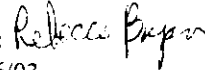


**Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Anabaena flos-aquae***  
PMRA Submission #: {.....} EPA MRID #: 45386315


**Data Requirement:** PMRA DATA CODE {.....}  
EPA DP Barcode D284719  
OECD Data Point {.....}  
EPA MRID 45386315  
EPA Guideline 123-2

**Test material:** AE F130060 Technical Purity: 95.7%  
**Common name:** Mesosulfuron-methyl  
**Chemical name:** IUPAC: methyl-2-[3-(4,6-dimethoxyprimidin-2-yl) ureidosulfonyl]-4-methanesulfonamidomethylbenzoate  
CAS name: methyl 2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amin]sulfonyl]-4-[[methylsulfonyl]amino]methyl]benzoate  
CAS No.: 208465-21-8  
Synonyms: Not reported


**Primary Reviewer:** Rebecca Bryan  
Staff Scientist, Dynamac Corporation

**Signature:**   
**Date:** 9/26/03

**QC Reviewer:** Teri Myers, Ph.D.  
Staff Scientist, Dynamac Corporation

**Signature:**   
**Date:** 9/26/03

**Primary Reviewer:** ~~Tim Bargar~~  
{EPA/OECD/PMRA}

**Date:** 04/09/04 

**Secondary Reviewer(s):** {.....}  
{EPA/OECD/PMRA}

**Date:** {.....}

**Company Code** {.....} [For PMRA]  
**Active Code** {.....} [For PMRA]  
**EPA PC Code** 122009

**Date Evaluation Completed:** {dd-mmm-yyyy}

**CITATION:** Abedi, J., Christ, M.T., and Young, B. M. 2000. Effect to *Anabaena flos-aquae* (Blue-Green Alga) in a Growth Inhibition Test. AE F130060, Technical, 95.7% w/w. Unpublished study performed by Aventis CropScience, Ecotoxicology Department, Pikeville, NC. Laboratory Study No. CK99W503, and sponsored by Aventis CropScience, Ecotoxicology Department, Research Triangle Park, NC. Experimental start date June 12, 2000 and experimental termination date June 16, 2000. Completed March 23, 2000.



2013003

### EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Anabaena flos-aquae* were exposed to Mesosulfuron-methyl under static conditions. Nominal concentrations were 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L. Mean measured concentrations over the study period were 0.6, 1.1, 2.1, 4.3, 8.6 mg/L; these treatment groups were compared to a dilution water control. Mean cell densities decreased in the 2.1, 4.3, and 8.6 mg/L treatment groups by 45, 77, and 91%, respectively, compared to the dilution water control. There was significant algal growth rate and biomass inhibitions in the 2.1, 4.3, and 8.6 mg/L treatment groups. The NOEC based on cell density, growth rate, and biomass was 1.1 mg/L. Cell density and biomass were the most sensitive parameters, with an  $EC_{50}$  of 2.4 mg/L; the  $EC_{05}$  for cell density and biomass was 0.64 and 0.65 mg/L.

The study is scientifically sound and it satisfies the guidelines for an aquatic nonvascular plant study with *Anabaena flos-aquae*. This study is classified as Core.

### Results Synopsis

Test Organism: *Anabaena flos-aquae*

Test Type: Static

#### Cell Density:

NOEC: 1.1 mg/L

$EC_{05}$ : 0.64 mg/L

$EC_{50}$ : 2.4 mg/L

Slope:  $2.87 \pm 0.491$

95% C.I.: 0.31-1.3 mg/L

95% C.I.: 1.7-3.3 mg/L

#### Growth rate:

NOEC: 1.1 mg/L

$EC_{05}$ : 1.1 mg/L

$EC_{50}$ : 4.1 mg/L

Slope:  $2.68 \pm 0.246$

95% C.I.: 0.75-1.5 mg/L

95% C.I.: 3.6-4.6 mg/L

#### Area Under the Growth Curve (Biomass):

NOEC: 1.1 mg/L

$EC_{05}$ : 0.65 mg/L

$EC_{50}$ : 2.4 mg/L

Slope:  $2.76 \pm 0.394$

95% C.I.: 0.35-1.2 mg/L

95% C.I.: 1.8-3.1 mg/L

Endpoint(s) Affected: Cell density (most sensitive), growth rate, and area under the growth curve (biomass)

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The test was based on the following guidelines: OECD Guideline no. 201 and US-EPA Subdivision J, §123-2. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The test vessels were agitated during testing. Agitation is not recommended for this species.
2. The cell density was not determined at 24 hours, but was measured at 48, 72, and 96 hours.
3. The length of the acclimation period was not reported.

