Record

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

PMRA Submission Number {........}   EPA MRID Number 465789-38

Data Requirement:	PMRA DATA CODE	{............}
	EPA DP Barcode	D319377
	OECD Data Point	{............}
	EPA MRID	465789-38
	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   **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:** 3/7/06

Primary Reviewer: Christopher J. Salice   **Date:** 6/30/06
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]
Active Code	{............}	[For PMRA]
Use Site Category:	{............}	[For PMRA]
EPA PC Code	108209	

Date Evaluation Completed: 31-07-2006

CITATION: Desjardins, D., T.Z. Kendall and H.O. Krueger. 2003. IR5878 50 WG ALONE: A 96 hour Toxicity Test with the Freshwater Alga *Anabaena flos-aquae*. Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-118 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued June 27, 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 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L.

By 96 hours, cell density percent inhibitions were 30, 15, -4.4, -2.8, 11, 58 and 93% for the 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L treatment groups, respectively, compared to the control. Cell density and biomass were equally sensitive, with EC_{50} values of 4.7 mg/L and NOAEC values of 2.2 mg/L. By 96 hours, biomass (area under the curve) inhibitions were 29, 5.6, 11, 9.8, 19, 60 and 89% for the 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were 6.9, 3.4, -0.56, 0.072, 3.2, 17 and 48% for the 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L treatment groups, respectively, compared to the control. The growth rate EC_{50} was >11 mg/L and the NOAEC was 2.2 mg/L.

Enlarged cells were observed in the 11 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}: 1.8 mg/L (0.90 mg ai/L) 95% C.I.: 0.87-3.7 mg/L (0.43-1.8 mg ai/L)
    EC_{50}: 4.7 mg/L (2.3 mg ai/L) 95% C.I.: 3.5-6.5 mg/L (1.7-3.2 mg ai/L)
    NOAEC: 2.2 mg/L (1.1 mg ai/L)
    Probit Slope: 3.92±0.954

    Growth rate (0-96 hours):
    EC_{05}: 3.0 mg/L (1.5 mg ai/L) 95% C.I.: 1.9-4.6 mg/L (0.95-2.3 mg ai/L)
    EC_{50}: 12 mg/L (6.0 mg ai/L) 95% C.I.: 10-13 mg/L (5.0-6.5 mg ai/L)
    NOAEC: 2.2 mg/L (1.1 mg ai/L)
    Probit Slope: 2.76±0.456

    Biomass (0-96 hours):
    EC_{05}: 1.5 mg/L (0.75 mg ai/L) 95% C.I.: 0.84-2.6 mg/L (0.42-1.3 mg ai/L)
    EC_{50}: 4.7 mg/L (2.3 mg ai/L) 95% C.I.: 3.7-5.9 mg/L (1.8-2.9 mg ai/L)
    NOAEC: 2.2 mg/L (1.1 mg ai/L)
    Probit Slope: 3.27±0.532

  Endpoint(s) Affected: Cell density, biomass, and growth rates.
  Most sensitive endpoint(s): Cell density and 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 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 <u>No</u> 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 102-106% of nominal at Hour 0 and 92.8-96.4% at 96 hours.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound) Only the water solubility was reported.

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

Physicochemical properties of orthosulfamuron

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

2. Test organism:

Name:	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. 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 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: 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	

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Parameter	Details	Remarks
		Criteria
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Erlenmeyer flasks 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	7.4-7.5 7.4-7.8 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 NA 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:	 0.091, 0.20, 0.45, 1.0, 2.2, 5.0 and 11 mg/L 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	At 0 and 96 hours samples were analyzed by HPLC	

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Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	22.9-24.1°C continuous 1960-2340 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	

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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	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Hemacytometer and microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	24, 48, 72 and 96 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
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	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96 hours, cell density percent inhibitions were 30, 15, -4.4, -2.8, 11, 58 and 93% for the 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L treatment groups, respectively, compared to the control. By 96 hours, biomass (area under the curve) inhibitions were 29, 5.6, 11, 9.8, 19, 60 and 89% for the 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were 6.9, 3.4, -0.56, 0.072, 3.2, 17 and 48% for the 0.089, 0.20, 0.45, 0.99, 2.2, 4.9 and 11 mg/L treatment groups, respectively, compared to the control.

