

Record

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Freshwater Algae, *Anabaena flos-aquae*

PMRA Submission Number {.....}

EPA MRID Number 465789-37

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D319377
OECD Data Point	{.....}
EPA MRID	465789-37
EPA Guideline	123-2

Test material: Orthosulfamuron **Purity:** 49.96 a.i.%
Common name
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: IR5878 50WG

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

Signature: *Dana Worcester*
Date: 2/24/06

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.

Signature: *Teri S. Myers*
Date: 3/11/06

Primary Reviewer: Christopher J. Salice
EPA/OPP/EFED/ERB IV

Date: 6/30/06 *C. J. Salice*

Secondary Reviewer(s): Christopher J. Salice
EPA/OPP/EFED/ERB IV

Date: 7/31/06 *C. J. Salice*

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 50WG: A 96 hour Toxicity Test with the Freshwater Alga *Anabaena flos-aquae*. Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-113 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued June 20, 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.



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EXECUTIVE SUMMARY:

In a 96 hour acute toxicity study, cultures of the freshwater algae, *Anabaena flos-aquae* were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.96%) at nominal concentrations of 0.091, 0.20, 0.45, 1.0, 2.2, 5.0 and 11 mg/L under static conditions. The measured (mean) concentrations were 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L. Both a negative and an adjuvant control were tested in this study.

By 96 hours, cell density percent inhibitions were -21, -43, -40, -7.8, -9.5, 60 and 84% for the 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L treatment groups, respectively, compared to the control. Cell density was the most sensitive endpoint, with an EC₅₀ of 3.8 mg/L and a NOAEC of 2.2 mg/L. By 96 hours, biomass (area under the curve) inhibitions were -1.5, -22, -20, -7.5, 6.6, 48 and 76% for the 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L treatment groups, respectively, compared to the control. The biomass EC₅₀ was 4.7 mg/L and the NOEC was 2.2 mg/L. By 96 hours growth rate inhibitions were -3.7, -6.8, -7.5, -1.1, -2.0, 19 and 37% for the 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L treatment groups, respectively, compared to the control. The growth rate EC₅₀ was >10 mg/L and the NOEC was 2.2 mg/L.

Enlarged cells were observed in the 10 mg/L treatment group.

This toxicity study is scientifically sound and satisfies the guideline requirement for an aquatic nonvascular plant study with the freshwater alga, *Anabaena flos-aquae*. This study is classified ACCEPTABLE.

Results Synopsis

Test Organism: *Anabaena flos-aquae*

Test Type (Flow-through, Static, Static Renewal): Static

Cell density (96 Hours):

EC ₀₅ :	1.0 mg/L (0.50 mg ai/L)	95% C.I.: 0.37-2.8 mg/L (0.18-1.4 mg ai/L)
EC ₅₀ :	3.8 mg/L (1.9 mg ai/L)	95% C.I.: 2.5-5.6 mg/L (1.2-2.8 mg ai/L)
NOAEC:	2.2 mg/L (1.1 mg ai/L)	
Probit Slope:	2.89±0.750	

Growth rate (0-96 hours):

EC ₀₅ :	1.1 mg/L (0.50 mg ai/L)	95% C.I.: 0.53-2.1 mg/L (0.26-1.0 mg ai/L)
EC ₅₀ :	4.7 mg/L (2.3 mg ai/L)	95% C.I.: 3.7-6.0 mg/L (1.8-3.0 mg ai/L)
NOAEC:	2.2 mg/L (1.1 mg ai/L)	
Probit Slope:	2.55±0.444	

Biomass (0-96 hours):

EC ₀₅ :	1.8 mg/L (0.90 mg ai/L)	95% C.I.: 0.91-3.5 mg/L (0.45-1.7 mg ai/L)
EC ₅₀ :	>10 mg/L (>5.0 mg ai/L)	95% C.I.: N/A
NOAEC:	2.2 mg/L (1.1 mg ai/L)	
Probit Slope:	1.89±0.377	

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

Most sensitive endpoint(s): Cell density

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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 deviation from these guidelines are:

The dilution water characteristics of TOC, particulate matter, and chlorine content were not reported.

