

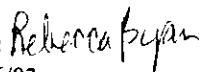
US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Marine Diatom *Skeletonema costatum*
PMRA Submission #: {.....} EPA MRID #: 45386314

Data Requirement: PMRA DATA CODE: {.....}
EPA DP Barcode: D284719
OECD Data Point: {.....}
EPA MRID: 45386314
EPA Guideline: 123-2

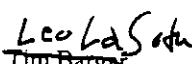
Test material: AE F130060 Technical Purity: 95.7%
Common name: Mesosulfuron-methyl
Chemical name: IUPAC: methyl-2-[3-(4,6-dimethoxyprimidin-2-yl) ureidosulfonyl]-4-methanesulfonamidomethylbenzoate
CAS name: methyl 2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amin]sulfonyl]-4-[[[(methylsulfonyl)amino]methyl]benzoate
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: 
Leo LaSota
Tim Bargar
{EPA/OECD/PMRA}

Date: 
01/09/04

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: Young, B. M. and Abedi, J. 2001. Effect to *Skeletonema costatum* (Marine Diatom) in a Growth Inhibition Test, AE F130060, Technical, 95.7 w/w. Unpublished study performed by Aventis CropScience, Ecotoxicology Department, Pikeville, NC.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. CK99W502, and sponsored by Aventis CropScience, Ecotoxicology Department, Research Triangle Park, NC. Experimental start date June 5, 2000 and experimental termination date June 9, 2000. Completed February 16, 2001.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Skeletonema costatum* were exposed to Mesosulfuron-methyl under static conditions. Nominal concentrations were 13, 22, 36, 60, and 100 mg/L. Mean measured concentrations over the study period were 12.3, 22.6, 37.8, 62.3, and 106.2 mg/L; these treatment groups were compared to a dilution water control. Cell density and growth rate were significantly inhibited in the 106.2 mg/L treatment group. Biomass was significantly inhibited in the 62.3 and 106.2 mg/L treatment groups. Biomass was the most sensitive parameter; the NOEC was 37.8 mg/L and the EC₅₀ was 98 mg/L.

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

Results Synopsis

Test Organism: *Skeletonema costatum*
Test Type: Static

Cell Density:

NOEC: 62.3 mg/L
EC₀₅: 70 mg/L 95% C.I.: 43-120 mg/L
EC₅₀: 110 mg/L 95% C.I.: 100-130 mg/L
Slope: 7.72±4.41

Growth rate:

NOEC: 62.3 mg/L
EC₀₅: 88 mg/L 95% C.I.: 68-120 mg/L
EC₅₀: >106 mg/L 95% C.I.: N/A
Slope: 5.90±3.98

Biomass (Area under growth curve):

NOEC: 37.8 mg/L
EC₀₅: 45 mg/L 95% C.I.: 34-61 mg/L
EC₅₀: 98 mg/L 95% C.I.: 91-110 mg/L
Slope: 4.92±0.833

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

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 flasks were agitated by shaking once daily and the EPA recommends continuous agitation at ~60 rpm for this species.
2. The length of the acclimation period was not reported.
3. The carbon source of the growth medium 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 94 to 106% 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: *Skeletonema costatum*

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: CCMP1332

Source: Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow Laboratory for

Ocean Sciences, West Boothbay Harbor, Maine
 Age of inoculum: 7 days old
 Method of cultivation: Marine algal assay (MAA) nutrient medium

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 test exposed marine alga, *Skeletonema costatum* to nominal concentrations of 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 2, 9, 0, and 26% biomass inhibition in the 0.1, 1.0, 10, and 100 mg/L treatment groups, respectively, compared to the control. The growth rate inhibition was 0.5, 2.4, 0, and 7.6% biomass inhibition in the 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 Marine algal assay (MAA) nutrient 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	
Duration of the test	96 hours	<i>EPA requires: 96 - 120 hours</i> <i>OECD: 72 hours</i>

Parameter	Details	Remarks
		Criteria
Test vessel material: (glass/polystyrene) size: fill volume:	Glass 250 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>
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	Marine algal assay (MAA) nutrient medium 7.6-8.0 8.8-9.2 Na ₂ EDTA•2H ₂ O Not reported 30-31‰	Appendix 1, p. 42. <i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i> <i>EPA recommends 20X-AAP medium</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes (Appendix 1, p. 42)	
Dilution water source: type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Seawater from Wood's Hole, MA, and laboratory well water Filtered natural seawater 8.1 ± 0.1 30-31‰ Not reported <1.0 mg/L <11.0 mg/L (total suspended solids) ≤27.6 mg/L (Appendix 2, p. 44) not detected not reported	Seawater was not analyzed. The laboratory well water was analyzed. <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> <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	

