Data Evaluation Report on the acute toxicity of Mesosulfuron-methyl metabolite (AE F147447) on the Freshwater Alga *Pseudokirchneriella subcapitata*

PMRA Submission #: {.....}

EPA MRID #: 45386308

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D284719
OECD Data Point	{.....}
EPA MRID	45386308
EPA Guideline	123-2

Test material: AE F147447 Technical **Purity:** 93.1%

Common name: Mesosulfuron-methyl metabolite

Chemical name: IUPAC: 6-methanesulfonamidomethyl-1,2-benzisothiazol-3(2H)-one1,1-dioxide

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 S Myers
Date: 9/26/03

Primary Reviewer: ~~Tim Bargar~~ LaSata
{EPA/OECD/PMRA}

Date: {01/9/04} LaSata

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 F147447; substance, technical (Metabolite of AE F130060). Unpublished study performed by Aventis CropScience GmbH, Frankfort, Germany. Laboratory Study Identification No. CE00/082. Study submitted by Aventis CropScience, Research Triangle Park, NC. Experimental start date August 10, 2000 and experimental termination date August 14, 2000. The final report issued October 16, 2000.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* (syn *Selenastrum capricomutum)* were exposed to Mesosulfuron-methyl metabolite (AE F147447) under static conditions. Nominal concentrations were 10, 18, 32, 56, and 100 mg/L. Mean measured concentrations over the study period were 9.1, 16.4, 29.2, 51.1, and 92.0 mg/L; these treatment groups were compared to a dilution water control. No significant inhibitions in mean cell densities, growth rates, and biomass were observed in any treatment group. The 96-hour NOEC based on cell density, growth rate, and biomass was 92.0 mg/L, the highest concentration tested; the EC_{50} was >92.0 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: 92.0 mg/L
EC_{05}: 26 mg/L 95% C.I.: 0.048-14000 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: 0.798±1.74

Growth rate:
NOEC: 92.0 mg/L
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: could not determine

Area Under the Growth Curve (Biomass):
NOEC: 92.0 mg/L
EC_{05}: 16 mg/L 95% C.I.: 0.35-770 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: 0.878±0.910

Endpoint(s) Affected: None

2

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 metabolite (AE F147447 Technical)

Description: Light yellow powder

Lot No./Batch No. : AE F147447 00 1C93 0001

Purity: 93.1%

Stability of Compound
Under Test Conditions: The day 0 measured concentrations were 97.1-98.8% of nominal and the day 4 measured concentrations were 97.3-99.7% of nominal. Mean measured concentrations of Mesosulfuron-methyl ranged from 97.9 to 98.8% 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 capricomutum)*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 61.81

3

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	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator-water bath	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with pressed paper stoppers 300 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

4

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 6.8-7.5 8.2-9.6 $Na_2EDTA \cdot 2H_2O$ $NaHCO_3$ N/A	*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: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 rpm	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

Parameter	Details	Remarks *Criteria*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Pseudokirchneriella subcapitata, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 6 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*

6

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	10, 18, 32, 56, and 100 mg/L 9.1, 16.4, 29.2, 51.1,and 92.0 mg/L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; 0 and 96 hours	
Test conditions temperature: photoperiod: light intensity and quality:	24.2-25.1°C Continuous 58.99-74.63 $\mu E*m^{-2}*s^{-1}$, white fluorescent lighting	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell count, growth rate, and biomass	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell counting chamber with a microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water control group cell density at test termination was 190X greater than the dilution water control group cell density at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

8

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

No significant inhibitions in mean cell densities, growth rates, and biomass were observed in any treatment group.

Table 3: Effect of AE F147447 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	39,000	1,900,000	--
9.1 (10)	~10,000	38,000	1,593,000	16
16.4 (18)	~10,000	43,000	2,039,000	-7
29.2 (32)	~10,000	29,000	1,948,000	-3
51.1 (56)	~10,000	36,000	1,507,000	21
92.0 (100)	~10,000	33,000	1,733,000	8
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Mean measured concentrations of Mesosulfuron-methyl metabolite. Nominal concentrations are in parentheses.
[b] Reviewer calculated % inhibition by comparing the treatment groups to the dilution water control.