Enlarged cells were observed in the 11 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	19,333	155,333	2,575,000	---
Solvent control (if used)	N/A	N/A	N/A	N/A	N/a
0.091 (0.089)	10,000	13,000	157,000	1,793,333	30
0.20 (0.20)	10,000	6,000	120,333	2,176,667	15
0.45 (0.45)	10,000	17,333	91,667	2,688,333	-4.4
1.0 (0.99)	10,000	69,333	146,000	2,648,333	-2.8
2.2 (2.2)	10,000	34,333	199,000	2,281,667	11
5.0 (4.9)	10,000	18,000	77,333	1,075,000	58
11 (11)	10,000	15,000	29,667	192,667	93
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)	2.2	2.2	2.2
EC_{50} (mg/L)	4.7	4.7	>11
IC_{50} or EC_{50} (mg/L) (95% C.I.)	4.7 (3.5-6.5)	4.7 (3.7-5.9)	>11
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

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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_{50} value was determined by non-linear regression or linear interpolation. The reported toxicity values were determined in terms of the mean measured test concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

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

Cell density (96 Hours):

EC_{05}:   1.8 mg/L (0.90 mg ai/L)   95% C.I.: 0.87-3.7 mg/L (0.43-1.8 mg ai/L)
EC_{50}:   4.7 mg/L (2.3 mg ai/L)   95% C.I.: 3.5-6.5 mg/L (1.7-3.2 mg ai/L)
NOAEC: 2.2 mg/L (1.1 mg ai/L)
Probit Slope: 3.92 ± 0.954

Growth rate (0-96 hours):

EC_{05}:   3.0 mg/L (1.5 mg ai/L)   95% C.I.: 1.9-4.6 mg/L (0.95-2.3 mg ai/L)
EC_{50}:   12 mg/L (6.0 mg ai/L)   95% C.I.: 10-13 mg/L (5.0-6.5 mg ai/L)
NOAEC: 2.2 mg/L (1.1 mg ai/L)
Probit Slope: 2.76 ± 0.456

Biomass (0-96 hours):

EC_{05}:   1.5 mg/L (0.75 mg ai/L)   95% C.I.: 0.84-2.6 mg/L (0.42-1.3 mg ai/L)
EC_{50}:   4.7 mg/L (2.3 mg ai/L)   95% C.I.: 3.7-5.9 mg/L (1.8-2.9 mg ai/L)
NOAEC: 2.2 mg/L (1.1 mg ai/L)
Probit Slope: 3.27 ± 0.532

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

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 11, 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 and biomass were the most sensitive endpoints. The EC_{50} was 4.7 mg/L (2.3 mg ai/L); the NOAEC value was 2.2 mg/L (1.1 mg ai/L).

Cell density (96 Hours):
EC_{05}:   1.8 mg/L (0.90 mg ai/L)   95% C.I.: 0.87-3.7 mg/L (0.43-1.8 mg ai/L)
EC_{50}:   4.7 mg/L (2.3 mg ai/L)   95% C.I.: 3.5-6.5 mg/L (1.7-3.2 mg ai/L)
NOAEC: 2.2 mg/L (1.1 mg ai/L)
Probit Slope: 3.92±0.954

Growth rate (0-96 hours):
EC_{05}:   3.0 mg/L (1.5 mg ai/L)   95% C.I.: 1.9-4.6 mg/L (0.95-2.3 mg ai/L)
EC_{50}:   12 mg/L (6.0 mg ai/L)   95% C.I.: 10-13 mg/L (5.0-6.5 mg ai/L)
NOAEC: 2.2 mg/L (1.1 mg ai/L)
Probit Slope: 2.76±0.456

Biomass (0-96 hours):
EC_{05}:   1.5 mg/L (0.75 mg ai/L)   95% C.I.: 0.84-2.6 mg/L (0.42-1.3 mg ai/L)
EC_{50}:   4.7 mg/L (2.3 mg ai/L)   95% C.I.: 3.7-5.9 mg/L (1.8-2.9 mg ai/L)
NOAEC: 2.2 mg/L (1.1 mg ai/L)
Probit Slope: 3.27±0.532

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

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.

