Recent

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae
Pseudokirchneriella subcapitata

PMRA Submission Number {........}   EPA MRID Number 465789-59

Data Requirement:	PMRA DATA CODE	{............}
	EPA DP Barcode	D319377
	OECD Data Point	{............}
	EPA MRID	465789-59
	EPA Guideline	123-2

Test material: Orthosulfamuron   **Purity:** 49.80 a.i.%
Common name
Chemical name: IUPAC: Not reported
                 CAS name: Not reported
                 CAS No.: Not reported
                 Synonyms: IR5878 50WG

Primary Reviewer: Dana Worcester   **Signature:** _Dana Worcester_
Staff Scientist, Cambridge Environmental Inc.   **Date:** 2/24/06

Secondary Reviewer: Teri S. Myers   **Signature:** _Teri S Myers_
Senior Scientist, Cambridge Environmental Inc.   **Date:** 3/15/06

Primary Reviewer: Christopher J. Salice   **Date:** 6/28/06
EPA/OPP/EFED/ERB-IV

Secondary Reviewer(s): Christopher J. Salice   **Date:** 7/31/06
EPA/OPP/EFED/ERB-IV

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

Company Code	{............}	[For PMRA]
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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 50WG + ADJ2506: A 72 hour Toxicity Test with the Freshwater Alga (*Pseudokircheriella subcapitata*). Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-132 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued April 12, 2005.

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.

2057792

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to *Pseudokirchneriella subcapitata*

EXECUTIVE SUMMARY:

In a 72 hour acute toxicity study, cultures of the freshwater green algae, *Pseudokircheriella subcapitata* were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.80%) at nominal concentrations of 0.28, 0.62, 1.4, 3.0, 6.8 and 15 mg/L under static conditions. The measured (mean) concentrations were 0.27, 0.57, 1.3, 2.9, 6.4 and 15 mg/L. The test concentrations were not corrected for the percent active ingredient in the test substance.

Cell density was the most sensitive endpoint, with a 72-hour EC_{50} of 7.0 mg/L; the 72-hour NOAEC was 0.57 mg/L. By 72 hours, biomass (area under the curve) inhibitions were −5.4, -5.6, 22, 15, 37 and 75% for the 0.27, 0.57, 1.3, 2.9, 6.4 and 15 mg/L treatment groups, respectively, compared to the pooled control. The biomass EC_{50} was 8.0 mg/L and the NOAEC was 0.57 mg/L. By 72 hours growth rate inhibitions were −1.7, -0.79, 5.1, 3.0, 12 and 31% for the 0.27, 0.57, 1.3, 2.9, 6.4 and 15 mg/L treatment groups, respectively, compared to the control. The growth rate EC_{50} was >15 mg/L and the NOAEC was 2.9 mg/L.

At 72 hours, aggregation/flocculation were observed in the 2.9, 6.8 and 15 mg/L treatment groups.

The study is scientifically sound but does not satisfy the guideline requirement for an aquatic nonvascular plant study with *Pseudokircheriella subcapitata*. This study was only conducted for 72 hours and, according to EPA guidelines, should be considered for Tier I screening purposes only. The study is classified SUPPLEMENTAL.

Results Synopsis
Test Organism: *Pseudokircheriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density (72 Hours):
EC_{05}:   1.3 mg/L (0.65 mg ai/L)   95% C.I.: 0.49-3.7 mg/L (0.24-1.8 mg ai/L)
EC_{50}:   7.0 mg/L (3.5 mg ai/L)   95% C.I.: 4.9-9.8 mg/L (2.4-4.9 mg ai/L)
NOAEC: 0.57 mg/L (0.28 mg ai/L)
Probit Slope: 2.30±0.548

Growth rate (0-72 hours):
EC_{05}:   3.5 mg/L (1.7 mg ai/L)   95% C.I.: 2.1-5.6 mg/L (1.0-2.8 mg ai/L)
EC_{50}:   >15 mg/L (>7.5 mg ai/L)   95% C.I.: N/A
NOAEC: 2.9 mg/L (1.4 mg ai/L)
Probit Slope: 1.78±0.299

Biomass (0-72 hours):
EC_{05}:   1.5 mg/L (0.75 mg ai/L)   95% C.I.: 0.60-3.9 mg/L (0.3-1.9 mg ai/L)
EC_{50}:   8.0 mg/L (4.0 mg ai/L)   95% C.I.: 5.9-11 mg/L (3.0-5.5 mg ai/L)
NOAEC: 0.57 mg/L (0.28 mg ai/L)
Probit Slope: 2.29±0.523

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

I. MATERIALS AND METHODS

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

1. The study was only conducted for 72 hours. According to a memo issued by US EPA entitled, "Closure on Nontarget Plant Phytotoxicity Policy Issues" on October 1994, three day OECD studies will be considered for Tier I screening purposes only.

