

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: {.....}

EPA MRID #: 45386306

Data Requirement: PMRA DATA CODE {.....}
EPA DP Barcode D284719
OECD Data Point {.....}
EPA MRID 45386306
EPA Guideline 123-2

Test material: AE F130060 Technical Purity: 94.6%
Common name: Mesosulfuron-methyl
Chemical name: IUPAC: methyl-2-[3-(4,6-dimethoxyprimidin-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: *Les LaSota*
Tim Dargatzis
{EPA/OECD/PMRA}

Date: *10/08/03*
Les LaSota

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

Date: {.....}

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

Date Evaluation Completed: {dd-mm-yy}

CITATION: Heusel, R., Weller, O., and Gosch, H. 1998. AE F130060, substance, technical, 94.6%, Code :AE F130060 00 1C95 0001, Algal growth inhibition- *Pseudokirchneriella subcapitata*. Unpublished study performed by Hoechst Schering AgrEvo, Frankfurt, Germany. Laboratory Study Identification No. CE97/024. Study submitted by Aventis CropScience, Research Triangle Park, NC. Experimental start date February 24, 1997 and experimental termination date June 23, 1997. The final report issued June 23, 1998.



2013013

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Pseudokirchneriella subcapitata*

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* (syn *Selenastrum capricornutum*) were exposed to Mesosulfuron-methyl under static conditions. Nominal concentrations were 0.032, 0.056, 0.1, 0.18, and 0.32 mg/L. Measured concentrations at test initiation were 0.029, 0.048, 0.088, 0.164, and 0.288 mg/L. These treatment groups were compared to a dilution water control. Mean cell density was significantly reduced in the 0.088, 0.164, and 0.288 mg/L treatment groups by 16, 11, and 70%, respectively, compared to the dilution water control. The algal growth rates were significantly inhibited in the 0.088, 0.164, and 0.288 mg/L treatment groups. The biomass % inhibition was significant in the 0.048, 0.088, 0.164, and 0.288 mg/L treatment groups. Biomass was the most sensitive endpoint, with an EC₅₀ of 0.21 mg/L.

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: 0.048 mg/L
EC₀₅: 0.15 mg/L 95% C.I.: 0.12-0.18 mg/L
EC₅₀: 0.25 mg/L 95% C.I.: 0.24-0.26 mg/L
Slope: 7.62±1.10

Growth rate:

NOEC: 0.048 mg/L
EC₀₅: 0.20 mg/L 95% C.I.: 0.18-0.23 mg/L
EC₅₀: >0.288 mg/L 95% C.I.: N/A
Slope: 5.51±0.849

Area Under the Growth Curve (Biomass):

NOEC: 0.029 mg/L
EC₀₅: 0.11 mg/L 95% C.I.: 0.091-0.13 mg/L
EC₅₀: 0.21 mg/L 95% C.I.: 0.20-0.23 mg/L
Slope: 5.61±0.518

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

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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/EWG 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 (AE F130060 Technical)

Description: Light beige powder

Lot No./Batch No.: AE F130060 00 1C95 0001

Purity: 94.6%

Stability of Compound

Under Test Conditions: The day 0 measured concentrations were 90.7-98.0% of nominal and the day 4 measured concentrations were 14.9-87.4% of nominal. Mean measured concentrations of Mesosulfuron-methyl ranged from 54.8 to 91.6% 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

Strain: 61.81 (=CCAP 278.4=UTEX 1648=ATCC 22662)

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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 7.5 8.2-9.3 Na ₂ EDTA·2H ₂ O NaHCO ₃ 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. <hr/> 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:	0.032, 0.056, 0.1, 0.18, and 0.32 mg/L 0.018, 0.041, 0.084, 0.165, and 0.292 mg/L (study author-reported mean measured concentrations) 0.029, 0.048, 0.088; 0.164, and 0.288 mg/L (measured concentrations at test initiation)	Measured concentrations at test initiation were used by the reviewer for toxicity estimates. <hr/> 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:	24.5-24.9°C Continuous 64.2-69.8 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, white fluorescent lighting	<hr/> 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 ($\pm 15\%$), Others: 4 - 5 Klux ($\pm 15\%$) OECD recommended the temperature in the range of 21 to 25°C maintained at $\pm 2^\circ\text{C}$ 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, 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	Counting chamber	<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 228X greater than the dilution water control group cell density at test initiation.	<i>EPA requires control cell count at termination to be $\geq 2X$ 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	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Mean cell densities decreased in the 0.088, 0.164, and 0.288 mg/L treatment groups by 16, 11, and 70%, respectively, compared to the dilution water control. The algal growth rates had significant inhibition in the 0.088, 0.164, and 0.288 mg/L treatment groups. The biomass % inhibition was significant in the 0.048, 0.088, 0.164, and 0.288 mg/L treatment groups.

