

Record

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

PMRA Submission Number {.....}

EPA MRID Number 465789-31

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D319377
OECD Data Point	{.....}
EPA MRID	465789-31
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

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

Date: 6/30/06

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

Date: 7/31/06

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CITATION: Desjardins, D., T.Z. Kendall and H.O. Krueger. 2003. IR5878 50 WG Alone: A 96 hour Toxicity Test with the Freshwater Alga *Selenastrum capricornutum*. Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-121 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued March 25, 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 1.9, 3.8, 7.5, 15 and 30 mg/L under static conditions. The measured (mean) concentrations were 1.9, 3.7, 7.4, 15 and 30 mg/L.

By 96 hours, cell density percent inhibitions were 14, 29, 60, 78, and 84% for the 1.9, 3.7, 7.4, 15 and 30 mg/L treatment groups, respectively, compared to the control. Cell density was the most sensitive endpoint, with an EC₅₀ of 6.2 mg/L and a NOAEC of <1.9 mg/L. By 96 hours, biomass (area under the curve) inhibitions were 14, 26, 56, 77, and 91% for the 1.9, 3.7, 7.4, 15 and 30 mg/L treatment groups, respectively, compared to the control. The biomass EC₅₀ was 6.6 mg/L and the NOAEC was <1.9 mg/L. By 96 hours growth rate inhibitions were 2.4, 5.4, 15, 24 and 44% for the 1.9, 3.7, 7.4, 15 and 30 mg/L treatment groups, respectively, compared to the control. The growth rate EC₅₀ was >30 mg/L and the NOAEC was 1.9 mg/L.

There were no compound related phytotoxic effects.

This toxicity study is scientifically sound and satisfies the guideline requirement for an aquatic nonvascular plant toxicity study with the freshwater green algae species, *Selenastrum capricornutum*. This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism: *Selenastrum capricornutum*

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

Cell density (96 Hours):

EC ₀₅ :	1.1 mg/L (0.55 mg ai/L)	95% C.I.: 0.78-1.6 mg/L (0.39-0.80 mg ai/L)
EC ₅₀ :	6.2 mg/L (3.1 mg ai/L)	95% C.I.: 5.3-7.2 mg/L (2.6-3.6 mg ai/L)
NOAEC:	<1.9 mg/L (<0.95 mg ai/L)	
Probit Slope:	2.20±0.140	

Growth rate (0-96 hours):

EC ₀₅ :	3.7 mg/L (1.8 mg ai/L)	95% C.I.: 3.0-4.6 mg/L (1.5-2.3 mg ai/L)
EC ₅₀ :	>30 mg/L (>15 mg ai/L)	95% C.I.: N/A
NOAEC:	1.9 mg/L (0.95 mg ai/L)	
Probit Slope:	1.63±0.0859	

Biomass (0-96 hours):

EC ₀₅ :	1.1 mg/L (0.55 mg ai/L)	95% C.I.: 0.74-1.6 mg/L (0.37-0.80 mg ai/L)
EC ₅₀ :	6.6 mg/L (3.3 mg ai/L)	95% C.I.: 5.7-7.8 mg/L (2.8-3.9 mg ai/L)
NOAEC:	<1.9 mg/L (<0.95 mg ai/L)	
Probit Slope:	2.08±0.135	

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 deviations from these guidelines are:

1. Guidelines recommend sub-culture of this algal species at a pH of 7.5 (± 0.1). The pH of the freshwater algal medium was 8.0 and the pH during the test period ranged from 7.9-9.4.
2. The light intensity (5410-6540 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.

These deviations did not affect the validity of the study.

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

A. MATERIALS:

1. Test material IR5878 50WG (Orthosulfamuron)

Description: Brown granular solid

Lot No./Batch No.: G038/02

Purity: 49.96%

Stability of compound under test conditions: The measured concentrations of orthosulfamuron were 99.6-102% of nominal at Hour 0 and 96.4-101% 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
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	7.9-8.0 8.1-9.3 disodium EDTA NaHCO ₃ N/A	<p style="text-align: center;"><i>Criteria</i></p> <p><i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i></p> <p><i>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).</i></p>
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	<p><i>EPA pH: <u>Skeletonema costatum</u> = ~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.</i></p> <p><i>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.</i></p>
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 N/A 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:	1.9, 3.8, 7.5, 15 and 30 mg/L 1.9, 3.7, 7.4, 15 and 30 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 <i>Criteria</i>
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	23.2-24.3°C continuous 5410-6540 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	

