

Receipt

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....}

EPA MRID Number 465789-34

**Data Requirement:**

PMRA DATA CODE	{.....}
EPA DP Barcode	D319377
OECD Data Point	{.....}
EPA MRID	465789-34
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:** 3/7/06

**Primary Reviewer:** Christopher Salice  
**EPA/EFED/ERB-IV**

**Date:** 6/30/06

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

**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 50WG: A 96 hour Toxicity Test with the Freshwater Alga *Selenastrum capricornutum*. Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-116 and submitted by ISAGRO S.p.A., Milano, Italy.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.



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## EXECUTIVE SUMMARY:

In a 96 hour acute toxicity study, cultures of the freshwater green algae, *Selenastrum capricornutum* were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.96%) at nominal concentrations of 0.12, 0.26, 0.58, 1.3, 2.8, 6.2, 14 and 30 mg/L under static conditions. The measured (mean) concentrations were 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 mg/L. The test concentrations were not corrected for the percent active ingredient in the test substance. Both a negative and an adjuvant control were tested in this study. Significant inhibition of all parameters occurred in the adjuvant control; as a result, response in the treated levels was compared to the negative control group.

By 96 hours, cell density inhibitions were 10, 12, 15, 35, 60, 59, 76 and 96% for the 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 mg/L treatment groups, respectively, compared to the control. The cell density EC<sub>50</sub> was 3.1 mg/L and the NOEC was <0.13 mg/L. By 96 hours, biomass (area under the curve) inhibitions were 6.8, 5.5, 18, 35, 54, 62, 81 and 94% for the 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 mg/L treatment groups, respectively, compared to the control. Biomass was the most sensitive endpoint, with an EC<sub>50</sub> of 2.9 mg/L and a NOAEC of 0.27 mg/L. By 96 hours growth rate inhibitions were 2.0, 2.4, 2.8, 7.6, 17, 16, 25 and 55% for the 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 mg/L treatment groups, respectively, compared to the control. The growth rate EC<sub>50</sub> was 30 mg/L and the NOAEC was 0.60 mg/L.

Enlarged cells were observed in the adjuvant control and the 0.31 mg/L treatment group.

This toxicity study is scientifically sound, however does not satisfy the guideline requirement for an aquatic nonvascular plant toxicity study with the freshwater green algae species, *Selenastrum capricornutum* since there was significant inhibition in the adjuvant control. The information in this study useful for risk assessment purposes, however there is uncertainty associated with the effects of the adjuvant, therefore this study is classified SUPPLEMENTAL.

## Results Synopsis

Test Organism: *Selenastrum capricornutum*

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

### Cell density (96 Hours):

EC<sub>05</sub>: 0.17 mg/L (0.08 mg ai/L) 95% C.I.: 0.072-0.41 mg/L (0.036-0.20 mg ai/L)

EC<sub>50</sub>: 3.1 mg/L (1.5 mg ai/L) 95% C.I.: 2.1-4.4 mg/L (1.0-2.2 mg ai/L)

NOAEC: <0.13 mg/L (<0.06 mg ai/L)

Probit Slope: 1.32±0.129

### Growth rate (0-96 hours):

EC<sub>05</sub>: 3.1 mg/L (1.5 mg ai/L) 95% C.I.: 2.0-4.9 mg/L (1.0-2.4 mg ai/L)

EC<sub>50</sub>: 30 mg/L (15 mg ai/L) 95% C.I.: 26-34 mg/L (13-17 mg ai/L)

NOAEC: 0.6 mg/L (0.3 mg ai/L)

Probit Slope: 1.67±0.177

### Biomass (0-96 hours):

EC<sub>05</sub>: 0.17 mg/L (0.08 mg ai/L) 95% C.I.: 0.095-0.32 mg/L (0.047-0.16 mg ai/L)

EC<sub>50</sub>: 2.9 mg/L (1.4 mg ai/L) 95% C.I.: 2.3-3.8 mg/L (1.1-1.9 mg ai/L)

NOAEC: 0.27 mg/L (0.13 mg ai/L)

Probit Slope: 1.35±0.0936

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

Most sensitive endpoint(s): Biomass

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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 deviations from these guidelines are:

1. Guidelines recommend sub-culture of this algal species at a pH of 7.5 ( $\pm 0.1$ ). The pH of the freshwater algal medium was 8.0 and the pH during the test period ranged from 7.7-9.8.
2. The light intensity (5350-6600 lux) was higher than recommended for this algal species (4-5 Klux  $\pm 15\%$ ).
3. The dilution water characteristics of TOC, particulate matter, and chlorine content were not reported.
4. There was 95% inhibition in the adjuvant control after 96 hours. As a result, all treatment responses were compared to the negative control. Given the toxic effects of the adjuvant, it is impossible to attribute inhibition in the treated groups to orthosulfamuron alone.

