

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

1. CHEMICAL: SAN 582H PC Code No.: 129051

2. TEST MATERIAL: Dimethenamid Purity: 97%

3. CITATION

Authors: Catherine M. Holmes and James P. Swigert  
Title: A Flow-through Life-Cycle Toxicity Test  
With the Cladoceran (Daphnia magna)  
Study Completion Date: 1992  
Laboratory: Wildlife International LTD., Easton, MD  
Sponsor: Sandoz Agro, Inc.  
Laboratory Report ID: 131A-147A  
MRID No.: 43914301  
DP Barcode: D223359

4. REVIEWED BY: Joanne S. Edwards, Entomologist, EEB, EFED

Signature: Date:

5. APPROVED BY: Leslie Touart, Head of Section 1, EEB, EFED

Signature: Date:

6. STUDY PARAMETERS

Scientific Name of Test Organism: Daphnia magna  
Age of Test Organism: Instars <24-hr old  
Definitive Test Duration: 21 days  
Study Method: Flow-through  
Type of Concentrations: Mean measured

7. CONCLUSIONS:

Results Synopsis based on survival and growth (weight)  
NOEC: 1.36 ppm LOEC: 2.51 ppm  
MATC: 1.85 ppm

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

No major guideline deviations noted.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<b>Species</b> <i>Daphnia magna</i>	<i>Daphnia magna</i>
<b>Source</b>	laboratory-reared culture
<b>Parental Acclimation Conditions</b> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	yes
<b>Parental Acclimation Period</b> At least 21 days.	daphnids were cultured under test conditions
<b>Age of Parental Stock</b> At least 10-12 days old at the beginning of the acclimation period.	not specified
<b>Food</b> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	daphnids were fed a mixture of yeast, Cerophyll and trout chow, and a suspension of freshwater green alga ( <i>S. capricornutum</i> )
<b>Food Concentration</b> 5 mg/L (dry wt.) of synthetic food or 10 <sup>8</sup> cells/L of algae is recommended.	food concentration was not specified; daphnids were fed 3X daily.
<b>Were daphnids in good health during acclimation period?</b>	yes

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>well water located on site which was filtered through a sand filter (25 um) and stored in a tank, then aerated and filtered prior to delivery to the system.</p> <p>analysis for pesticides and metals in well water was made June, 1990.</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>20 ± 1°C throughout the study</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>7.6 to 7.9</p>
<p><b><u>Total Hardness</u></b>  160 to 180 mg/L as CaCO<sub>3</sub> is recommended.</p>	<p>136 - 144 mg/L as CaCO<sub>3</sub></p>
<p><b><u>Dissolved Oxygen</u></b>  <u>Renewal</u>: must not drop below 50% for more than 48 hours.  <u>Flow-through</u>: • 60% throughout test.</p>	<p>&gt;60% throughout the study</p>
<p><b><u>Test Vessels or Compartments</u></b>  1. <u>Material</u>: Glass, No. 316 stainless steel, or perfluorocarbon plastics  2. <u>Size</u>: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.</p>	<p>300 mL glass beakers</p>
<p><b><u>Covers</u></b>  <u>Renewal</u>: Test vessels should be covered with a glass plate.  <u>Flow-through</u>: openings in test compartments should be covered with mesh nylon or stainless steel screen.</p>	<p>Nytex screen covers were used</p>
<p><b><u>Type of Dilution System</u></b>  Must provide reproducible supply of toxicant. Inter-mittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>continuous-flow diluter system</p>

Guideline Criteria	Reported Information
<p><b>Flow Rate</b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.</p>	<p>approx. 14 volume additions daily</p> <p>flow rates varied by no more than <math>\pm 10\%</math></p>
<p><b>Aeration</b> Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	<p>dilution water was aerated prior to delivery to the test system; it was not indicated if the test tanks were aerated</p>
<p><b>Photoperiod</b> 16 hours light, 8 hours dark.</p>	<p>16 hours light, 8 hours dark</p>
<p><b>Solvents</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: DMF maximum conc.: 0.1 ml/L</p>

### C. Test Design

Guideline Criteria	Reported Information
<p><b>Duration</b> 21 days</p>	<p>21 days</p>
<p><b>Nominal Concentrations</b> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.</p>	<p>five nominal concentrations of 0.312, 0.625, 1.25, 2.50 and 5.00 ppm; solvent control and dilution water control</p>
<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>for each treatment level and control: 7 test chambers containing 1 daphnid each, and 3 test chambers containing 5 daphnids each.</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>yes</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>N/A</p>