Parameter	Details	Remarks
		Criteria
Aeration or agitation	Agitation, test flasks were swirled once per day	Continuous agitation not performed. <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>
Initial cells density	10,000 cells/mL	<i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <u>Anabaena flos-aquae</u>, cell counts on day 2 are not required. OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <u>S. capricornutum</u> and <u>S. subspicatus</u>. When other species are used the biomass should be comparable.</i>
Number of replicates control: solvent control: treated ones:	6 N/A 3	<i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <u>Navicula</u> 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.</i>

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Parameter	Details	Remarks
		Criteria
Test concentrations nominal: measured:	13, 22, 36, 60, and 100 mg/L. 12.3, 22.6, 37.8, 62.3, and 106.2 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:	17.9-21.6°C 14 hours light, 10 hours dark 4300 lux ± 15%, cool-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 (±15%), Others: 4 - 5 Klux (±15%)</i></p> <p><i>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.</i></p>
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)	<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> <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 73.1X greater than the 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	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Cell density percent inhibitions were 1, 0, -5, 1, and 39% in the 12.3, 22.6, 37.8, 62.3, and 106.2 mg/L treatment groups, respectively, compared to the dilution water control. The algal growth rates had significant inhibition in the 106.2 mg/L treatment group. The biomass percent inhibition was significant in the 62.3 and 106.2 mg/L treatment groups.

Table 3: Effect of Mesosulfuron-methyl on diatom growth (*Skeletonema costatum*)

Measured and nominal concentrations ^a (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition ^b
Negative control	~10,000	29,000	731,000	--
12.3 (13)	~10,000	32,000	726,000	1
22.6 (22)	~10,000	33,000	733,000	0
37.8 (36)	~10,000	26,000	766,000	-5
62.3 (60)	~10,000	23,000	723,000	1
106.2 (100)	~10,000	14,000	447,000	39
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 reviewer-calculated by comparing the treatment groups to the negative (dilution water) control.

Table 4: Effect of Mesosulfuron-methyl on the Marine Diatom *Skeletonema costatum*

Measured and nominal concentrations ^a (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)
Negative control	~10,000	0.0447	--	24,500,000	--
12.3 (13)	~10,000	0.0446	0	25,200,000	-3
22.6 (22)	~10,000	0.0447	0	25,800,000	-5
37.8 (36)	~10,000	0.0452	-1	25,700,000	-5
62.3 (60)	~10,000	0.0446	0	20,700,000	16*
106.2 (100)	~10,000	0.0394	12*	10,900,000	55*

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Measured and nominal concentrations ^a (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)	37.8	62.3	62.3
LOEC (mg/L)	62.3	106.2	106.2
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	93 (86 to 100) ¹	>106.2	Not reported
other (IC ₂₅ /EC ₂₅)	N/A	N/A	N/A
Reference chemical, if used NOEC IC ₅₀ /EC ₅₀	N/A	N/A	N/A

N/A = Not applicable.

¹ The EC₅₀ for biomass was calculated using nominal test concentrations.

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[®] Procedure NLIN (Version 8.0) or TOXSTAT (Version 3.4). The EC₅₀ was determined using the non-linear regression procedure (Bruce and Versteeg). Toxicity calculations were performed using nominal concentrations.

Cell Density:

NOEC: 60 mg/L

LOEC: 100 mg/L

EC₅₀: Not reported 95% C.I.: N/A

Growth rate:

NOEC: 60 mg/L

LOEC: 100 mg/L

EC₅₀: >100 mg/L 95% C.I.: N/A

Biomass (Area under growth curve):

NOEC: 36 mg/L

LOEC: 60 mg/L
EC₅₀: 93 mg/L 95% C.I.: 86-100 mg/L

Most Sensitive Endpoint: Biomass

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 Bonferroni's t-test via TOXSTAT statistical software. The EC₀₅ and EC₅₀ values were determined using the Probit method via Nuthatch statistical software. Toxicity calculations were performed using mean measured concentrations.

