Data Evaluation Report on the acute toxicity of AE F132345 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus*
PMRA Submission #:{.....} EPA MRID #: 46203517

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D294536
	OECD Data Point	{.....}
	EPA MRID	46203517
	EPA Guideline	123-2 (OPPTS 850.5400)

Test material: AE F132345 (Thidiazuron Metabolite) **Purity:** 91% w/w a.i.
Common name: Thidiazuron Metabolite (1,2,3-thiadiazol-5-yhurea)
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 4/28/2004

QC Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 4/29/2004

Primary Reviewer: William Evans
{EPA/OECD/PMRA}
Date: 11/16/04

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 120301

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Desjardins, D., Kendall, T., and Krueger, H. 2003. AE F132345: A 72-Hour Toxicity Test with the Freshwater Alga (*Scenedesmus subspicatus*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 149A-150. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Experimental start date July 28, 2003 and experimental termination date July 31, 2003. The final report issued August 13, 2003.

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Date: {.....}

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

Date: {.....}

Company Code {.....} [For PMRA]
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EXECUTIVE SUMMARY:

In a 72-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to AE F132345 (Thidiazuron Metabolite) under static conditions at nominal concentrations of 0 (negative control), 0.28, 0.62, 1.4, 3.0, 6.8, and 15 ppm AE F132345. The day-0 measured concentrations were <0.150 (<LOQ, negative control), 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F132345. Cell density percent inhibition was 4, 0, 5, 35, 91, and 97% at the 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F132345 treatment levels, respectively. Biomass (area under the growth curve, 0 to 72 hours) percent inhibition was 6.7, -1.2, 3.6, 35, 86, and 94% at the 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F132345 treatment levels, respectively. Growth rate (0 to 72 hours) percent inhibition was -0.18, -1.1, 1.1, 8.9, 47, and 69% at the 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F132345 treatment levels, respectively. Biomass was significantly reduced at the 3.0, 6.7 and 15 ppm AE F132345 treatment levels compared to the control. Growth rate was significantly reduced at the 6.7 and 15 ppm AE F132345 treatment levels. Cell density, growth rate and biomass EC_{50} values were 3.4, 8.3 and 3.6 ppm AE F132345, respectively. The NOEC for AE F132345 was 1.3 ppm, based on cell density and biomass.

The study is scientifically sound but does not satisfy the guideline requirements for a Tier II aquatic nonvascular plant study with *Scenedesmus subspicatus*. According to US EPA Memorandum (Oct. 21, 1994), Closure on Nontarget Plant Phytotoxicity Policy Issues, three day OECD studies will be reviewed as Tier I screening studies only, therefore this study is classified as Supplemental due the shorter than recommended definitive test duration.

Results Synopsis

Test Organism: *Scenedesmus subspicatus*
Test Type: Static

Cell density:
NOEC/EC_{05}: 1.3 ppm AE F132345
EC_{05}: 1.2 ppm AE F132345 95% C.I.: 0.56-2.5 ppm AE F132345
EC_{50}/IC_{50}: 3.4 ppm AE F132345 95% C.I.: 2.4-4.8 ppm AE F132345
Slope: 3.56

Growth rate:
NOEC/EC_{05}: 3.0 ppm AE F132345
EC_{05}: 1.5 ppm AE F132345 95% C.I.: 0.97-2.3 ppm AE F132345
EC_{50}/IC_{50}: 8.3 ppm AE F132345 95% C.I.: 7.2-9.6 ppm AE F132345
Slope: 2.22

Biomass (area under the growth curve):
NOEC/EC_{05}: 1.3 ppm AE F132345
EC_{05}: Not determined 95% C.I.: Not determined
EC_{50}/IC_{50}: 3.6 ppm AE F132345 95% C.I.: 2.5-5.3 ppm AE F132345
Slope: Not reported

Endpoint(s) Affected: Cell density 9the most sensitive), biomass and growth rate

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: OECD Guideline for Testing of Chemicals, 201: *Algal Growth Inhibition Test* and Official Journal of the European Communities No. L383, Method C.3: *Algal Growth Inhibition Test*. The following deviations from U.S. EPA Guideline, §123-2 were noted:

1. The dilution water total organic carbon, particulate matter and residual chlorine concentrations were not reported.
2. Light intensity (6550-8100 lux) was higher than recommended (~43000 lux) and the photoperiod was continuous rather than 14:10 as recommended.
3. The test duration was 72-hours rather than the recommended 96-120 hours.