2. The light intensity was 6450-7720 lux, which is higher than the recommendation for this species (4-5 ±15% Klux).

3. The pH of the algal medium (8.0±0.1) was higher than recommended for this species (7.5±0.1).

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: Solid

Lot No./Batch No.:   G009/03

Purity: 49.80 ± 0.5%

**Stability of compound
under test conditions:** The measured concentrations of orthosulfamuron were 91.9-97.0% of nominal at Hour 0 and 89.6-95.9% at 72 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: Green algae *Pseudokircheriella subcapitata*

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: UTCC 37
Source: Current in-house laboratory cultures, originally obtained from University of Toronto Culture.
Age of inoculum: Two weeks
Method of cultivation: Algal Assay Procedure (AAP) 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 Algal Assay Procedure (AAP) 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	72 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**):	7.8-7.9 8.0-8.7 disodium EDTA 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 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	10,000	*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 3 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:	 0.28, 0.62, 1.4, 3.0, 6.8 and 15 mg/L 0.27, 0.57, 1.3, 2.9, 6.4 and 15 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 72 hours samples were analyzed by HPLC	

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Parameter	Details	Remarks
		Criteria
Test conditions Temperature: Photoperiod: Light intensity and quality:	23.2-24.4°C continuous 6450-7720 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), and 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	Electronic Coulter 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	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 72 hours, biomass (area under the curve) inhibitions were –5.4, –5.6, 22, 15, 37 and 75% for the 0.27, 0.57, 1.3, 2.9, 6.4 and 15 mg/L treatment groups, respectively, compared to the pooled control. The biomass EC_{50} was 7.9 mg/L and the NOAEC was 2.9 mg/L; the EC_{10} was reported to be 2.2 mg/L. By 72 hours growth rate inhibitions were –1.7, –0.79, 5.1, 3.0, 12 and 31% for the 0.27, 0.57, 1.3, 2.9, 6.4 and 15 mg/L treatment groups, respectively, compared to the control. The growth rate EC_{50} was >15 mg/L and the NOAEC was 2.9 mg/L; the EC_{10} was reported to be 5.5 mg/L.

At 72 hours, aggregation/flocculation were observed in the 2.9, 6.8 and 15 mg/L treatment groups.

Table 3: Effect of orthosulfamuron on algal growth green algae *Pseudokircheriella subcapitata*

Treatment (record measured and nominal concentration (mg/L)	Initial cell density	Cell density at			
		24 hours	48 hours	72 hours	
				cell count	% inhibition
Negative control	10,000	28,152	197,911	1,560,185	--
Solvent control (if used)	10,000	24,753	196,181	1,423,227	---
Pooled control	---	26,453	197,046	1,491,706	--
0.28 (0.27)	10,000	27,316	188,102	1,609,152	-8
0.62 (0.57)	10,000	27,327	194,304	1,600,774	-7
1.4 (1.3)	10,000	23,845	155,609	1,155,055	23
3.0 (2.9)	10,000	23,832	151,360	1,305,566	12
6.8 (6.4)	10,000	22,900	190,649	821,483	45
15 (15)	10,000	21,094	76,191	319,226	79
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Reviewer calculated percent inhibitions using replicate data in Appendix 4, p. 45. These are relative to the pooled control group.

Table 4: Statistical endpoint values.

Statistical Endpoint	biomass	growth rate	cell density
NOAEC or EC_{05} (mg/L)	2.9	2.9	NR
EC_{50} (mg/L)	7.9	>15	NR
IC_{50} or EC_{50} (mg/L) (95% C.I.)	5.8 to 11	N/A	NR
Other (IC_{10}/EC_{10}) mg/L	2.2	5.5	NR
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	NA