None of these deviations affected the acceptability or the validity of the study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

### A. MATERIALS:

**1. Test Material** Mesosulfuron-methyl

**Description:** Light beige powder

**Lot No./Batch No.:** AE F130060 00 1C95 0001 (Pfl. 35316)

**Purity:** 95.7%

#### Stability of Compound

**Under Test Conditions:** Mean measured concentrations of Mesosulfuron-methyl ranged from 105 to 120% of nominal concentrations for test solutions. OECD requirements were not reported.

*(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)*

**Storage conditions of test chemicals:** The test substance was stored at  $25 \pm 5^\circ\text{C}$  in the dark.

### 2. Test organism:

**Name:** *Anabaena flos-aquae*

*EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricornutum, and a freshwater diatom is tested*

*OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported*

**Strain:** UTEX#1444

**Source:** University of Texas

**Age of inoculum:** 7 days

Method of cultivation: Algal Assay Procedure nutrient medium (AAP)

**B. STUDY DESIGN:**

a) Range-finding Study: A static range-finding study with Mesosulfuron-methyl was conducted in order to estimate the nominal concentration range for the definitive study. The 4 day static tests exposed freshwater alga, *Anabaena flos-aquae* to nominal concentrations of 0.01, 0.1, 1.0, 10, and 100 mg/L. Response to these conditions was compared to a dilution water control. At test termination of the test (96 hours), there was 3, -20, 25, 101, and 102% biomass inhibition in the 0.01, 0.1, 1.0, 10, and 100 mg/L treatment groups, respectively, compared to the control. The growth rate inhibition was 3, -9, 9, 107, and 114% biomass inhibition in the 0.01, 0.1, 1.0, 10, and 100 mg/L treatment groups, respectively, compared to the control.

b) Definitive Study

**Table 1. Experimental Parameters**

| Parameter   | Details   | Remarks  |
|---|---|--|
|   |   | Criteria   |
| Acclimation period:<br>culturing media and conditions: (same as test or not)<br>health: (any toxicity observed) | Not reported<br>Algal Assay Procedure nutrient medium (AAP); same as test<br>Algae for the test were taken from a stock culture in the log phase of growth, with a cell density of $2.94 \times 10^6$ cells/mL. | <i>EPA recommends two week acclimation period.</i><br><br><i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i> |
| Test system<br>static/static renewal:<br>renewal rate for static renewal:                                       | Static  |  |
| Incubation facility   | Environmental chamber   |  |
| Duration of the test  | 96 hours  | <i>EPA requires: 96 - 120 hours</i><br><br><i>OECD: 72 hours</i>   |

4

| Parameter   | Details  | Remarks   |
|---|--|---|
|   |  | Criteria  |
| Test vessel material: (glass/polystyrene)<br>size:<br>fill volume:  | Glass<br>250 mL<br>100 mL  | <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>  |
| Details of growth medium name:<br>pH at test initiation:<br>pH at test termination:<br>Chelator used:<br>Carbon source:<br>Salinity (for marine algae):                                       | Algal Assay Procedure nutrient medium (AAP)<br>7.1-7.3<br>7.4-7.5<br>Na <sub>2</sub> EDTA·2H <sub>2</sub> O<br>NaHCO <sub>3</sub><br>N/A                                   | Appendix 1, p. 40<br><i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i><br><br><i>EPA recommends 20X-AAP medium.</i>   |
| If non-standard nutrient medium was used, detailed composition provided (Yes/No)  | N/A  |   |
| Dilution water source:<br>type:<br>pH:<br>salinity (for marine algae):<br>water pretreatment (if any):<br>Total Organic Carbon:<br>particulate matter:<br>metals:<br>pesticides:<br>chlorine: | Laboratory well water<br>Deionized enriched medium<br>7.5±0.1<br>N/A<br>Not reported<br><1.0 mg/L<br>Not detected<br>See Appendix 2, p. 42<br>Not detected<br>Not reported | <i>EPA pH: <u>Skeletonema costatum</u> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.</i><br><br><i>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</i> |
| Indicate how the test material is added to the medium (added directly or used stock solution)   | Stock solution   |   |
| Aeration or agitation   | Agitation, 100 rpm   | <i>Agitation is not recommended for this species.</i><br><i>EPA recommends agitation only for <u>Selenastrum</u> at 100 cycles per min and <u>Skeletonema</u> at ~60 cycles per min. Aeration is not recommended.</i>   |

| Parameter  | Details                       | Remarks  |
|--|-------------------------------|--|
|  |                               | Criteria   |
| Initial cells density  | Approximately 10,000 cells/mL | <p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>  |
| Number of replicates control:<br>solvent control:<br>treated ones: | 6<br>N/A<br>3                 | <p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</p> <p>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.</p> |