This deviation 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 96.1-101% of nominal at Hour 0 and 83.9-94.3% 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	

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2. Test organism:

Name: Algae *Anabaena flos-aquae*

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. capricornutum, 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 67
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:	Continuous	<i>EPA recommends two week acclimation period.</i> <i>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.</i>
Culturing media and conditions: (same as test or not)	Algal Assay Procedure (AAP) medium; same as test.	
Health: (any mortality observed)	Not reported	
<u>Test system</u> Static/static renewal	Static	<i>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).</i>
Renewal rate for static renewal		
Incubation facility	Environmental chamber	

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Parameter	Details	Remarks <i>Criteria</i>
Duration of the test	96 hours	EPA requires: 96-120 hours OECD: 72 hours
<u>Test vessel</u> 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.
<u>Details of growth medium name</u> pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	7.4-7.6 7.6-7.7 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, E1415-91 and D 3978-80 (reapproved 1987).
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
<u>Dilution water</u> 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: <i>Skeletonema costatum</i> = ~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	

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Parameter	Details	Remarks ----- Criteria
Initial cells density	10,000	<p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>
<u>Number of replicates</u> Control: Solvent control: Treatments:	3 3 3	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula sp.</i> tests should be conducted with four replicate.</p> <p>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.</p>
<u>Test concentrations</u> Nominal: Measured:	0.091, 0.20, 0.45, 1.0, 2.2, 5.0 and 11 mg/L 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L	<p>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</p> <p>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.</p>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	At 0 and 96 hours samples were analyzed by HPLC	

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Parameter	Details	Remarks <i>Criteria</i>
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	23.6-24.5°C continuous 1980-2220 lux, cool white light	----- <i>EPA temperature: <u>Skeletonema</u>: 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%)</i> <i>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i>
<u>Reference chemical (if used)</u> name: concentrations:	None	
Other parameters, if any	None	

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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	<i>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.</i>
Measurement technique for cell density and other end points	Hemocytometer and microscope	<i>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).</i>
Observation intervals	24, 48, 72 and 96 hours	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes	<i>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.</i>
Were raw data included?	Replicate data were provided	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96 hours, cell density percent inhibitions were -21, -43, -40, -7.8, -9.5, 60 and 84% for the 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L treatment groups, respectively, compared to the control. By 96 hours, biomass (area under the curve) inhibitions were -1.5, -22, -20, -7.5, 6.6, 48 and 76% for the 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were -3.7, -6.8, -7.5, -1.1, -2.0, 19 and 37% for the 0.082, 0.19, 0.43, 0.96, 2.2, 4.6 and 10 mg/L treatment groups, respectively, compared to the control.

Enlarged cells were observed in the 10 mg/L treatment group.

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Table 3: Effect of Orthosulfamuron on algal growth *Anabaena flos-aquae*

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	10,000	33,667	114,333	1,390,000	---
Adjuvant control	10,000	10,000	29,000	1,288,333	7.3
0.091 (0.082)	10,000	11,000	119,667	1,686,667	-21
0.20 (0.19)	10,000	9,333	97,333	1,983,333	-43
0.45 (0.43)	10,000	16,333	102,667	1,946,667	-40
1.0 (0.96)	10,000	15,000	91,333	1,498,333	-7.8
2.2 (2.2)	10,000	16,667	105,333	1,521,667	-9.5
5.0 (4.6)	10,000	11,667	138,667	553,333	60
11 (10)	10,000	10,000	34,333	228,667	84
Reference chemical (if used)	N/A				

Table 4: Statistical endpoint values.

Statistical Endpoint	biomass	growth rate	cell density
NOAEC or EC ₀₅ (mg/L)	2.2	2.2	2.2
EC ₅₀ (mg/L)	3.8	4.7	>10
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	3.8 (2.5-5.6)	4.7 (3.7-6.0)	>10
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 EC₅₀ value was determined by non-linear regression or linear interpolation. The reported toxicity values were determined in terms of the mean measured test concentrations.

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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 transformation to satisfy the assumptions of ANOVA. The NOAEC values were determined using ANOVA, followed by Dunnett's or William's test. These analyses were conducted using TOXSTAT statistical software. The response in the treatment groups was compared to the negative control. Student's t-tests were used to compare response in the negative control to that in the adjuvant control for all parameters; with the exception of biomass, there were no differences. 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 :	1.0 mg/L (0.50 mg ai/L)	95% C.I.: 0.37-2.8 mg/L (0.18-1.4 mg ai/L)
EC ₅₀ :	3.8 mg/L (1.9 mg ai/L)	95% C.I.: 2.5-5.6 mg/L (1.2-2.8 mg ai/L)
NOAEC:	2.2 mg/L (1.1 mg ai/L)	
Probit Slope:	2.89±0.750	