These deviations, particularly inhibition in the adjuvant control, effect the validity of this 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 98.9-108% of nominal at Hour 0 and 95.3-105% 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.

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## 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 *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. 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 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

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**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	-----  <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>
<u>Test system</u> Static/static renewal  Renewal rate for 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>
Incubation facility	Environmental chamber	
Duration of the test	96 hours	-----  <i>EPA requires: 96-120 hours</i> <i>OECD: 72 hours</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Erlenmeyer flasks 250 mL 100 mL	-----  <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
<u>Details of growth medium name</u>		-----

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Parameter	Details	Remarks ----- Criteria
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	7.7-7.8 8.0-9.8 disodium EDTA NaHCO <sub>3</sub> 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</i> sp. 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:	0.12, 0.26, 0.58, 1.3, 2.8, 6.2, 14 and 30 mg/L 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 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, 72 and 96 hours samples were analyzed by HPLC	

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Parameter	Details	Remarks ----- Criteria
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	23.1-23.8°C continuous 5350-6600 lux, cool white light	EPA temperature: <i>Skeletonema</i> : 20EC, Others: 24-25EC; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <i>Anabaena</i> : 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)  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.
<u>Reference chemical (if used)</u> 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	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	

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Parameters	Details	Remarks
		Criteria
Indicate whether there was an exponential growth in the control	Yes	<p><i>EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.</i></p> <p><i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i></p>
Were raw data included?	Replicate data were provided	

**II. RESULTS and DISCUSSION:**

**A. INHIBITORY EFFECTS:**

By 96 hours, cell density percent inhibitions were 10, 12, 15, 35, 60, 59, 76 and 96% for the 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 mg/L treatment groups, respectively, compared to the control. By 96 hours, biomass (area under the curve) inhibitions were 6.8, 5.5, 18, 35, 54, 62, 81 and 94% for the 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were 2.0, 2.4, 2.8, 7.6, 17, 16, 25 and 55% for the 0.13, 0.27, 0.60, 1.3, 2.8, 6.3, 14 and 31 mg/L treatment groups, respectively, compared to the control.

The pH increased from 7.7-7.8 to 8.0-9.8 at 96 hours. Enlarged cells were observed in the solvent control and 31 mg/L treatment group.

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**Table 3: Effect of Orthosulfamuron on algal growth green algae *Selenastrum capricornutum***

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	46,503	245,575	2,924,676	--
Adjuvant control	10,000	16,345	31,381	148,560	95
0.12 (0.13)	10,000	43,588	258,306	2,620,606	10
0.26 (0.27)	10,000	44,231	275,739	2,563,022	12
0.58 (0.60)	10,000	46,923	232,414	2,495,203	15
1.3 (1.3)	10,000	44,504	213,326	1,903,033	35
2.8 (2.8)	10,000	49,296	201,759	1,164,227	60
6.2 (6.3)	10,000	50,364	187,402	1,203,603	59
14 (14)	10,000	38,949	66,764	713,264	76
30 (31)	10,000	49,926	54,299	126,375	96
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 <sub>05</sub> (mg/L)	0.13	0.27	0.60
EC <sub>50</sub> (mg/L)	3.0	2.9	30
IC <sub>50</sub> or EC <sub>50</sub> (mg/L) (95% C.I.)	3.0 (2.1-4.3)	2.9 (2.7-3.8)	30 (26-34)
Other (IC <sub>25</sub> /EC <sub>25</sub> )	NR	NR	NR
Reference chemical, if used NOAEC IC <sub>50</sub> /EC <sub>50</sub>	N/A	N/A	NA

NR Not reported

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## 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<sub>50</sub> 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. Data for biomass were natural log-transformed to satisfy the assumptions of ANOVA, while transformations of cell density and growth rate data were not necessary. The NOAEC values were determined using ANOVA, followed by William's test. These analyses were conducted using TOXSTAT statistical software. The EC<sub>x</sub> 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 <sub>05</sub> :	0.17 mg/L (0.08 mg ai/L)	95% C.I.: 0.072-0.41 mg/L (0.036-0.20 mg ai/L)
EC <sub>50</sub> :	3.1 mg/L (1.5 mg ai/L)	95% C.I.: 2.1-4.4 mg/L (1.0-2.2 mg ai/L)
NOAEC:	<0.13 mg/L (<0.06 mg ai/L)	
Probit Slope:	1.32±0.129	