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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.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density (96 h)
File: 8938c   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	16378892.625	2339841.804	7.255
Within (Error)	16	5160372.000	322523.250	
Total	23	21539264.625		

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

cell density (96 h)
File: 8938c   Transform: NO TRANSFORMATION

DUNNETTS TEST   -   TABLE 1 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	2575.000	2575.000		
2	0.089	1793.333	1793.333	1.686	
3	0.2	2176.667	2176.667	0.859	
4	0.45	2688.333	2688.333	-0.244	
5	0.99	2648.333	2648.333	-0.158	
6	2.2	2281.667	2281.667	0.633	
7	4.9	1075.000	1075.000	3.235	*
8	11	192.667	192.667	5.138	*

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

cell density (96 h)
File: 8938c   Transform: NO TRANSFORMATION

DUNNETTS TEST   -   TABLE 2 OF 2   Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	0.089	3	1187.066	46.1	781.667
3	0.2	3	1187.066	46.1	398.333
4	0.45	3	1187.066	46.1	-113.333
5	0.99	3	1187.066	46.1	-73.333

6	2.2	3	1187.066	46.1	293.333
7	4.9	3	1187.066	46.1	1500.000
8	11	3	1187.066	46.1	2382.333

cell density (96 h)
File: 8938c   Transform: NO TRANSFORMATION

               WILLIAMS TEST   (Isotonic regression model)   TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	2575.000	2575.000	2575.000
2	0.089	3	1793.333	1793.333	2326.667
3	0.2	.3	2176.667	2176.667	2326.667
4	0.45	3	2688.333	2688.333	2326.667
5	0.99	3	2648.333	2648.333	2326.667
6	2.2	3	2281.667	2281.667	2281.667
7	4.9	3	1075.000	1075.000	1075.000
8	11	3	192.667	192.667	192.667

cell density (96 h)
File: 8938c   Transform: NO TRANSFORMATION

               WILLIAMS TEST   (Isotonic regression model)   TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	2575.000				
0.089	2326.667	0.536		1.75	k= 1, v=16
0.2	2326.667	0.536		1.83	k= 2, v=16
0.45	2326.667	0.536		1.86	k= 3, v=16
0.99	2326.667	0.536		1.87	k= 4, v=16
2.2	2281.667	0.633		1.88	k= 5, v=16
4.9	1075.000	3.235	*	1.89	k= 6, v=16
11	192.667	5.138	*	1.89	k= 7, v=16

s = 567.911
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	1.8	0.87	3.7	0.15	0.48
EC10	2.2	1.2	4.2	0.13	0.53
EC25	3.2	2.0	5.1	0.097	0.63

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EC50              4.7        3.5        6.5      0.065      0.73

        Slope =    3.92  Std.Err. =     0.954


Goodness of fit: p =      0.45  based on DF=       5.0        16.
----------------------------------------------------------------------
8938C : cell density (96 h)
----------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
----------------------------------------------------------------------
     Dose      #Reps.     Obs.       Pred.      Obs.      Pred.     %Change
                          Mean       Mean      -Pred.    %Control

     0.00      3.00    2.58e+03    2.39e+03    182.      100.       0.00
   0.0890      3.00    1.79e+03    2.39e+03   -599.      100.      6.63e-10
    0.200      3.00    2.18e+03    2.39e+03   -216.      100.      3.60e-06
    0.450      3.00    2.69e+03    2.39e+03    296.      100.      0.00311
    0.990      3.00    2.65e+03    2.38e+03    265.      99.6      0.389
     2.20      3.00    2.28e+03    2.16e+03    120.      90.3      9.66
     4.90      3.00    1.08e+03    1.14e+03   -60.9      47.5      52.5
     11.0      3.00     193.        179.       13.8      7.48      92.5

biomass (0-96)
File: 8938b         Transform: NO TRANSFORMATION

                         ANOVA TABLE
----------------------------------------------------------------------

SOURCE            DF            SS               MS            F
----------------------------------------------------------------------
Between            7      6728940522.000    961277217.429     20.506

Within (Error)    16       750048672.000     46878042.000
----------------------------------------------------------------------
Total             23      7478989194.000
----------------------------------------------------------------------

