

US EPA ARCHIVE DOCUMENT

7 Days

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) to aquatic vascular plants *Lemna gibba*

PMRA Submission #: {.....}

EPA MRID#: 45386311

Data Requirement:
PMRA Data Code: {.....}
EPA DP Barcode: D284719
OECD Data Point: {.....}
EPA MRID: 45386311
EPA Guideline: 123-2

Test material: AE F160459 Purity: 96.8%
Common name: Mesosulfuron-methyl metabolite
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: *Leo LaSota*
Tim Bargar
{EPA/OECD/PMRA}

Date: *01/09/04*
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. and Weller, O. 2000. Duckweed (*Lemna gibba* G3) Growth Inhibition Test, AE F160459, substance, pure. (Metabolite of AE F130060). Unpublished study performed by Aventis CropScience GmbH, Frankfurt, Germany. Laboratory Study Identification No. CE00/058. Study submitted by Aventis CropScience, Research Triangle Park, NC. Experimental start date July 14, 2000 and experimental termination date July 21, 2000. The final report issued August 28, 2000.

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) to aquatic vascular plants *Lemna gibba*

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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater floating aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to Mesosulfuron-methyl metabolite (AE F160459) at mean measured concentrations of 0.1, 0.17, 0.29, 0.53, 0.93, 1.7, 3.0, 5.3, and 9.6 mg/L under static-renewal conditions. Nominal concentrations were 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg/L. Mean frond number decreased as test concentrations increased, when compared to the dilution water control: frond number was significantly reduced at treatment groups equal to and greater than 0.53 mg/L. Mean percent inhibition was 0, 0, 2, 8, 12, 65, 79, 86, and 88% in the 0.1, 0.17, 0.29, 0.53, 0.93, 1.7, 3.0, 5.3, and 9.6 mg/L treatment groups, respectively. By day 7, the mean dry weights were 23.4, 23.3, 23.1, 23.1, 22.7, 21.9, 10.8, 5.7, 5.8, and 3.9 mg in the 0.1, 0.17, 0.29, 0.53, 0.93, 1.7, 3.0, 5.3, and 9.6 mg/L treatment groups, respectively. Dry weight and growth rate were significantly reduced at the 0.93, 1.7, 3.0, 5.3, and 9.6 mg/L treatment groups. By day 7, yellow colored fronds were observed in the 0.93 and 1.7 mg/L treatment groups. The 3.0, 5.3, and 9.6 mg/L treatment groups had yellow fronds and fronds not fully separated. Frond number was the most sensitive endpoint: the NOEC was 0.29 mg/L and the EC₅₀ was 1.5 mg/L.

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core.

Results Synopsis

Test Organism: *Lemna gibba* G3

Test Type: Static-renewal

Number of fronds:

NOEC: 0.29 mg/L

EC₀₅: 0.20 mg/L

EC₅₀: 1.5 mg/L

Slope: 1.89±0.195

95% C.I.: 0.10-0.39 mg/L

95% C.I.: 1.1-2.0 mg/L

Dry Weight:

NOEC: 0.53 mg/L

EC₀₅: 0.22 mg/L

EC₅₀: 1.9 mg/L

Slope: 1.75±0.194

95% C.I.: 0.11-0.46 mg/L

95% C.I.: 1.4-2.6 mg/L

Growth Rate:

NOEC: 0.53 mg/L

EC₀₅: 0.31 mg/L

EC₅₀: 2.8 mg/L

Slope: 1.71±0.132

95% C.I.: 0.19-0.50 mg/L

95% C.I.: 2.4-3.4 mg/L

Endpoint(s) Affected: Frond number (most sensitive), dry weight, and growth rate

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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 American Society for Testing and Materials Guide E 1415-91. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The pretest health of the test organism was not reported.
2. The number of plants tested (3-4 plants) was less than the required 5 plants; therefore, there were 12 fronds per replicate, instead of the 15 fronds per replicate that is recommended.
3. 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: Measured concentrations (days 0, 3, and 5) for new test solutions ranged from 91.9 to 115.1% of nominal concentrations and measured concentrations (days 3, 5, and 7) of old test concentrations ranged from 87.7 to 101.9% of nominal concentrations, showing that the test material was stable under test conditions. 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: Duckweed, *Lemna gibba* EPA requires a vascular species: *Lemna gibba*.

