Data Evaluation ~~Report~~ Record on the Acute Toxicity of Orthosulfamuron Marine Diatom, *Skeletonema costatum*

PMRA Submission Number {.....} EPA MRID Number 465789-33

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D319377
	OECD Data Point	{.....}
	EPA MRID	465789-33
	EPA Guideline	123-2

Test material: Orthosulfamuron
Common name
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: IR5878 50WG

Purity: 49.96 a.i.%

Primary Reviewer: Dana Worcester
Staff Scientist, Cambridge Environmental Inc.

Signature: Dana Worcester
Date: 2/24/06

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Date: 6/30/06

Secondary Reviewer(s): Christopher J. Salice
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Date: 7/31/06

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	108209	

Date Evaluation Completed: 31-07-2006

CITATION: Desjardins, D., T.Z. Kendall and H.O. Krueger. 2003. IR5878 50 WG Alone: A 96 hour Toxicity Test with the Marine Diatom (*Skeletonema costatum)*. Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-122 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued April 21, 2003.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96 hour acute toxicity study, cultures of the marine diatom, *Skeletonema costatum* were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.96%) at nominal concentrations of 3.8, 7.5, 15, 30 and 60 mg/L under static conditions. The measured (mean) concentrations were 3.6, 7.2, 15, 29 and 59 mg/L.

By 96 hours, cell density percent inhibitions were 5.9, 13, 20, 28 and 97% for the 3.6, 7.2, 15, 29 and 59 mg/L treatment groups, respectively, compared to the control. The cell density EC_{50} was 36 mg/L and the NOAEC was 7.2 mg/L. By 96 hours, biomass (area under the curve) inhibitions were 0.94, -1.8, 13, 68 and 100% for 3.6, 7.2, 15, 29 and 59 mg/L treatment groups, respectively, compared to the control. Biomass was the most sensitive endpoint, with an EC_{50} of 24 mg/L and a NOAEC of 15 mg/L. By 96 hours growth rate inhibitions were 2.2, 6.0, 8.2, 12 and 100% for the 3.6, 7.2, 15, 29 and 59 mg/L treatment groups, respectively, compared to the control. The growth rate EC_{50} was 42 mg/L and the NOEC was 7.2 mg/L.

At 96 hours in the 59 mg/L, there were aggregations and flocculations.

The study is scientifically sound and satisfies the guideline requirement for an aquatic nonvascular plant study with the marine diatom, *Skeletonema costatum*. This study is classified ACCEPTABLE.

Results Synopsis
Test Organism: *Skeletonema costatum*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density (96 Hours):

EC_{05}:	23 mg/L (11.5 mg ai/L)	95% C.I.: 19-28 mg/L (9.5-14.0 mg ai/L)
EC_{50}:	36 mg/L (18.0 mg ai/L)	95% C.I.: 33-40 mg/L (16.5-20.0 mg ai/L)
NOAEC:	7.2 mg/L (3.6 mg ai/L)	
Probit Slope:	8.52±0.943	

Growth rate (0-96 hours)(study-author reported):

EC_{05}:	7.0 mg/L (3.5 mg ai/L)	95% C.I.: 1.3-38 mg/L (0.65-19.0 mg ai/L)
EC_{50}:	42 mg/L (21 mg ai/L)	95% C.I.: 41-43 mg/L (20.5-21.5 mg ai/L)
NOAEC:	7.2 mg/L (3.6 mg ai/L)	
Probit Slope:	0.805±0.370	

Biomass (0-96 hours):

EC_{05}:	12 mg/L (6.0 mg ai/L)	95% C.I.: 8.8-17 mg/L (4.4-8.5 mg ai/L)
EC_{50}:	24 mg/L (12 mg ai/L)	95% C.I.: 22-26 mg/L (11-13 mg ai/L)
NOAEC:	15 mg/L (7.5 mg ai/L)	
Probit Slope:	5.56±0.939	

Endpoint(s) Affected: Cell density, biomass, and growth rates.
Most sensitive endpoint(s): Biomass