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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	Electronic Coulter particle counter	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>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.</i> <i>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	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96 hours, cell density inhibitions were 14, 29, 60, 78 and 94% for the mean measured 1.9, 3.7, 7.4, 15 and 30 mg/L treatment groups, respectively, compared to the control. By 96 hours biomass inhibitions were 14, 26, 56, 77 and 91% for the mean measured 1.9, 3.7, 7.4, 15 and 30 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were 2.4, 5.4, 15, 24 and 44% for the mean measured 1.9, 3.7, 7.4, 15 and 30 mg/L treatment groups, respectively, compared to the control.

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The pH increased from 7.9-8.0 to 8.1-9.3 at 96 hours. There were no compound related phytotoxic effects.

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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	39,682	239,214	5,629,687	--
Solvent control (if used)	N/A	N/A	N/A	N/A	N/A
1.9 (1.9)	10,000	43,749	244,999	4,819,656	14
3.8 (3.7)	10,000	42,901	222,466	4,022,509	29
7.5 (7.4)	10,000	37,011	150,099	2,256,991	60
15 (15)	10,000	33,437	82,266	1,238,564	78
30 (30)	10,000	51,075	75,134	339,669	94
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Statistical endpoint values.

Statistical Endpoint	biomass	growth rate	cell density
NOAEC or EC ₀₅ (mg/L)	1.1	1.9	1.1
EC ₅₀ (mg/L)	6.6	>30	6.2
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	6.6 (5.7-7.8)	>30 (not calculable)	6.2 (5.3-7.2)
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 for cell density and biomass were natural log-transformed to satisfy the assumptions of ANOVA, while transformation of growth rate data was unsuccessful. The NOAEC for cell density and biomass were determined using ANOVA, followed by William's test. The NOAEC for growth rate was determined using the non-parametric Kruskal Wallis test. These analyses were conducted using TOXSTAT statistical software. The EC_x values were determined using non-linear regression via Nuthatch statistical software. Mean-measured concentrations were used to compute these estimates.

Cell density (96 Hours):

EC ₀₅ :	1.1 mg/L (0.55 mg ai/L)	95% C.I.: 0.78-1.6 mg/L (0.39-0.80 mg ai/L)
EC ₅₀ :	6.2 mg/L (3.1 mg ai/L)	95% C.I.: 5.3-7.2 mg/L (2.6-3.6 mg ai/L)
NOAEC:	<1.9 mg/L (<0.95 mg ai/L)	
Probit Slope:	2.20±0.140	

Growth rate (0-96 hours):

EC ₀₅ :	3.7 mg/L (1.8 mg ai/L)	95% C.I.: 3.0-4.6 mg/L (1.5-2.3 mg ai/L)
EC ₅₀ :	>30 mg/L (>15 mg ai/L)	95% C.I.: N/A
NOAEC:	7.4 mg/L (3.7 mg ai/L)	
Probit Slope:	1.63±0.0859	

Biomass (0-96 hours):

EC ₀₅ :	1.1 mg/L (0.55 mg ai/L)	95% C.I.: 0.74-1.6 mg/L (0.37-0.80 mg ai/L)
EC ₅₀ :	6.6 mg/L (3.3 mg ai/L)	95% C.I.: 5.7-7.8 mg/L (2.8-3.9 mg ai/L)
NOAEC:	<1.9 mg/L (<0.95 mg ai/L)	
Probit Slope:	2.08±0.135	

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:

With the exception of the NOAEC for growth rate, the reviewer's conclusions were identical to the study authors'. Differences between these estimates are attributed to the different statistical methods used to calculate them. The study authors' more conservative NOAEC for growth rate is reported in the Executive Summary and Conclusions sections.

The experimental start date was February 14, 2003 and the experimental termination date was February 18, 2003.

F. CONCLUSIONS:

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The study is scientifically sound and is classified as acceptable. The most sensitive endpoint was cell density, with an EC₅₀ of 6.2 mg/L (3.1 mg ai/L); the EC₀₅ and NOAEC values were 1.1 mg/L (0.55 mg ai/L) and <1.9 mg/L (<0.95 mg ai/L), respectively.

