean # of Young per female Reprod. Day*	Length (mm)	Weight (mg)
Nominal	Mean Measured					
Control	N/D	100% (10/10)	132.4	9.5	N/M	N/M
0.78	N/M	100% (10/10)	129.6	9.3	N/M	N/M
1.56	N/M	100% (10/10)	133.5	9.5	N/M	N/M
3.13	3.03	100% (10/10)	129.1	9.4	N/M	N/M
6.25	N/M	100% (10/10)	127.8	9.1	N/M	N/M
12.5	12.6	100% (10/10)	116.5	8.3	N/M	N/M
25	N/M	30% (3/10)	138.3	8.0	N/M	N/M
50	N/M	0% (0/10)	0	3.6	N/M	N/M
100	100.7	0% (0/10)	0	0	N/M	N/M

\* = Reviewer's calculation of reproduction.  
 N/D = Non detectable.  
 N/M = Not measured.

Toxicity Observations: None reported.

**B. Statistical Results:**

Most sensitive endpoint: Survival.

Endpoint	Method	NOEC (ppm)	LOEC (ppm)
Survival	Not reported.	12.5	25
Reproduction	Duncan's multiple range test	≥25	>25

Endpoint	Method	NOEC (ppm)	LOEC (ppm)
Weight	Not measured.	-	-
Length	Not measured.	-	-

**13. VERIFICATION OF STATISTICAL RESULTS:**

Most sensitive endpoint: Survival.

Endpoint	Method	NOEC (ppm)	LOEC (ppm)
Survival	Visual inspection	12.5	25
Reproduction	ANOVA with Dunnett's and Bonferroni's test	25	50
Weight	-	-	-
Length	-	-	-

**14. REVIEWER'S COMMENTS:** This study is scientifically sound but does not fulfill the guideline requirements for a freshwater invertebrate life-cycle test using *Daphnia magna*. While the test consisted of eight concentrations, chemical analysis was conducted on only three test concentrations. Also, the growth of first generation daphnids was not measured at test termination. This study is classified as **SUPPLEMENTAL**.

Based on the most sensitive endpoint measured in this test (survival), the MATC was between 12.5 and 25 ppm nominal concentrations. The geometric mean MATC was 17.7 ppm.