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

1. CHEMICAL: Azoxystrobin  
   PC Code No.: 128810

2. TEST MATERIAL: ICIA5504 technical  
   Purity: 96.2%

3. CITATION
   Authors: D.V. Smyth, S.J. Kent, S.A. Sankey and J.M. Shearing
   Title: ICIA5504: Toxicity to the Blue-Green Alga (Anabaena flos-aquae)
   Study Completion Date: December 11, 1993
   Laboratory: Brixham Environmental Laboratory, Brixham Devon, UK
   Sponsor: Zeneca Ag Products, Wilmington, DE
   Laboratory Report ID: BL5054/B
   MRID No.: 436781-62

4. REVIEWED BY:
   William Erickson  
   Biologist  
   EEB/EFED/EPA  
   Signature:  
   Date: 4/04/96

5. APPROVED BY:
   Harry Craven  
   Section Head 4  
   EEB/EFED/EPA  
   Signature:  
   Date: 6/21/96

6. STUDY PARAMETERS
   Definitive Test Duration: 120 hours
   Type of Concentrations: Mean measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test.

   Results Synopsis
   EC₃₀: 13 ppm  
   NOEC: 9 ppm  
   95% C.I.: 12 - 14 ppm  
   Probit Slope: N/A

8. ADEQUACY OF THE STUDY
   A. Classification: Core
B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

1. Initial cell density (20,000 cells/mL) was greater than recommended (3,000 cells/mL).

10. SUBMISSION PURPOSE:

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></td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td></td>
</tr>
<tr>
<td>Anabaena flos-aquae</td>
<td></td>
</tr>
<tr>
<td>Selenastrum capricornutum</td>
<td></td>
</tr>
<tr>
<td>Navicula pelliculosa</td>
<td></td>
</tr>
<tr>
<td><strong>Initial Number of Cells</strong></td>
<td>20,000 cells/mL</td>
</tr>
<tr>
<td>3,000 - 10,000 cells/mL</td>
<td></td>
</tr>
<tr>
<td><strong>Nutrients</strong></td>
<td>CCAP 1403/13A</td>
</tr>
<tr>
<td>Standard formula, e.g. 20XAAP</td>
<td></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>Dimethylformamide (DMF)</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td></td>
</tr>
<tr>
<td>Skeletonema: 20°C</td>
<td>24°C</td>
</tr>
<tr>
<td>Others: 24-25°C</td>
<td></td>
</tr>
<tr>
<td><strong>Light Intensity</strong></td>
<td>3.0 KLux</td>
</tr>
<tr>
<td>Anabaena: 2.0 KLux (±15%)</td>
<td></td>
</tr>
<tr>
<td>Others: 4.0-5.0 KLux (±15%)</td>
<td></td>
</tr>
<tr>
<td><strong>Photoperiod</strong></td>
<td>Continuous</td>
</tr>
<tr>
<td>Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark</td>
<td></td>
</tr>
<tr>
<td>Others: Continuous</td>
<td>2</td>
</tr>
</tbody>
</table>
DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 123-2 (TIER II)

1. CHEMICAL: Actomyostatin
   PC Code No.: 123810

2. TEST MATERIAL: ICIA5504 technical
   Purity: 96.2%

3. CITATION
   Authors: D.V. Smyth, S.J. Kent, S.A. Sankey and J.M. Shearing
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   Sponsor: Zeneca Ag Products, Wilmington, DE
   Laboratory Report ID: BL5054/B
   MRID No.: 436781-62

4. REVIEWED BY: Max Feken, M.S., Environmental Toxicologist,
   KBN Engineering and Applied Sciences, Inc.
   Signature: [Signature] Date: 12/1/95

   APPROVED BY: Mark Mossler, M.S., Toxicologist,
   KBN Engineering and Applied Sciences, Inc.
   Signature: [Signature] Date: 12/5/95

5. APPROVED BY:
   Signature: Date:

6. STUDY PARAMETERS
   Definitive Test Duration: 120 hours
   Type of Concentrations: Mean measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test.

   Results Synopsis
   EC₅₀: 13 ppm
   NOEC: 9 ppm
   95% C.I.: 12 - 14 ppm
   Probit Slope: N/A

8. ADEQUACY OF THE STUDY
   A. Classification: Core
### C. Test Design

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td></td>
</tr>
<tr>
<td>Skeletonema: approx. 8.0</td>
<td>Initial 7.4 - 7.5</td>
</tr>
<tr>
<td>Others: approx. 7.5</td>
<td>Final 7.2 - 7.6</td>
</tr>
<tr>
<td><strong>Dose range</strong></td>
<td></td>
</tr>
<tr>
<td>2X or 3X progression</td>
<td>2X</td>
</tr>
<tr>
<td><strong>Doses</strong></td>
<td></td>
</tr>
<tr>
<td>at least 5</td>
<td>6</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
</tr>
<tr>
<td>negative and/or solvent</td>
<td>Negative and solvent control</td>
</tr>
<tr>
<td><strong>Replicates per dose</strong></td>
<td></td>
</tr>
<tr>
<td>3 or more</td>
<td>3 (6 for solvent control)</td>
</tr>
<tr>
<td><strong>Duration of test</strong></td>
<td></td>
</tr>
<tr>
<td>120 hours</td>
<td>120 hours</td>
</tr>
<tr>
<td><strong>Daily observations were made?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Method of Observations</strong></td>
<td>Algal cell absorbance</td>
</tr>
<tr>
<td><strong>Maximum Labeled Rate</strong></td>
<td>Not reported</td>
</tr>
</tbody>
</table>

### 12. REPORTED RESULTS

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial and 120 h cell densities were measured?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Control cell count at 120 hr &gt;2X initial count?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Initial chemical concentrations measured?</strong> (Optional)</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Raw data included?</strong></td>
<td>Yes</td>
</tr>
</tbody>
</table>
## Dose Response