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study followed OECD Guideline 201 and U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400, *Algal Toxicity, Tiers I and II*. The following deviations from these guidelines are:

1. The dilution water characteristics of TOC, particulate matter, and chlorine content were not reported.
2. Cells were agitated at a higher rate (100 cycles/min) than is recommended for this species (60 cycles/min). However, there were no signs of adherence of cells to test chambers or changes in cell morphology during the test. Aside from aggregations/flocculations in the 59 mg/L treatment group, there were no other noticed abnormal signs.
3. The photoperiod (16h light: 8h dark) was slightly longer than recommended for this algal species (14h light: 10h dark).

These deviations did not affect the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice.

A. MATERIALS:

1. Test material IR5878 50WG (Orthosulfamuron)

Description: Brown granular solid

Lot No./Batch No.: G038/02

Purity: 49.96%

Stability of compound under test conditions: The measured concentrations of Orthosulfamuron were 98.5-103% of nominal at Hour 0 and 90.1-94.2% at 96 hours.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound) Only the water solubility was reported.

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

Physicochemical properties of orthosulfamuron

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Marine diatom *Skeletonema costatum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. 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: CCMP 1332
Source: Current in-house laboratory cultures originally obtained from Provasoli Guillard National Center of Marine.
Age of inoculum: Two weeks old
Method of cultivation: Artificially Enriched Seawater (AES) medium

B. STUDY DESIGN:

1. Experimental Conditions

a. 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 mortality observed)	Continuous Artificially Enriched Seawater (AES) 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	*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).*
Incubation facility	Environmental chamber	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Erlenmeyer flasks 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		

Parameter	Details	Remarks *Criteria*
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	7.9 8.0-8.8 disodium EDTA None 30‰	*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 and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
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 Not reported Not reported 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 solution	
Aeration or agitation	Agitation, 100 rpm	

Parameter	Details	Remarks *Criteria*
Initial cells density	77,000	*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.*
Number of replicates Control: Solvent control: Treatments:	 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.*
Test concentrations Nominal: Measured:	 3.8, 7.5, 15, 30 and 60 mg/L 3.6, 7.2, 15, 29 and 59 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	At 0 and 96 hours samples were analyzed by HPLC	

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	20.2-21.9°C 16h light: 8h dark 3690-4200 lux, cool white light	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; 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:	None	
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 density, biomass (area under the curve), growth rate	*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 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	24, 48, 72 and 96 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	

Parameters	Details	Remarks Criteria
Indicate whether there was an exponential growth in the control	Yes	*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?	Replicate data were provided	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96 hours, cell density inhibitions were 5.9, 13, 20, 28 and 97% for the mean measured 3.6, 7.2, 15, 29 and 59 mg/L treatment groups, respectively, compared to the control. By 96 hours biomass inhibitions were 0.94, -1.8, 13, 68 and 100% for the mean measured 3.6, 7.2, 15, 29 and 59 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were 2.2, 6.0, 8.2, 12 and 100% for the mean measured 3.6, 7.2, 15, 29 and 59 mg/L treatment groups, respectively, compared to the control.

At 96 hours in the 59 mg/L, there were aggregations and flocculations.

Table 3: Effect of Orthosulfamuron on marine diatom *Skeletonema costatum*

Treatment (record measured and nominal concentration (mg/L)	Initial cell density	Cell density at			
		24 hours	48 hours	96 hours	
				cell count	% inhibition
Negative control	77,000	260,667	643,333	1,066,667	--
Solvent control (if used)	N/A	N/A	N/A	N/A	N/A
3.8 (3.6)	77,000	235,667	656,667	1,033,333	5.9
7.5 (7.2)	77,000	222,000	665,000	925,000	13
15 (15)	77,000	216,667	596,667	858,333	20
30 (29)	77,000	84,333	195,000	766,667	28
60 (59)	77,000	32,000	46,667	34,000	97
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Statistical endpoint values.

