DATA EVALUATION RECORD
ALGAE OR DIATOM EC50 TEST
GUIDELINE 123-2 (TIER II)

1. CHEMICAL: Captan
   PC Code No.: 081301

2. TEST MATERIAL: Captan technical
   Purity: 99.8%

3. CITATION:
   Authors: K.R. Drottar and H.O. Krueger
   Title: Captan: A 96-Hour Toxicity Test with the Freshwater Alga (Anabaena flos-aquae)
   Study Completion Date: April 8, 1999
   Laboratory: Wildlife International Ltd., Easton, MD
   Laboratory Report ID: 493A-101A
   DP Barcode: D255807
   MRID No.: 448065-01

4. REVIEWED BY: Mark Mossler, M.S., Environmental Scientist, Golder Associates Inc.
   Signature: Date:

   APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.
   Signature: Date:

5. APPROVED BY: Brian Montague, Fisheries Biologist
   Environmental Fate and Effects Division, 7507C
   Signature: Date: Oct. 29, 1999

6. STUDY PARAMETERS:
   Definitive Test Duration: 96 hours
   Type of Concentrations: Initial measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test using Anabaena flos-aquae. Core classification.
   Results Synopsis
   EC50: 1.2 ppm ai
   95% C.I.: 0.83 - 1.6 ppm ai
   Probit Slope: N/A
   NOEC: < 0.23 ppm ai
8. **ADEQUACY OF THE STUDY:**
   A. **Classification:** Core.
   B. **Rationale:** N/A.
   C. **Repairability:** N/A.

9. **GUIDELINE DEVIATIONS:**
   1. The test length (96 hours) was less than recommended (120 hours).

10. **SUBMISSION PURPOSE:** To support captan use on crops where aquatic habitats and plantlife is expected to be subject to exposure.

11. **MATERIALS AND METHODS:**
    A. **Test Organisms**

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td><em>Anabaena flos-aquae</em></td>
</tr>
<tr>
<td><strong>Initial Number of Cells</strong></td>
<td></td>
</tr>
<tr>
<td>3,000 - 10,000 cells/mL</td>
<td>10,000 cells/mL</td>
</tr>
<tr>
<td><strong>Nutrients</strong></td>
<td></td>
</tr>
<tr>
<td>Standard formula, e.g. 20XAAP</td>
<td>Freshwater algal medium</td>
</tr>
</tbody>
</table>

    B. **Test System**

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvent</strong></td>
<td>DMF (100 μL/L)</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td></td>
</tr>
<tr>
<td>Skeletonema: 20°C</td>
<td>24.0-25.8°C</td>
</tr>
<tr>
<td>Others: 24-25°C</td>
<td></td>
</tr>
<tr>
<td><strong>Light Intensity</strong></td>
<td></td>
</tr>
<tr>
<td>Anabaena: 2.0 KLux (±15%)</td>
<td>1.9-2.4 KLux</td>
</tr>
<tr>
<td>Others: 4.0-5.0 KLux (±15%)</td>
<td></td>
</tr>
</tbody>
</table>
### Guideline Criteria | Reported Information
---|---
**Photoperiod**
Skeletonema:
14 h light, 10 h dark or 16 h light, 8 h dark
Others: Continuous | Continuous lighting
**pH**
Skeletonema: approx. 8.0
Others: approx. 7.5 | Initial: 7.4 - 7.5
| Final: 7.5 - 8.2

### C. Test Design

| Guideline Criteria | Reported Information |
---|---
**Dose range**
2X or 3X progression | 2X
**Doses**
at least 5 | 0.32, 0.64, 1.3, 2.6, and 5.1 mg ai/L
**Controls**
negative and/or solvent | Negative and solvent controls
**Replicates per dose**
3 or more | 3
**Duration of test**
120 hours | 96 hours
**Daily observations were made?** | Yes
**Method of Observations** | Cellular counts
**Maximum Labeled Rate** | Not reported