The shorter than recommended test duration affected the acceptability of the study, consequently this Tier II test is acceptable as a Tier I test. All other deviations were considered minor.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the U.S. CFR Title 40, parts 160 and 792 (August 17, 1989).

A. MATERIALS:

1. Test Material Thidiazuron (AE F132345)

Description: Light Yellow Powder

Lot No./Batch No. : JV0585+JV0585A/Product Code AE F132345 00 1C91 0001

Purity: 91%

Stability of Compound Under Test Conditions: The 0-hour measured test concentrations were 96.0-100% of the nominal concentrations and the 72 hour measured test concentrations were 61.1-76.6% of the nominal concentrations (Table 1, p. 20).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored under frozen conditions.

2. Test organism:

Name: *Scenedesmus subspicatus*

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: CCAP 276/22

Source: Originally from Culture Collection of Algae and Protozoa in the United Kingdom. Current in-house laboratory cultures.
Age of inoculum: ≥ 14 days
Method of cultivation: Freshwater algal medium

B. STUDY DESIGN:

a) Range-finding Study: A previous range-finding study was conducted in order to estimate the nominal concentration range for the definitive study. The results were 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)	≥ 14 days Freshwater algal medium; same as test Algal cells were actively growing.	*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	Environmental chamber	
Duration of the test	72 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass 250 mL (Erlenmeyer flask) 100 mL	Test vessels were plugged with foam stoppers. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

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):**	 Freshwater algal medium 7.8 7.5-7.6 Yes $NaHCO_3$ N/A	See Appendix 2, p. 32. *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:	 Well water, NANOpure® filtered with reagent grade chemicals Filter -sterilized (0.22 µm) 7.5 N/A pH adjusted with 10% HCl Not reported Not reported <LOD <LOD 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 solutions	
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.*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, 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.*

Parameter	Details	Remarks *Criteria*
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 replicates.* *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.*
Test concentrations nominal: measured:	0 (negative control), 0.28, 0.62, 1.4, 3.0, 6.8, and 15 ppm AE F13245 <0.150 (<LOQ, negative control), 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F13245	The measured concentrations are from day-0 samples. *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 72 hours.	

Parameter	Details	Remarks *Criteria*
Test conditions temperature: photoperiod: light intensity and quality:	22.5-23.9°C Continuous 6550-8100 lux, cool-white fluorescent light.	*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 (biomass and growth rate were calculated).	*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.*

Parameters	Details	Remarks/Criteria
Measurement technique for cell density and other end points	Cell counts using a electronic particle counter.	*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 cell density at test termination was 163X greater than the dilution water control cell density at test initiation.	Mean cell densities were reviewer-calculated. *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	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The cell density percent inhibition was 4, 0, 5, 35, 91, and 97% (reviewer-determined) at the 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F13245 treatment levels, respectively. Biomass (area under the growth curve, 0 to 72 hours) percent inhibition was 6.7, -1.2, 3.6, 35, 86, and 94% in the 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F13245 treatment levels, respectively. Growth rate (0 to 72 hours) percent inhibition was -0.18, -1.1, 1.1, 8.9, 47, and 69% at the 0.28, 0.62, 1.3, 3.0, 6.7, and 15 ppm AE F13245 treatment levels, respectively. Biomass and growth rate were significantly reduced at the 6.7 and 15 ppm AE F13245 treatment levels compared to the control. The observed biomass reduction at the 3.0 ppm AE F13245 treatment level was considered treatment-related by the study authors (pp. 17-18).