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Algal cell absorbance</th>
<th>% reduction in area under growth curve</th>
<th>120-Hour pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal Mean Measured</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control &lt;0.007</td>
<td>2.337</td>
<td>0(+12)</td>
<td>7.6</td>
</tr>
<tr>
<td>Solvent &lt;0.007</td>
<td>1.947</td>
<td>--</td>
<td>7.5</td>
</tr>
<tr>
<td>1.3 1.4</td>
<td>2.133</td>
<td>0(+8)</td>
<td>7.5</td>
</tr>
<tr>
<td>2.4 2.4</td>
<td>1.910</td>
<td>7</td>
<td>7.5</td>
</tr>
<tr>
<td>4.3 3.9</td>
<td>2.033</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>7.8 8.5</td>
<td>1.730</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>14 14</td>
<td>1.303</td>
<td>63</td>
<td>7.5</td>
</tr>
<tr>
<td>25 21</td>
<td>&lt;0.016</td>
<td>98</td>
<td>7.3</td>
</tr>
</tbody>
</table>

* Inoculum (Day 0) calculated cell absorbance = 0.005 (2000 cells/mL)
** compared to the solvent control

Other Significant Results: Area under the growth curve was the most sensitive end-point measured in the study.

### Statistical Results for Areas Under the Growth Curve

Statistical Method: Probit analysis and Dunnett's test for mean separation.

EC$_{50}$: 10 ppm  
95% C.I.: 6.4 - >21 ppm  
Probit Slope: Not reported  
NOEC: 8.5 ppm

### Statistical Results for Growth Rate

Statistical Method: Probit analysis and Dunnett's test for mean separation.

EC$_{50}$: >21 ppm  
95% C.I.: N/A  
Probit Slope: N/A  
NOEC: 8.5 ppm

#### 13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Moving average method and Williams' test for solvent control comparisons. Results based on mean measured concentrations.
14. **REVIEWER’S COMMENTS**: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC$_{50}$ and NOEC for *A. flos-aquae* exposed to ICIA5504 were 13 and 9 ppm, respectively. This study can be categorized as Core.
<table>
<thead>
<tr>
<th>CONC.</th>
<th>NUMBER EXPOSED</th>
<th>NUMBER DEAD</th>
<th>PERCENT DEAD</th>
<th>BINOMIAL PROB. (PERCENT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>100</td>
<td>99</td>
<td>99</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>33</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>8.5</td>
<td>100</td>
<td>11</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>3.9</td>
<td>100</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2.4</td>
<td>100</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1.4</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The binomial test shows that 14 and 21 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 15.21465

Results calculated using the moving average method

<table>
<thead>
<tr>
<th>SPAN</th>
<th>G</th>
<th>LC50</th>
<th>95 percent confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.155227E-02</td>
<td>13.3062</td>
<td>12.50765 14.20901</td>
</tr>
</tbody>
</table>

Results calculated using the probit method

<table>
<thead>
<tr>
<th>ITERATIONS</th>
<th>G</th>
<th>H</th>
<th>Goodness of fit probability</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.677712</td>
<td>25.68942</td>
<td>0</td>
<td>0.0122</td>
</tr>
</tbody>
</table>

A probability of 0 means that it is less than 0.001.

Since the probability is less than 0.05, results calculated using the probit method probably should not be used.

Slope = 4.259726
95 percent confidence limits = -1.257749 and 9.7772

LC50 = 13.40221
95 percent confidence limits = 0 and +infinity

LC10 = 6.745826
95 percent confidence limits = 0 and 12.11624

*******************************************************************************

From Absorbance (# of Cells)

Mean measured 7
### WILLIAMS TEST (Isotonic regression model) - Table 1 of 2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>N</th>
<th>ORIGINAL MEAN</th>
<th>TRANSFORMED MEAN</th>
<th>ISOTONIZED MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOLVENT</td>
<td>6</td>
<td>1.947</td>
<td>1.947</td>
<td>2.009</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>3</td>
<td>2.133</td>
<td>2.133</td>
<td>2.009</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>3</td>
<td>1.910</td>
<td>1.910</td>
<td>1.972</td>
</tr>
<tr>
<td>4</td>
<td>3.9</td>
<td>3</td>
<td>2.033</td>
<td>2.033</td>
<td>1.972</td>
</tr>
<tr>
<td>5</td>
<td>8.5</td>
<td>3</td>
<td>1.730</td>
<td>1.730</td>
<td>1.730</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>3</td>
<td>1.303</td>
<td>1.303</td>
<td>1.303</td>
</tr>
</tbody>
</table>

### SULFENTRAZONE

File: 43678162

### WILLIAMS TEST (Isotonic regression model) - Table 2 of 2

<table>
<thead>
<tr>
<th>IDENTIFICATION</th>
<th>ISOTONIZED MEAN</th>
<th>CALC. WILLIAMS</th>
<th>SIG P=.05</th>
<th>TABLE WILLIAMS</th>
<th>DEGREES OF FREEDOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOLVENT</td>
<td>2.009</td>
<td>0.431</td>
<td>1.75</td>
<td>k= 1, v=15</td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>2.009</td>
<td>0.173</td>
<td>1.84</td>
<td>k= 2, v=15</td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>1.972</td>
<td>0.173</td>
<td>1.87</td>
<td>k= 3, v=15</td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>1.972</td>
<td>1.500</td>
<td>1.88</td>
<td>k= 4, v=15</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>1.730</td>
<td>4.454</td>
<td>1.89</td>
<td>k= 5, v=15</td>
<td></td>
</tr>
</tbody>
</table>

$s = 0.204$

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

Noel - 8.5 ppm