### 12. REPORTED RESULTS:

| Guideline Criteria | Reported Information |
---|---
Initial and terminal cell densities were |
<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>measured?</td>
<td>Yes</td>
</tr>
<tr>
<td>Control cell count at termination ≥2X initial count?</td>
<td>Yes</td>
</tr>
<tr>
<td>Initial chemical concentrations measured? (Optional)</td>
<td>Yes, samples collected at test initiation were analyzed by GC.</td>
</tr>
<tr>
<td>Raw data included?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Measured Concentrations**

<table>
<thead>
<tr>
<th>Nominal</th>
<th>Toxicant Concentration (mg ai/L)</th>
<th>Percent of Nominal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hour</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>&lt;LOQ</td>
<td>N/A</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>&lt;LOQ</td>
<td>N/A</td>
</tr>
<tr>
<td>0.32</td>
<td>0.23</td>
<td>73</td>
</tr>
<tr>
<td>0.64</td>
<td>0.51</td>
<td>80</td>
</tr>
<tr>
<td>1.3</td>
<td>0.88</td>
<td>68</td>
</tr>
<tr>
<td>2.6</td>
<td>2.16</td>
<td>83</td>
</tr>
<tr>
<td>5.1</td>
<td>4.89</td>
<td>96</td>
</tr>
</tbody>
</table>

Note: Mean method validation recovery = 98% and LOQ = 0.125 ppm ai

**Dose Response**

<table>
<thead>
<tr>
<th>Initial Measured Concentration (mg ai/L)</th>
<th>96-hr. Average Cell Density (x 10^4 cells/mL)</th>
<th>Inhibition* (%)</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>161.7</td>
<td>N/A</td>
<td>8.2</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>148.0</td>
<td>N/A</td>
<td>8.0</td>
</tr>
<tr>
<td>0.23</td>
<td>137.7</td>
<td>11</td>
<td>7.9</td>
</tr>
<tr>
<td>0.51</td>
<td>100.0a</td>
<td>35</td>
<td>7.9</td>
</tr>
<tr>
<td>Initial Measured Concentration (mg ai/L)</td>
<td>96-hr. Average Cell Density (x 10^4 cells/mL)</td>
<td>Inhibition* (%)</td>
<td>Final pH</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>---------------------------------------------</td>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>0.88</td>
<td>84.3a</td>
<td>46</td>
<td>7.8</td>
</tr>
<tr>
<td>2.16</td>
<td>51.8a</td>
<td>67</td>
<td>7.5</td>
</tr>
<tr>
<td>4.89</td>
<td>7.4a</td>
<td>95</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Compared to the pooled control.

aSignificantly reduced when compared to the pooled control (p<0.05).

Other Significant Results: The only sign of test material toxicity was enlarged cells at the 4.89 ppm ai treatment level. Cells from this solution were observed to recover to control levels after three days of reculturing in untreated algal medium, indicating algistatic effects.

Statistical Results for Cell Density

Statistical Method: Linear interpolation was used for EC₅₀ estimation and Bonferroni's test was used for NOEC determination.

EC₅₀: 1.2 ppm ai
Probit Slope: N/A
95% C.I.: 0.91 - 1.7 ppm ai
NOEC: 0.23 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Nonlinear regression was used for EC₅₀ estimation and Williams' test was used for NOEC determination. Comparison was made to the solvent control.

EC₅₀: 1.2 ppm ai
Probit Slope: N/A
95% C.I.: 0.83 - 1.6 ppm ai
Observed NOEC: <0.23 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. Based on initial measured concentrations, the 96-hour EC₅₀ was 1.2 ppm ai. The observed NOEC was determined to be < 0.23 ppm ai, based on 11% cell density reductions beginning at this dosage level which appeared dose related and were below both pooled and solvent control cell density levels. This study can be categorized as Core.
### Anabaena cell density