NR Not reported

B. REPORTED STATISTICS:

The 72-Hour treatment and control response data passed the tests for normality (Shapiro-Wilks) and homogeneity of variance (Levene's). The 72-Hour EC_{50} value was determined by non-linear regression. 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. The negative and adjuvant control groups were compared for all endpoints and pooled for analysis upon detecting no significant differences using a Student's paired t-test. Data for cell density and biomass required a square-root transformation to satisfy the assumptions of ANOVA. The NOAEC values were determined using ANOVA, followed by William's test. These analyses were conducted using TOXSTAT statistical software. For cell density and biomass, the reviewer (and study authors) visually assigned a lower NOAEC than that revealed through statistical analysis (i.e., 2.9 mg/L), based on significant inhibition (12-23%) at the 1.3 and 2.9 mg/L treatment levels. The EC_x values were determined using non-linear regression via Nuthatch statistical software. Mean-measured concentrations were used to compute these estimates. Because the test concentrations were not corrected for the purity of the test material, the reviewer additionally calculated values based on active ingredient by multiplying estimates by 49.8%.

Cell density (72 Hours):
EC_{05}:   1.3 mg/L (0.65 mg ai/L)   95% C.I.: 0.49-3.7 mg/L (0.24-1.8 mg ai/L)
EC_{50}:   7.0 mg/L (3.5 mg ai/L)   95% C.I.: 4.9-9.8 mg/L (2.4-4.9 mg ai/L)
NOAEC: 0.57 mg/L (0.28 mg ai/L)
Probit Slope: 2.30±0.548

Growth rate (0-72 hours):
EC_{05}:   3.5 mg/L (1.7 mg ai/L)   95% C.I.: 2.1-5.6 mg/L (1.0-2.8 mg ai/L)
EC_{50}:   >15 mg/L (>7.5 mg ai/L)   95% C.I.: N/A
NOAEC: 2.9 mg/L (1.4 mg ai/L)
Probit Slope: 1.78±0.299

Biomass (0-72 hours):
EC_{05}:   1.5 mg/L (0.75 mg ai/L)   95% C.I.: 0.60-3.9 mg/L (0.3-1.9 mg ai/L)
EC_{50}:   8.0 mg/L (4.0 mg ai/L)   95% C.I.: 5.9-11 mg/L (3.0-5.5 mg ai/L)
NOAEC: 0.57 mg/L (0.28 mg ai/L)
Probit Slope: 2.29±0.523

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

D. STUDY DEFICIENCIES:

The study was conducted for 72 hours. Three-day OECD tests will be considered as Tier 1 screening studies.

E. REVIEWER∢S COMMENTS:

The reviewer's conclusions regarding biomass and growth rate were identical to the study authors'. The reviewer additionally provides results for cell density, which was the most sensitive endpoint; this endpoint was not statistically analyzed by the study authors.

The experimental start date was November 29, 2004 and the experimental termination date was December 2, 2004.

F. CONCLUSIONS:

The study is scientifically sound classified SUPPLEMENTAL, since it was conducted for 72 hours; EPA guidelines require a longer exposure. Cell density was the most sensitive endpoint. The EC_{50} was 7.0 mg/L (3.5 mg ai/L); the EC_{05} and NOAEC values were 1.3 mg/L (0.65 mg ai/L) and 0.57 mg/L (0.28 mg ai/L), respectively.

Cell density (72 Hours):
EC_{05}:	1.3 mg/L (0.65 mg ai/L)	95% C.I.: 0.49-3.7 mg/L (0.24-1.8 mg ai/L)
EC_{50}:	7.0 mg/L (3.5 mg ai/L)	95% C.I.: 4.9-9.8 mg/L (2.4-4.9 mg ai/L)
NOAEC:	0.57 mg/L (0.28 mg ai/L)	
Probit Slope:	2.30±0.548	

Growth rate (0-72 hours):
EC_{05}:	3.5 mg/L (1.7 mg ai/L)	95% C.I.: 2.1-5.6 mg/L (1.0-2.8 mg ai/L)
EC_{50}:	>15 mg/L (>7.5 mg ai/L)	95% C.I.: N/A
NOAEC:	2.9 mg/L (1.4 mg ai/L)	
Probit Slope:	1.78±0.299	

Biomass (0-72 hours):
EC_{05}:	1.5 mg/L (0.75 mg ai/L)	95% C.I.: 0.60-3.9 mg/L (0.3-1.9 mg ai/L)
EC_{50}:	8.0 mg/L (4.0 mg ai/L)	95% C.I.: 5.9-11 mg/L (3.0-5.5 mg ai/L)
NOAEC:	0.57 mg/L (0.28 mg ai/L)	
Probit Slope:	2.29±0.523	

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

III. REFERENCES:

ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae.* American Society for Testing and Materials. Philadelphia, PA.