6

| Parameter   | Details  | Remarks   |
|---|--|---|
|   |  | Criteria  |
| Test concentrations<br>nominal:<br><br>measured:                                | 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L<br><br>0.6, 1.1, 2.1, 4.3, and 8.6 mg/L     | EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.<br><br>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely. |
| Solvent (type, percentage, if used)   | N/A  |   |
| Method and interval of analytical verification                                  | HPLC; 0 and 96 hours   |   |
| Test conditions<br>temperature:<br>photoperiod:<br>light intensity and quality: | 22.7-25.4°C<br>Continuous<br>2200 lux ± 15%, cool-white fluorescent lighting | EPA temperature: <i>Skeletonema</i> : 20°C, Others: 24-25°C; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <i>Anabaena</i> : 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)<br><br>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.  |
| Reference chemical (if used)<br>name:<br>concentrations:                        | N/A  |   |
| Other parameters, if any  | None   |   |

2. Observations:

Table 2: Observation parameters

| Parameters  | Details   | Remarks/Criteria   |
|---|---|--|
| Parameters measured including the growth inhibition/other toxicity symptoms | Cell count (cell density), Growth rate (average specific growth rate), and Area under the growth curves (biomass)   | <i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i>   |
| Measurement technique for cell density and other end points                 | Haemocytometer and compound microscope  | <i>EPA recommends the measurement technique of cell counts or chlorophyll a</i><br><br><i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i> |
| Observation intervals   | 48, 72, and 96 hours  | The cell density was not determined at 24 hours.<br><i>EPA and OECD: every 24 hours.</i>   |
| Other observations, if any  | None  |  |
| Indicate whether there was exponential growth in the control                | Yes, dilution water control group cell density at test termination was 23.4X greater than the dilution water control group cell density at test initiation. | <i>EPA requires control cell count at termination to be <math>\geq 2X</math> initial count or by a factor of at least 16 during the test.</i><br><br><i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>   |
| Were raw data included?   | Yes   |  |

8



**II. RESULTS and DISCUSSION:**

**A. INHIBITORY EFFECTS:**

Mean cell densities decreased in the 2.1, 4.3, and 8.6 mg/L treatment groups by 45, 77, and 91%, respectively, compared to the dilution water control. There was significant algal growth rate and biomass inhibitions in the 2.1, 4.3, and 8.6 mg/L treatment groups.

**Table 3: Effect of Mesosulfuron-methyl on freshwater alga (*Anabaena flos-aquae*)**

| Treatment measured and nominal concentration <sup>a</sup> (mg/L) | Initial cell density (cells/mL) | Mean Cell density (cells/mL) at |            |                           |
|--|---------------------------------|---------------------------------|------------|---------------------------|
|  |                                 | 48 hours                        | 96 hours   |                           |
|  |                                 |                                 | cell count | % inhibition <sup>b</sup> |
| Dilution water control   | ~10,000                         | 28,000                          | 234,000    | --                        |
| 0.6 (0.5)  | ~10,000                         | 32,000                          | 308,000    | -31                       |
| 1.1 (1.0)  | ~10,000                         | 29,000                          | 237,000    | -1                        |
| 2.1 (2.0)  | ~10,000                         | 18,000                          | 129,000    | 45                        |
| 4.3 (4.0)  | ~10,000                         | 16,000                          | 53,000     | 77                        |
| 8.6 (8.0)  | ~10,000                         | 13,000                          | 21,000     | 91                        |
| Reference chemical (if used)                                     | N/A                             | N/A                             | N/A        | N/A                       |

<sup>a</sup> Mean measured concentrations of Mesosulfuron-methyl. Nominal concentrations are in parentheses.

<sup>b</sup> % inhibition was determined by comparing the treatment groups to the dilution water control.