Cell density (96 Hours):

EC ₀₅ :	1.1 mg/L (0.55 mg ai/L)	95% C.I.: 0.78-1.6 mg/L (0.39-0.80 mg ai/L)
EC ₅₀ :	6.2 mg/L (3.1 mg ai/L)	95% C.I.: 5.3-7.2 mg/L (2.6-3.6 mg ai/L)
NOAEC:	<1.9 mg/L (<0.95 mg ai/L)	
Probit Slope:	2.20±0.140	

Growth rate (0-96 hours):

EC ₀₅ :	3.7 mg/L (1.8 mg ai/L)	95% C.I.: 3.0-4.6 mg/L (1.5-2.3 mg ai/L)
EC ₅₀ :	>30 mg/L (>15 mg ai/L)	95% C.I.: N/A
NOAEC:	1.9 mg/L (0.95 mg ai/L)	
Probit Slope:	1.63±0.0859	

Biomass (0-96 hours):

EC ₀₅ :	1.1 mg/L (0.55 mg ai/L)	95% C.I.: 0.74-1.6 mg/L (0.37-0.80 mg ai/L)
EC ₅₀ :	6.6 mg/L (3.3 mg ai/L)	95% C.I.: 5.7-7.8 mg/L (2.8-3.9 mg ai/L)
NOAEC:	<1.9 mg/L (<0.95 mg ai/L)	
Probit Slope:	2.08±0.135	

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.
- 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 (96h)

File: 8931c

Transform: NATURAL LOG(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	19.373	3.875	387.500
Within (Error)	15	0.154	0.010	
Total	20	19.527		

Critical F value = 2.90 (0.05,5,15)

Since F > Critical F REJECT Ho:All groups equal

cell density (96h)

File: 8931c

Transform: NATURAL LOG(Y)

BONFERRONI T-TEST

TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	8.633	5629.687		
2	1.9	8.478	4819.656	2.181	
3	3.7	8.294	4022.509	4.793	*
4	7.4	7.714	2256.991	12.996	*
5	15	7.122	1238.564	21.368	*
6	30	5.825	339.669	39.707	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

cell density (96h)

File: 8931c

Transform: NATURAL LOG(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	control	6			
2	1.9	3	943.418	16.8	810.031
3	3.7	3	943.418	16.8	1607.178
4	7.4	3	943.418	16.8	3372.696
5	15	3	943.418	16.8	4391.123
6	30	3	943.418	16.8	5290.018

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cell density (96h)
File: 8931c Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	5629.687	8.633	8.633
2	1.9	3	4819.656	8.478	8.478
3	3.7	3	4022.509	8.294	8.294
4	7.4	3	2256.991	7.714	7.714
5	15	3	1238.564	7.122	7.122
6	30	3	339.669	5.825	5.825

cell density (96h)
File: 8931c 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
control	8.633				
1.9	8.478	2.155	*	1.75	k= 1, v=15
3.7	8.294	4.735	*	1.84	k= 2, v=15
7.4	7.714	12.836	*	1.87	k= 3, v=15
15	7.122	21.105	*	1.88	k= 4, v=15
30	5.825	39.220	*	1.89	k= 5, v=15

s = 0.101

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	0.78	1.6	0.073	0.70
EC10	1.6	1.2	2.2	0.063	0.74
EC25	3.1	2.4	3.8	0.047	0.80
EC50	6.2	5.3	7.2	0.032	0.86

Slope = 2.20 Std.Err. = 0.140

Goodness of fit: p = 0.43 based on DF= 3.0 15.

8931C : cell density (96h)

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

PMRA Submission Number {.....}

EPA MRID Number 465789-31

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	5.63e+03	5.62e+03	8.17	100.	0.00
1.90	3.00	4.82e+03	4.89e+03	-74.3	87.1	12.9
3.70	3.00	4.02e+03	3.87e+03	150.	68.9	31.1
7.40	3.00	2.26e+03	2.43e+03	-177.	43.3	56.7
15.0	3.00	1.24e+03	1.12e+03	117.	19.9	80.1
30.0	3.00	340.	372.	-32.1	6.61	93.4

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

!!!Warning: EC10 not bracketed by doses evaluated.

biomass (0-96)

File: 8931b

Transform: NATURAL LOG(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	14.687	2.937	293.700
Within (Error)	15	0.156	0.010	
Total	20	14.843		

Critical F value = 2.90 (0.05,5,15)