Table 3: Effect of Mesosulfuron-methyl on freshwater alga (*Pseudokirchneriella subcapitata*)

Treatment measured and nominal concentration ^a (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition ^b
Dilution water control	~10,000	38,000	2,266,000	--
0.029 (0.032)	~10,000	43,000	2,307,000	-1
0.048 (0.056)	~10,000	37,000	2,256,000	0
0.088 (0.1)	~10,000	29,000	1,913,000	16
0.164 (0.18)	~10,000	17,000	2,022,000	11
0.288 (0.32)	~10,000	18,000	691,000	70
Reference chemical (if used)	N/A	N/A	N/A	N/A

^a Measured concentrations of Mesosulfuron-methyl at test initiation. Nominal concentrations are in parentheses.

^b Reviewer calculated % inhibition by comparing the treatment groups to the dilution water control.

Table 4: Effect of Mesosulfuron-methyl on the Freshwater alga *Pseudokirchneriella subcapitata*

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	0.05648	--	59,132,000	--
0.029 (0.032)	~10,000	0.05668	-0.3	59,504,000	-1
0.048 (0.056)	~10,000	0.05644	0.1	55,608,000	6
0.088 (0.1)	~10,000	0.05473	3	48,104,000	19
0.164 (0.18)	~10,000	0.05530	2	43,072,000	27

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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)
0.288 (0.32)	~10,000	0.04412	22	12,576.000	79
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

^a Measured concentrations of Mesosulfuron-methyl at test initiation. Nominal concentrations are in parentheses.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC (mg/L)	0.018	0.041	Not reported
EC ₅₀ (mg/L)	0.21	>0.29	Not reported
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	0.16-0.29	N/A	Not reported
other (IC ₂₅ /EC ₂₅)	N/A	N/A	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: The statistical software, SAS 1989, was used to calculate growth inhibitions. The EC₅₀ for biomass was calculated using the C.E. Stephan software (method not specified). The NOEC was verified using Analysis of Variance, General Linear Models with DUNCAN's Multiple Range Test Procedures.

Cell Density:

NOEC: Not reported

EC₅₀: Not reported 95% C.I.: N/A

Growth rate:

NOEC: 0.041 mg/L

EC₅₀: >0.29 mg/L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):

NOEC: 0.018 mg/L

EC₅₀: 0.21 mg/L 95% C.I.: 0.16-0.29 mg/L

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, growth rate, and biomass data satisfied the assumptions of ANOVA, so this test was used to determine the NOEC, followed by William's multiple comparison test via TOXSTAT statistical software. The EC₀₅ and EC₅₀ values were determined using the Probit method via Nuthatch statistical software.

Cell Density:

NOEC: 0.048 mg/L

EC₀₅: 0.15 mg/L 95% C.I.: 0.12-0.18 mg/L

EC₅₀: 0.25 mg/L 95% C.I.: 0.24-0.26 mg/L

Slope: 7.62±1.10

Growth rate:

NOEC: 0.048 mg/L

EC₀₅: 0.20 mg/L 95% C.I.: 0.18-0.23 mg/L

EC₅₀: >0.288 mg/L 95% C.I.: N/A

Slope: 5.51±0.849

Area Under the Growth Curve (Biomass):

NOEC: 0.029 mg/L

EC₀₅: 0.11 mg/L 95% C.I.: 0.091-0.13 mg/L

EC₅₀: 0.21 mg/L 95% C.I.: 0.20-0.23 mg/L

Slope: 5.61±0.518

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

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'. Biomass was the most sensitive endpoint, with an EC₅₀ value of 0.21 mg/L; the EC₀₅ value for this endpoint was 0.11 mg/L.

The reviewer used the measured concentrations at test initiation (day 0), which are reported in Table 6.2 on p. 23, for calculating the toxicity values in this study because the test material declined over the study period. Even though these concentrations differ slightly from the mean measured concentrations reportedly used by the study authors, the reviewer's EC₅₀ estimate for biomass (the most sensitive endpoint) was identical to that of the study authors.

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). Biomass was the most sensitive endpoint. This study is classified as Core.