**Table 4: Effect of Mesosulfuron-methyl on the Freshwater alga *Anabaena flos-aquae***

| Mean Measured and Nominal <sup>a</sup> Treatment Concentrations (mg/L) | Initial cell density (cells/mL) | Mean Growth Rate per day | % inhibition (Mean Growth Rate per day) | Mean Area Under Growth Curve | % inhibition (Mean Area Under Growth Curve) |
|--|---------------------------------|--------------------------|---|------------------------------|---|
| Dilution water control   | ~10,000                         | 0.0326                   | --                                      | 5,160,000                    | --  |
| 0.6 (0.5)  | ~10,000                         | 0.0357                   | -10                                     | 6,200,000                    | -20   |
| 1.1 (1.0)  | ~10,000                         | 0.0327                   | 0                                       | 5,180,000                    | 0   |
| 2.1 (2.0)  | ~10,000                         | 0.0267                   | 18*                                     | 3,030,000                    | 41*   |
| 4.3 (4.0)  | ~10,000                         | 0.017                    | 47*                                     | 1,300,000                    | 74*   |
| 8.6 (8.0)  | ~10,000                         | 0.0075                   | 77*                                     | 540,000                      | 89*   |

| Mean Measured and Nominal <sup>a</sup> Treatment Concentrations (mg/L) | Initial cell density (cells/mL) | Mean Growth Rate per day | % inhibition (Mean Growth Rate per day) | Mean Area Under Growth Curve | % inhibition (Mean Area Under Growth Curve) |
|--|---------------------------------|--------------------------|---|------------------------------|---|
| Reference chemical (if used)   | Not reported                    | Not reported             | Not reported                            | Not reported                 | Not reported                                |

<sup>a</sup> Mean measured concentrations of Mesosulfuron-methyl. Nominal concentrations are in parentheses.  
 \* Significantly different from the control ( $P \leq 0.05$ ).

Table 5: Statistical endpoint values.

| Statistical Endpoint   | Biomass          | Growth rate      | Cell density |
|--|------------------|------------------|--------------|
| NOEC or EC <sub>05</sub> (mg/L)  | 1.1              | 1.1              | 1.1          |
| LOEC (mg/L)  | 2.1              | 2.1              | 2.1          |
| IC <sub>50</sub> or EC <sub>50</sub> (mg/L) (95% C.I.)                     | 2.4 (1.8 to 3.1) | 4.1 (3.6 to 4.6) | Not reported |
| other (IC <sub>25</sub> /EC <sub>25</sub> )                                | Not reported     | Not reported     | Not reported |
| Reference chemical, if used<br>NOAEC<br>IC <sub>50</sub> /EC <sub>50</sub> | N/A              | N/A              | N/A          |

N/A = Not applicable.

**B. REPORTED STATISTICS:**

Statistical Method: Shapiro-Wilk's test and Bartlett's test were employed to evaluate the normality and homogeneity of the variances, respectively, and data were analyzed using a one-way analysis of variance (ANOVA) and a Bonferroni t-test. The NOEC was calculated using SAS<sup>®</sup> Procedure NLIN (Version 8.0) or TOXSTAT (Version 3.4). The EC<sub>50</sub> was determined using the non-linear regression procedure (Bruce and Versteeg).

**Cell Density:**

NOEC: 1.1 mg/L  
 LOEC: 2.1 mg/L  
 EC<sub>50</sub>: Not reported      95% C.I.: N/A

**Growth rate:**

NOEC: 1.1 mg/L  
 LOEC: 2.1 mg/L  
 EC<sub>50</sub>: 4.1 mg/L      95% C.I.: 3.6 to 4.6

**Area Under the Growth Curve (Biomass):**

NOEC: 1.1 mg/L

LOEC: 2.1 mg/L  
EC<sub>50</sub>: 2.4 mg/L                      95% C.I.: 1.8 to 3.1

Endpoint(s) Affected: Cell density, growth rate, and area under the growth curve (biomass)

### C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Growth rate, and biomass data satisfied the assumptions of ANOVA and cell density data were square root-transformed to satisfy these assumptions; this test was used to determine the NOEC, followed by Bonferroni's t-test (cell density) and William's multiple comparison tests via TOXSTAT statistical software. The EC<sub>05</sub> and EC<sub>50</sub> values were determined using the Probit method via Nuthatch statistical software.