Since F > Critical F REJECT Ho:All groups equal

biomass (0-96)

File: 8931b

Transform: NATURAL LOG(Y)

BONFERRONI T-TEST

TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	11.585	107942.804		
2	1.9	11.441	93287.564	2.030	
3	3.7	11.282	79506.556	4.282	*
4	7.4	10.764	47752.128	11.615	*
5	15	10.099	24307.276	21.020	*
6	30	9.205	9976.464	33.662	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

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

PMRA Submission Number {.....}

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biomass (0-96)

File: 8931b

Transform: NATURAL LOG(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	control	6			
2	1.9	3	18065.773	16.7	14655.240
3	3.7	3	18065.773	16.7	28436.248
4	7.4	3	18065.773	16.7	60190.676
5	15	3	18065.773	16.7	83635.528
6	30	3	18065.773	16.7	97966.340

biomass (0-96)

File: 8931b

Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	107942.804	11.585	11.585
2	1.9	3	93287.564	11.441	11.441
3	3.7	3	79506.556	11.282	11.282
4	7.4	3	47752.128	10.764	10.764
5	15	3	24307.276	10.099	10.099
6	30	3	9976.464	9.205	9.205

biomass (0-96)

File: 8931b

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
control	11.585				
1.9	11.441	1.992	*	1.75	k= 1, v=15
3.7	11.282	4.201	*	1.84	k= 2, v=15
7.4	10.764	11.395	*	1.87	k= 3, v=15
15	10.099	20.622	*	1.88	k= 4, v=15
30	9.205	33.024	*	1.89	k= 5, v=15

s = 0.102

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 465789-31

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	1.1	0.74	1.6	0.077	0.69
EC10	1.6	1.2	2.2	0.066	0.73
EC25	3.1	2.5	4.0	0.049	0.79
EC50	6.6	5.7	7.8	0.033	0.85

Slope = 2.08 Std.Err. = 0.135

Goodness of fit: p = 0.71 based on DF= 3.0 15.

8931B : biomass (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	1.08e+05	1.08e+05	-275.	100.	0.00
1.90	3.00	9.33e+04	9.42e+04	-928.	87.1	12.9
3.70	3.00	7.95e+04	7.59e+04	3.64e+03	70.1	29.9
7.40	3.00	4.78e+04	4.99e+04	-2.11e+03	46.1	53.9
15.0	3.00	2.43e+04	2.50e+04	-661.	23.1	76.9
30.0	3.00	9.98e+03	9.37e+03	611.	8.65	91.3

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

!!!Warning: EC10 not bracketed by doses evaluated.

growth rate (0-96)

File: 8931g Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	6.593	6.593	109.000
2	1.9	6.433	6.433	43.000
3	3.7	6.240	6.240	34.000
4	7.4	5.637	5.637	24.000
5	15	5.020	5.020	15.000
6	30	3.667	3.667	6.000

Calculated H Value = 18.746 Critical H Value Table = 11.070
 Since Calc H > Crit H REJECT Ho: All groups are equal.

growth rate (0-96)

File: 8931g Transform: NO TRANSFORM

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

PMRA Submission Number {.....}

EPA MRID Number 465789-31

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP						
				0	0	0	0	0	0	
6	30	3.667	3.667	\						
5	15	5.020	5.020	.	\					
4	7.4	5.637	5.637	.	.	\				
3	3.7	6.240	6.240	.	.	.	\			
2	1.9	6.433	6.433	\		
1	control	6.593	6.593	*	*	\

* = significant difference (p=0.05)
Table q value (0.05,6) = 2.936

. = no significant difference
Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	3.7	3.0	4.6	0.046	0.80
EC10	6.2	5.2	7.3	0.035	0.84
EC25	15.	13.	16.	0.019	0.91
EC50	38.	35.	41.	0.016	0.92

Slope = 1.63 Std.Err. = 0.0859

!!!Poor fit: p = 0.039 based on DF= 3.0 15.

8931G : 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	6.59	6.57	0.0240	100.	0.00
1.90	3.00	6.43	6.46	-0.0232	98.3	1.72
3.70	3.00	6.24	6.24	-0.000593	95.0	5.00
7.40	3.00	5.64	5.75	-0.116	87.6	12.4
15.0	3.00	5.02	4.88	0.136	74.3	25.7
30.0	3.00	3.67	3.71	-0.0442	56.5	43.5

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