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



biomass (0-96)
File: 8938b         Transform: NO TRANSFORMATION

      DUNNETTS TEST    -    TABLE 1 OF 2              Ho:Control<Treatment
----------------------------------------------------------------------
                          TRANSFORMED   MEAN CALCULATED IN
GROUP    IDENTIFICATION      MEAN       ORIGINAL UNITS    T STAT   SIG
-----  ----------------   -----------  ------------------  ------   ---
  1            control    57532.000        57532.000
  2              0.089    40668.000        40668.000        3.017    *
  3                0.2    54292.000        54292.000        0.580
  4               0.45    51060.000        51060.000        1.158
```

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Freshwater Algae,
Anabaena flos-aquae
PMRA Submission Number {........}   EPA MRID Number 465789-38

```
5                   0.99  51900.000        51900.000        1.007
6                   2.2   46652.000        46652.000        1.946
7                   4.9   22804.000        22804.000        6.212   *
8                   11     6200.000         6200.000        9.182   *
-------------------------------------------------------------------------------
Dunnett table value =  2.56    (1 Tailed Value, P=0.05,  df=16,7)
```

biomass (0-96)
File: 8938b   Transform: NO TRANSFORMATION

```
        DUNNETTS 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              control    3
  2                0.089    3           14311.299      24.9      16864.000
  3                  0.2    3           14311.299      24.9       3240.000
  4                 0.45    3           14311.299      24.9       6472.000
  5                 0.99    3           14311.299      24.9       5632.000
  6                  2.2    3           14311.299      24.9      10880.000
  7                  4.9    3           14311.299      24.9      34728.000
  8                   11    3           14311.299      24.9      51332.000
-------------------------------------------------------------------------------
```

biomass (0-96)
File: 8938b   Transform: NO TRANSFORMATION

```
        WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
-------------------------------------------------------------------------------
GROUP                              ORIGINAL     TRANSFORMED    ISOTONIZED
         IDENTIFICATION     N       MEAN          MEAN           MEAN
------  -------------------  ---  -----------  -----------   -----------
  1              control    3    57532.000    57532.000     57532.000
  2                0.089    3    40668.000    40668.000     49480.000
  3                  0.2    3    54292.000    54292.000     49480.000
  4                 0.45    3    51060.000    51060.000     49480.000
  5                 0.99    3    51900.000    51900.000     49480.000
  6                  2.2    3    46652.000    46652.000     46652.000
  7                  4.9    3    22804.000    22804.000     22804.000
  8                   11    3     6200.000     6200.000      6200.000
-------------------------------------------------------------------------------
```

biomass (0-96)
File: 8938b   Transform: NO TRANSFORMATION

```
        WILLIAMS TEST  (Isotonic regression model)    TABLE 2 OF 2
-------------------------------------------------------------------------------
                    ISOTONIZED    CALC.     SIG     TABLE     DEGREES OF
```

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

PMRA Submission Number {........}   EPA MRID Number 465789-38

IDENTIFICATION	MEAN	WILLIAMS	P=.05	WILLIAMS	FREEDOM
control	57532.000				
0.089	49480.000	1.440		1.75	k= 1, v=16
0.2	49480.000	1.440		1.83	k= 2, v=16
0.45	49480.000	1.440		1.86	k= 3, v=16
0.99	49480.000	1.440		1.87	k= 4, v=16
2.2	46652.000	1.946	*	1.88	k= 5, v=16
4.9	22804.000	6.212	*	1.89	k= 6, v=16
11	6200.000	9.182	*	1.89	k= 7, v=16

s = 6846.754
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	0.84	2.6	0.12	0.57
EC10	1.9	1.2	3.1	0.10	0.62
EC25	2.9	2.1	4.1	0.073	0.70
EC50	4.7	3.7	5.9	0.047	0.80

Slope = 3.27   Std.Err. = 0.532

Goodness of fit: p =   0.18   based on DF=   5.0   16.

8938B : biomass (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	5.75e+04	5.15e+04	6.05e+03	100.	0.00
0.0890	3.00	4.07e+04	5.15e+04	-1.08e+04	100.	8.90e-07
0.200	3.00	5.43e+04	5.15e+04	2.81e+03	100.	0.000371
0.450	3.00	5.11e+04	5.15e+04	-399.	100.	0.0436
0.990	3.00	5.19e+04	5.08e+04	1.12e+03	98.6	1.36
2.20	3.00	4.67e+04	4.42e+04	2.45e+03	85.9	14.1
4.90	3.00	2.28e+04	2.44e+04	-1.62e+03	47.4	52.6
11.0	3.00	6.20e+03	5.79e+03	407.	11.3	88.7

growth rate (0-96)
File: 8938g   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	18.975	2.711	22.975
Within (Error)	16	1.891	0.118	

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Anabaena flos-aquae
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```
------------------------------------------------------------------------
Total              23              20.866
------------------------------------------------------------------------
```

