

9/26/03

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: {.....}

EPA MRID #: 45386309

Data Requirement; PMRA DATA CODE {.....}  
EPA DP Barcode D284719  
OECD Data Point {.....}  
EPA MRID 45386309  
EPA Guideline 123-2

Test material: AE F160459 Purity: 96.8%  
Common name: Mesosulfuron-methyl metabolite  
Chemical name: IUPAC: Methyl 2-[3-(4-hydroxy-6-methoxyprimidin-2-yl)ureidosulfonyl]-4-methanesulfonamidomethylbenzoate  
CAS name: Not reported  
CAS No.: Not reported  
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

Signature: Teri Myers  
Date: 9/26/03

Primary Reviewer: Leo LaSota  
Tim Bargar  
{EPA/OECD/PMRA}

Date: 11/09/04  
Signature: Leo LaSota

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: Sowig, P., Gosch, H., and Weller, O. 2000. Algal growth inhibition- *Pseudokirchneriella subcapitata*, AE F160459; substance, pure (Metabolite of AE F130060). Unpublished study performed by Aventis CropScience GmbH, Frankfurt, Germany. Laboratory Study Identification No. CE00/081. Study submitted by Aventis CropScience, Research Triangle Park, NC. Experimental start date August 7, 2000 and experimental termination date October 2, 2000. The final report issued October 16, 2000.

**EXECUTIVE SUMMARY:**

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* (syn *Selenastrum capricornutum*) were exposed to Mesosulfuron-methyl metabolite (AE F160459) under static conditions. Nominal concentrations were 10, 18, 32, 56, and 100 mg/L. Mean measured concentrations over the study period were 9.8, 17.9, 30.6, 54.6, and 98.4 mg/L; these treatment groups were compared to a dilution water control. There was significant growth inhibition at the 98.4 mg/L treatment level for cell density, biomass, and growth rate; the NOEC was 54.6 mg/L. The study authors determined that the EC<sub>50</sub> for biomass was 98 mg/L (based on nominal concentrations); a 95% confidence interval was not provided for this estimate and the reviewer's analysis could not confirm this value. Inhibition of cell density and growth rate did not exceed 50% in this study.

The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata* (U.S. EPA Guideline 123-2). This study is classified as Core.

**Results Synopsis**

Test Organism: *Pseudokirchneriella subcapitata*  
Test Type: Static

**Cell Density:**

NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: >98.4 mg/L 95% C.I.: N/A  
Slope: could not determine

**Growth rate:**

NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: >98.4 mg/L 95% C.I.: N/A  
Slope: could not determine

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

NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: 98 mg/L 95% C.I.: not reported  
Slope: could not determine

Endpoint(s) Affected: cell density, growth rate, and biomass

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The test was based on the following guidelines: OECD Guideline no. 201, US-EPA Subdivision J, §123-2, and EU directive 92/69/EEG Annex Part C: C.3. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The acclimation period (4 days) was less than the recommended 2 weeks.
2. The storage conditions of the test chemical, carbon source of the growth medium, and some dilution water characteristics were not reported.

These deviations did not affect 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 metabolite (AE F160459)

**Description:** White powder

**Lot No./Batch No. :** AE F160459 00 1B97 0001

**Purity:** 96.8%

#### Stability of Compound

**Under Test Conditions:** The day 0 measured concentrations were 96.7-103.2% of nominal and the day 4 measured concentrations were 98.2-103.4% of nominal. Mean measured concentrations of Mesosulfuron-methyl ranged from 98.8 to 102.8% 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:** Not reported.

### 2. Test organism:

**Name:** *Pseudokirchneriella subcapitata* (syn *Selenastrum capricornutum*)

*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*

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**Strain:** 61.81

**Source:** University of Goettingen ,Germany

**Age of inoculum:** Four days

**Method of cultivation:** Standard algal medium (OECD and EPA guidelines)

**B. STUDY DESIGN:**

a) Range-finding Study: A range-finding study was not reported.