Cell Density:

NOEC: 0.048 mg/L

EC₀₅: 0.15 mg/L

EC₅₀: 0.25 mg/L

Slope: 7.62±1.10

95% C.I.: 0.12-0.18 mg/L

95% C.I.: 0.24-0.26 mg/L

Growth rate:

NOEC: 0.048 mg/L

EC₀₅: 0.20 mg/L

EC₅₀: >0.288 mg/L

Slope: 5.51±0.849

95% C.I.: 0.18-0.23 mg/L

95% C.I.: N/A

Area Under the Growth Curve (Biomass):

NOEC: 0.029 mg/L

EC₀₅: 0.11 mg/L

EC₅₀: 0.21 mg/L

Slope: 5.61±0.518

95% C.I.: 0.091-0.13 mg/L

95% C.I.: 0.20-0.23 mg/L

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

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/EWG Annex part C.3. Algae growth inhibition test; 29. Dec. 1992.
- Kuhl, A. and Lorenzen, H. 1964. Handling and Culturing of Chlorella, in Methods of Cell Physiology, Vol. I. New York-London, pp. 159-187.
- 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.
- Stephan, C.E., 1982. A Computer Program for Calculating an LC_{50} , U.S. Environmental Protection Agency, Duluth, Mn. Letter to Dr. Lowell Bahner, Chairman of the ASTM Task Group on Calculating LC_{50} s: September 10, 1982
- SAS Institute Inc., 1989. Release 6.08 TS 407. Cary, North Carolina 27511.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density

File: 6306cd

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	60319.719	12063.944	199.200
Within (Error)	15	908.433	60.562	
Total	20	61228.152		

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

Since F > Critical F REJECT Ho: All groups equal

cell density

File: 6306cd

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	226.567	226.567		
2	0.029	230.733	230.733	-0.757	
3	0.048	225.600	225.600	0.176	
4	0.088	191.333	191.333	6.403	*
5	0.164	202.200	202.200	4.428	*
6	0.288	69.133	69.133	28.610	*

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

cell density

File: 6306cd

Transform: NO TRANSFORMATION

BONFERRONI I-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	0.029	3	14.324	6.3	-4.167
3	0.048	3	14.324	6.3	0.967

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4	0.088	3	14.324	6.3	35.233
5	0.164	3	14.324	6.3	24.367
6	0.288	3	14.324	6.3	157.433

cell density

File: 6306cd

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	226.567	226.567	227.956
2	0.029	3	230.733	230.733	227.956
3	0.048	3	225.600	225.600	225.600
4	0.088	3	191.333	191.333	196.767
5	0.164	3	202.200	202.200	196.767
6	0.288	3	69.133	69.133	69.133

cell density

File: 6306cd

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	227.956				
0.029	227.956	0.252		1.75	k= 1, v=15
0.048	225.600	0.176		1.84	k= 2, v=15
0.088	196.767	5.415	*	1.87	k= 3, v=15
0.164	196.767	5.415	*	1.88	k= 4, v=15
0.288	69.133	28.610	*	1.89	k= 5, v=15

s = 7.782

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

Estimates of EC₅

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.15	0.12	0.18	0.040	0.82
EC10	0.17	0.14	0.20	0.033	0.85

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EC25	0.20	0.18	0.23	0.022	0.90
EC50	0.25	0.24	0.26	0.012	0.95

Slope = 7.62 Std.Err. = 1.10

!!!Poor fit: p < 0.001 based on DF= 3.00 15.0

6306CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	227.	220.	6.31	100.	0.00
0.0290	3.00	231.	220.	10.5	100.	5.78e-11
0.0480	3.00	226.	220.	5.35	100.	2.61e-06
0.0880	3.00	191.	220.	-28.9	100.	0.0293
0.164	3.00	202.	202.	0.450	91.6	8.40
0.285	3.00	69.1	69.2	-0.0489	31.4	68.6

growth rate 96

File: 6306g

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	373.672	74.734	458.491
Within (Error)	15	2.448	0.163	
Total	20	376.120		

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

growth rate 96

File: 6306g

Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2

Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
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GROUP	IDENTIFICATION	NUM OF REPS	ORIGINAL MEAN	TRANSFORMED MEAN	DIFFERENCE FROM CONTROL
1	control	6	56.483	56.483	
2	0.029	3	56.677	56.677	-0.677
3	0.048	3	56.437	56.437	0.163
4	0.088	3	54.723	54.723	6.165 *
5	0.164	3	55.300	55.300	4.145 *
6	0.288	3	44.120	44.120	43.307 *

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

growth rate 96

File: 6306g

Transform: NO TRANSFORM

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	0.029	3	0.743	1.3	-0.193
3	0.048	3	0.743	1.3	0.047
4	0.088	3	0.743	1.3	1.760
5	0.164	3	0.743	1.3	1.183
6	0.288	3	0.743	1.3	12.363

growth rate 96

File: 6306g

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	56.483	56.483	56.548
2	0.029	3	56.677	56.677	56.548
3	0.048	3	56.437	56.437	56.437
4	0.088	3	54.723	54.723	55.012
5	0.164	3	55.300	55.300	55.012
6	0.288	3	44.120	44.120	44.120

growth rate 96

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Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #:.....