Growth rate (0-96 hours):

EC ₀₅ :	1.1 mg/L (0.50 mg ai/L)	95% C.I.: 0.53-2.1 mg/L (0.26-1.0 mg ai/L)
EC ₅₀ :	4.7 mg/L (2.3 mg ai/L)	95% C.I.: 3.7-6.0 mg/L (1.8-3.0 mg ai/L)
NOAEC:	2.2 mg/L (1.1 mg ai/L)	
Probit Slope:	2.55±0.444	

Biomass (0-96 hours):

EC ₀₅ :	1.8 mg/L (0.90 mg ai/L)	95% C.I.: 0.91-3.5 mg/L (0.45-1.7 mg ai/L)
EC ₅₀ :	>10 mg/L (>5.0 mg ai/L)	95% C.I.: N/A
NOAEC:	2.2 mg/L (1.1 mg ai/L)	
Probit Slope:	1.89±0.377	

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

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'.

The experimental start date was April 17, 2003 and the experimental termination date was April 24, 2003.

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F. CONCLUSIONS:

The study is scientifically sound and is classified ACCEPTABLE. Cell density was the most sensitive endpoint. The EC₅₀ was 3.8 mg/L (1.9 mg ai/L); the EC₀₅ and NOAEC values were 1.0 mg/L (0.50 mg ai/L) and 2.2 mg/L (1.1 mg ai/L), respectively.

Cell density (96 Hours):

EC₀₅: 1.0 mg/L (0.50 mg ai/L) 95% C.I.: 0.37-2.8 mg/L (0.18-1.4 mg ai/L)

EC₅₀: 3.8 mg/L (1.9 mg ai/L) 95% C.I.: 2.5-5.6 mg/L (1.2-2.8 mg ai/L)

NOAEC: 2.2 mg/L (1.1 mg ai/L)

Probit Slope: 2.89±0.750

Growth rate (0-96 hours):

EC₀₅: 1.1 mg/L (0.50 mg ai/L) 95% C.I.: 0.53-2.1 mg/L (0.26-1.0 mg ai/L)

EC₅₀: 4.7 mg/L (2.3 mg ai/L) 95% C.I.: 3.7-6.0 mg/L (1.8-3.0 mg ai/L)

NOAEC: 2.2 mg/L (1.1 mg ai/L)

Probit Slope: 2.55±0.444

Biomass (0-96 hours):

EC₀₅: 1.8 mg/L (0.90 mg ai/L) 95% C.I.: 0.91-3.5 mg/L (0.45-1.7 mg ai/L)

EC₅₀: >10 mg/L (>5.0 mg ai/L) 95% C.I.: N/A

NOAEC: 2.2 mg/L (1.1 mg ai/L)

Probit Slope: 1.89±0.377

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

Most sensitive endpoint(s): Cell density

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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.
- 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.
- Official Journal of the European Communities. 1992. No. L383. Method C.3.: *Algal Inhibition Test*.
- OECD. 1984. OECD Guidelines for Testing of Chemicals 201. Alga, Growth Inhibition Test.
- The SAS System for Windows. 1999. Version 8.02. SAS Institute Inc. Cary, NC.
- U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400.: *Algal Toxicity, Tiers I and II*.
- West, Inc. and D.D. Gulley. TOXSTAT Version 3.5. Copyright 1996. Western Ecosystems Technology, Inc. Cheyenne, WY.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density (96 h)

File: 8937c

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	8446787.167	1206683.881	6.148
Within (Error)	16	3140380.667	196273.792	
Total	23	11587167.833		

Critical F value = 2.66 (0.05,7,16)

Since F > Critical F REJECT Ho:All groups equal

cell density (96 h)

File: 8937c

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	neg control	1390.000	1390.000		
2	0.082	1686.667	1686.667	-0.820	
3	0.19	1983.333	1983.333	-1.640	
4	.43	1946.667	1946.667	-1.539	
5	0.96	1498.333	1498.333	-0.299	
6	2.2	1521.667	1521.667	-0.364	
7	4.6	553.333	553.333	2.313	
8	10	228.667	228.667	3.210	*

Dunnnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

cell density (96 h)

File: 8937c

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	neg control	3			
2	0.082	3	926.031	66.6	-296.667
3	0.19	3	926.031	66.6	-593.333
4	.43	3	926.031	66.6	-556.667
5	0.96	3	926.031	66.6	-108.333