#### Cell Density:

NOEC: 1.1 mg/L  
EC<sub>05</sub>: 0.64 mg/L                      95% C.I.: 0.31-1.3 mg/L  
EC<sub>50</sub>: 2.4 mg/L                      95% C.I.: 1.7-3.3 mg/L  
Slope: 2.87±0.491

#### Growth rate:

NOEC: 1.1 mg/L  
EC<sub>05</sub>: 1.1 mg/L                      95% C.I.: 0.75-1.5 mg/L  
EC<sub>50</sub>: 4.4 mg/L                      95% C.I.: 3.9-5.0 mg/L  
Slope: 2.68±0.246

#### Area Under the Growth Curve (Biomass):

NOEC: 1.1 mg/L  
EC<sub>05</sub>: 0.65 mg/L                      95% C.I.: 0.35-1.2 mg/L  
EC<sub>50</sub>: 2.6 mg/L                      95% C.I.: 2.0-3.4 mg/L  
Slope: 2.76±0.394

### D. STUDY DEFICIENCIES:

The deviations did not affect the acceptability or validity of the study.

### E. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study authors'; however the study authors' EC<sub>50</sub> estimates for biomass and growth rate were slightly lower than the reviewer's and provided slightly narrower 95% confidence intervals. The reviewer determined toxicity values for cell density, as well as the EC<sub>05</sub> values for all endpoints, and these are reported in the Executive Summary and Conclusions sections. Based on the reviewer's and study authors' conclusions, cell density and biomass were the most sensitive endpoints, with an EC<sub>50</sub> value of 2.4 mg/L.

**F. CONCLUSIONS:** The study is scientifically sound and it satisfies the guidelines for an aquatic nonvascular plant study with *Anabaena flos-aquae*. This study is classified as Core.

#### Cell Density:

NOEC: 1.1 mg/L

EC<sub>05</sub>: 0.64 mg/L                      95% C.I.: 0.31-1.3 mg/L  
EC<sub>50</sub>: 2.4 mg/L                        95% C.I.: 1.7-3.3 mg/L  
Slope: 2.87±0.491

**Growth rate:**

NOEC: 1.1 mg/L  
EC<sub>05</sub>: 1.1 mg/L                        95% C.I.: 0.75-1.5 mg/L  
EC<sub>50</sub>: 4.1 mg/L                        95% C.I.: 3.6-4.6 mg/L  
Slope: 2.68±0.246

**Area Under the Growth Curve (Biomass):**

NOEC: 1.1 mg/L  
EC<sub>05</sub>: 0.65 mg/L                      95% C.I.: 0.35-1.2 mg/L  
EC<sub>50</sub>: 2.4 mg/L                        95% C.I.: 1.8-3.1 mg/L  
Slope: 2.76±0.394

Endpoint(s) Affected: Cell density (most sensitive), growth rate, and area under the growth curve (biomass)

12

### III. REFERENCES:

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- West, Inc., and D.D. Gulley (1994) "TOXSTAT\* 3.4", University of Wyoming.

**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

cell density

File: 6315cd Transform: SQUARE ROOT(Y)

ANOVA TABLE

| SOURCE         | DF | SS    | MS    | F      |
|----------------|----|-------|-------|--------|
| Between        | 5  | 4.193 | 0.839 | 41.950 |
| Within (Error) | 15 | 0.293 | 0.020 |        |
| Total          | 20 | 4.486 |       |        |

Critical F value = 2.90 (0.05,5,15)  
 Since F > Critical F REJECT Ho:All groups equal

cell density

File: 6315cd Transform: SQUARE ROOT(Y)

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT        | SIG |
|-------|----------------|------------------|-----------------------------------|---------------|-----|
| 1     | control        | 1.519            | 2.338                             |               |     |
| 2     | 0.6            | 1.755            | 3.080                             | -2.350        |     |
| 3     | 1.1            | <b>1.528</b>     | <b>2.367</b>                      | <b>-0.087</b> |     |
| 4     | 2.1            | 1.137            | 1.293                             | 3.826         | *   |
| 5     | 4.3            | 0.725            | 0.530                             | 7.944         | *   |
| 6     | 8.6            | 0.454            | 0.207                             | 10.657        | *   |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

cell density

File: 6315cd Transform: SQUARE ROOT(Y)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1     | control        | 6           |                                   |              |                         |
| 2     | 0.6            | 3           | 0.723                             | 30.9         | -0.742                  |
| 3     | 1.1            | 3           | 0.723                             | 30.9         | -0.028                  |
| 4     | 2.1            | 3           | 0.723                             | 30.9         | 1.045                   |