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)	Four days Standard algal medium; same as test Not reported	<i>EPA recommends two week acclimation period.</i>  <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 static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator-water bath	
Duration of the test	96 hours	<i>EPA requires: 96 - 120 hours</i>  <i>OECD: 72 hours</i>
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with pressed paper stoppers 300 mL 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>

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Parameter	Details	Remarks
		Criteria
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	Standard algal medium 6.9-7.3 7.9-9.9 Na <sub>2</sub> EDTA·2H <sub>2</sub> O NaHCO <sub>3</sub> N/A	<hr/> 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 water Deionized 7.5 N/A Not reported Not reported Not reported Not reported Not detected Not reported	Dilution water characteristics were not reported.  EPA pH: <i>Skeletonema costatum</i> = ~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	<hr/> EPA recommends agitation only for <i>Selenastrum</i> at 100 cycles per min and <i>Skeletonema</i> at ~60 cycles per min. Aeration is not recommended.

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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>Pseudokirchneriella subcapitata</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: solvent control: treated ones:	6 N/A 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>

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Parameter	Details	Remarks
		Criteria
Test concentrations nominal:  measured:	10, 18, 32, 56, and 100 mg/L  9.8, 17.9, 30.6, 54.6, and 98.4 mg/L	<p><i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i></p> <p><i>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.</i></p>
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:	24.3-25.0°C Continuous 59.44-71.48 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , white fluorescent lighting	<p><i>EPA temperature: <u>Skeletonema</u>: 20°C, Others: 24-25°C; EPA photoperiod: <u>S. costatum</u> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <u>Anabaena</u>: 2.0 Klux (<math>\pm 15\%</math>), Others: 4 - 5 Klux (<math>\pm 15\%</math>)</i></p> <p><i>OECD recommended the temperature in the range of 21 to 25°C maintained at <math>\pm 2^\circ\text{C}</math> and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i></p>
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

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2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell count, growth rate, and 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	Cell counting chamber with a microscope	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i>  <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	Every 24 hours	<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 169X 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>  <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	

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**II. RESULTS and DISCUSSION:**

**A. INHIBITORY EFFECTS:**

There was significant growth inhibition in the 98.4 mg/L treatment group. No significant inhibitions in growth were observed in the 9.8, 17.9, 30.6, or 54.6 mg/L treatment groups.

**Table 3: Effect of AE F160459 on freshwater alga (*Pseudokirchneriella subcapitata*)**

Treatment measured and nominal concentration <sup>a</sup> (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition <sup>b</sup>
Dilution water control	~10,000	32,000	1,688,000	--
9.8 (10)	~10,000	26,000	1,729,000	-2
17.9 (18)	~10,000	34,000	1,951,000	-15
30.6 (32)	~10,000	29,000	1,984,000	-17
54.6 (56)	~10,000	32,000	1,997,000	-18
98.4 (100)	~10,000	18,000	924,000	45
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> Reviewer calculated % inhibition by comparing the treatment groups to the dilution water control.

**Table 4: Effect of AE F160459 on the Freshwater alga *Pseudokirchneriella subcapitata***

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	0.05342	--	34,355,400	--
9.8 (10)	~10,000	0.05359	-0.31	34,855,600	-1.46
17.9 (18)	~10,000	0.05493	-2.82	40,713,200	-18.51
30.6 (32)	~10,000	0.05511	-3.16	39,321,600	-14.46
54.6 (56)	~10,000	0.05516	-3.26	38,648,000	-12.49
98.4 (100)	~10,000	0.04708	11.86	16,196,800	52.86

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

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Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC <sub>05</sub> (mg/L)	54.6	54.6	Not reported
EC <sub>50</sub> (mg/L)	98	>98.4	Not reported
IC <sub>50</sub> or EC <sub>50</sub> (mg/L) (95% C.I.)	Not reported	N/A	Not reported
other (IC <sub>25</sub> /EC <sub>25</sub> )	N/A	N/A	Not reported
Reference chemical, if used NOAEC IC <sub>50</sub> /EC <sub>50</sub>	N/A	N/A	N/A

N/A = Not applicable.

**B. REPORTED STATISTICS:**

Statistical Method: The statistical software, SAS 1989, was used to calculate growth inhibitions. The EC<sub>50</sub> was estimated using growth inhibition data compared to the control. The biomass EC<sub>50</sub> was calculated using nominal test concentrations. The NOEC was verified using Analysis of Variance, General Linear Models with DUNCAN's Multiple Range Test Procedures.

