Data Evaluation Report on the acute toxicity of Penoxsulam, GF-443, on the Freshwater Alga *Selenastrum capricornutum*

PMRA Submission #:{.....} EPA MRID #: 45831107

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	{.....}
EPA MRID	45831107
EPA Guideline	123-2

Test material: Penoxsulam Purity: 21.9%
Common name: GF-443
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: Rebecca Bryan
Date: 12/29/03

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Date: 12/29/03

Primary Reviewer: J. GOODYEAR
{EPA/OECD/PMRA}
Date: {.....} Goodyear

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

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code ~~199031~~ 119031
Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Hahne, R. 2002. GF-443: Growth Inhibition Test with the Green Alga, *Selenastrum capricornutum*. Unpublished study performed by ABC Laboratories, Inc., Columbia, Missouri. Laboratory Project Identification No. 47287/Dow Study No. 021048. Study submitted by The Dow Chemical Company, Midland, Michigan for Dow AgroSciences, LLC, Indianapolis, Indiana. Experimental start date April 15, 2002 and experimental termination date April 19, 2002. The final report issued March 7, 2002.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to GF-443 under static conditions. The nominal concentrations were 5.0, 10, 20, 40, 80, and 160 µg a.i./L with dilution water control. The mean measured concentrations were <1.77 (control), 8.76, 15.8, 27.9, 41.2, 86.7, and 168 µg a.i./L. The cell density percent inhibitions were -1, 2, 18, 28, 47, and 60% reduced in the 8.76, 15.8, 27.9, 41.2, 86.7, and 168 µg a.i./L treatment groups, respectively. The growth rate percent inhibitions were 0, 0, 4, 6, 11, and 17% reduced in the 8.76, 15.8, 27.9, 41.2, 86.7, and 168 µg a.i./L treatment groups, respectively. The mean area under the growth curve (biomass) percent inhibitions were 4, 11, 20, 33, 51, and 60% reduced in the 8.76, 15.8, 27.9, 41.2, 86.7, and 168 µg a.i./L treatment groups, respectively. Biomass was the most sensitive endpoint, with an EC_{50} of 94 µg a.i./L; the NOAEC for biomass was 8.76 µg a.i./L.

The study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Selenastrum capricornutum*. This study is classified as Core.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell Density:
NOAEC: **15.8** µg a.i./L
EC_{05}: **8.2** µg a.i./L **95% C.I.**: 4.9-14 µg a.i./L
EC_{50}: 100 µg a.i./L **95% C.I.**:85-120 µg a.i./L
Slope: 1.52±0.125

Growth rate:
NOAEC: **168** µg a.i./L
EC_{05}: **30** µg a.i./L **95% C.I.**: 21-43 µg a.i./L
EC_{50}: >168 µg a.i./L **95% C.I.**:N/A
Slope: 1.05±0.0954

Area Under the Growth Curve (Biomass):
NOAEC: **8.76** µg a.i./L
EC_{05}: **5.1** µg a.i./L **95% C.I.**: 2.2-12 µg a.i./L
EC_{50}: 94 µg a.i./L **95% C.I.**:73-120 µg a.i./L
Slope: 1.30±0.150

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision J (122-2 and 123-2); and method 201 of the OECD Guideline for Testing of Chemicals. The following deviation from U.S. EPA Guideline 123-2 are noted:

1. A detailed description of freshwater algal nutrient medium, FWAM, was not provided.

This deviation 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 Penoxsulam, GF-443

Description: Not reported

Lot No.: E-828-59

Purity: 21.9%

Stability of Compound Under Test Conditions: The measured concentrations of GF-443 were 103-178% of nominal at hour 0 and 102-169% of nominal at hour 96 (Table 3, p. 23).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound. OECD requirements were not reported.)

Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:

Name: *Selenastrum capricornutum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. 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: Not reported.
Source: Originally from Department of Botany, Culture Collection of Algae, University of Texas at Austin, Austin, Texas. In-house laboratory cultures.
Age of inoculum: 3 days old.
Method of cultivation: Freshwater algal nutrient medium, FWAM

B. STUDY DESIGN:

a) Range-finding Study: A range-finding study with GF-443 was conducted in order to estimate the nominal test concentrations for the definitive study. The range-finding test concentrations were 0.0 (control), 0.50, 1.0, 10, 50, 100, and 1000 µg a.i./L. The percent inhibitions in cell growth compared to the control ranged from 0% in the 0.5 and 1.0 µg a.i./L treatment groups to 99% in the 1000 µg a.i./L treatment group.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	3 days	*EPA recommends two week acclimation period.*
culturing media and conditions: (same as test or not)	Freshwater algal nutrient medium, FWAM; same as test.	
health: (any toxicity observed)	Not reported	*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:		

Parameter	Details	Remarks Criteria
renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber.	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Erlenmeyer flasks with foam stoppers. 250 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.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	Freshwater algal nutrient medium, FWAM 7.41-7.48 8.10-8.69 Not reported Not reported 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)	No	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Laboratory reagent water Not reported 7.5 ± 0.1 N/A pH adjusted using 0.1 N NaOH and filtered through a 0.45-µm Millipore filter. Not reported Not reported Not detected (Appendix B, p.40) Not detected (Appendix B, p.40) 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.*

Parameter	Details	Remarks Criteria
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation rate of 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 Selenastrum capricornutum, 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:	 3 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.*
Test concentrations nominal: measured:	 control, 5.0, 10, 20, 40, 80, and 160 µg a.i./L <1.77 (control), 8.76, 15.8,	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five*

Parameter	Details	Remarks Criteria
	27.9, 41.2, 86.7, and 168 µg a.i./L	*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:	23.8-25.7°C Continuous 4650-4699 lux, cool-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	Cell count, growth rate, and area under the growth curve	

Parameters	Details	Remarks/Criteria
symptoms		*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	Hemacytometer and optical 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 186X 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	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The cell density percent inhibitions were -1, 2, 18, 28, 47, and 60% reduced in the 8.76, 15.8, 27.9, 41.2, 86.7, and 168 µg a.i./L treatment groups, respectively. The growth rate percent inhibitions were 0, 0, 4, 6, 11, and 17% reduced in the 8.76, 15.8, 27.9, 41.2, 86.7, and 168 µg a.i./L treatment groups, respectively. The mean area under the growth curve percent inhibitions were 4, 11, 20, 33, 51, and 60% reduced in the 8.76, 15.8, 27.9, 41.2, 86.7, and 168 µg a.i./L treatment groups, respectively. The percent reductions in cell density, growth rate, and mean area under the growth curve were statistically significant in the 27.9, 41.2, 86.7, and 168 µg a.i./L treatment groups.

Table 3: Effect of Penoxsulam, GF-443, on freshwater alga (*Selenastrum capricornutum*)

Treatment mean measured and nominal concentrations [a] (µg a.i./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	32,000	1,860,000	--
8.76 (5.0)	~10,000	28,000	1,870,000	-1
15.8 (10)	~10,000	29,000	1,820,000	2
27.9 (20)	~10,000	21,000*	1,530,000*	18
41.2 (40)	~10,000	26,000	1,330,000*	28
86.7 (80)	~10,000	28,000	990,000*	47
168 (160)	~10,000	28,000	740,000*	60
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.
[b] Percent inhibition based on control (using percent change data, Table 4, p. 24).
*Statistically significant reduction compared to the control ($p \leq 0.05$).

Table 4: Effect of Penoxsulam, GF-443, on the freshwater alga *Selenastrum capricornutum*

Mean Measured and Nominal Treatment Concentrations [a] (µg a.i./L)	Initial cell density (cells/mL)	Mean Growth Rate (cells/mL/hour)	% inhibition (Mean Growth Rate per day)	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)
Dilution water control	~10,000	0.054	--	4500	--
8.76 (5.0)	~10,000	0.054	0	4300	4