H

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Anabaena flos-aquae*  
 PMRA Submission #: [.....] EPA MRID #: 45386315

|   |     |   |       |      |       |
|---|-----|---|-------|------|-------|
| 5 | 4.3 | 3 | 0.723 | 30.9 | 1.808 |
| 6 | 8.6 | 3 | 0.723 | 30.9 | 2.132 |

cell density  
 File: 6315cd Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|----------------|---|---------------|------------------|-----------------|
| 1     | control        | 6 | 2.338         | 1.519            | 1.598           |
| 2     | 0.6            | 3 | 3.080         | 1.755            | 1.598           |
| 3     | 1.1            | 3 | 2.367         | 1.528            | 1.528           |
| 4     | 2.1            | 3 | 1.293         | 1.137            | 1.137           |
| 5     | 4.3            | 3 | 0.530         | 0.725            | 0.725           |
| 6     | 8.6            | 3 | 0.207         | 0.454            | 0.454           |

cell density  
 File: 6315cd Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|----------------|-----------------|----------------|-----------|----------------|--------------------|
| control        | 1.598           |                |           |                |                    |
| 0.6            | 1.598           | 0.793          |           | 1.75           | k= 1, v=15         |
| 1.1            | 1.528           | 0.088          |           | 1.84           | k= 2, v=15         |
| 2.1            | 1.137           | 3.872          | *         | 1.87           | k= 3, v=15         |
| 4.3            | 0.725           | 8.039          | *         | 1.88           | k= 4, v=15         |
| 8.6            | 0.454           | 10.784         | *         | 1.89           | k= 5, v=15         |

s = 0.140

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

Estimates of EC%

| Parameter | Estimate | 95% Bounds |       | Std.Err. | Lower Bound /Estimate |
|-----------|----------|------------|-------|----------|-----------------------|
|           |          | Lower      | Upper |          |                       |
| EC5       | 0.64     | 0.31       | 1.3   | 0.15     | 0.48                  |
| EC10      | 0.86     | 0.45       | 1.6   | 0.13     | 0.53                  |
| EC25      | 1.4      | 0.86       | 2.3   | 0.099    | 0.62                  |
| EC50      | 2.4      | 1.7        | 3.3   | 0.067    | 0.72                  |

15

**Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Anabaena flos-aquae***  
 PMRA Submission #:{.....} EPA MRID #: 45386315

Slope = 2.87 Std.Err. = 0.491

!!!Poor fit: p = 0.047 based on DF= 3.0 15.

6315CD : cell density

Observed vs. Predicted Treatment Group Means

| Dose  | #Reps. | Obs. Mean | Pred. Mean | Obs. -Pred. | Pred. %Control | %Change |
|-------|--------|-----------|------------|-------------|----------------|---------|
| 0.00  | 6.00   | 2.34      | 2.62       | -0.278      | 100.           | 0.00    |
| 0.600 | 3.00   | 3.08      | 2.51       | 0.575       | 95.8           | 4.24    |
| 1.10  | 3.00   | 2.37      | 2.18       | 0.186       | 83.4           | 16.6    |
| 2.10  | 3.00   | 1.29      | 1.48       | -0.184      | 56.5           | 43.5    |
| 4.30  | 3.00   | 0.530     | 0.609      | -0.0794     | 23.3           | 76.7    |
| 8.60  | 3.00   | 0.207     | 0.146      | 0.0611      | 5.57           | 94.4    |

growth rate

File: 6315g

Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE         | DF | SS     | MS    | F      |
|----------------|----|--------|-------|--------|
| Between        | 5  | 19.399 | 3.880 | 90.233 |
| Within (Error) | 15 | 0.648  | 0.043 |        |
| Total          | 20 | 20.047 |       |        |

Critical F value = 2.90 (0.05, 5, 15)

Since F > Critical F REJECT Ho: All groups equal

growth rate

File: 6315g

Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2

Ho: Control < Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|------------------|-----------------------------------|--------|-----|
| 1     | control        | 3.258            | 3.258                             |        |     |
| 2     | 0.6            | 3.573            | 3.573                             | -2.148 |     |
| 3     | 1.1            | 3.267            | 3.267                             | -0.057 |     |