**File:** ana  
**Transform:** NO TRANSFORMATION

#### WILLIAMS TEST (Isotonic regression model)  
**TABLE 1 OF 2**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ORIGINAL</th>
<th>TRANSFORMED</th>
<th>ISOTONIZED</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDENTIFICATION</td>
<td>N</td>
<td>MEAN</td>
<td>MEAN</td>
</tr>
<tr>
<td>Sol. Con.</td>
<td>3</td>
<td>1480000.000</td>
<td>1480000.000</td>
</tr>
<tr>
<td>0.23 ppm ai</td>
<td>3</td>
<td>1376666.667</td>
<td>1376666.667</td>
</tr>
<tr>
<td>0.51 ppm ai</td>
<td>3</td>
<td>1000000.000</td>
<td>1000000.000</td>
</tr>
<tr>
<td>0.88 ppm ai</td>
<td>3</td>
<td>843333.333</td>
<td>843333.333</td>
</tr>
<tr>
<td>2.16 ppm ai</td>
<td>3</td>
<td>518333.333</td>
<td>518333.333</td>
</tr>
<tr>
<td>4.89 ppm ai</td>
<td>3</td>
<td>74333.333</td>
<td>74333.333</td>
</tr>
</tbody>
</table>

#### WILLIAMS TEST (Isotonic regression model)  
**TABLE 2 OF 2**

<table>
<thead>
<tr>
<th>IDENTIFICATION</th>
<th>MEAN</th>
<th>WILLIAMS</th>
<th>DEGREES OF FREEDOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol. Con.</td>
<td>1480000.000</td>
<td>1.78</td>
<td>k= 1, v=12</td>
</tr>
<tr>
<td>0.23 ppm ai</td>
<td>1376666.667</td>
<td>*1.87</td>
<td>k= 2, v=12</td>
</tr>
<tr>
<td>0.51 ppm ai</td>
<td>1000000.000</td>
<td>*1.90</td>
<td>k= 3, v=12</td>
</tr>
<tr>
<td>0.88 ppm ai</td>
<td>843333.333</td>
<td>*1.92</td>
<td>k= 4, v=12</td>
</tr>
<tr>
<td>2.16 ppm ai</td>
<td>518333.333</td>
<td>*1.93</td>
<td>k= 5, v=12</td>
</tr>
<tr>
<td>4.89 ppm ai</td>
<td>74333.333</td>
<td>*1.98</td>
<td>k= 6, v=12</td>
</tr>
</tbody>
</table>

$s = 105235.714$

Note: df used for table values are approximate when $v > 20.$

---

**anabaena cell density**  

**3**  

16:53 Wednesday, September 15, 1999

<table>
<thead>
<tr>
<th>OBS</th>
<th>CONC</th>
<th>LOG_CONC</th>
<th>Y1</th>
<th>Y2</th>
<th>Y3</th>
<th>Y4</th>
<th>Y5</th>
<th>Y6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>1630000</td>
<td>1400000</td>
<td>1410000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.23</td>
<td>-0.63827</td>
<td>1340000</td>
<td>1420000</td>
<td>1370000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.51</td>
<td>-0.29243</td>
<td>1120000</td>
<td>1030000</td>
<td>850000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.88</td>
<td>-0.05552</td>
<td>845000</td>
<td>825000</td>
<td>860000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.16</td>
<td>0.33445</td>
<td>695000</td>
<td>365000</td>
<td>495000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.89</td>
<td>0.68931</td>
<td>111000</td>
<td>48000</td>
<td>64000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**anabaena cell density**  

**4**  

16:53 Wednesday, September 15, 1999

**MODEL:**  
COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)

**WEIGHTED REGRESSION**

16:53 Wednesday, September 15, 1999

Non-Linear Least Squares Iterative Phase
NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics  

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Weighted SS</th>
<th>Weighted MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>3</td>
<td>15878000.00</td>
<td>5292666.67</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>432753.75</td>
<td>28850.25</td>
</tr>
<tr>
<td>Uncorrected Total</td>
<td>18</td>
<td>16310753.75</td>
<td></td>
</tr>
<tr>
<td>(Corrected Total)</td>
<td>17</td>
<td>9119342.76</td>
<td></td>
</tr>
</tbody>
</table>