Strain, if provided: G3

Source: AgrEvo USA Company, Pikeville, NC (original supplier: Plant Hormone Laboratory, USDA, Beltsville, MD)

Age of inoculum: 6 weeks

Method of cultivation: 20X AAP culture medium

B. STUDY DESIGN:

- a) Range-finding Study: No range-finding study was 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)	Approximately 6 weeks 20X AAP medium; same as test. Not reported.	
Test system static/static renewal/ renewal rate for static renewal:	Static-renewal	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Environmental chamber-water bath	
Duration of the test	7 days	<i>EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.</i>
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer-flasks 300 mL 150 mL	
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP medium 7.6 8.6-8.9 Na ₂ EDTA·2H ₂ O NaHCO ₃	<i>EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP. Chelators are not recommended.</i>

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Parameter	Details	Remarks
		Criteria
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Reagent grade water 7.5±0.1 Deionized water which is additionally filtered by an ultrafiltration, ion exchange and a charcoal unit. Not reported Not reported Not reported Not reported Not reported	<i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported	
Sediment used (for rooted aquatic vascular plants) origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	3 N/A 3	
Number of plants/replicate	3-4 plants per replicate	The number of plants tested (3-4 plants) were less than the required 5 plants. <i>EPA requires 5 plants.</i>
Number of fronds/plant	12 fronds per replicate at test initiation	There were probably three fronds per plant. <i>EPA requires 3 fronds per plant.</i>

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Parameter	Details	Remarks
		Criteria
Test concentrations nominal: measured:	0.1, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg/L 0.1, 0.17, 0.29, 0.53, 0.93, 1.7, 3.0, 5.3, and 9.6 mg/L	Mean measured concentrations were reviewer-calculated from mean fresh water and mean aged water values. <i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; new test solutions at 0, 3, and 5 days and old test solutions at 3, 5 and 7 days.	
Test conditions temperature: photoperiod: light intensity and quality:	24.0-24.5°C continuous light 105-108 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, white fluorescent lighting	<i>EPA temperature: 25°C EPA photoperiod: continuous EPA light: 5.0 Klux ($\pm 15\%$)</i>
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, dry weights, growth rates, and toxicity symptoms (yellow colored fronds and fronds not fully separated).	
Measurement technique for frond number and other end points	Direct counts	
Observation intervals	3, 5, and 7 days.	

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Table 3: Effect of Mesosulfuron-methyl metabolite (AE F160459) on frond number of Duckweed, *Lemna gibba*

Treatment ¹ (estimated measured and nominal concentration) mg/L	Initial frond number/test solution	Mean frond number at ²				Mean Growth Rate at Day 7	Mean Dry Weight of Fronds (biomass) (mg) ²
		3 days	5 days	7 days	% inhibition at 7 days ³		
Negative control (dilution water)	12	39	86	192	---	0.396	23.4
0.1 (0.10)	12	40	85	191	0	0.395	23.3
0.17 (0.18)	12	40	86	191	0	0.395	23.1
0.29 (0.32)	12	37	82	189	2	0.393	23.1
0.53 (0.56)	12	34	78	177	8	0.384	22.7
0.93 (1.0)	12	34	70	168	12	0.377	21.9
1.7 (1.8)	12	26	37	68	65	0.247	10.8
3.0 (3.2)	12	20	32	40	79	0.171	5.7
5.3 (5.6)	12	18	27	28	86	0.119	5.8
9.6 (10.0)	12	18	23	23	88	0.093	3.9
Reference chemical (if used)	Not applicable						

¹ Mean measured concentrations of Mesosulfuron-methyl metabolite were reviewer-calculated. Nominal concentrations are in parentheses.