### Growth rate (0-96 hours):

EC <sub>05</sub> :	3.1 mg/L (1.5 mg ai/L)	95% C.I.: 2.0-4.9 mg/L (1.0-2.4 mg ai/L)
EC <sub>50</sub> :	30 mg/L (15 mg ai/L)	95% C.I.: 26-34 mg/L (13-17 mg ai/L)
NOAEC:	0.6 mg/L (0.3 mg ai/L)	
Probit Slope:	1.67±0.177	

### Biomass (0-96 hours):

EC <sub>05</sub> :	0.17 mg/L (0.08 mg ai/L)	95% C.I.: 0.095-0.32 mg/L (0.047-0.16 mg ai/L)
EC <sub>50</sub> :	2.9 mg/L (1.4 mg ai/L)	95% C.I.: 2.3-3.8 mg/L (1.1-1.9 mg ai/L)
NOAEC:	0.27 mg/L (0.13 mg ai/L)	
Probit Slope:	1.35±0.0936	

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

Most sensitive endpoint(s): Biomass

## D. STUDY DEFICIENCIES:

At 96 hours the cell density inhibition in the adjuvant control was 95%, showing that the adjuvant had toxic properties for the green algae *Selenastrum capricornutum*. The study authors concluded that it is impossible to discern the effects of the adjuvant from the effects of the test material, Orthosulfamuron.

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### E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'. Given the toxicity of the adjuvant, it is impossible to attribute inhibition in the treated groups to Orthosulfamuron alone.

The experimental start date was February 21, 2003 and the experimental termination date was March 3, 2003.

### F. CONCLUSIONS:

The study is scientifically sound although there is uncertainty associated with the high inhibitions (95%) in the adjuvant control. The information in this study is useful for risk assessment purposes therefore the study is classified SUPPLEMENTAL. The most sensitive endpoint was biomass, with an EC<sub>50</sub> of 2.9 mg/L (1.4 mg ai/L); the EC<sub>05</sub> and NOAEC values were 0.17 mg/L (0.08 mg ai/L) and 0.27 mg/L (0.13 mg ai/L), respectively.

#### Cell density (96 Hours):

EC <sub>05</sub> :	0.17 mg/L (0.08 mg ai/L)	95% C.I.: 0.072-0.41 mg/L (0.036-0.20 mg ai/L)
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EC <sub>05</sub> :	0.17 mg/L (0.08 mg ai/L)	95% C.I.: 0.095-0.32 mg/L (0.047-0.16 mg ai/L)
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Endpoint(s) Affected: Cell density, biomass, and growth rates.

Most sensitive endpoint(s): Biomass

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**III. REFERENCES:**

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

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**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

cell density (96h)  
File: 8934c Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	26835544.495	3354443.062	87.963
Within (Error)	21	800824.568	38134.503	
Total	29	27636369.063		

Critical F value = 2.42 (0.05,8,21)  
Since F > Critical F REJECT Ho:All groups equal

cell density (96h)  
File: 8934c Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	2916.343	2916.343		
2	0.13	2620.606	2620.606	2.142	
3	0.27	2563.022	2563.022	2.559	
4	0.6	2495.203	2495.203	3.050	*
5	1.3	1903.033	1903.033	7.338	*
6	2.8	1164.227	1164.227	12.689	*
7	6.3	1203.603	1203.603	12.404	*
8	14	713.264	713.264	15.955	*
9	31	126.375	126.375	20.205	*

Bonferroni T table value = 2.73 (1 Tailed Value, P=0.05, df=21,8)

cell density (96h)  
File: 8934c Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	6			
2	0.13	3	377.246	12.9	295.737
3	0.27	3	377.246	12.9	353.321

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....}

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4	0.6	3	377.246	12.9	421.139
5	1.3	3	377.246	12.9	1013.309
6	2.8	3	377.246	12.9	1752.115
7	6.3	3	377.246	12.9	1712.740
8	14	3	377.246	12.9	2203.078
9	31	3	377.246	12.9	2789.968

cell density (96h)

File: 8934c

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	2916.343	2916.343	2916.343
2	0.13	3	2620.606	2620.606	2620.606
3	0.27	3	2563.022	2563.022	2563.022
4	0.6	3	2495.203	2495.203	2495.203
5	1.3	3	1903.033	1903.033	1903.033
6	2.8	3	1164.227	1164.227	1183.915
7	6.3	3	1203.603	1203.603	1183.915
8	14	3	713.264	713.264	713.264
9	31	3	126.375	126.375	126.375

cell density (96h)