Table 3: Effect of AE F132345 (Thidiazuron Metabolite) on Algae (*Scenedesmus subspicatus*)

Treatment Day-0 Measured and (Nominal Concn.); ppm AE F132345	Initial Cell Density (cells/mL)	Observation Period		
		24-Hours	72-Hours	
			Cell Count[a]	% Inhibition[a]
Dilution water control	10,000	33,287	1,629,731	--
0.28 (0.28)	10,000	34,653	1,566,680	4
0.62 (0.62)	10,000	34,362	1,630,360	0
1.3 (1.4)	10,000	34,757	1,542,180	5
3.0 (3.0)	10,000	26,912	1,059,272	35
6.7 (6.8)	10,000	27,165	148,198	91
15 (15)	10,000	27,556	46,926	97
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The cell density means and % inhibition compared to the control were reviewer-calculated based on data provided in Appendix 5, p. 47.

Table 4: Effect of AE F132345 (Thidiazuron Metabolite) on Algae *(Scenedesmus subspicatus)*

Treatment Day-0 Measured and (Nominal Concn.); ppm AE F132345	Initial Cell Density (cells/mL)	Mean Growth Rate per Day	% Inhibition, Mean Growth Rate per Day	Mean Area Under Growth Curve (Biomass)	% Inhibition, Biomass
Dilution water control	10,000	0.0699	--	24,450,076	--
0.28 (0.28)	10,000	0.0700	-0.18	22,816,260	6.7
0.62 (0.62)	10,000	0.0707	-1.1	24,753,536	-1.2
1.3 (1.4)	10,000	0.0692	1.1	23,558,796	3.6
3.0 (3.0)	10,000	0.0637	8.9	15,893,004**	35
6.7 (6.8)	10,000	0.0370*	47	3,485,856*	86
15 (15)	10,000	0.0214*	69	1,576,628*	94
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

* Statistically significant difference ($p<0.05$) from the control using Dunnett's test.
** Not statistically significant, but considered treatment related by the study authors.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC_{05} (mg a.i./L)	1.3	3.0	Not Reported
EC_{50} (mg a.i./L)	3.6	8.3	Not Reported
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	2.3-5.3	7.2-9.6	Not Reported
IC_{25}/EC_{25} (mg a.i./L) (95% C.I.)	Not Reported	Not Reported	Not Reported
Reference chemical, if used NOAEC IC_{25}/EC_{25}	N/A	N/A	N/A

N/A = Not applicable.

B. REPORTED STATISTICS:

Statistical Method: Biomass (area under the growth curve) and growth rate formulas are found on pages 15-16. Percent inhibition was determined for biomass and growth rate. Data were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Levene's test. The data met the assumptions of ANOVA, therefore, Dunnett's test was used to determine NOEC values for the biomass and growth rate endpoints. Non-linear regression or linear interpolation was used to determine the 72-hour EC50. All toxicity values were determined via The SAS System for Windows statistical software using Day-0 measured treatment concentrations (pp. 20).
Cell density: Not reported

Growth rate:
$NOEC/EC_{05}$: 3.0 mg a.i./L
EC_{50}/IC_{50}: 8.3 mg a.i./L 95% C.I.: 7.2-9.6 mg a.i./L

Biomass (area under the growth curve):
$NOEC/EC_{05}$: 1.3 mg a.i./L
EC_{50}/IC_{50}: 3.6 mg a.i./L 95% C.I.: 2.5-5.3 mg a.i./L

Endpoint(s) Affected: Biomass and growth rates

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, biomass (area under the growth curve), and dry weight data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). NOEC and LOEC values were determined using ANOVA and William's multiple comparison test. The analyses described above were conducted using TOXSTAT statistical software and the day-0 measured concentrations were used for all calculations. The EC_{05} and EC_{50} values based on cell density and growth rate data were determined using the Probit method via Nuthatch statistical software. An EC_{50} value based on biomass data could not be calculated/verified by the reviewer using the Probit method via Nuthatch statistical software, consequently, the study authors' reported value for this endpoint is reported in the Executive Summary and Conclusion sections of this DER. An EC_5 value was not reported by the study authors and could not be determined by the reviewer for biomass due to the software limitation mentioned above.