² Mean frond number and dry weights were reviewer-calculated from replicate data.

³ % inhibition was determined by comparing the treatment groups to the dilution water control.

* Significantly different from dilution water control.

Table 4: Statistical endpoint values.

Statistical Endpoint ^a	frond No.	growth rate	dry weight (biomass)
NOEC or EC ₀₅ (mg/L)	0.56	0.56	0.56
LOEC (mg/L)	1.0	1.0	1.0
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	Not reported	2.6 (1.8 to 3.2)	1.7 (1.0 to 1.8)
other (IC ₂₅ /EC ₂₅)	Not reported	Not reported	Not reported
Reference chemical NOAEC IC ₅₀ /EC ₅₀	Not applicable	Not applicable	Not reported

^a Statistical data based on nominal test concentrations.

B. REPORTED STATISTICS: The formulas used for growth rates, doubling time, and mean percent inhibitions on found on pages 18 and 19. The NOEC was verified using Analysis of Variance, General Linear Models with DUNCAN's Multiple Range Test Procedures (SAS 1989). The EC₅₀ values were determined using binomial probability.

Biomass:

NOEC: 0.56 mg/L
 EC₅₀: 1.7 mg/L 95% C.I.: 1.0-1.8 mg/L
 Slope: N/A

Growth Rate:

NOEC: 0.56 mg/L
 EC₅₀: 2.6 mg/L 95% C.I.: 1.8-3.2 mg/L
 Slope: N/A

C. VERIFICATION OF STATISTICAL RESULTS:

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

Number of fronds:

NOEC: 0.29 mg/L
 EC₀₅: 0.20 mg/L 95% C.I.: 0.10-0.39 mg/L
 EC₅₀: 1.5 mg/L 95% C.I.: 1.1-2.0 mg/L
 Slope: 1.89±0.195

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Dry Weight:

NOEC: 0.53 mg/L
EC₀₅: 0.22 mg/L 95% C.I.: 0.11-0.46 mg/L
EC₅₀: 1.9 mg/L 95% C.I.: 1.4-2.6 mg/L
Slope: 1.75±0.194

Growth Rate:

NOEC: 0.53 mg/L
EC₀₅: 0.31 mg/L 95% C.I.: 0.19-0.50 mg/L
EC₅₀: 2.8 mg/L 95% C.I.: 2.4-3.4 mg/L
Slope: 1.71±0.132

Endpoint(s) Affected: Frond number (most sensitive), dry weight, and growth rate

D. STUDY DEFICIENCIES:

The deviations, including the reduced replicate size, were not considered to have impacted the study results, so they 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 frond number, 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 conclusions, frond number was the most sensitive endpoint, with an EC₅₀ value of 1.5 mg/L.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core.

Number of fronds:

NOEC: 0.29 mg/L
EC₀₅: 0.20 mg/L 95% C.I.: 0.10-0.39 mg/L
EC₅₀: 1.5 mg/L 95% C.I.: 1.1-2.0 mg/L
Slope: 1.89±0.195

Dry Weight:

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Growth Rate:

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Slope: 1.71 ± 0.132

Endpoint(s) Affected: Frond number (most sensitive), dry weight, and growth rate

III. REFERENCES:

- Organization of Economic Co-operation and Development, Draft OECD Guideline for Testing of Chemicals Guideline: *Lemna*, Growth Inhibition Test, April 1997.
- U.S. Environmental Protection Agency (EPA), 1982 Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants; Tier 2 of nontarget area testing; §123-2 Growth and reproduction of aquatic plants.
- U.S. Environmental Protection Agency (EPA), April 1996, Ecological Effects Test Guidelines; OPPTS 850.4400 Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II; EPA 712-C-96-156, Public Draft.
- ASTM (1991). Standard Guide for Conducting Static Toxicity Test With *Lemna gibba* G3. American Society for Testing and Materials. E 1415-91
- 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.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

frond number

File: 6311fn

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	156256.133	17361.793	706.712
Within (Error)	20	491.333	24.567	
Total	29	156747.467		