File: 8934c

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	2916.343				
0.13	2620.606	2.142	*	1.72	k= 1, v=21
0.27	2563.022	2.559	*	1.80	k= 2, v=21
0.6	2495.203	3.050	*	1.83	k= 3, v=21
1.3	1903.033	7.338	*	1.84	k= 4, v=21
2.8	1183.915	12.546	*	1.85	k= 5, v=21
6.3	1183.915	12.546	*	1.85	k= 6, v=21
14	713.264	15.955	*	1.85	k= 7, v=21
31	126.375	20.205	*	1.86	k= 8, v=21

s = 195.281

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

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....}

EPA MRID Number 465789-34

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.17	0.072	0.41	0.18	0.42
EC10	0.32	0.15	0.68	0.16	0.48
EC25	0.94	0.54	1.6	0.12	0.58
EC50	3.1	2.1	4.4	0.075	0.70

Slope = 1.32 Std.Err. = 0.129

!!!Poor fit: p < 0.001 based on DF= 6.00 21.0

8934C : cell density (96h)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	2.92e+03	2.85e+03	67.4	100.	0.00
0.130	3.00	2.62e+03	2.75e+03	-127.	96.4	3.57
0.270	3.00	2.56e+03	2.61e+03	-49.6	91.7	8.30
0.600	3.00	2.50e+03	2.35e+03	149.	82.4	17.6
1.30	3.00	1.90e+03	1.96e+03	-54.6	68.7	31.3
2.80	3.00	1.16e+03	1.48e+03	-317.	52.0	48.0
6.30	3.00	1.20e+03	967.	236.	34.0	66.0
14.0	3.00	713.	548.	166.	19.2	80.8
31.0	3.00	126.	264.	-138.	9.28	90.7

biomass (0-96)

File: 8934b

Transform: NATURAL LOG(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	21.733	2.717	226.417
Within (Error)	21	0.247	0.012	
Total	29	21.980		

Critical F value = 2.42 (0.05,8,21)

Since F > Critical F REJECT Ho:All groups equal

biomass (0-96)

File: 8934b

Transform: NATURAL LOG(Y)

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....}

EPA MRID Number 465789-34

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

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GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	11.284	79803.102		
2	0.13	11.213	74377.388	0.921	
3	0.27	11.225	75390.352	0.762	
4	0.6	11.094	65817.796	2.457	
5	1.3	10.861	52241.704	5.464	*
6	2.8	10.491	36430.248	10.234	*
7	6.3	10.301	30039.920	12.692	*
8	14	9.647	15475.988	21.141	*
9	31	8.546	5146.196	35.354	*

Bonferroni T table value = 2.73 (1 Tailed Value, P=0.05, df=21,8)

biomass (0-96)

File: 8934b

Transform: NATURAL LOG(Y)

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

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GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	6			
2	0.13	3	15172.326	19.0	5425.714
3	0.27	3	15172.326	19.0	4412.750
4	0.6	3	15172.326	19.0	13985.306
5	1.3	3	15172.326	19.0	27561.398
6	2.8	3	15172.326	19.0	43372.854
7	6.3	3	15172.326	19.0	49763.182
8	14	3	15172.326	19.0	64327.114
9	31	3	15172.326	19.0	74656.906

biomass (0-96)

File: 8934b

Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

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GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	79803.102	11.284	11.284
2	0.13	3	74377.388	11.213	11.219
3	0.27	3	75390.352	11.225	11.219
4	0.6	3	65817.796	11.094	11.094
5	1.3	3	52241.704	10.861	10.861
6	2.8	3	36430.248	10.491	10.491
7	6.3	3	30039.920	10.301	10.301

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....}

EPA MRID Number 465789-34

8	14	3	15475.988	9.647	9.647
9	31	3	5146.196	8.546	8.546

biomass (0-96)

File: 8934b

Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	11.284				
0.13	11.219	0.851		1.72	k= 1, v=21
0.27	11.219	0.851		1.80	k= 2, v=21
0.6	11.094	2.483	*	1.83	k= 3, v=21
1.3	10.861	5.522	*	1.84	k= 4, v=21
2.8	10.491	10.342	*	1.85	k= 5, v=21
6.3	10.301	12.826	*	1.85	k= 6, v=21
14	9.647	21.364	*	1.85	k= 7, v=21
31	8.546	35.728	*	1.86	k= 8, v=21

s = 0.108

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	0.17	0.095	0.32	0.13	0.55
EC10	0.33	0.19	0.55	0.11	0.59
EC25	0.92	0.63	1.4	0.082	0.68
EC50	2.9	2.3	3.8	0.053	0.78

Slope = 1.35 Std.Err. = 0.0936

Goodness of fit: p = 0.26 based on DF= 6.0 21.