Cell density:
NOEC/EC_{05}: 1.3 ppm AE F132345
EC_{05}: 1.2 ppm AE F132345 95% C.I.: 0.56-2.5 ppm AE F132345
EC_{50}/IC_{50}: 3.4 ppm AE F132345 95% C.I.: 2.4-4.8 ppm AE F132345
Slope: 3.56

Growth rate:
NOEC/EC_{05}: 3.0 ppm AE F132345
EC_{05}: 1.5 ppm AE F132345 95% C.I.: 0.97-2.3 ppm AE F132345
EC_{50}/IC_{50}: 8.3 ppm AE F132345 95% C.I.: 7.2-9.6 ppm AE F132345
Slope: 2.22

Biomass (area under the growth curve):
NOEC/EC_{05}: 1.3 ppm AE F132345
EC_{05}: Not determined 95% C.I.: Not determined
EC_{50}/IC_{50}: Not determined 95% C.I.: Not determined
Slope: N/A

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

D. STUDY DEFICIENCIES:

The duration of the definitive study affected the acceptability of this study as a Tier II Aquatic Plant Growth Study. According to US EPA Memorandum (Oct. 21, 1994), Closure on Nontarget Plant Phytotoxicity Policy Issues:

> "Aquatic Plant Growth Studies (122-2, 123-2), 1.) Four or 5 day algal studies will be accepted for review by the agency. Three day OECD studies will be reviewed as Tier I screening studies only. (This is a harmonization issue)."

Consequently, this study is classified as SUPPLEMENTAL.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from those of the study authors'. The study authors did not report toxicity values based on cell density data, only toxicity values based on growth rate and biomass. Therefore, the reviewer determined toxicity values for cell density are reported in the Executive Summary and Conclusion sections of this DER. Cell density was significantly reduced at the day-0 measured 3.0, 6.7 and 15 ppm AE F132345 treatment levels. The study authors detected statistically significant reductions in biomass and growth rate at the day-0 measured 6.7 and 15 ppm AE F132345 treatment levels, however, the observed biomass reduction at the 3.0 ppm AE F13245 treatment level was considered treatment-related by the study authors (pp. 17-18). Results of the reviewer's statistical verification for biomass indicated a statistically significant reduction at the 3.0 ppm AE F13245 treatment level, presumably due to the different statistical method used. Consequently, the reviewer's NOEC values are identical to those of the study authors' for biomass and growth rate. The EC_{50} value based on biomass data could not be calculated/verified by the reviewer using the Probit method via Nuthatch statistical software, consequently, the study authors' reported value for this endpoint is reported in the Executive Summary and Conclusion sections of this DER. The reviewer's EC_{50} value based on growth rate data was identical to that of the study authors'. An EC_5 value was not reported by the study authors and could not be determined by the reviewer for biomass due to the software limitations. EC_5 values based on cell density and growth rate data were determined by the reviewer and are reported in the Executive Summary and Conclusion sections of this DER.

F. CONCLUSIONS:

The study is scientifically sound but does not satisfy the guideline requirements for a Tier II aquatic nonvascular plant study with *Scenedesmus subspicatus*. According to US EPA Memorandum (Oct. 21, 1994), Closure on Nontarget Plant Phytotoxicity Policy Issues, three day OECD studies will be reviewed as Tier I screening studies only, therefore this study is classified as Supplemental due the shorter than recommended definitive test duration.

Cell density:
NOEC/EC_{05}: 1.3 ppm AE F132345
EC_{05}: 1.2 ppm AE F132345 95% C.I.: 0.56-2.5 ppm AE F132345
EC_{50}/IC_{50}: 3.4 ppm AE F132345 95% C.I.: 2.4-4.8 ppm AE F132345
Slope: 3.56

Growth rate:
NOEC/EC_{05}: 3.0 ppm AE F132345
EC_{05}: 1.5 ppm AE F132345 95% C.I.: 0.97-2.3 ppm AE F132345
EC_{50}/IC_{50}: 8.3 ppm AE F132345 95% C.I.: 7.2-9.6 ppm AE F132345
Slope: 2.22

Biomass (area under the growth curve):
NOEC/EC_{05}: 1.3 ppm AE F132345
EC_{05}: Not determined 95% C.I.: Not determined
EC_{50}/IC_{50}: 3.6 ppm AE F132345 95% C.I.: 2.5-5.3 ppm AE F132345
Slope: Not reported

Endpoint(s) Affected: Cell density 9the most sensitive), biomass and growth rate

III. REFERENCES:

Organisation for Economic Cooperation and Development. 1984. OECD Guideline for Testing of Chemicals, 201: *Alga, Growth Inhibition Test.*

Official Journal of the European Communities. 1992. No. L383. Method C.3.: *Algal Inhibition Test.*

The SAS System for Windows. 1996. Release 8.02, TS Level 0020. SAS Institute, Inc. Cary, North Carolina.

Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry*. 11: 1485-1494.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density
File: 3517cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	9740139029808.000	1623356504968.000	9.648
Within (Error)	17	2860393862336.000	168258462490.250	
Total	23	12600532892144.000		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

Cell density
File: 3517cd 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	neg control	1629730.667	1629730.667		
2	0.28	1566679.667	1566679.667	0.217	
3	0.62	1630360.000	1630360.000	-0.002	
4	1.3	1542180.333	1542180.333	0.302	
5	3.0	1059271.667	1059271.667	1.967	
6	6.7	148198.667	148198.667	5.108	*
7	15	46926.333	46926.333	5.457	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

Cell density
File: 3517cd 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	neg control	6			
2	0.28	3	770083.797	47.3	63051.000
3	0.62	3	770083.797	47.3	-629.333
4	1.3	3	770083.797	47.3	87550.333
5	3.0	3	770083.797	47.3	570459.000
6	6.7	3	770083.797	47.3	1481532.000
7	15	3	770083.797	47.3	1582804.333

Cell density
File: 3517cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	1629730.667	1629730.667	1629730.667
2	0.28	3	1566679.667	1566679.667	1598519.833
3	0.62	3	1630360.000	1630360.000	1598519.833
4	1.3	3	1542180.333	1542180.333	1542180.333
5	3.0	3	1059271.667	1059271.667	1059271.667
6	6.7	3	148198.667	148198.667	148198.667
7	15	3	46926.333	46926.333	46926.333

Cell density
File: 3517cd 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
neg control	1629730.667				
0.28	1598519.833	0.108		1.74	k= 1, v=17
0.62	1598519.833	0.108		1.82	k= 2, v=17
1.3	**1542180.333**	**0.302**		**1.85**	**k= 3, v=17**
3.0	1059271.667	1.967	*	1.87	k= 4, v=17
6.7	148198.667	5.108	*	1.87	k= 5, v=17
15	46926.333	5.457	*	1.88	k= 6, v=17

s = 410193.201
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.2	0.56	2.5	0.16	0.47
EC10	1.5	0.78	2.9	0.14	0.52
EC25	2.2	1.4	3.6	0.10	0.61
EC50	3.4	2.4	4.8	0.072	0.71

Slope = 3.56 Std.Err. = 0.775

Goodness of fit: p = 0.66 based on DF= 4.0 17.

3517CD : Cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	1.63e+06	1.63e+06	-1.07e+03	100.	0.00
0.280	3.00	1.57e+06	1.63e+06	-6.40e+04	100.	0.00523
0.620	3.00	1.63e+06	1.62e+06	6.14e+03	99.6	0.403
1.30	3.00	1.54e+06	1.52e+06	1.96e+04	93.4	6.64
3.00	3.00	1.06e+06	9.50e+05	1.09e+05	58.3	41.7
6.70	3.00	1.48e+05	2.45e+05	-9.70e+04	15.0	85.0
15.0	3.00	4.69e+04	1.83e+04	2.86e+04	1.12	98.9

biomass
File: 3517bd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between		61999811644930048.000	33301940821504.000	10.150
Within (Error)	17	558258160547840.000	32838715326336.000	
Total		232558069805477888.000		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

biomass
File: 3517bd 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	neg control	24450076.000	24450076.000		
2	0.28	22816260.000	22816260.000	0.403	
3	0.62	24753536.000	24753536.000	-0.075	
4	1.3	23558796.000	23558796.000	0.220	
5	3.0	15893004.000	15893004.000	2.112	
6	6.7	3485856.000	3485856.000	5.174	*
7	15	1576628.000	1576628.000	5.645	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