Critical F value = 2.39 (0.05, 9, 20)

Since F > Critical F REJECT Ho: All groups equal

frond number

File: 6311fn

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	191.667	191.667		
2	0.10	191.000	191.000	0.165	
3	0.17	190.667	190.667	0.247	
4	0.29	188.667	188.667	0.741	
5	0.53	177.000	177.000	3.624	*
6	0.93	168.000	168.000	5.848	*
7	1.7	67.667	67.667	30.640	*
8	3.0	40.000	40.000	37.477	*
9	5.3	27.667	27.667	40.524	*
10	9.6	23.000	23.000	41.677	*

Dunnett table value = 2.60 (1 Tailed Value, P=0.05, df=20, 9)

frond number

File: 6311fn

Transform: NO TRANSFORMATION

DUNNETTS 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	3			
2	0.10	3	10.522	5.5	0.667
3	0.17	3	10.522	5.5	1.000
4	0.29	3	10.522	5.5	3.000
5	0.53	3	10.522	5.5	14.667
6	0.93	3	10.522	5.5	23.667
7	1.7	3	10.522	5.5	124.000
8	3.0	3	10.522	5.5	151.667
9	5.3	3	10.522	5.5	164.000
10	9.6	3	10.522	5.5	168.667

frond number
File: 6311fn

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	191.667	191.667	191.667
2	0.10	3	191.000	191.000	191.000
3	0.17	3	190.667	190.667	190.667
4	0.29	3	188.667	188.667	188.667
5	0.53	3	177.000	177.000	177.000
6	0.93	3	168.000	168.000	168.000
7	1.7	3	67.667	67.667	67.667
8	3.0	3	40.000	40.000	40.000
9	5.3	3	27.667	27.667	27.667
10	9.6	3	23.000	23.000	23.000

frond number
File: 6311fn

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	191.667				
0.10	191.000	0.165		1.72	k= 1, v=20
0.17	190.667	0.247		1.81	k= 2, v=20
0.29	188.667	0.741		1.83	k= 3, v=20
0.53	177.000	3.624	*	1.85	k= 4, v=20
0.93	168.000	5.848	*	1.86	k= 5, v=20
1.7	67.667	30.640	*	1.86	k= 6, v=20
3.0	40.000	37.477	*	1.86	k= 7, v=20

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5.3	27.667	40.524	*	1.87	k= 8, v=20
9.6	23.000	41.678	*	1.87	k= 9, v=20

s = 4.956

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	0.20	0.10	0.39	0.14	0.52
EC10	0.31	0.18	0.56	0.12	0.56
EC25	0.66	0.43	1.0	0.091	0.65
EC50	1.5	1.1	2.0	0.060	0.75

Slope = 1.89 Std.Err. = 0.195

!!!Poor fit: p < 0.001 based on DF= 7.00 20.0

6311FN : frond number

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	192.	202.	-10.0	100.	0.00
0.100	3.00	191.	199.	-8.01	98.7	1.32
0.170	3.00	191.	194.	-3.51	96.3	3.72
0.290	3.00	189.	184.	4.97	91.1	8.91
0.530	3.00	177.	162.	15.1	80.3	19.7
0.930	3.00	168.	131.	36.5	65.2	34.8
1.70	3.00	67.7	92.5	-24.8	45.9	54.1
3.00	3.00	40.0	57.4	-17.4	28.4	71.6
5.30	3.00	27.7	30.2	-2.58	15.0	85.0
9.60	3.00	23.0	12.9	10.1	6.38	93.6

dry weight

File: 6311dw

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	2008.014	223.113	637.466
Within (Error)	20	7.000	0.350	
Total	29	2015.014		