Mean Measured and Nominal Treatment Concentrations [a] (µg a.i./L)	Initial cell density (cells/mL)	Mean Growth Rate (cells/mL/hour)	% inhibition (Mean Growth Rate per day)	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)
15.8 (10)	~10,000	0.054	0	4000	11
27.9 (20)	~10,000	0.052*	4	3600*	20
41.2 (40)	~10,000	0.051*	6	3000*	33
86.7 (80)	~10,000	0.048*	11	2200*	51
168 (160)	~10,000	0.045*	17	1800*	60
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

[a] The nominal test concentrations are presented in parentheses.
[b] Percent inhibition based on control (using percent change data, Tables 6-7, pp. 26-27).
*Statistically significant reduction compared to the control ($p \leq 0.05$).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC or EC_{05} (µg a.i./L)	15.8	15.8	15.8
IC_{50} or EC_{50} (µg a.i./L) (95% C.I.)	99.1	>168	108
other (IC_{25}/EC_{25}) (µg a.i./L)	30.4	>168	39.1
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 treatment group data were analyzed using Shapiro-Wilk's test for normality and Levene's test for homogeneity of variance. The NOAEC was calculated using the analysis of variance and one-way Dunnett's comparison to the control. The data for all endpoints were analyzed using nonparametric analysis. Percent inhibitions were calculated for every endpoint. The EC_{25} and EC_{50} values were calculated by SAS using nonlinear (weighted) regression. The reported statistics were based on the mean measured test concentrations.

Cell Density:
$NOAEC/EC_{05}$: 15.8 µg a.i./L
EC_{50}: 108 µg a.i./L **95% C.I.**: 98.2-118 µg a.i./L

Growth rate:
NOAEC/EC_{05}: 15.8 µg a.i./L
EC_{50}: >168 µg a.i./L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):
NOAEC/EC_{05}: 15.8 µg a.i./L
EC_{50}: 99.1 µg a.i./L 95% C.I.: 88.2-110 µg a.i./L

Endpoints Affected: Cell density, growth rates and area under the growth curve.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density and biomass data satisfied the assumptions of ANOVA, while growth rate data did not. The NOAEC for cell density and biomass was determined using ANOVA, followed by William's multiple comparison test via TOXSTAT statistical software. The NOAEC for growth rate was determined using the non-parametric Kruskal-Wallis test via TOXSTAT software. The EC_x values were determined using the Probit method via Nuthatch statistical software. The reviewer used the mean measured concentrations to calculate toxicity values.

Cell Density:
NOAEC: 15.8 µg a.i./L
EC_{05}: 8.2 µg a.i./L 95% C.I.: 4.9-14 µg a.i./L
EC_{50}: 100 µg a.i./L 95% C.I.:85-120 µg a.i./L
Slope: 1.52±0.125

Growth rate:
NOAEC: 168 µg a.i./L
EC_{05}: 30 µg a.i./L 95% C.I.: 21-43 µg a.i./L
EC_{50}: >168 µg a.i./L 95% C.I.:N/A
Slope: 1.05±0.0954

Area Under the Growth Curve (Biomass):
NOAEC: 8.76 µg a.i./L
EC_{05}: 5.1 µg a.i./L 95% C.I.: 2.2-12 µg a.i./L
EC_{50}: 94 µg a.i./L 95% C.I.:73-120 µg a.i./L
Slope: 1.30±0.150

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

D. STUDY DEFICIENCIES:

The deviation (failure to provide details regarding the nutrient medium) was minor, so it did not affect the acceptability or validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's toxicity values differed slightly from those of the study author, presumably due to the different statistical methods used to estimate these values. While the study author's EC_{50} estimates for cell density and biomass were associated with slightly narrower 95% confidence intervals, the reviewer's toxicity values are reported

in the Executive Summary and Conclusions sections because the reviewer's analysis provided EC_{05} values and slopes for all endpoints.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata* [§123-2]. This study is classified as Core. Biomass was the most sensitive endpoint, with an EC_{50} of 94 µg a.i./L; the NOAEC for biomass was 8.76 µg a.i./L.