Statistical Endpoint	biomass	growth rate	cell density
NOAEC or EC_{05} (mg/L)	15	15	15
EC_{50} (mg/L)	36	25	42
IC_{50} or EC_{50} (mg/L) (95% C.I.)	36 (33-40)	25 (23-27)	42 (41-43)
Other (IC_{25}/EC_{25})	NR	NR	NR
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	NA

NR Not reported

B. REPORTED STATISTICS:

The 96-Hour treatment and control response data passed the tests for normality (Shapiro-Wilks) and homogeneity of variance (Levene's). The 96-Hour EC_{50} value was determined using non-linear regression or linear interpolation. The reported toxicity values were determined in terms of the mean measured test concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, growth rate, and biomass data were analyzed using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett's tests for homogeneity of variances. Data did not require transformations to satisfy the assumptions of ANOVA; for biomass and growth rate, the highest treatment group data were excluded from the analysis because response was "0" for each replicate. The NOAEC values were determined using ANOVA, followed by William's test. These analyses were conducted using TOXSTAT statistical software. The EC_x values were determined using non-linear regression via Nuthatch statistical software. Mean-measured concentrations were used to compute these estimates.

Cell density (96 Hours):

EC_{05}:	23 mg/L (11.5 mg ai/L)	95% C.I.: 19-28 mg/L (9.5-14.0 mg ai/L)
EC_{50}:	36 mg/L (18.0 mg ai/L)	95% C.I.: 33-40 mg/L (16.5-20.0 mg ai/L)
NOAEC:	7.2 mg/L (3.6 mg ai/L)	
Probit Slope:	8.52±0.943	

Growth rate (0-96 hours):

EC_{05}:	7.0 mg/L (3.5 mg ai/L)	95% C.I.: 1.3-38 mg/L (0.65-19.0 mg ai/L)
EC_{50}:	>29 mg/L (14.5 mg ai/L)	95% C.I.: N/A
NOAEC:	7.2 mg/L (3.6 mg ai/L)	
Probit Slope:	0.805±0.370	

Biomass (0-96 hours):

EC_{05}:	12 mg/L (6.0 mg ai/L)	95% C.I.: 8.8-17 mg/L (4.4-8.5 mg ai/L)
EC_{50}:	24 mg/L (12 mg ai/L)	95% C.I.: 22-26 mg/L (11-13 mg ai/L)
NOAEC:	15 mg/L (7.5 mg ai/L)	
Probit Slope:	5.56±0.939	

Endpoint(s) Affected: Cell density, biomass, and growth rates.
Most sensitive endpoint(s): Biomass

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's analysis detected a lower NOAEC value for cell density and growth rate than the study authors' analysis. Additionally, the study authors' analysis identified an EC_{50} value for growth rate, while the reviewer's Probit model was not an appropriate fit to this data set. Aside from these differences, the reviewer's conclusions were identical to the study authors'. The reviewer's more conservative NOAEC for cell density and growth rate and the study authors' EC_{50} value for growth rate are reported in the Executive Summary and Conclusions sections.

The experimental start date was March 7, 2003 and the experimental termination date was March 17, 2003.

F. CONCLUSIONS:

The study is scientifically sound and is classified acceptable. Biomass was the most sensitive endpoint. The EC_{50} was 24 mg/L (12 mg ai/L); the EC_{05} and NOAEC values were 12 mg/L (6.0 mg ai/L) and 15 mg/L (7.5 mg ai/L), respectively.

Cell density (96 Hours):

EC_{05}:	23 mg/L (11.5 mg ai/L)	95% C.I.: 19-28 mg/L (9.5-14.0 mg ai/L)
EC_{50}:	36 mg/L (18.0 mg ai/L)	95% C.I.: 33-40 mg/L (16.5-20.0 mg ai/L)
NOAEC:	7.2 mg/L (3.6 mg ai/L)	
Probit Slope:	8.52±0.943	

Growth rate (0-96 hours):