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Critical F value = 2.39 (0.05, 9, 20)

Since F > Critical F REJECT Ho: All groups equal

dry weight

File: 6311dw

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	23.400	23.400		
2	0.10	23.267	23.267	0.276	
3	0.17	23.067	23.067	0.690	
4	0.29	23.067	23.067	0.690	
5	0.53	22.667	22.667	1.518	
6	0.93	21.900	21.900	3.105	*
7	1.7	10.833	10.833	26.015	*
8	3.0	5.700	5.700	36.642	*
9	5.3	5.767	5.767	36.504	*
10	9.6	3.900	3.900	40.369	*

Dunnett table value = 2.60 (1 Tailed Value, P=0.05, df=20, 9)

dry weight

File: 6311dw

Transform: NO TRANSFORMATION

DUNNETTS 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	3			
2	0.10	3	1.256	5.4	0.133
3	0.17	3	1.256	5.4	0.333
4	0.29	3	1.256	5.4	0.333
5	0.53	3	1.256	5.4	0.733
6	0.93	3	1.256	5.4	1.500
7	1.7	3	1.256	5.4	12.567
8	3.0	3	1.256	5.4	17.700
9	5.3	3	1.256	5.4	17.633
10	9.6	3	1.256	5.4	19.500

dry weight

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File: 6311dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	23.400	23.400	23.400
2	0.10	3	23.267	23.267	23.267
3	0.17	3	23.067	23.067	23.067
4	0.29	3	23.067	23.067	23.067
5	0.53	3	22.667	22.667	22.667
6	0.93	3	21.900	21.900	21.900
7	1.7	3	10.833	10.833	10.833
8	3.0	3	5.700	5.700	5.733
9	5.3	3	5.767	5.767	5.733
10	9.6	3	3.900	3.900	3.900

dry weight
File: 6311dw 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	23.400				
0.10	23.267	0.276		1.72	k= 1, v=20
0.17	23.067	0.690		1.81	k= 2, v=20
0.29	23.067	0.690		1.83	k= 3, v=20
0.53	22.667	1.518		1.85	k= 4, v=20
0.93	21.900	3.105	*	1.86	k= 5, v=20
1.7	10.833	26.015	*	1.86	k= 6, v=20
3.0	5.733	36.573	*	1.86	k= 7, v=20
5.3	5.733	36.573	*	1.87	k= 8, v=20
9.6	3.900	40.369	*	1.87	k= 9, v=20

s = 0.592

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

Estimates of ECs

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.22	0.11	0.46	0.15	0.48
EC10	0.36	0.19	0.67	0.13	0.54
EC25	0.79	0.50	1.3	0.097	0.63
EC50	1.9	1.4	2.6	0.061	0.75

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Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) to aquatic vascular plants *Lemna gibba*

PMRA Submission #: !.....!

EPA MRID#: 45386311

Slope = 1.75 Std.Err. = 0.194

!!!Poor fit: p < 0.001 based on DF= 7.00 20.0

6311DW : dry weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. - Pred.	Pred. %Control	%Change
0.00	3.00	23.4	24.5	-1.13	100.	0.00
0.100	3.00	23.3	24.2	-0.959	98.8	1.23
0.170	3.00	23.1	23.7	-0.662	96.7	3.26
0.290	3.00	23.1	22.7	0.381	92.5	7.51
0.530	3.00	22.7	20.5	2.14	83.7	16.3
0.930	3.00	21.9	17.4	4.48	71.0	29.0
1.70	3.00	10.8	13.2	-2.38	53.9	46.1
3.00	3.00	5.70	9.05	-3.35	36.9	63.1
5.30	3.00	5.77	5.44	0.327	22.2	77.8
9.60	3.00	3.90	2.74	1.16	11.2	88.8

growth rate

File: 6311g

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	0.4316	0.0480	480.000
Within (Error)	20	0.0012	0.0001	
Total	29	0.4328		

Critical F value = 2.39 (0.05, 9, 20)
 Since F > Critical F REJECT Ho: All groups equal

growth rate

File: 6311g

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.396	0.396		

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) to aquatic vascular plants *Lemma gibba*

PMRA Submission #: !.....!