biomass
File: 3517bd 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	neg control	6			
2	0.28	3	10758274.148	44.0	1633816.000
3	0.62	3	10758274.148	44.0	-303460.000
4	1.3	3	10758274.148	44.0	891280.000
5	3.0	3	10758274.148	44.0	8557072.000
6	6.7	3	10758274.148	44.0	20964220.000
7	15	3	10758274.148	44.0	22873448.000

biomass
File: 3517bd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	24450076.000	24450076.000	24450076.000
2	0.28	4	22816260.000	22816260.000	23784898.000
3	0.62	4	24753536.000	24753536.000	23784898.000
4	1.3	4	23558796.000	23558796.000	23558796.000
5	3.0	4	15893004.000	15893004.000	15893004.000
6	6.7	3	3485856.000	3485856.000	3485856.000
7	15	3	1576628.000	1576628.000	1576628.000

biomass
File: 3517bd 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
neg control	24450076.000				
0.28	23784898.000	0.164		1.74	k= 1, v=17
0.62	23784898.000	0.164		1.82	k= 2, v=17
1.3	**23558796.000**	**0.220**		**1.85**	**k= 3, v=17**
3.0	15893004.000	2.112	*	1.87	k= 4, v=17
6.7	3485856.000	5.174	*	1.87	k= 5, v=17
15	1576628.000	5.645	*	1.88	k= 6, v=17

s = 5730507.423
Note: df used for table values are approximate when v > 20.

Estimates of EC%

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

growth rate
File: 3517gd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.756	0.126	63.000
Within (Error)	17	0.038	0.002	
Total	23	0.794		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

growth rate
File: 3517gd 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	neg control	0.699	0.699		
2	0.28	0.700	0.700	-0.037	
3	0.62	0.707	0.707	-0.248	
4	1.3	0.692	0.692	0.237	
5	3.0	0.637	0.637	1.966	
6	6.7	0.370	0.370	10.420	*
7	15	0.214	0.214	15.332	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

growth rate
File: 3517gd 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	neg control	6			
2	0.28	3	0.084	12.0	-0.001
3	0.62	3	0.084	12.0	-0.008
4	1.3	3	0.084	12.0	0.007
5	3.0	3	0.084	12.0	0.062
6	6.7	3	0.084	12.0	0.329
7	15	3	0.084	12.0	0.485

growth rate
File: 3517gd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	0.699	0.699	0.701
2	0.28	3	0.700	0.700	0.701
3	0.62	3	0.707	0.707	0.701
4	1.3	3	0.692	0.692	0.692
5	3.0	3	0.637	0.637	0.637
6	6.7	3	0.370	0.370	0.370
7	15	3	0.214	0.214	0.214

growth rate
File: 3517gd 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
neg control	0.701				
0.28	0.701	0.067		1.74	k= 1, v=17

0.62	0.701	0.067		1.82	k= 2, v=17
1.3	0.692	0.225		1.85	k= 3, v=17
3.0	**0.637**	**1.862**		**1.87**	**k= 4, v=17**
6.7	0.370	9.868	*	1.87	k= 5, v=17
15	0.214	14.520	*	1.88	k= 6, v=17

s = 0.047
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.5	0.97	2.3	0.093	0.64
EC10	2.2	1.5	3.2	0.077	0.69
EC25	4.1	3.2	5.3	0.051	0.78
EC50	8.3	7.2	9.6	0.029	0.87

Slope = 2.22 Std.Err. = 0.230

Goodness of fit: p = 0.16 based on DF= 4.0 17.

3517GD : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	0.699	0.708	-0.00926	100.	0.00
0.280	3.00	0.700	0.708	-0.00770	99.9	0.0548
0.620	3.00	0.707	0.704	0.00298	99.4	0.622
1.30	3.00	0.692	0.682	0.00946	96.3	3.70
3.00	3.00	0.637	0.593	0.0442	83.7	16.3
6.70	3.00	0.370	0.413	-0.0429	58.2	41.8
15.0	3.00	0.214	0.202	0.0125	28.5	71.5