**Cell Density:**

NOEC: Not reported

EC<sub>50</sub>: Not reported      95% C.I.: N/A

**Growth rate:**

NOEC: 54.6 mg/L

EC<sub>50</sub>: >98.4 mg/L      95% C.I.: N/A

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

NOEC: 54.6 mg/L

EC<sub>50</sub>: 98 mg/L      95% C.I.: Not reported

Endpoint(s) Affected: cell density, growth rate, and biomass

**C. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Cell density, growth rate, and biomass data satisfied the assumptions of ANOVA, so this test, followed by Bonferroni's t-test was used to determine the NOEC via TOXSTAT statistical software. The EC<sub>05</sub> and EC<sub>50</sub> (biomass) values could not be determined using the Probit method via Nuthatch statistical software; the EC<sub>50</sub> values for cell density and growth rate could be determined visually, because inhibition did not exceed 50%.

**Cell Density:**

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NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: >98.4 mg/L 95% C.I.: N/A  
Slope: could not determine

**Growth rate:**

NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: >98.4 mg/L 95% C.I.: N/A  
Slope: could not determine

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

NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: 98 mg/L 95% C.I.: not reported  
Slope: could not determine

Endpoint(s) Affected: cell density, growth rate, and biomass

**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'; there was significant inhibition of all endpoints at the highest treatment level. The reviewer's method of EC<sub>50</sub> estimation could not be used to confirm the study authors' value for biomass and the study authors did not report a 95% confidence interval for this estimate; the reviewer's method of analysis was also inappropriate to determine the EC<sub>05</sub> estimate for all endpoints.

**F. CONCLUSIONS:** The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata* (U.S. EPA Guideline 123-2). This study is classified as Core.

**Cell Density:**

NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: >98.4 mg/L 95% C.I.: N/A  
Slope: could not determine

**Growth rate:**

NOEC: 54.6 mg/L  
EC<sub>05</sub>: could not determine 95% C.I.: N/A  
EC<sub>50</sub>: >98.4 mg/L 95% C.I.: N/A  
Slope: could not determine

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

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NOEC: 54.6 mg/L

EC<sub>05</sub>: could not determine 95% C.I.: N/A

EC<sub>50</sub>: 98 mg/L 95% C.I.: not reported

Slope: could not determine

Endpoint(s) Affected: cell density, growth rate, and biomass

**III. REFERENCES:**

Organization of Economic Co-operation and Development, 1984 OECD-Guidelines for Testing of Chemicals Guideline No. 201: Alga, Growth Inhibition Test, 07 June 1984.

EU directive 92/69/EEG Annex part C.3. Algae growth inhibition test; 29. Dec. 1992.

U.S. Environmental Protection Agency (EPA), 1982, Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants.

U.S. Environmental Protection Agency (EPA). 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR Part 792) Fed. Reg., Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

SAS Institute Inc., 1989. Release 6.08 TS 407. Cary, North Carolina 27511.

Stephan, C.E., 1982. A Computer Program for Calculating an LC<sub>50</sub>, U.S. Environmental Protection Agency, Duluth, Mn. Letter to Dr. Lowell Bahner, Chairman of the ASTM Task Group on Calculating LC50s; September 10, 1982

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

cell density

File: 6309cd

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	25045.576	5009.115	35.012
Within (Error)	15	2146.023	143.068	
Total	20	27191.599		

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

cell density

File: 6309cd

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	168.782	168.782		
2	9.75	172.897	172.897	-0.487	
3	17.92	195.117	195.117	-3.114	
4	30.61	198.413	198.413	-3.503	
5	<b>54.59</b>	<b>199.653</b>	<b>199.653</b>	<b>-3.650</b>	
6	98.41	92.380	92.380	9.033	*

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

cell density

File: 6309cd

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			

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2	9.75	3	22.016	13.0	-4.115
3	17.92	3	22.016	13.0	-26.335
4	30.61	3	22.016	13.0	-29.632
5	54.59	3	22.016	13.0	-30.572
6	98.41	3	22.016	13.0	76.402

cell density

File: 6309cd

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	168.782	168.782	183.941
2	9.75	3	172.897	172.897	183.941
3	17.92	3	195.117	195.117	183.941
4	30.61	3	198.413	198.413	183.941
5	54.59	3	199.653	199.653	183.941
6	98.41	3	92.380	92.380	92.380

cell density

File: 6309cd

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	183.941				
9.75	183.941	1.792	*	1.75	k= 1, v=15
17.92	183.941	1.792		1.84	k= 2, v=15
30.61	183.941	1.792		1.87	k= 3, v=15
54.59	183.941	1.792		1.88	k= 4, v=15
98.41	92.380	9.033	*	1.89	k= 5, v=15

s = 11.961

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

EC<sub>x</sub>

!!!Failure #3: Data not suitable for probit model fit.