EPA MRID #: 45386306

File: 6306g

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	56.548				
0.029	56.548	0.226		1.75	k= 1, v=15
0.048	56.437	0.163		1.84	k= 2, v=15
0.088	55.012	5.152	*	1.87	k= 3, v=15
0.164	55.012	5.152	*	1.88	k= 4, v=15
0.288	44.120	43.280	*	1.89	k= 5, v=15

s = 0.404

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

Estimates of EC_x

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.20	0.18	0.23	0.024	0.89
EC10	0.23	0.22	0.25	0.015	0.93
EC25	0.30	0.29	0.31	0.0056	0.97
EC50	0.40	0.36	0.45	0.022	0.90

Slope = 5.51 Std.Err. = 0.849

!!!Poor fit: p < 0.001 based on DF= 3.00 15.0

6306G : growth rate 96

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	56.5	56.2	0.313	100.	0.00
0.0290	3.00	56.7	56.2	0.507	100.	1.62e-08
0.0480	3.00	56.4	56.2	0.267	100.	1.88e-05
0.0880	3.00	54.7	56.2	-1.44	100.	0.0142
0.164	3.00	55.3	55.3	0.0411	98.4	1.62
0.288	3.00	44.1	44.1	-0.00240	78.6	21.4

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

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Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: [REDACTED]

EPA MRID #: 45386306

area under growth curve 96

File: 6306b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	51511216.045	10302243.209	458.182
Within (Error)	15	337275.841	22485.056	
Total	20	51848491.886		

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

area under growth curve 96

File: 6306b 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	5913.200	5913.200		
2	0.029	5950.400	5950.400	-0.351	
3	0.048	5560.800	5560.800	3.324	*
4	0.088	4810.400	4810.400	10.401	*
5	0.164	4307.200	4307.200	15.147	*
6	0.288	1257.600	1257.600	43.908	*

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

area under growth curve 96

File: 6306b 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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Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: [.....]

EPA MRID #: 45386306

2	0.029	3	275.998	4.7	-37.200
3	0.048	3	275.998	4.7	352.400
4	0.088	3	275.998	4.7	1102.800
5	0.164	3	275.998	4.7	1606.000
6	0.288	3	275.998	4.7	4655.600

area under growth curve 96

File: 6306b

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	5913.200	5913.200	5925.600
2	0.029	3	5950.400	5950.400	5925.600
3	0.048	3	5560.800	5560.800	5560.800
4	0.088	3	4810.400	4810.400	4810.400
5	0.164	3	4307.200	4307.200	4307.200
6	0.288	3	1257.600	1257.600	1257.600

area under growth curve 96

File: 6306b

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	5925.600				
0.029	5925.600	0.117		1.75	k= 1, v=15
0.048	5560.800	3.324	*	1.84	k= 2, v=15
0.088	4810.400	10.401	*	1.87	k= 3, v=15
0.164	4307.200	15.147	*	1.88	k= 4, v=15
0.288	1257.600	43.908	*	1.89	k= 5, v=15

s = 149.950

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

Estimates of EC:

Parameter	Estimate	95% Bounds	Std.Err.	Lower Bound
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Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: {.....}

EPA MRID #: 45386306

		Lower	Upper		/Estimate
EC5	0.11	0.091	0.13	0.036	0.84
EC10	0.13	0.11	0.14	0.030	0.86
EC25	0.16	0.14	0.18	0.021	0.90
EC50	0.21	0.20	0.23	0.013	0.94

Slope = 5.61 Std.Err. = 0.518

!!!Poor fit: p < 0.001 based on DF= 3.00 15.0

6306B : area under growth curve 96

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	5.91e+03	5.67e+03	240.	100.	0.00
0.0290	3.00	5.95e+03	5.67e+03	278.	100.	6.38e-05
0.0480	3.00	5.56e+03	5.67e+03	-111.	100.	0.0150
0.0880	3.00	4.81e+03	5.58e+03	-770.	98.4	1.62
0.164	3.00	4.31e+03	4.16e+03	150.	73.3	26.7
0.288	3.00	1.26e+03	1.28e+03	-26.6	22.6	77.4

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