EPA MRID#: 45386311

2	0.10	0.395	0.395	0.041
3	0.17	0.395	0.395	0.082
4	0.29	0.393	0.393	0.286
5	0.53	0.385	0.385	1.347
6	0.93	0.377	0.377	2.286
7	1.7	0.247	0.247	18.167 *
8	3.0	0.172	0.172	27.434 *
9	5.3	0.119	0.119	33.844 *
10	9.6	0.093	0.093	37.110 *

Dunnett table value = 2.60 (1 Tailed Value, P=0.05, df=20,9)

growth rate

File: 6311g

Transform: NO TRANSFORMATION

DUNNETTS 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	3			
2	0.10	3	0.021	5.4	0.000
3	0.17	3	0.021	5.4	0.001
4	0.29	3	0.021	5.4	0.002
5	0.53	3	0.021	5.4	0.011
6	0.93	3	0.021	5.4	0.019
7	1.7	3	0.021	5.4	0.148
8	3.0	3	0.021	5.4	0.224
9	5.3	3	0.021	5.4	0.276
10	9.6	3	0.021	5.4	0.303

growth rate

File: 6311g

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	0.396	0.396	0.396
2	0.10	3	0.395	0.395	0.395
3	0.17	3	0.395	0.395	0.395
4	0.29	3	0.393	0.393	0.393
5	0.53	3	0.385	0.385	0.385
6	0.93	3	0.377	0.377	0.377
7	1.7	3	0.247	0.247	0.247
8	3.0	3	0.172	0.172	0.172

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) to aquatic vascular plants *Lemna gibba*

PMRA Submission #: !.....!

EPA MRID#: 45386311

9	5.3	3	0.119	0.119	0.119
10	9.6	3	0.093	0.093	0.093

growth rate
File: 6311g

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	0.396				
0.10	0.395	0.053		1.72	k= 1, v=20
0.17	0.395	0.105		1.81	k= 2, v=20
0.29	0.393	0.369		1.83	k= 3, v=20
0.53	0.385	1.739		1.85	k= 4, v=20
0.93	0.377	2.951	*	1.86	k= 5, v=20
1.7	0.247	23.454	*	1.86	k= 6, v=20
3.0	0.172	35.418	*	1.86	k= 7, v=20
5.3	0.119	43.692	*	1.87	k= 8, v=20
9.6	0.093	47.909	*	1.87	k= 9, v=20

s = 0.008

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	0.31	0.19	0.50	0.10	0.62
EC10	0.51	0.34	0.75	0.085	0.67
EC25	1.1	0.87	1.5	0.059	0.76
EC50	2.8	2.4	3.4	0.035	0.85

Slope = 1.71 Std.Err. = 0.132

!!!Poor fit: p < 0.001 based on DF= 7.00 20.0

6311G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	0.396	0.409	-0.0136	100.	0.00
0.100	3.00	0.395	0.407	-0.0113	99.3	0.653
0.170	3.00	0.395	0.402	-0.00679	98.2.	1.84

Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F160459) to aquatic vascular plants *Lemna gibba*

PMRA Submission #: {.....}

EPA MRID#: 45386311

0.290	3.00	0.393	0.391	0.00253	95.5	4.52
0.530	3.00	0.385	0.366	0.0189	89.4	10.6
0.930	3.00	0.377	0.326	0.0509	79.7	20.3
1.70	3.00	0.247	0.266	-0.0183	64.9	35.1
3.00	3.00	0.172	0.198	-0.0268	48.5	51.5
5.30	3.00	0.119	0.132	-0.0128	32.3	67.7
9.60	3.00	0.0927	0.0754	0.0173	18.4	81.6

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