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6	2.2	3	926.031	66.6	-131.667
7	4.6	3	926.031	66.6	836.667
8	10	3	926.031	66.6	1161.333

cell density (96 h)

File: 8937c

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	1390.000	1390.000	1751.667
2	0.082	3	1686.667	1686.667	1751.667
3	0.19	3	1983.333	1983.333	1751.667
4	.43	3	1946.667	1946.667	1751.667
5	0.96	3	1498.333	1498.333	1510.000
6	2.2	3	1521.667	1521.667	1510.000
7	4.6	3	553.333	553.333	553.333
8	10	3	228.667	228.667	228.667

cell density (96 h)

File: 8937c

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	1751.667				
0.082	1751.667	1.000		1.75	k= 1, v=16
0.19	1751.667	1.000		1.83	k= 2, v=16
.43	1751.667	1.000		1.86	k= 3, v=16
0.96	1510.000	0.332		1.87	k= 4, v=16
2.2	1510.000	0.332		1.88	k= 5, v=16
4.6	553.333	2.313	*	1.89	k= 6, v=16
10	228.667	3.210	*	1.89	k= 7, v=16

s = 443.028

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.0	0.37	2.8	0.21	0.37
EC10	1.4	0.58	3.2	0.18	0.42
EC25	2.2	1.2	4.1	0.13	0.54

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EC50 3.8 2.5 5.6 0.084 0.67

Slope = 2.89 Std.Err. = 0.750

Goodness of fit: p = 0.42 based on DF= 5.0 16.

8937C : cell density (96 h)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	1.39e+03	1.74e+03	-349.	100.	0.00
0.0820	3.00	1.69e+03	1.74e+03	-51.9	100.	7.74e-05
0.190	3.00	1.98e+03	1.74e+03	245.	100.	0.00883
0.430	3.00	1.95e+03	1.73e+03	214.	99.7	0.321
0.960	3.00	1.50e+03	1.66e+03	-166.	95.7	4.29
2.20	3.00	1.52e+03	1.31e+03	216.	75.1	24.9
4.60	3.00	553.	699.	-145.	40.2	59.8
10.0	3.00	229.	192.	36.3	11.1	88.9

biomass

File: 8937b

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	2424342816.000	346334688.000	13.736
Within (Error)	16	403418304.000	25213644.000	
Total	23	2827761120.000		

Critical F value = 2.66 (0.05,7,16)

Since F > Critical F REJECT Ho:All groups equal

biomass

File: 8937b

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	neg control	31232.000	31232.000		
2	0.082	31696.000	31696.000	-0.113	
3	0.19	37996.000	37996.000	-1.650	
4	.43	37624.000	37624.000	-1.559	
5	0.96	33588.000	33588.000	-0.575	

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Freshwater Algae,
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6	2.2	29168.000	29168.000	0.503
7	4.6	16332.000	16332.000	3.634 *
8	10	7436.000	7436.000	5.804 *

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

biomass
File: 8937b 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	neg control	3			
2	0.082	3	10495.718	33.6	-464.000
3	0.19	3	10495.718	33.6	-6764.000
4	.43	3	10495.718	33.6	-6392.000
5	0.96	3	10495.718	33.6	-2356.000
6	2.2	3	10495.718	33.6	2064.000
7	4.6	3	10495.718	33.6	14900.000
8	10	3	10495.718	33.6	23796.000

biomass
File: 8937b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	31232.000	31232.000	34637.000
2	0.082	3	31696.000	31696.000	34637.000
3	0.19	3	37996.000	37996.000	34637.000
4	.43	3	37624.000	37624.000	34637.000
5	0.96	3	33588.000	33588.000	33588.000
6	2.2	3	29168.000	29168.000	29168.000
7	4.6	3	16332.000	16332.000	16332.000
8	10	3	7436.000	7436.000	7436.000

biomass
File: 8937b 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
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Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Freshwater Algae, *Anabaena flos-aquae*

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neg control	34637.000					
0.082	34637.000	0.831		1.75	k= 1, v=16	
0.19	34637.000	0.831		1.83	k= 2, v=16	
.43	34637.000	0.831		1.86	k= 3, v=16	
0.96	33588.000	0.575		1.87	k= 4, v=16	
2.2	29168.000	0.503		1.88	k= 5, v=16	
4.6	16332.000	3.634	*	1.89	k= 6, v=16	
10	7436.000	5.804	*	1.89	k= 7, v=16	

s = 5021.319

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.1	0.53	2.1	0.15	0.50
EC10	1.5	0.82	2.7	0.12	0.56
EC25	2.6	1.7	3.9	0.085	0.66
EC50	4.7	3.7	6.0	0.051	0.78

Slope = 2.55 Std.Err. = 0.444

Goodness of fit: p = 0.44 based on DF= 5.0 16.