Table 4: Effect of AE F147447 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	0.05430	--	45,883, 400	--
9.1 (10)	~10,000	0.05268	2.98	40,499,200	11.73
16.4 (18)	~10,000	0.05527	-1.77	46,940,800	-2.30
29.2 (32)	~10,000	0.05488	-1.05	45,162,400	1.57
51.1 (56)	~10,000	0.05204	4.17	35,993,600	21.55
92.0 (100)	~10,000	0.05367	1.16	39,678,000	13.52

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

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC_{05} (mg/L)	92.0	92.0	Not reported
EC_{50} (mg/L)	>92.0	>92.0	Not reported
IC_{50} or EC_{50} (mg/L) (95% C.I.)	N/A	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 EC_{50} was estimated using growth inhibition data compared to the control. The NOEC was verified using Analysis of Variance, General Linear Models with DUNCAN's Multiple Range Test Procedures.

Cell Density:
NOEC: 92.0 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A

Growth rate:
NOEC:92.0 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):
NOEC:92.0 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

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 via TOXSTAT statistical software. The EC_{05} values were determined using the Probit method via Nuthatch statistical software; the EC_{50} values could be determined visually, as no inhibition was greater than 50%.

Cell Density:
NOEC: 92.0 mg/L
EC_{05}: 26 mg/L 95% C.I.: 0.048-14000 mg/L

EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: 0.798±1.74

Growth rate:
NOEC: 92.0 mg/L
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: could not determine

Area Under the Growth Curve (Biomass):
NOEC: 92.0 mg/L
EC_{05}: 16 mg/L 95% C.I.: 0.35-770 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: 0.878±0.910

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

The deviations did not affect the acceptability or validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'; there was no significant inhibition in this study. The reviewer's analysis provided and EC_{05} estimate for cell density and biomass.

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: 92.0 mg/L
EC_{05}: 26 mg/L 95% C.I.: 0.048-14000 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: 0.798±1.74

Growth rate:
NOEC: 92.0 mg/L
EC_{05}: could not determine 95% C.I.: N/A
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: could not determine

Area Under the Growth Curve (Biomass):
NOEC: 92.0 mg/L
EC_{05}: 16 mg/L 95% C.I.: 0.35-770 mg/L
EC_{50}: >92.0 mg/L 95% C.I.: N/A
Slope: 0.878±0.910

Endpoint(s) Affected: None

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.

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_{50}, U.S. Environmental Protection Agency, Duluth, Mn. Letter to Dr. Lowell Bahner, Chairman of the ASTM Task Group on Calculating LC50s; September 10, 1982

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 6308cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	6967.657	1393.531	1.017
Within (Error)	15	20558.861	1370.591	
Total	20	27526.518		

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

cell density
File: 6308cd 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	190.035	190.035		
2	9.12	159.300	159.300	1.174	
3	16.42	203.853	203.853	-0.528	
4	29.19	194.767	194.767	-0.181	
5	51.13	150.653	150.653	1.504	
6	91.96	173.297	173.297	0.639	

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

cell density
File: 6308cd 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	9.12	3	68.142	35.9	30.735
3	16.42	3	68.142	35.9	-13.818

4	29.19	3	68.142	35.9	-4.732
5	51.13	3	68.142	35.9	39.382
6	91.96	3	68.142	35.9	16.738

cell density
File: 6308cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	190.035	190.035	190.035
2	9.12	3	159.300	159.300	185.973
3	16.42	3	203.853	203.853	185.973
4	29.19	3	194.767	194.767	185.973
5	51.13	3	150.653	150.653	161.975
6	91.96	3	173.297	173.297	161.975

cell density
File: 6308cd 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	190.035				
9.12	185.973	0.155		1.75	k= 1, v=15
16.42	185.973	0.155		1.84	k= 2, v=15
29.19	185.973	0.155		1.87	k= 3, v=15
51.13	161.975	1.072		1.88	k= 4, v=15
91.96	161.975	1.072		1.89	k= 5, v=15

s = 37.021
Note: df used for table values are approximate when v > 20.

Estimates of EC5

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	**26.**	**0.048**	**1.4E+04**	**1.3**	**0.0018**
EC10	75.	1.7	3.3E+03	0.78	0.023

EC25	4.3E+02	0.078	2.4E+06	1.8	0.00018
EC50	3.0E+03	0.00012	7.9E-10	3.5	3.9E-08

Slope = 0.798 Std.Err. = 1.74

Goodness of fit: p = 0.26 based on DF= 3.0 15.