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

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

OECD. 2004. Working Draft of OECD Proposal for Updating Guideline 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test.* Circulated April 9, 2004.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 8959c   Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1293.431	215.572	13.437
Within (Error)	23	368.986	16.043	
Total	29	1662.417		

Critical F value =   2.53 (0.05,6,23)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 8959c   Transform: SQUARE ROOT(Y)

BONFERRONI T-TEST   -   TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	38.465	1491.706		
2	0.27	40.033	1609.152	-0.607	
3	0.57	39.540	1600.774	-0.416	
4	1.3	33.869	1155.055	1.777	
5	2.9	35.850	1305.566	1.011	
6	6.4	28.648	821.483	3.797	*
7	15	17.829	319.226	7.982	*

Bonferroni T table value = 2.58   (1 Tailed Value, P=0.05, df=23,6)

cell density
File: 8959c   Transform: SQUARE ROOT(Y)

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	GRPS 1&2 POOLED	12			
2	0.27	3	469.153	31.5	-117.446
3	0.57	3	469.153	31.5	-109.068
4	1.3	3	469.153	31.5	336.651
5	2.9	3	469.153	31.5	186.140
6	6.4	3	469.153	31.5	670.223

```
   7              15    3          469.153      31.5      1172.479
```

```
cell density
File: 8959c          Transform: SQUARE ROOT(Y)

        WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
-----------------------------------------------------------------------------
GROUP                               ORIGINAL    TRANSFORMED   ISOTONIZED
            IDENTIFICATION     N      MEAN         MEAN         MEAN
------   -----------------    ---   ---------   -----------   ----------
    1       GRPS 1&2 POOLED   12    1491.706      38.465       38.905
    2                 0.27     3    1609.152      40.033       38.905
    3                 0.57     3    1600.774      39.540       38.905
    4                 1.3      3    1155.055      33.869       34.860
    5                 2.9      3    1305.566      35.850       34.860
    6                 6.4      3     821.483      28.648       28.648
    7                 15       3     319.226      17.829       17.829
-----------------------------------------------------------------------------


cell density
File: 8959c          Transform: SQUARE ROOT(Y)

        WILLIAMS TEST  (Isotonic regression model)    TABLE 2 OF 2
-----------------------------------------------------------------------------
                    ISOTONIZED   CALC.      SIG      TABLE      DEGREES OF
    IDENTIFICATION     MEAN     WILLIAMS   P=.05    WILLIAMS     FREEDOM
-----------------   ----------  --------   -----   ----------  -----------
    GRPS 1&2 POOLED   38.905
              0.27    38.905     0.170               1.72      k= 1,  v=23
              0.57    38.905     0.170               1.80      k= 2,  v=23
              1.3     34.860     1.394               1.83      k= 3,  v=23
              2.9     34.860     1.394               1.84      k= 4,  v=23
              6.4     28.648     3.797       *       1.85      k= 5,  v=23
              15      17.829     7.982       *       1.85      k= 6,  v=23
-----------------------------------------------------------------------------

s =    4.005
Note: df used for table values are approximate when v > 20.


Estimates of EC%
-----------------------------------------------------------------------------
Parameter   Estimate     95% Bounds        Std.Err.   Lower Bound
                       Lower    Upper                 /Estimate
EC5           1.3      0.49      3.7         0.21        0.36
EC10          1.9      0.83      4.5         0.18        0.43
EC25          3.6      2.0       6.3         0.12        0.56
EC50          7.0      4.9       9.8         0.073       0.71

       Slope =    2.30  Std.Err. =    0.548
```

Goodness of fit: p =   0.50 based on DF=   4.0   23.
- -
8959C : cell density
- -
Observed vs. Predicted Treatment Group Means
- -

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	12.0	1.49e+03	1.50e+03	-9.32	100.	0.00
0.270	3.00	1.61e+03	1.50e+03	109.	99.9	0.0574
0.570	3.00	1.60e+03	1.49e+03	109.	99.4	0.613
1.30	3.00	1.16e+03	1.43e+03	-276.	95.4	4.65
2.90	3.00	1.31e+03	1.22e+03	89.9	81.0	19.0
6.40	3.00	821.	802.	19.5	53.4	46.6
15.0	3.00	319.	333.	-13.9	22.2	77.8

biomass (0-72h)
File: 8959b   Transform: SQUARE ROOT(Y)