Guideline Criteria	Reported Information
<p><b><u>Water Parameter Measurements</u></b></p> <p>1. Dissolved oxygen must be measured at each concentration at least once a week.</p> <p>2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control.</p> <p>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>all criteria were met</p>
<p><b><u>Chemical Analysis</u></b></p> <p>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>from each individual test chamber, the concentration of SAN 582H was determined prior to test initiation, on day 0, and at weekly intervals thereafter</p>

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	yes
Control Mortality • 30%	<10%
Did daphnids in each control produce at least 40 young after 21 days?	yes
Were no ehippia produced in any of the controls?	not indicated
<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.	mortality of 1st generation daphnids  average # young produced per female  length and dry weight of each first generation daphnid  observations of other effects or clinical signs.
Raw data included?	excerpted

Effects Data

Toxicant Concentration (ppm)		% Dead or Immobile (21 Days)	Mean Young per Female per Repro. Day	Mean Length (mm) / Dry Weight (mg)
Nominal	Measured			
Control	-	9	64	3.83/0.45
Solvent Control	-	0	77	3.87/0.49
0.312	0.33	5	91	3.08/0.50
0.625	0.72	0	77	4.01/0.55
1.25	1.36	9	77	3.90/0.43
2.50	2.51	68	78	3.74/0.29
5.00	4.94	100	-	-

Toxicity Observations:

**B. Statistical Results:** Kruskal-Wallis test (since dry weight data failed to meet assumptions of normality and homogeneity of variances a square root transformation was performed). Due to the level of mortality at the 2.51 and 4.94 ppm test concentrations, reproduction, length and dry weight data were not analyzed at these test concentration levels. Solvent and negative control data were pooled since no statistically significant difference between the two was noted using a t-test.

Survival:

The 21-day survival values in the two highest groups were significantly lower when compared to the control (p <0.05).

Reproduction:

There was no treatment-related effects at the 0.33, 0.72 and 1.36 ppm levels that was statistically significant.

Growth:

There was no treatment-related effects (length and weight) at the 0.33, 0.72 and 1.36 ppm levels that was statistically significant. The authors reported that, although mortality in the 2.51 ppm test concentration precluded a full evaluation of effects upon growth, there appeared to be a reduction in dry weight, but that effects on length were not noted at that level.

The study authors concluded that based on marked effects on survival at the 2.51 and 4.94 ppm test concentrations, the NOEC for the study is 1.36 ppm and the LOEC is 2.51 ppm (MATC = 1.85 ppm).

### 13. VERIFICATION OF STATISTICAL RESULTS

Most sensitive endpoint:

Endpoint	Method	NOEC (ppm)	LOEC (ppm)	MATC (ppm)
Survival	visual observation of the data <sup>1</sup>	1.36	2.51	1.85
Reproduction (total # of progeny produced per replicate)	William's	2.51	-	-
Weight	William's <sup>2</sup>	1.36	2.51	1.85
Length	William's <sup>2</sup>	2.51	-	-

<sup>1</sup> significant mortality - after 21 days, 100% mortality in 4.94 ppm treatment group and 68% mortality in 2.51 ppm treatment group.

<sup>2</sup> length/weight/neonate production analyses did not include 2.51 , due to 100% mortality at that level.

### 14. REVIEWER'S COMMENTS:

The following study deviations were noted:

- o The recommended hardness of water is 160 to 180 mg/L as CaCO<sub>3</sub>. The hardness of the water in this test was slightly lower (136 - 144 as CaCO<sub>3</sub>).
- o the approximate age of the parental stock used was not specified.
- o the food concentration was not specified.
- o the well water analysis for pesticides and metals was made almost two years prior to study initiation.
- o it was not indicated if the test tanks were aerated; they should not have been aerated during the study.
- o it was not indicated if any ehippia were produced in the controls.

Based on a statistically significant reduction (p <0.05) in reproduction capacity as measured by growth (weight) and significant mortality of daphnid progeny, the NOEC, LOEC, and MATC for daphnids exposed to SAN 582H for 21 days is 1.36 ppm, 2.51 ppm, and 1.85 ppm, respectively.