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: *Rebecca Bryan*
Date: 9/26/03

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation
Leo LaSoh
Primary Reviewer: ~~Tim Bargar~~
{EPA/OECD/PMRA}

Signature: *[signature]*
Date: 9/26/03

Date: 04/09/04 *[signature]*

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 AE F130060, Technical, 95.7% w/w. Unpublished study performed by Aventis CropScience, Ecotoxicology Department, Pikeville, NC.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.

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   95% C.I.: 0.31-1.3 mg/L
EC_{50}: 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_{05}: 1.1 mg/L   95% C.I.: 0.75-1.5 mg/L
EC_{50}: 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_{05}: 0.65 mg/L   95% C.I.: 0.35-1.2 mg/L
EC_{50}: 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)

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Anabaena flos-aquae*
PMRA Submission #: |.........|

EPA MRID #: 45386315

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 <u>No</u> 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 ± 5°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. capricorntum, 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: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Not reported Algal Assay Procedure nutrient medium (AAP); same as test Algae for the test were taken from a stock culture in the log phase of growth, with a cell density of 2.94 x 10^6 cells/mL.	*EPA recommends two week acclimation period.* *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.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*

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Parameter	Details	Remarks
		Criteria
Test vessel material: (glass/polystyrene) size: fill volume:	Glass 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	Algal Assay Procedure nutrient medium (AAP) 7.1-7.3 7.4-7.5 Na$_2$EDTA·2H$_2$O NaHCO$_3$ N/A	Appendix 1, p. 40 *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Laboratory well water Deionized enriched medium 7.5±0.1 N/A Not reported <1.0 mg/L Not detected See Appendix 2, p. 42 Not detected Not reported	*EPA pH: Skeletonema costatum= ~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.* *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.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 rpm	Agitation is not recommended for this species. *EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

Parameter	Details	Remarks
		Criteria
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	6 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *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.*

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Anabaena flos-aquae*
PMRA Submission #: ┆........┆

EPA MRID #: 45386315

Parameter	Details	Remarks
		Criteria
Test concentrations nominal: measured:	0.5, 1.0, 2.0, 4.0, and 8.0 mg/L 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.* *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 temperature: photoperiod: light intensity and quality:	22.7-25.4°C Continuous 2200 lux ± 15%, cool-white fluorescent lighting	*EPA temperature: Skeletonema: 20°C. Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark. Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: 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)	*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.*
Measurement technique for cell density and other end points	Haemocytometer and compound microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *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).*
Observation intervals	48, 72, and 96 hours	The cell density was not determined at 24 hours. *EPA and OECD: every 24 hours.*
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.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

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[a] (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		48 hours	96 hours	
			cell count	% inhibition[b]
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

[a] Mean measured concentrations of Mesosulfuron-methyl. Nominal concentrations are in parentheses.
[b] % 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[a] 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[a] 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

[a] Mean measured concentrations of Mesosulfuron-methyl. Nominal concentrations are in parentheses.
* Significantly different from the control (P≤0.05).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC_{05} (mg/L)	1.1	1.1	1.1
LOEC (mg/L)	2.1	2.1	2.1
IC_{50} or EC_{50} (mg/L) (95% C.I.)	2.4 (1.8 to 3.1)	4.1 (3.6 to 4.6)	Not reported
other (IC_{25}/EC_{25})	Not reported	Not reported	Not reported
Reference chemical, if used NOAEC IC_{50}/EC_{50}	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 homogenity 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 [*] Procedure NLIN (Version 8.0) or TOXSTAT (Version 3.4). The EC_{50} was determined using the non-linear regression procedure (Bruce and Versteeg).

Cell Density:
NOEC: 1.1 mg/L
LOEC: 2.1 mg/L
EC_{50}: Not reported   95% C.I.: N/A

Growth rate:
NOEC: 1.1 mg/L
LOEC: 2.1 mg/L
EC_{50}: 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$_{50}$: 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$_{05}$ and EC$_{50}$ values were determined using the Probit method via Nuthatch statistical software.