ANOVA TABLE
- -

SOURCE	DF	SS	MS	F
Between	6	16425.856	2737.643	15.341
Within (Error)	23	4104.511	178.457	
Total	29	20530.367		

   Critical F value =   2.53   (0.05,6,23)
   Since F > Critical F REJECT Ho:All groups equal

biomass (0-72h)
File: 8959b   Transform: SQUARE ROOT(Y)

      BONFERRONI T-TEST   -   TABLE 1 OF 2   Ho:Control<Treatment
- -

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	150.153	22664.511		
2	0.27	154.314	23879.852	-0.483	
3	0.57	153.181	23928.416	-0.351	
4	1.3	132.246	17567.564	2.077	
5	2.9	137.911	19271.384	1.420	
6	6.4	119.852	14382.964	3.514	*
7	15	74.511	5565.564	8.772	*

Bonferroni T table value = 2.58   (1 Tailed Value, P=0.05, df=23,6)

```
biomass (0-72h)
File: 8959b           Transform: SQUARE ROOT(Y)

     BONFERRONI T-TEST    -    TABLE 2 OF 2           Ho:Control<Treatment
  ---------------------------------------------------------------------------
                          NUM OF   Minimum Sig Diff   % of     DIFFERENCE
  GROUP    IDENTIFICATION  REPS    (IN ORIG. UNITS)  CONTROL   FROM CONTROL
  -----  --------------- -------  ----------------- -------   ------------
    1     GRPS 1&2 POOLED   12
    2               0.27    3         6192.710        27.3      -1215.341
    3               0.57    3         6192.710        27.3      -1263.905
    4               1.3     3         6192.710        27.3       5096.947
    5               2.9     3         6192.710        27.3       3393.127
    6               6.4     3         6192.710        27.3       8281.547
    7               15      3         6192.710        27.3      17098.947
  ---------------------------------------------------------------------------



biomass (0-72h)
File: 8959b           Transform: SQUARE ROOT(Y)

     WILLIAMS TEST   (Isotonic regression model)     TABLE 1 OF 2
  ---------------------------------------------------------------------------
  GROUP                          ORIGINAL    TRANSFORMED    ISOTONIZED
          IDENTIFICATION    N      MEAN         MEAN           MEAN
  ------  --------------- ---  ----------- ------------- ------------
    1     GRPS 1&2 POOLED  12   22664.511     150.153        151.351
    2               0.27    3   23879.852     154.314        151.351
    3               0.57    3   23928.416     153.181        151.351
    4               1.3     3   17567.564     132.246        135.079
    5               2.9     3   19271.384     137.911        135.079
    6               6.4     3   14382.964     119.852        119.852
    7               15      3    5565.564      74.511         74.511
  ---------------------------------------------------------------------------



biomass (0-72h)
File: 8959b           Transform: SQUARE ROOT(Y)

     WILLIAMS TEST   (Isotonic regression model)     TABLE 2 OF 2
  ---------------------------------------------------------------------------
                   ISOTONIZED   CALC.      SIG     TABLE     DEGREES OF
   IDENTIFICATION     MEAN     WILLIAMS  P=.05   WILLIAMS     FREEDOM
  --------------- ----------- --------- -----  ----------- -------------
   GRPS 1&2 POOLED   151.351
             0.27    151.351    0.139              1.72      k= 1,  v=23
             0.57    151.351    0.139              1.80      k= 2,  v=23
             1.3     135.079    1.748              1.83      k= 3,  v=23
             2.9     135.079    1.748              1.84      k= 4,  v=23
             6.4     119.852    3.514      *       1.85      k= 5,  v=23
```

| | 15 | 74.511 | 8.772 | * | 1.85 | k= 6, v=23 |

s = 13.359
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	1.5	0.60	3.9	0.20	0.39
EC10	2.2	1.0	4.8	0.16	0.46
EC25	4.0	2.4	6.8	0.11	0.60
EC50	8.0	5.9	11.	0.064	0.74

Slope = 2.29 Std.Err. = 0.523

Goodness of fit: p = 0.37 based on DF= 4.0 23.