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

growth rate (0-96)
File: 8938g   Transform: NO TRANSFORMATION

```
          DUNNETTS TEST    -    TABLE 1 OF 2              Ho:Control<Treatment
--------------------------------------------------------------------------
                             TRANSFORMED   MEAN CALCULATED IN
GROUP    IDENTIFICATION         MEAN        ORIGINAL UNITS     T STAT  SIG
-----  -----------------    -----------   -----------------   ------  ---
  1           control         5.780            5.780
  2             0.089         5.380            5.380           1.426
  3             0.2           5.583            5.583           0.701
  4             0.45          5.813            5.813          -0.119
  5             0.99          5.777            5.777           0.012
  6             2.2           5.590            5.590           0.677
  7             4.9           4.807            4.807           3.470    *
  8            11             3.010            3.010           9.876    *
--------------------------------------------------------------------------
Dunnett table value =  2.56      (1 Tailed Value, P=0.05,  df=16,7)
```

growth rate (0-96)
File: 8938g   Transform: NO TRANSFORMATION

```
          DUNNETTS 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           control        3
  2             0.089        3          0.718           12.4        0.400
  3             0.2          3          0.718           12.4        0.197
  4             0.45         3          0.718           12.4       -0.033
  5             0.99         3          0.718           12.4        0.003
  6             2.2          3          0.718           12.4        0.190
  7             4.9          3          0.718           12.4        0.973
  8            11            3          0.718           12.4        2.770
--------------------------------------------------------------------------
```

growth rate (0-96)
File: 8938g   Transform: NO TRANSFORMATION

```
          WILLIAMS TEST   (Isotonic regression model)    TABLE 1 OF 2
--------------------------------------------------------------------------
```

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	5.780	5.780	5.780
2	0.089	3	5.380	5.380	5.638
3	0.2	3	5.583	5.583	5.638
4	0.45	3	5.813	5.813	5.638
5	0.99	3	5.777	5.777	5.638
6	2.2	3	5.590	5.590	5.590
7	4.9	3	4.807	4.807	4.807
8	11	3	3.010	3.010	3.010

growth rate (0-96)
File: 8938g   Transform: NO TRANSFORMATION

WILLIAMS TEST   (Isotonic regression model)   TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	5.780				
0.089	5.638	0.505		1.75	k= 1, v=16
0.2	5.638	0.505		1.83	k= 2, v=16
0.45	5.638	0.505		1.86	k= 3, v=16
0.99	5.638	0.505		1.87	k= 4, v=16
2.2	5.590	0.677		1.88	k= 5, v=16
4.9	4.807	3.468	*	1.89	k= 6, v=16
11	3.010	9.868	*	1.89	k= 7, v=16

s =   0.344
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	3.0	1.9	4.6	0.091	0.65
EC10	4.0	2.9	5.6	0.070	0.72
EC25	6.7	5.6	8.0	0.038	0.83
EC50	12.	10.	13.	0.027	0.88

Slope =   2.76 Std.Err. =   0.456

Goodness of fit: p =   0.72 based on DF=   5.0   16.

8938G : growth rate (0-96)

Observed vs. Predicted Treatment Group Means

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Freshwater Algae,
Anabaena flos-aquae
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Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	5.78	5.67	0.106	100.	0.00
0.0890	3.00	5.38	5.67	-0.294	100.	2.54e-07
0.200	3.00	5.58	5.67	-0.0902	100.	5.46e-05
0.450	3.00	5.81	5.67	0.140	100.	0.00475
0.990	3.00	5.78	5.66	0.112	99.8	0.154
2.20	3.00	5.59	5.55	0.0449	97.7	2.26
4.90	3.00	4.81	4.83	-0.0246	85.2	14.8
11.0	3.00	3.01	3.01	0.00483	53.0	47.0

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