Cell Density:

NOEC: 62.3 mg/L
EC₀₅: 70 mg/L 95% C.I.: 43-120 mg/L
EC₅₀: 110 mg/L 95% C.I.: 100-130 mg/L
Slope: 7.72±4.41

Growth rate:

NOEC: 62.3 mg/L
EC₀₅: 88 mg/L 95% C.I.: 68-120 mg/L
EC₅₀: >106 mg/L 95% C.I.: N/A
Slope: 5.90±3.98

Biomass (Area under growth curve):

NOEC: 37.8 mg/L
EC₀₅: 45 mg/L 95% C.I.: 34-61 mg/L
EC₅₀: 98 mg/L 95% C.I.: 91-110 mg/L
Slope: 4.92±0.833

Endpoints 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'; however the reviewer's toxicity calculations were based on the mean measured concentrations, while the study authors based calculations on the nominal concentrations. In addition, the reviewer determined toxicity values for cell density, as well as the EC₀₅ values for all endpoints, and these are reported in the Executive Summary and Conclusions sections. Based on the reviewer's and study authors' conclusions, biomass was the most sensitive endpoint, with an EC₅₀ value of 98 mg/L.

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

Cell Density:

NOEC: 62.3 mg/L

EC₀₅: 70 mg/L

EC₅₀: 110 mg/L

Slope: 7.72±4.41

95% C.I.: 43-120 mg/L

95% C.I.: 100-130 mg/L

Growth rate:

NOEC: 62.3 mg/L

EC₀₅: 88 mg/L

EC₅₀: >106 mg/L

Slope: 5.90±3.98

95% C.I.: 68-120 mg/L

95% C.I.: N/A

Biomass (Area under growth curve):

NOEC: 37.8 mg/L

EC₀₅: 45 mg/L

EC₅₀: 98 mg/L

Slope: 4.92±0.833

95% C.I.: 34-61 mg/L

95% C.I.: 91-110 mg/L

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

III. REFERENCES:

- Organization of Economic Cooperation and Development (1989) "Alga, Growth Inhibition Test", OECD Guideline for Testing of Chemicals, Guideline 201, Paris.
- U.S. Environmental Protection Agency (1986) *Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Wildlife and Aquatic Organisms*; Office of Pesticide Programs, Guideline 123-2, EPA 540/9-86-134. 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.
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- 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.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density

File: 6314cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	21.740	4.348	14.209
Within (Error)	15	4.587	0.306	
Total	20	26.327		

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

cell density

File: 6314cd 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	7.310	7.310		
2	12.25	7.260	7.260	0.128	
3	22.55	7.327	7.327	-0.043	
4	37.75	7.663	7.663	-0.903	
5	62.25	7.223	7.223	0.222	
6	106.15	4.467	4.467	7.269	*

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

cell density

File: 6314cd 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	12.25	3	1.018	13.9	0.050
3	22.55	3	1.018	13.9	-0.017
4	37.75	3	1.018	13.9	-0.353
5	62.25	3	1.018	13.9	0.087

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6 106.15 3 1.018 13.9 2.843

cell density
 File: 6314cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	7.310	7.310	7.374
2	12.25	3	7.260	7.260	7.374
3	22.55	3	7.327	7.327	7.374
4	37.75	3	7.663	7.663	7.374
5	62.25	3	7.223	7.223	7.223
6	106.15	3	4.467	4.467	4.467

cell density
 File: 6314cd 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	7.374				
12.25	7.374	0.164		1.75	k= 1, v=15
22.55	7.374	0.164		1.84	k= 2, v=15
37.75	7.374	0.164		1.87	k= 3, v=15
62.25	7.223	0.222		1.88	k= 4, v=15
106.15	4.467	7.271	*	1.89	k= 5, v=15

s = 0.553

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	70.	43.	1.2E+02	0.10	0.61
EC10	78.	54.	1.1E+02	0.076	0.69
EC25	94.	80.	1.1E+02	0.033	0.85
EC50	1.1E+02	1.0E+02	1.3E+02	0.023	0.89

Slope = 7.72 Std.Err. = 4.41

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Goodness of fit: p = 0.84 based on DF= 3.0 15.

6314CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. - Pred.	Pred. %Control	%Change
0.00	6.00	7.31	7.37	-0.0633	100.	0.00
12.3	3.00	7.26	7.37	-0.113	100.	2.92e-12
22.6	3.00	7.33	7.37	-0.0467	100.	2.32e-06
37.8	3.00	7.66	7.37	0.291	100.	0.00934
62.3	3.00	7.22	7.23	-0.00423	98.0	1.98
106.	3.00	4.47	4.47	0.000156	60.6	39.4

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

growth rate

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Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.735	0.147	13.364
Within (Error)	15	0.169	0.011	
Total	20	0.904		

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

growth rate

File: 6314g

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	4.470	4.470		
2	12.25	4.463	4.463	0.090	
3	22.55	4.473	4.473	-0.045	
4	37.75	4.520	4.520	-0.674	
5	62.25	4.453	4.453	0.225	
6	106.15	3.943	3.943	7.102	*