8934B : biomass (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	7.98e+04	7.93e+04	476.	100.	0.00
0.130	3.00	7.44e+04	7.66e+04	-2.21e+03	96.5	3.46
0.270	3.00	7.54e+04	7.28e+04	2.59e+03	91.8	8.22
0.600	3.00	6.58e+04	6.52e+04	596.	82.2	17.8

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....} EPA MRID Number 465789-34

1.30	3.00	5.22e+04	5.41e+04	-1.82e+03	68.2	31.8
2.80	3.00	3.64e+04	4.04e+04	-3.98e+03	50.9	49.1
6.30	3.00	3.00e+04	2.59e+04	4.15e+03	32.6	67.4
14.0	3.00	1.55e+04	1.43e+04	1.22e+03	18.0	82.0
31.0	3.00	5.15e+03	6.63e+03	-1.49e+03	8.36	91.6

growth rate (0-96)

File: 8934g Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	1.6622	0.2078	259.750
Within (Error)	21	0.0169	0.0008	
Total	29	1.6792		

Critical F value = 2.42 (0.05,8,21)

Since F > Critical F REJECT Ho:All groups equal

growth rate (0-96)

File: 8934g 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	neg control	2.432	5.913		
2	0.13	2.408	5.800	1.172	
3	0.27	2.403	5.773	1.450	
4	0.6	2.397	5.747	1.724	
5	1.3	2.337	5.463	4.720	*
6	2.8	2.219	4.927	10.638	*
7	6.3	2.231	4.977	10.057	*
8	14	2.108	4.443	16.190	*
9	31	1.626	2.643	40.298	*

Bonferroni T table value = 2.73 (1 Tailed Value, P=0.05, df=21,8)

growth rate (0-96)

File: 8934g 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

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....}

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1	neg control	6			
2	0.13	3	0.055	0.9	0.113
3	0.27	3	0.055	0.9	0.140
4	0.6	3	0.055	0.9	0.167
5	1.3	3	0.055	0.9	0.450
6	2.8	3	0.055	0.9	0.987
7	6.3	3	0.055	0.9	0.937
8	14	3	0.055	0.9	1.470
9	31	3	0.055	0.9	3.270

growth rate (0-96)

File: 8934g

Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	6	5.913	2.432	2.432
2	0.13	3	5.800	2.408	2.408
3	0.27	3	5.773	2.403	2.403
4	0.6	3	5.747	2.397	2.397
5	1.3	3	5.463	2.337	2.337
6	2.8	3	4.927	2.219	2.225
7	6.3	3	4.977	2.231	2.225
8	14	3	4.443	2.108	2.108
9	31	3	2.643	1.626	1.626

growth rate (0-96)

File: 8934g

Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	2.432				
0.13	2.408	1.165		1.72	k= 1, v=21
0.27	2.403	1.441		1.80	k= 2, v=21
0.6	2.397	1.714		1.83	k= 3, v=21
1.3	2.337	4.691	*	1.84	k= 4, v=21
2.8	2.225	10.283	*	1.85	k= 5, v=21
6.3	2.225	10.283	*	1.85	k= 6, v=21
14	2.108	16.090	*	1.85	k= 7, v=21
31	1.626	40.049	*	1.86	k= 8, v=21

s = 0.028

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

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Algae *Selenastrum capricornutum***

PMRA Submission Number {.....}

EPA MRID Number 465789-34

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	3.1	2.0	4.9	0.096	0.63
EC10	5.1	3.6	7.3	0.075	0.70
EC25	12.	9.7	14.	0.042	0.82
EC50	30.	26.	34.	0.030	0.87

Slope = 1.67 Std.Err. = 0.177

!!!Poor fit: p < 0.001 based on DF= 6.00 21.0

8934G : growth rate (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	5.91	5.73	0.187	100.	0.00
0.130	3.00	5.80	5.73	0.0737	100.	0.00393
0.270	3.00	5.77	5.72	0.0486	100.	0.0316
0.600	3.00	5.75	5.71	0.0332	99.8	0.228
1.30	3.00	5.46	5.66	-0.198	98.9	1.14
2.80	3.00	4.93	5.48	-0.554	95.7	4.29
6.30	3.00	4.98	4.99	-0.00863	87.1	12.9
14.0	3.00	4.44	4.06	0.386	70.8	29.2
31.0	3.00	2.64	2.80	-0.155	48.9	51.1