8959B : biomass (0-72h)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	12.0	2.27e+04	2.26e+04	103.	100.	0.00
0.270	3.00	2.39e+04	2.26e+04	1.33e+03	100.	0.0380
0.570	3.00	2.39e+04	2.25e+04	1.47e+03	99.6	0.434
1.30	3.00	1.76e+04	2.18e+04	-4.19e+03	96.4	3.56
2.90	3.00	1.93e+04	1.90e+04	255.	84.3	15.7
6.40	3.00	1.44e+04	1.32e+04	1.15e+03	58.7	41.3
15.0	3.00	5.57e+03	5.98e+03	-414.	26.5	73.5

growth rate (0-72h)
File: 8959g   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	12.849	2.141	24.330
Within (Error)	23	2.014	0.088	
Total	29	14.863		

Critical F value = 2.53 (0.05,6,23)
Since F > Critical F REJECT Ho:All groups equal

```
growth rate (0-72h)
File: 8959g      Transform: NO TRANSFORMATION

      BONFERRONI T-TEST  -   TABLE 1 OF 2           Ho:Control<Treatment
------------------------------------------------------------------------
                           TRANSFORMED   MEAN CALCULATED IN
                             MEAN        ORIGINAL UNITS    T STAT  SIG
GROUP    IDENTIFICATION
------  ---------------    -----------   -----------------  ------  ---
  1      GRPS 1&2 POOLED     6.930           6.930
  2              0.27        7.047           7.047          -0.609
  3              0.57        6.987           6.987          -0.296
  4               1.3        6.573           6.573           1.863
  5               2.9        6.723           6.723           1.079
  6               6.4        6.120           6.120           4.230    *
  7                15        4.797           4.797          11.141    *
------------------------------------------------------------------------
Bonferroni T table value =  2.58     (1 Tailed Value, P=0.05,  df=23,6)
```

```
growth rate (0-72h)
File: 8959g      Transform: NO TRANSFORMATION

      BONFERRONI T-TEST  -   TABLE 2 OF 2           Ho:Control<Treatment
------------------------------------------------------------------------
                        NUM OF  Minimum Sig Diff  % of :  DIFFERENCE
                        REPS    (IN ORIG. UNITS)  CONTROL FROM CONTROL
GROUP    IDENTIFICATION
------  ---------------  -------  ---------------- -------  ------------
  1      GRPS 1&2 POOLED   12
  2              0.27       3        0.495          7.1       -0.117
  3              0.57       3        0.495          7.1       -0.057
  4               1.3       3        0.495          7.1        0.357
  5               2.9       3        0.495          7.1        0.207
  6               6.4       3        0.495          7.1        0.810
  7                15       3        0.495          7.1        2.133
------------------------------------------------------------------------
```

```
growth rate (0-72h)
File: 8959g      Transform: NO TRANSFORMATION

      WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
------------------------------------------------------------------------
GROUP                        ORIGINAL    TRANSFORMED   ISOTONIZED
        IDENTIFICATION    N    MEAN         MEAN          MEAN
------  ---------------  ---  --------   -----------   -----------
  1      GRPS 1&2 POOLED  12   6.930        6.930         6.959
  2              0.27      3   7.047        7.047         6.959
  3              0.57      3   6.987        6.987         6.959
  4               1.3      3   6.573        6.573         6.648
  5               2.9      3   6.723        6.723         6.648
  6               6.4      3   6.120        6.120         6.120
  7                15      3   4.797        4.797         4.797
------------------------------------------------------------------------
```

growth rate (0-72h)
File: 8959g   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
GRPS 1&2 POOLED	6.959				
0.27	6.959	0.151		1.72	k= 1, v=23
0.57	6.959	0.151		1.80	k= 2, v=23
1.3	6.648	1.475		1.83	k= 3, v=23
2.9	6.648	1.475		1.84	k= 4, v=23
6.4	6.120	4.240	*	1.85	k= 5, v=23
15	4.797	11.168	*	1.85	k= 6, v=23

s =   0.296
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	3.5	2.1	5.6	0.10	0.62
EC10	5.5	4.0	7.7	0.070	0.72
EC25	12.	10.	14.	0.033	0.86
EC50	29.	21.	40.	0.067	0.73

Slope =   1.78 Std.Err. =   0.299

Goodness of fit: p =   0.46   based on DF=   4.0   23.

8959G : growth rate (0-72h)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	12.0	6.93	6.92	0.00539	100.	0.00
0.270	3.00	7.05	6.92	0.123	100.	0.0148
0.570	3.00	6.99	6.92	0.0702	99.9	0.118
1.30	3.00	6.57	6.87	-0.295	99.2	0.814
2.90	3.00	6.72	6.67	0.0580	96.3	3.74
6.40	3.00	6.12	6.08	0.0355	87.9	12.1
15.0	3.00	4.80	4.81	-0.0134	69.5	30.5

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