8937B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	3.12e+04	3.48e+04	-3.57e+03	100.	0.00
0.0820	3.00	3.17e+04	3.48e+04	-3.10e+03	100.	0.000365
0.190	3.00	3.80e+04	3.48e+04	3.20e+03	100.	0.0190
0.430	3.00	3.76e+04	3.47e+04	2.97e+03	99.6	0.403
0.960	3.00	3.36e+04	3.34e+04	154.	96.1	3.92
2.20	3.00	2.92e+04	2.78e+04	1.33e+03	80.0	20.0
4.60	3.00	1.63e+04	1.77e+04	-1.41e+03	51.0	49.0
10.0	3.00	7.44e+03	7.02e+03	418.	20.2	79.8

growth rate

File: 8937g

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	13.543	1.935	15.117

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Within (Error)	16	2.051	0.128
Total	23	15.593	

Critical F value = 2.66 (0.05,7,16)
 Since F > Critical F REJECT Ho:All groups equal

growth rate
 File: 8937g 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	neg control	5.110	5.110		
2	0.082	5.297	5.297	-0.639	
3	0.19	5.457	5.457	-1.187	
4	.43	5.493	5.493	-1.312	
5	0.96	5.163	5.163	-0.183	
6	2.2	5.210	5.210	-0.342	
7	4.6	4.127	4.127	3.366	*
8	10	3.200	3.200	6.538	*

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

growth rate
 File: 8937g 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	neg control	3			
2	0.082	3	0.748	14.6	-0.187
3	0.19	3	0.748	14.6	-0.347
4	.43	3	0.748	14.6	-0.383
5	0.96	3	0.748	14.6	-0.053
6	2.2	3	0.748	14.6	-0.100
7	4.6	3	0.748	14.6	0.983
8	10	3	0.748	14.6	1.910

growth rate
 File: 8937g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Freshwater Algae,
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GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	5.110	5.110	5.339
2	0.082	3	5.297	5.297	5.339
3	0.19	3	5.457	5.457	5.339
4	.43	3	5.493	5.493	5.339
5	0.96	3	5.163	5.163	5.187
6	2.2	3	5.210	5.210	5.187
7	4.6	3	4.127	4.127	4.127
8	10	3	3.200	3.200	3.200

growth rate
File: 8937g

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	5.339				
0.082	5.339	0.784		1.75	k= 1, v=16
0.19	5.339	0.784		1.83	k= 2, v=16
.43	5.339	0.784		1.86	k= 3, v=16
0.96	5.187	0.262		1.87	k= 4, v=16
2.2	5.187	0.262		1.88	k= 5, v=16
4.6	4.127	3.364	*	1.89	k= 6, v=16
10	3.200	6.534	*	1.89	k= 7, v=16

s = 0.358

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.8	0.91	3.5	0.14	0.51
EC10	2.8	1.7	4.6	0.11	0.60
EC25	5.8	4.5	7.5	0.053	0.77
EC50	13.	10.	17.	0.056	0.76

Slope = 1.89 Std.Err. = 0.377

Goodness of fit: p = 0.53 based on DF= 5.0 16.

8937G : growth rate

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Freshwater Algae,
*Anabaena flos-aquae***

PMRA Submission Number {.....}

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Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	5.11	5.34	-0.234	100.	0.00
0.0820	3.00	5.30	5.34	-0.0475	100.	0.00149
0.190	3.00	5.46	5.34	0.114	100.	0.0247
0.430	3.00	5.49	5.33	0.162	99.8	0.245
0.960	3.00	5.16	5.26	-0.0973	98.4	1.56
2.20	3.00	5.21	4.97	0.242	93.0	7.05
4.60	3.00	4.13	4.31	-0.185	80.7	19.3
10.0	3.00	3.20	3.15	0.0457	59.0	41.0

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

Control t-tests

cell density	biomass	growth rate
negative control	negative control	negative control
1005000	29700000	0.048
1345000	31476000	0.0511
1820000	32520000	0.0542

adjuvant control	adjuvant control	adjuvant control
980000	20712000	0.0478
1430000	25368000	0.0517
1455000	25482000	0.0519

t-test 0.531171 t-test 0.012972 t-test 0.540128