EC_{05}:	7.0 mg/L (3.5 mg ai/L)	95% C.I.: 1.3-38 mg/L (0.65-19.0 mg ai/L)
EC_{50}:	42 mg/L (21 mg ai/L)	95% C.I.: 41-43 mg/L (20.5-21.5 mg ai/L)
NOAEC:	7.2 mg/L (3.6 mg ai/L)	
Probit Slope:	0.805±0.370	

Biomass (0-96 hours):

EC_{05}:	12 mg/L (6.0 mg ai/L)	95% C.I.: 8.8-17 mg/L (4.4-8.5 mg ai/L)
EC_{50}:	24 mg/L (12 mg ai/L)	95% C.I.: 22-26 mg/L (11-13 mg ai/L)
NOAEC:	15 mg/L (7.5 mg ai/L)	
Probit Slope:	5.56±0.939	

Endpoint(s) Affected: Cell density, biomass, and growth rates.
Most sensitive endpoint(s): Biomass

III. REFERENCES:

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Norgerg-King, T.J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: the Inhibition Concentration (Icp) Approach.* Version 2.0. U.S. Environmental Protection Agency. National Effluent Toxicity Assessment Center. Duluth, MN. Technical Report 03-93.

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West, Inc. and D.D. Gulley. TOXSTAT Version 3.5. Copyright 1996. Western Ecosystems Technology, Inc. Cheyenne, WY.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density (96h)
File: 8933c Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2147393.333	429478.667	41.265
Within (Error)	12	124894.667	10407.889	
Total	17	2272288.000		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

cell density (96h)
File: 8933c 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	1066.667	1066.667		
2	3.6	1003.333	1003.333	0.760	
3	7.2	925.000	925.000	1.701	
4	15	858.333	858.333	2.501	*
5	29	766.667	766.667	3.602	*
6	59	34.000	34.000	12.397	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

cell density (96h)
File: 8933c 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	3.6	3	208.246	19.5	63.333
3	7.2	3	208.246	19.5	141.667
4	15	3	208.246	19.5	208.333
5	29	3	208.246	19.5	300.000
6	59	3	208.246	19.5	1032.667

cell density (96h)
File: 8933c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	1066.667	1066.667	1066.667
2	3.6	3	1003.333	1003.333	1003.333
3	7.2	3	925.000	925.000	925.000
4	15	3	858.333	858.333	858.333
5	29	3	766.667	766.667	766.667
6	59	3	34.000	34.000	34.000

cell density (96h)
File: 8933c 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	1066.667				
3.6	1003.333	0.760		1.78	k= 1, v=12
7.2	925.000	1.701		1.87	k= 2, v=12
15	858.333	2.501	*	1.90	k= 3, v=12
29	766.667	3.602	*	1.92	k= 4, v=12
59	34.000	12.397	*	1.93	k= 5, v=12

s = 102.019
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	23.	19.	28.	0.038	0.83
EC10	26.	22.	30.	0.033	0.85
EC25	30.	26.	34.	0.027	0.88
EC50	36.	33.	40.	0.021	0.90

Slope = 8.52 Std.Err. = 0.943

Goodness of fit: p = 0.14 based on DF= 3.0 12.

8933C : cell density (96h)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	1.07e+03	964.	103.	100.	0.00
3.60	3.00	1.00e+03	964.	39.6	100.	2.36e-14
7.20	3.00	925.	964.	-38.7	100.	1.13e-07
15.0	3.00	858.	963.	-105.	99.9	0.0555
29.0	3.00	767.	765.	1.17	79.4	20.6
59.0	3.00	34.0	34.1	-0.103	3.54	96.5

biomass (0-96)
File: 8933b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	2308132128.000	577033032.000	35.757
Within (Error)	10	161377056.000	16137705.600	
Total	14	2469509184.000		

Critical F value = 3.48 (0.05,4,10)
Since F > Critical F REJECT Ho:All groups equal

biomass (0-96)
File: 8933b 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	46908.000	46908.000		
2	3.6	46468.000	46468.000	0.134	
3	7.2	47760.000	47760.000	-0.260	
4	15	40912.000	40912.000	1.828	
5	29	15092.000	15092.000	9.700	*