16



Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Anabaena flos-aquae*  
 PMRA Submission #: {.....} EPA MRID #: 45386315

|   |     |       |       |          |
|---|-----|-------|-------|----------|
| 4 | 2.1 | 2.667 | 2.667 | 4.035 *  |
| 5 | 4.3 | 1.717 | 1.717 | 10.514 * |
| 6 | 8.6 | 0.747 | 0.747 | 17.129 * |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

growth rate  
 File: 6315g Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1     | control        | 6           |                                   |              |                         |
| 2     | 0.6            | 3           | 0.382                             | 11.7         | -0.315                  |
| 3     | 1.1            | 3           | 0.382                             | 11.7         | -0.008                  |
| 4     | 2.1            | 3           | 0.382                             | 11.7         | 0.592                   |
| 5     | 4.3            | 3           | 0.382                             | 11.7         | 1.542                   |
| 6     | 8.6            | 3           | 0.382                             | 11.7         | 2.512                   |

growth rate  
 File: 6315g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|----------------|---|---------------|------------------|-----------------|
| 1     | control        | 6 | 3.258         | 3.258            | 3.363           |
| 2     | 0.6            | 3 | 3.573         | 3.573            | 3.363           |
| 3     | 1.1            | 3 | 3.267         | 3.267            | 3.267           |
| 4     | 2.1            | 3 | 2.667         | 2.667            | 2.667           |
| 5     | 4.3            | 3 | 1.717         | 1.717            | 1.717           |
| 6     | 8.6            | 3 | 0.747         | 0.747            | 0.747           |

growth rate  
 File: 6315g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| ISOTONIZED | CALC. | SIG | TABLE | DEGREES OF |
|------------|-------|-----|-------|------------|
|------------|-------|-----|-------|------------|

17

| IDENTIFICATION | MEAN         | WILLIAMS     | P=.05 | WILLIAMS    | FREEDOM           |
|----------------|--------------|--------------|-------|-------------|-------------------|
| control        | 3.363        |              |       |             |                   |
| 0.6            | 3.363        | 0.715        |       | 1.75        | k= 1, v=15        |
| 1.1            | <b>3.267</b> | <b>0.057</b> |       | <b>1.84</b> | <b>k= 2, v=15</b> |
| 2.1            | 2.667        | 4.026        | *     | 1.87        | k= 3, v=15        |
| 4.3            | 1.717        | 10.491       | *     | 1.88        | k= 4, v=15        |
| 8.6            | 0.747        | 17.092       | *     | 1.89        | k= 5, v=15        |

s = 0.208

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

Estimates of EC%

| Parameter | Estimate | 95% Bounds |       | Std.Err. | Lower Bound /Estimate |
|-----------|----------|------------|-------|----------|-----------------------|
|           |          | Lower      | Upper |          |                       |
| EC5       | 1.1      | 0.75       | 1.5   | 0.074    | 0.70                  |
| EC10      | 1.5      | 1.1        | 2.0   | 0.062    | 0.74                  |
| EC25      | 2.5      | 2.0        | 3.0   | 0.043    | 0.81                  |
| EC50      | 4.4      | 3.9        | 5.0   | 0.026    | 0.88                  |

Slope = 2.68 Std.Err. = 0.246

Goodness of fit: p = 0.26 based on DF= 3.0 15.

6315G : growth rate

Observed vs. Predicted Treatment Group Means

| Dose  | #Reps. | Obs. Mean | Pred. Mean | Obs. -Pred. | Pred. %Control | %Change |
|-------|--------|-----------|------------|-------------|----------------|---------|
| 0.00  | 6.00   | 3.26      | 3.38       | -0.120      | 100.           | 0.00    |
| 0.600 | 3.00   | 3.57      | 3.34       | 0.229       | 99.0           | 1.02    |
| 1.10  | 3.00   | 3.27      | 3.20       | 0.0677      | 94.7           | 5.32    |
| 2.10  | 3.00   | 2.67      | 2.72       | -0.0556     | 80.6           | 19.4    |
| 4.30  | 3.00   | 1.72      | 1.73       | -0.0105     | 51.1           | 48.9    |
| 8.60  | 3.00   | 0.747     | 0.737      | 0.00979     | 21.8           | 78.2    |

area under the curve

File: 6315b Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE  | DF | SS     | MS     | F      |
|---------|----|--------|--------|--------|
| Between | 5  | 85.865 | 17.173 | 28.960 |

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Anabaena flos-aquae*  
 PMRA Submission #: [.....] EPA MRID #: 45386315

|                |    |        |       |
|----------------|----|--------|-------|
| Within (Error) | 15 | 8.898  | 0.593 |
| Total          | 20 | 94.763 |       |