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

growth rate
 File: 6314g 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	12.25	3	0.193	4.3	0.007
3	22.55	3	0.193	4.3	-0.003
4	37.75	3	0.193	4.3	-0.050
5	62.25	3	0.193	4.3	0.017
6	106.15	3	0.193	4.3	0.527

growth rate
 File: 6314g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	4.470	4.470	4.479
2	12.25	3	4.463	4.463	4.479
3	22.55	3	4.473	4.473	4.479
4	37.75	3	4.520	4.520	4.479
5	62.25	3	4.453	4.453	4.453
6	106.15	3	3.943	3.943	3.943

growth rate
 File: 6314g 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	4.479				
12.25	4.479	0.124		1.75	k= 1, v=15
22.55	4.479	0.124		1.84	k= 2, v=15
37.75	4.479	0.124		1.87	k= 3, v=15
62.25	4.453	0.222		1.88	k= 4, v=15

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Marine Diatom *Skeletonema costatum*
 PMRA Submission #: [REDACTED] EPA MRID #: 45386314

106.15 3.943 7.019 * 1.89 k= 5, v=15

s = 0.106

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	88.	68.	1.2E+02	0.056	0.76
EC10	1.0E+02	94.	1.1E+02	0.017	0.92
EC25	1.3E+02	98.	1.7E+02	0.057	0.76
EC50	1.7E+02	88.	3.2E+02	0.13	0.52

Slope = 5.90 Std.Err. = 3.98

Goodness of fit: p = 0.91 based on DF= 3.0 15.

6314G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	4.47	4.48	-0.00913	100.	0.00
12.3	3.00	4.46	4.48	-0.0158	100.	9.57e-10
22.6	3.00	4.47	4.48	-0.00580	100.	1.32e-05
37.8	3.00	4.52	4.48	0.0412	100.	0.00648
62.3	3.00	4.45	4.45	-0.00133	99.5	0.546
106.	3.00	3.94	3.94	4.45e-05	88.0	12.0

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

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

area under growth curve

File: 6314b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	5.198	1.040	26.000
Within (Error)	15	0.594	0.040	
Total	20	5.792		

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

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Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Marine Diatom *Skeletonema costatum*
 PMRA Submission #: [REDACTED] EPA MRID #: 45386314

Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

area under growth curve
 File: 6314b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	2.448	2.448		
2	12.25	2.523	2.523	-0.530	
3	22.55	2.583	2.583	-0.955	
4	37.75	2.563	2.563	-0.813	
5	62.25	2.067	2.067	2.699	*
6	106.15	1.094	1.094	9.577	*

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

area under growth curve
 File: 6314b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	12.25	3	0.368	15.0	-0.075
3	22.55	3	0.368	15.0	-0.135
4	37.75	3	0.368	15.0	-0.115
5	62.25	3	0.368	15.0	0.382
6	106.15	3	0.368	15.0	1.354

area under growth curve
 File: 6314b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	2.448	2.448	2.513
2	12.25	3	2.523	2.523	2.513
3	22.55	3	2.583	2.583	2.513
4	37.75	3	2.563	2.563	2.513

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl on the Marine Diatom *Skeletonema costatum*
 PMRA Submission #: [.....] EPA MRID #: 45386314

5	62.25	3	2.067	2.067	2.067
6	106.15	3	1.094	1.094	1.094

area under growth curve
 File: 6314b 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	2.513				
12.25	2.513	0.462		1.75	k= 1, v=15
22.55	2.513	0.462		1.84	k= 2, v=15
37.75	2.513	0.462		1.87	k= 3, v=15
62.25	2.067	2.712	*	1.88	k= 4, v=15
106.15	1.094	9.622	*	1.89	k= 5, v=15

s = 0.199

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	45.	34.	61.	0.060	0.75
EC10	54.	43.	68.	0.048	0.79
EC25	71.	62.	82.	0.029	0.87
EC50	98.	91.	1.1E+02	0.016	0.93

Slope = 4.92 Std.Err. = 0.833

Goodness of fit: p = 0.65 based on DF= 3.0 15.

6314B : area under growth curve

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	2.45	2.52	-0.0702	100.	0.00
12.3	3.00	2.52	2.52	0.00484	100.	0.000438
22.6	3.00	2.58	2.52	0.0669	99.9	0.0841
37.8	3.00	2.56	2.47	0.0969	97.9	2.07
62.3	3.00	2.07	2.10	-0.0339	83.4	16.6
106.	3.00	1.09	1.09	0.00549	43.2	56.8

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Pages 21 through 25 are not included in this copy.

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- Identity of product impurities.
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- Description of quality control procedures.
- Identity of the source of product ingredients.
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