Parameter Estimate Asymptotic Asymptotic 95 %

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Std. Error</td>
<td>Confidence Interval</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOG_EC50</td>
<td>0.065</td>
<td>0.067591</td>
<td>-0.0790</td>
<td>0.2091</td>
</tr>
<tr>
<td>SIGMA</td>
<td>0.456</td>
<td>0.059148</td>
<td>0.3297</td>
<td>0.5818</td>
</tr>
<tr>
<td>C0</td>
<td>1434651.17</td>
<td>99410.618726</td>
<td>1222763.2605</td>
<td>1646539.0856</td>
</tr>
</tbody>
</table>

Asymptotic Correlation Matrix

<table>
<thead>
<tr>
<th>Corr</th>
<th>LOG_EC50</th>
<th>SIGMA</th>
<th>C0</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOG_EC50</td>
<td>1</td>
<td>-0.707185222</td>
<td>-0.787459251</td>
</tr>
<tr>
<td>SIGMA</td>
<td>-0.707185222</td>
<td>1</td>
<td>0.5840980055</td>
</tr>
<tr>
<td>C0</td>
<td>-0.787459251</td>
<td>0.5840980055</td>
<td>1</td>
</tr>
</tbody>
</table>

anabaena cell density 5

MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
16:53 Wednesday, September 15, 1999

Plot of COUNT*LOG_CONC. Symbol used is 'o'.
Plot of PRED*LOG_CONC. Symbol used is '.'.

NOTE: 1355 obs had missing values. 1284 obs hidden.
Comparision of Means for NOEL Determination
Test if Treatment is Less Than Control

Wednesday, September 15, 1999

General Linear Models Procedure
Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOSE</td>
<td>6</td>
<td>0 0.23 0.51 0.88 2.16 4.89</td>
</tr>
</tbody>
</table>

Number of observations in data set = 36

NOTE: Due to missing values, only 18 observations can be used in this analysis.

General Linear Models Procedure
Dependent Variable: RESPONSE

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>5</td>
<td>4.20689E+12</td>
<td>8.41378E+11</td>
<td>75.97</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>1.32895E+11</td>
<td>1.10746E+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>17</td>
<td>4.33979E+12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-Square  C.V.  Root MSE  RESPONSE Mean
0.969378  11.92998  105235.7  882111.1

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOSE</td>
<td>5</td>
<td>4.20689E+12</td>
<td>8.41378E+11</td>
<td>75.97</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOSE</td>
<td>5</td>
<td>4.20689E+12</td>
<td>8.41378E+11</td>
<td>75.97</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
16:53 Wednesday, September 15, 1999

General Linear Models Procedure

<table>
<thead>
<tr>
<th>DOSE</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>1480000.00</td>
<td>130000.000</td>
</tr>
<tr>
<td>0.23</td>
<td>3</td>
<td>1376666.67</td>
<td>40414.519</td>
</tr>
<tr>
<td>0.51</td>
<td>3</td>
<td>1000000.00</td>
<td>137477.271</td>
</tr>
<tr>
<td>0.88</td>
<td>3</td>
<td>843333.33</td>
<td>17559.423</td>
</tr>
<tr>
<td>2.16</td>
<td>3</td>
<td>518333.33</td>
<td>166232.769</td>
</tr>
<tr>
<td>4.89</td>
<td>3</td>
<td>743333.33</td>
<td>32746.501</td>
</tr>
</tbody>
</table>

anabaena cell density

COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 12 MSE= 1.107E10
Critical Value of Dunnett's T= 2.502
Minimum Significant Difference= 214997

Comparisons significant at the 0.05 level are indicated by '***'.

<table>
<thead>
<tr>
<th>Simultaneous</th>
<th>Simultaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Difference</td>
<td>Upper Difference</td>
</tr>
<tr>
<td>DOSE</td>
<td>Confidence Limit</td>
</tr>
<tr>
<td>Comparison</td>
<td>Limit</td>
</tr>
</tbody>
</table>

| 0.23 - 0 | -318330 | -103333 | 111663 |
| 0.51 - 0 | -694997 | -480000 | -265003 *** |
| 0.88 - 0 | -851663 | -636667 | -421670 *** |
| 2.16 - 0 | -1176663 | -961667 | -746670 *** |
| 4.89 - 0 | -1620663 | -1405667 | -1190670 *** |