Critical F value = 2.90 (0.05, 5, 15)  
 Since F > Critical F REJECT Ho: All groups equal

area under the curve  
 File: 6315b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho: Control < Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|------------------|-----------------------------------|--------|-----|
| 1     | control        | 5.160            | 5.160                             |        |     |
| 2     | 0.6            | 6.197            | 6.197                             | -1.904 |     |
| 3     | 1.1            | 5.187            | 5.187                             | -0.049 |     |
| 4     | 2.1            | 3.027            | 3.027                             | 3.918  | *   |
| 5     | 4.3            | 1.340            | 1.340                             | 7.015  | *   |
| 6     | 8.6            | 0.543            | 0.543                             | 8.478  | *   |

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15, 5)

area under the curve  
 File: 6315b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho: Control < Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|-------|----------------|-------------|-----------------------------------|--------------|-------------------------|
| 1     | control        | 6           |                                   |              |                         |
| 2     | 0.6            | 3           | 1.417                             | 27.5         | -1.037                  |
| 3     | 1.1            | 3           | 1.417                             | 27.5         | -0.027                  |
| 4     | 2.1            | 3           | 1.417                             | 27.5         | 2.133                   |
| 5     | 4.3            | 3           | 1.417                             | 27.5         | 3.820                   |
| 6     | 8.6            | 3           | 1.417                             | 27.5         | 4.617                   |

area under the curve  
 File: 6315b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|----------------|---|---------------|------------------|-----------------|
| 1     | control        | 6 | 5.160         | 5.160            | 5.506           |
| 2     | 0.6            | 3 | 6.197         | 6.197            | 5.506           |
| 3     | 1.1            | 3 | 5.187         | 5.187            | 5.187           |
| 4     | 2.1            | 3 | 3.027         | 3.027            | 3.027           |
| 5     | 4.3            | 3 | 1.340         | 1.340            | 1.340           |
| 6     | 8.6            | 3 | 0.543         | 0.543            | 0.543           |

area under the curve  
 File: 6315b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|----------------|-----------------|----------------|-----------|----------------|--------------------|
| control        | 5.506           |                |           |                |                    |
| 0.6            | 5.506           | 0.634          |           | 1.75           | k= 1, v=15         |
| 1.1            | 5.187           | 0.049          |           | 1.84           | k= 2, v=15         |
| 2.1            | 3.027           | 3.917          | *         | 1.87           | k= 3, v=15         |
| 4.3            | 1.340           | 7.014          | *         | 1.88           | k= 4, v=15         |
| 8.6            | 0.543           | 8.477          | *         | 1.89           | k= 5, v=15         |

s = 0.770

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

Estimates of EC%

| Parameter | Estimate | 95% Bounds |       | Std.Err. | Lower Bound /Estimate |
|-----------|----------|------------|-------|----------|-----------------------|
|           |          | Lower      | Upper |          |                       |
| EC5       | 0.65     | 0.35       | 1.2   | 0.13     | 0.53                  |
| EC10      | 0.89     | 0.52       | 1.5   | 0.11     | 0.58                  |
| EC25      | 1.5      | 0.98       | 2.2   | 0.084    | 0.67                  |
| EC50      | 2.6      | 2.0        | 3.4   | 0.056    | 0.76                  |

Slope = 2.76 Std.Err. = 0.394

Goodness of fit: p = 0.078 based on DF= 3.0 15.

6315B : area under the curve

Observed vs. Predicted Treatment Group Means

| Dose  | #Reps. | Obs.<br>Mean | Pred.<br>Mean | Obs.<br>-Pred. | Pred.<br>%Control | %Change |
|-------|--------|--------------|---------------|----------------|-------------------|---------|
| 0.00  | 6.00   | 5.16         | 5.60          | -0.441         | 100.              | 0.00    |
| 0.600 | 3.00   | 6.20         | 5.38          | 0.820          | 96.0              | 4.02    |
| 1.10  | 3.00   | 5.19         | 4.74          | 0.443          | 84.7              | 15.3    |
| 2.10  | 3.00   | 3.03         | 3.35          | -0.324         | 59.8              | 40.2    |
| 4.30  | 3.00   | 1.34         | 1.52          | -0.179         | 27.1              | 72.9    |
| 8.60  | 3.00   | 0.543        | 0.420         | 0.123          | 7.51              | 92.5    |

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