Cell Density:
NOAEC: **15.8** µg a.i./L
EC_{05}: **8.2** µg a.i./L **95% C.I.**: 4.9-14 µg a.i./L
EC_{50}: 100 µg a.i./L **95% C.I.**:85-120 µg a.i./L
Slope: 1.52±0.125

Growth rate:
NOAEC: **168** µg a.i./L
EC_{05}: **30** µg a.i./L **95% C.I.**: 21-43 µg a.i./L
EC_{50}: >168 µg a.i./L **95% C.I.**:N/A
Slope: 1.05±0.0954

Area Under the Growth Curve (Biomass):
NOAEC: **8.76** µg a.i./L
EC_{05}: **5.1** µg a.i./L **95% C.I.**: 2.2-12 µg a.i./L
EC_{50}: 94 µg a.i./L **95% C.I.**:73-120 µg a.i./L
Slope: 1.30±0.150

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

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 1107cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	36419.810	6069.968	122.450
Within (Error)	14	694.000	49.571	
Total	20	37113.810		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 1107cd 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	185.667	185.667		
2	8.76	186.667	186.667	-0.174	
3	15.8	182.333	182.333	0.580	
4	27.9	153.000	153.000	5.682	*
5	41.2	132.667	132.667	9.220	*
6	86.7	98.667	98.667	15.134	*
7	168	74.333	74.333	19.367	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

cell density
File: 1107cd 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	8.76	3	14.544	7.8	-1.000
3	15.8	3	14.544	7.8	3.333
4	27.9	3	14.544	7.8	32.667
5	41.2	3	14.544	7.8	53.000

6	86.7	3	14.544	7.8	87.000
7	168	3	14.544	7.8	111.333

cell density
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	185.667	185.667	186.167
2	8.76	3	186.667	186.667	186.167
3	15.8	3	182.333	182.333	182.333
4	27.9	3	153.000	153.000	153.000
5	41.2	3	132.667	132.667	132.667
6	86.7	3	98.667	98.667	98.667
7	168	3	74.333	74.333	74.333

cell density
File: 1107cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE WILLIAMS P=.05	DEGREES OF FREEDOM
control	186.167				
8.76	186.167	0.087		1.76	k= 1, v=14
15.8	**182.333**	**0.580**		**1.85**	**k= 2, v=14**
27.9	153.000	5.682	*	1.88	k= 3, v=14
41.2	132.667	9.219	*	1.89	k= 4, v=14
86.7	98.667	15.134	*	1.90	k= 5, v=14
168	74.333	19.367	*	1.91	k= 6, v=14

s = 7.041
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err. /Estimate	Lower Bound
EC5	**8.2**	**4.9**	**14.**	**0.11**	**0.60**
EC10	14.	9.4	22.	0.087	0.66
EC25	36.	27.	47.	0.057	0.76
EC50	**1.0E+02**	**85.**	**1.2E+02**	**0.032**	**0.86**

Slope = 1.52 Std.Err. = 0.125

!!!Poor fit: p = 0.013 based on DF= 4.0 14.

1107CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	186.	193.	-7.16	100.	0.00
8.76	3.00	187.	182.	4.33	94.6	5.44
15.8	3.00	182.	171.	11.1	88.8	11.2
27.9	3.00	153.	154.	-1.17	80.0	20.0
41.2	3.00	133.	139.	-6.17	72.0	28.0
86.7	3.00	98.7	104.	-4.84	53.7	46.3
168.	3.00	74.3	70.5	3.86	36.5	63.5

!!!Warning: EC5 not bracketed by doses evaluated.

area under the growth curve

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	19546666.666	3257777.778	30.817
Within (Error)	14	1480000.000	105714.286	
Total	20	21026666.667		

Critical F value = 2.85 (0.05,6,14)

Since F > Critical F REJECT Ho:All groups equal

area under the growth curve

File: 1107b 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	4533.333	4533.333		
2	8.76	4333.333	4333.333	0.753	
3	15.8	4033.333	4033.333	1.883	
4	27.9	3600.000	3600.000	3.516	*
5	41.2	2966.667	2966.667	5.901	*
6	86.7	2266.667	2266.667	8.538	*