Cell Density:
NOEC: 1.1 mg/L
EC$_{05}$: 0.64 mg/L   95% C.I.: 0.31-1.3 mg/L
EC$_{50}$: 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$_{05}$: 1.1 mg/L   95% C.I.: 0.75-1.5 mg/L
EC$_{50}$: 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$_{05}$: 0.65 mg/L   95% C.I.: 0.35-1.2 mg/L
EC$_{50}$: 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$_{50}$ 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$_{05}$ 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$_{50}$ 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_{05}: 0.64 mg/L   95% C.I.: 0.31-1.3 mg/L
EC_{50}: 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_{05}: 1.1 mg/L   95% C.I.: 0.75-1.5 mg/L
EC_{50}: 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_{05}: 0.65 mg/L   95% C.I.: 0.35-1.2 mg/L
EC_{50}: 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)

III. REFERENCES:

Organization of Economic Cooperation and Development (1989) "Alga, Growth Inhibition Test", OECD Guideline for Testing of Chemicals, Guideline 201," Paris.

Holst, R.W. and T.C. Ellwanger (1982) *Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants;* PB 83-153940; EPA 540/9-82-020. Series 123:§ 123-2. U.S. Environmental Protection Agency. Washington, D.C.

U.S. Environmental Protection Agency (1989) "Good Laboratory Practice Standards, Final Rule (40 CFR Part 160)", Federal Register Vol. 54, No. 158:34052-34074, Federal Insecticide, Fungicide, Rodenticide Act (FIFRA): Washington, D.C.

Walsh, G.E., Alexander, S.V. (1980) "A Marine Algal Bioassay Method: Results With Pesticides and Industrial Wastes" *Water, Air and Soil Pollution*, 13:45-55.

Bruce, R.D., Versteeg, D.J. (1992) "A Statistical Procedure for Modeling Continuous Toxicity Data" Env. Tox. & Chem. 11:1485-1494.

Shapiro, S.S., Wilk, M.B. (1965) "An analysis of variance test for normality (complete samples)" Biometrika 52:591-611.

Bartlett, M.S. (1937) "Some examples of statistical methods of research in agriculture and applied biology" J. Royal Statist. Soc. Suppl. 4:137-183.

Weber, C.I., et.al (1989) "Short-term methods for estimating chronic toxicity of effluents and receiving waters to freshwater organisms." U.S. Environmental Protection Agency, 2nd Ed. EPA/600/4-89/001

SAS Institute, Inc. (1999) SAS*/STAT, Version 8.0, SAS Institute, Inc., Cary, NC

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

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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	1.528	2.367	-0.087	
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

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```
    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
                                         ORIGINAL   TRANSFORMED   ISOTONIZED
           IDENTIFICATION   N   MEAN   MEAN   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

                    ISOTONIZED   CALC.   SIG   TABLE   DEGREES OF
    IDENTIFICATION   MEAN   WILLIAMS   P=.05   WILLIAMS   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 |
|-----------|----------|------------|-------|----------|-------------|
|           |          | Lower | Upper |          | /Estimate |
| **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 |

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**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 | |

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```
   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
    -------------------------------------------------------------------------
                            NUM OF   Minimum Sig Diff   % of      DIFFERENCE
    GROUP  IDENTIFICATION   REPS     (IN ORIG. UNITS)   CONTROL   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
                                      ORIGINAL   TRANSFORMED   ISOTONIZED
           IDENTIFICATION    N          MEAN        MEAN          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
```

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

EPA MRID #: 45386315

IDENTIFICATION	MEAN	WILLIAMS	P=.05	WILLIAMS	FREEDOM
control	3.363				
0.6	3.363	0.715		1.75	k= 1, v=15
1.1	**3.267**	**0.057**		**1.84**	**k= 2, v=15**
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
		Lower	Upper		/Estimate
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

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```
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
-------------------------------------------------------------------------------
                            TRANSFORMED   MEAN CALCULATED IN
GROUP    IDENTIFICATION        MEAN       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
-------------------------------------------------------------------------------
                         NUM OF   Minimum Sig Diff   % of     DIFFERENCE
GROUP    IDENTIFICATION  REPS     (IN ORIG. UNITS)  CONTROL   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
```

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```
            WILLIAMS TEST   (Isotonic regression model)    TABLE 1 OF 2
          ----------------------------------------------------------------------
GROUP                                   ORIGINAL    TRANSFORMED    ISOTONIZED
           IDENTIFICATION      N          MEAN          MEAN          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
          ----------------------------------------------------------------------
                        ISOTONIZED   CALC.      SIG      TABLE      DEGREES OF
           IDENTIFICATION   MEAN    WILLIAMS   P=.05    WILLIAMS     FREEDOM
          --------------- ---------- --------- ------   ---------   -----------
             control       5.506
               0.6         5.506     0.634                1.75
               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
                         Lower   Upper   /Estimate
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. Mean	Pred. Mean	Obs. -Pred.	Pred. %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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