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Criterion is 3 or more distinct isotone means.

growth rate

File: 6309g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	144.433	28.887	40.233
Within (Error)	15	10.775	0.718	
Total	20	155.208		

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

growth rate

File: 6309g 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	53.422	53.422		
2	9.75	53.587	53.587	-0.275	
3	17.92	54.927	54.927	-2.512	
4	30.61	55.110	55.110	-2.818	
5	<b>54.59</b>	<b>55.167</b>	<b>55.167</b>	<b>-2.912</b>	
6	98.41	47.083	47.083	10.579	*

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

growth rate

File: 6309g 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
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1	control	6			
2	9.75	3	1.560	2.9	-0.165
3	17.92	3	1.560	2.9	-1.505
4	30.61	3	1.560	2.9	-1.688
5	54.59	3	1.560	2.9	-1.745
6	98.41	3	1.560	2.9	6.338

growth rate

File: 6309g

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	53.422	53.422	54.272
2	9.75	3	53.587	53.587	54.272
3	17.92	3	54.927	54.927	54.272
4	30.61	3	55.110	55.110	54.272
5	54.59	3	55.167	55.167	54.272
6	98.41	3	47.083	47.083	47.083

growth rate

File: 6309g

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	54.272				
9.75	54.272	1.419		1.75	k= 1, v=15
17.92	54.272	1.419		1.84	k= 2, v=15
30.61	54.272	1.419		1.87	k= 3, v=15
54.59	54.272	1.419		1.88	k= 4, v=15
98.41	47.083	10.576	*	1.89	k= 5, v=15

s = 0.848

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

EC<sub>x</sub>

!!!Failure #3: Data not suitable for probit model fit.

16



Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: !.....!

EPA MRID #: 45386309

Criterion is 3 or more distinct isotone means.

area under the growth curve

File: 6309b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	12362470.187	2472494.037	93.110
Within (Error)	15	398320.043	26554.670	
Total	20	12760790.231		

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

area under the growth curve

File: 6309b 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	3435.540	3435.540		
2	9.75	3485.560	3485.560	-0.434	
3	17.92	4071.320	4071.320	-5.518	
4	30.61	3932.160	3932.160	-4.310	
5	<b>54.59</b>	<b>3855.800</b>	<b>3855.800</b>	<b>-3.647</b>	
6	98.41	1619.680	1619.680	15.759	*

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

area under the growth curve

File: 6309b 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

17

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: {.....}

EPA MRID #: 45386309

1	control	6			
2	9.75	3	299.937	8.7	-50.020
3	17.92	3	299.937	8.7	-635.780
4	30.61	3	299.937	8.7	-496.620
5	54.59	3	299.937	8.7	-420.260
6	98.41	3	299.937	8.7	1815.860

area under the growth curve

File: 6309b

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	3435.540	3435.540	3702.653
2	9.75	3	3485.560	3485.560	3702.653
3	17.92	3	4071.320	4071.320	3702.653
4	30.61	3	3932.160	3932.160	3702.653
5	54.59	3	3855.800	3855.800	3702.653
6	98.41	3	1619.680	1619.680	1619.680

area under the growth curve

File: 6309b

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	3702.653				
9.75	3702.653	2.318	*	1.75	k= 1, v=15
17.92	3702.653	2.318	*	1.84	k= 2, v=15
30.61	3702.653	2.318	*	1.87	k= 3, v=15
54.59	3702.653	2.318	*	1.88	k= 4, v=15
98.41	1619.680	15.759	*	1.89	k= 5, v=15

s = 162.956

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

EC<sub>x</sub>

18

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) on the Freshwater  
Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: {.....}

EPA MRID #: 45386309

!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.

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