7	168	1833.333	1833.333	10.171 *

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

area under the growth curve
File: 1107b 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	8.76	3	671.648	14.8	200.000
3	15.8	3	671.648	14.8	500.000
4	27.9	3	671.648	14.8	933.333
5	41.2	3	671.648	14.8	1566.667
6	86.7	3	671.648	14.8	2266.667
7	168	3	671.648	14.8	2700.000

area under the growth curve
File: 1107b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	4533.333	4533.333	4533.333
2	8.76	3	4333.333	4333.333	4333.333
3	15.8	3	4033.333	4033.333	4033.333
4	27.9	3	3600.000	3600.000	3600.000
5	41.2	3	2966.667	2966.667	2966.667
6	86.7	3	2266.667	2266.667	2266.667
7	168	3	1833.333	1833.333	1833.333

area under the growth curve
File: 1107b 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	4533.333				
8.76	4333.333	0.753		1.76	k= 1, v=14

15.8	4033.333	1.883	*	1.85	k= 2, v=14
27.9	3600.000	3.516	*	1.88	k= 3, v=14
41.2	2966.667	5.901	*	1.89	k= 4, v=14
86.7	2266.667	8.538	*	1.90	k= 5, v=14
168	1833.333	10.171	*	1.91	k= 6, v=14

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s = 325.137
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err. /Estimate	Lower Bound
		Lower	Upper		
EC5	**5.1**	**2.2**	**12.**	**0.17**	**0.43**
EC10	9.8	4.9	19.	0.14	0.50
EC25	29.	18.	45.	0.094	0.64
EC50	**94.**	**73.**	**1.2E+02**	**0.054**	**0.77**

Slope = 1.30 Std.Err. = 0.150

Goodness of fit: p = 0.38 based on DF= 4.0 14.

1107B : area under the growth curve

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	4.53e+03	4.64e+03	-107.	100.	0.00
8.76	3.00	4.33e+03	4.23e+03	108.	91.1	8.95
15.8	3.00	4.03e+03	3.92e+03	118.	84.4	15.6
27.9	3.00	3.60e+03	3.50e+03	99.5	75.4	24.6
41.2	3.00	2.97e+03	3.16e+03	-189.	68.0	32.0
86.7	3.00	2.27e+03	2.41e+03	-139.	51.8	48.2
168.	3.00	1.83e+03	1.72e+03	110.	37.1	62.9

!!!Warning: EC5 not bracketed by doses evaluated.

growth rate
File: 1107g Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	54.333	54.333	49.500
2	8.76	54.667	54.667	54.000
3	15.8	54.333	54.333	49.500
4	27.9	52.333	52.333	33.000
5	41.2	51.000	51.000	24.000
6	86.7	47.667	47.667	15.000

7	168	44.667	44.667	6.000

Calculated H Value = 18.802 Critical H Value Table = 12.590
Since Calc H > Crit H REJECT Ho:All groups are equal.

growth rate
File: 1107g Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 7 6 5 4 1 3 2
7	168	44.667	44.667	\
6	86.7	47.667	47.667	. \
5	41.2	51.000	51.000	. . \
4	27.9	52.333	52.333	. . . \
1	control	54.333	54.333	. . . . \
3	15.8	54.333	54.333	. . . . . \
2	8.76	54.667	54.667	* . . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 5.005

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err. /Estimate	Lower Bound
EC5	**30.**	**21.**	**43.**	**0.075**	**0.70**
EC10	67.	53.	84.	0.047	0.80
EC25	2.5E+02	2.2E+02	3.0E+02	0.031	0.86
EC50	1.1E+03	7.7E+02	1.6E+03	0.078	0.69

Slope = 1.05 Std.Err. = 0.0954

!!!Poor fit: p = 0.013 based on DF= 4.0 14.

1107G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	54.3	55.0	-0.630	100.	0.00
8.76	3.00	54.7	54.2	0.451	98.6	1.36
15.8	3.00	54.3	53.5	0.810	97.4	2.62
27.9	3.00	52.3	52.4	-0.0807	95.4	4.64

41.2	3.00	51.0	51.3	-0.317	93.4	6.64
86.7	3.00	47.7	48.3	-0.590	87.8	12.2
168.	3.00	44.7	44.3	0.357	80.6	19.4

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

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