Dunnett table value = 2.47 (1 Tailed Value, P=0.05, df=10,4)

biomass (0-96)
File: 8933b 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	3.6	3	8101.627	17.3	440.000
3	7.2	3	8101.627	17.3	-852.000
4	15	3	8101.627	17.3	5996.000
5	29	3	8101.627	17.3	31816.000

biomass (0-96)
File: 8933b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	46908.000	46908.000	47045.333
2	3.6	3	46468.000	46468.000	47045.333
3	7.2	3	47760.000	47760.000	47045.333
4	15	3	40912.000	40912.000	40912.000
5	29	3	15092.000	15092.000	15092.000

biomass (0-96)
File: 8933b 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	47045.333				
3.6	47045.333	0.042		1.81	k= 1, v=10
7.2	47045.333	0.042		1.91	k= 2, v=10
15	40912.000	1.828		1.94	k= 3, v=10
29	15092.000	9.700	*	1.96	k= 4, v=10

s = 4017.176
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	12.	8.8	17.	0.063	0.73
EC10	14.	11.	18.	0.053	0.77
EC25	18.	15.	22.	0.035	0.84

EC50 24. 22. 26. 0.019 0.91

Slope = 5.56 Std.Err. = 0.939

Goodness of fit: p = 0.93 based on DF= 2.0 10.

8933B : biomass (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	4.69e+04	4.71e+04	-155.	100.	0.00
3.60	3.00	4.65e+04	4.71e+04	-594.	100.	0.000237
7.20	3.00	4.78e+04	4.70e+04	785.	99.8	0.186
15.0	3.00	4.09e+04	4.10e+04	-40.5	87.0	13.0
29.0	3.00	1.51e+04	1.51e+04	4.64	32.1	67.9

growth rate (0-96)
File: 8933g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.217	0.054	3.600
Within (Error)	10	0.145	0.015	
Total	14	0.362		

Critical F value = 3.48 (0.05,4,10)
Since F > Critical F REJECT Ho:All groups equal

growth rate (0-96)
File: 8933g 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	2.733	2.733		
2	3.6	2.677	2.677	0.567	
3	7.2	2.570	2.570	1.633	
4	15	2.513	2.513	2.200	
5	29	2.393	2.393	3.400	*

Dunnett table value = 2.47 (1 Tailed Value, P=0.05, df=10,4)

growth rate (0-96)
File: 8933g 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	3.6	3	0.247	9.0	0.057
3	7.2	3	0.247	9.0	0.163
4	15	3	0.247	9.0	0.220
5	29	3	0.247	9.0	0.340

growth rate (0-96)
File: 8933g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	2.733	2.733	2.733
2	3.6	3	2.677	2.677	2.677
3	7.2	3	2.570	2.570	2.570
4	15	3	2.513	2.513	2.513
5	29	3	2.393	2.393	2.393

growth rate (0-96)
File: 8933g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	2.733				
3.6	2.677	0.576		1.81	k= 1, v=10
7.2	2.570	1.661		1.91	k= 2, v=10
15	2.513	2.237	*	1.94	k= 3, v=10
29	2.393	3.457	*	1.96	k= 4, v=10

s = 0.120
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	7.0	1.3	38.	0.34	0.18
EC10	20.	7.6	51.	0.19	0.39
EC25	1.1E+02	23.	5.4E+02	0.31	0.21
EC50	7.7E+02	27.	2.2E+04	0.67	0.035

Slope = 0.805 Std.Err. = 0.370

Goodness of fit: p = 0.88 based on DF= 2.0 10.

8933G : growth rate (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	2.73	2.74	-0.00366	100.	0.00
3.60	3.00	2.68	2.65	0.0227	97.0	3.04
7.20	3.00	2.57	2.60	-0.0269	94.9	5.12
15.0	3.00	2.51	2.51	0.00704	91.6	8.43
29.0	3.00	2.39	2.39	0.000740	87.4	12.6

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

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