6308CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	190.	188.	1.64	100.	0.00
9.12	3.00	159.	184.	-25.0	97.8	2.20
16.4	3.00	204.	182.	22.1	96.5	3.51
29.2	3.00	195.	178.	16.5	94.6	5.36
51.1	3.00	151.	174.	-23.0	92.2	7.83
92.0	3.00	173.	167.	6.11	88.7	11.3

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

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

growth rate
File: 6308g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	24.385	4.877	0.888
Within (Error)	15	82.356	5.490	
Total	20	106.741		

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

growth rate
File: 6308g 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	54.303	54.303		
2	9.12	52.683	52.683	0.978	
3	16.42	55.267	55.267	-0.581	
4	29.19	54.873	54.873	-0.344	
5	51.13	52.040	52.040	1.366	
6	91.96	53.673	53.673	0.380	

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

growth rate
File: 6308g 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	9.12	3	4.313	7.9	1.620
3	16.42	3	4.313	7.9	-0.963
4	29.19	3	4.313	7.9	-0.570
5	51.13	3	4.313	7.9	2.263
6	91.96	3	4.313	7.9	0.630

growth rate
File: 6308g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	54.303	54.303	54.303
2	9.12	3	52.683	52.683	54.274
3	16.42	3	55.267	55.267	54.274
4	29.19	3	54.873	54.873	54.274
5	51.13	3	52.040	52.040	52.857
6	91.96	3	53.673	53.673	52.857

growth rate
File: 6308g 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	54.303				
9.12	54.274	0.017		1.75	k= 1, v=15
16.42	54.274	0.017		1.84	k= 2, v=15
29.19	54.274	0.017		1.87	k= 3, v=15
51.13	52.857	0.873		1.88	k= 4, v=15
91.96	52.857	0.873		1.89	k= 5, v=15

s = 2.343
Note: df used for table values are approximate when v > 20.

EC_x
!!!Failure#1: near-singular matrix, model possibly unsuitable.

area under the growth curve
File: 6308b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3092452.720	618490.544	1.640
Within (Error)	15	5658395.176	377226.345	
Total	20	8750847.897		

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

area under the growth curve
File: 6308b 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

17

1	control	4588.340	4588.340		
2	9.12	4049.920	4049.920	1.240	
3	16.42	4694.080	4694.080	-0.243	
4	29.19	4516.240	4516.240	0.166	
5	51.13	3599.360	3599.360	2.277	
6	91.96	3967.800	3967.800	1.429	

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

area under the growth curve
File: 6308b 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	9.12	3	1130.473	24.6	538.420
3	16.42	3	1130.473	24.6	-105.740
4	29.19	3	1130.473	24.6	72.100
5	51.13	3	1130.473	24.6	988.980
6	91.96	3	1130.473	24.6	620.540

area under the growth curve
File: 6308b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	4588.340	4588.340	4588.340
2	9.12	3	4049.920	4049.920	4420.080
3	16.42	3	4694.080	4694.080	4420.080
4	29.19	3	4516.240	4516.240	4420.080
5	51.13	3	3599.360	3599.360	3783.580
6	91.96	3	3967.800	3967.800	3783.580

area under the growth curve
File: 6308b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

	ISOTONIZED	CALC.	SIG	TABLE	DEGREES OF

IDENTIFICATION	MEAN	WILLIAMS	P=.05	WILLIAMS	FREEDOM
control	4588.340				
9.12	4420.080	0.387		1.75	k= 1, v=15
16.42	4420.080	0.387		1.84	k= 2, v=15
29.19	4420.080	0.387		1.87	k= 3, v=15
51.13	3783.580	1.853		1.88	k= 4, v=15
91.96	3783.580	1.853		1.89	k= 5, v=15

s = 614.188
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	**16.**	**0.35**	**7.7E+02**	**0.79**	**0.021**
EC10	43.	4.6	4.0E+02	0.46	0.11
EC25	2.1E+02	13.	3.4E+03	0.58	0.061
EC50	1.2E+03	2.4	6.4E+05	1.3	0.0019

Slope = 0.878 Std.Err. = 0.910

Goodness of fit: p = 0.23 based on DF= 3.0 15.

6308B : area under the growth curve

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	4.59e+03	4.57e+03	20.9	100.	0.00
9.12	3.00	4.05e+03	4.43e+03	-377.	96.9	3.07
16.4	3.00	4.69e+03	4.34e+03	354.	95.0	4.98
29.2	3.00	4.52e+03	4.22e+03	299.	92.3	7.68
51.1	3.00	3.60e+03	4.05e+03	-454.	88.8	11.2
92.0	3.00	3.97e+03	3.83e+03	137.	83.9	16.1

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

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

Page ___ is not included in this copy.

Pages 30 through 34 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.