

Receipt

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron Marine Diatom,
*Skeletonema costatum***

PMRA Submission Number {.....}

EPA MRID Number 465789-36

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D319377
OECD Data Point	{.....}
EPA MRID	465789-36
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/OPP/EFED/ERB-IV

Date: 6/30/06 *Chris Salice*

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

Date: 7/31/06 *Chris Salice*

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

Company Code {.....} [For PMRA]
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EPA PC Code 108209

Date Evaluation Completed: 31-07-2006

CITATION: Desjardins, D., T.Z. Kendall and H.O. Krueger. 2003. IR5878 50 WG: A 96 hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-117A and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued May 15, 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 marine diatom, *Skeletonema costatum* were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.96%) at nominal concentrations of 0.018, 0.041, 0.091, 0.20, 0.45 and 1.0 mg/L under static conditions. The measured (mean) concentrations were 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 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 percent inhibitions were -18, -16, 33, 26, 93 and 94% for the 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 mg/L treatment groups, respectively, compared to the control. The cell density EC₅₀ was 0.19 mg/L and the NOAEC was 0.043 mg/L. By 96 hours, biomass (area under the curve) inhibitions were 4.4, 5.6, 51, 53, 100 and 100% for the 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 mg/L treatment groups, respectively, compared to the control. Biomass was the most sensitive endpoint, with an EC₅₀ of 0.16 mg/L and a NOAEC of 0.043 mg/L. By 96 hours growth rate inhibitions were -6.4, -5.7, 17, 12, 100 and 100% for the 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 mg/L treatment groups, respectively, compared to the control. The growth rate EC₅₀ was 0.26 mg/L and the NOAEC was 0.043 mg/L.

There were no compound related phytotoxic effects.

The study is scientifically sound but does not satisfy the guideline requirement for an aquatic nonvascular plant study with the marine diatom, *Skeletonema costatum*. There is uncertainty associated with the toxicity of the adjuvant control and, therefore, the study is classified as SUPPLEMENTAL.

Results Synopsis

Test Organism: *Skeletonema costatum*

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

Cell density (96 Hours):

EC₀₅: 0.046 mg/L (0.023 mg ai/L) 95% C.I.: 0.019-0.12 mg/L (0.009-0.06 mg ai/L)

EC₅₀: 0.19 mg/L (0.09 mg ai/L) 95% C.I.: 0.13-0.29 mg/L (0.06-0.14 mg ai/L)

NOAEC: 0.043 mg/L (0.021 mg ai/L)

Probit Slope: 2.68±0.518

Growth rate (0-96 hours):

EC₀₅: 0.18 mg/L (0.09 mg ai/L) 95% C.I.: 0.15-0.22 mg/L (0.07-0.11 mg ai/L)

EC₅₀: 0.26 mg/L (0.13 mg ai/L) 95% C.I.: 0.23-0.29 mg/L (0.11-0.14 mg ai/L)

NOAEC: 0.043 mg/L (0.021 mg ai/L)

Probit Slope: 11.5±1.51

Biomass (0-96 hours):

EC₀₅: 0.059 mg/L (0.029 mg ai/L) 95% C.I.: 0.027-0.13 mg/L (0.013-0.06 mg ai/L)

EC₅₀: 0.16 mg/L (0.08 mg ai/L) 95% C.I.: 0.11-0.22 mg/L (0.05-0.11 mg ai/L)

NOAEC: 0.043 mg/L (0.021 mg ai/L)

Probit Slope: 3.86±0.921

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. The dilution water characteristics of TOC, particulate matter, and chlorine content were not reported.
2. Cells were agitated at a higher rate (100 cycles/min) than is recommended for this species (60 cycles/min). However, there were no signs of adherence of cells to test chambers, aggregation/flocculation of algae in the controls or treatment, or changes in cell morphology during the test.
3. The photoperiod (16h light: 8h dark) was slightly longer than recommended for this algal species (14h light: 10h dark).
4. At 96 hours the cell density inhibition in the adjuvant control was 95%, showing that the adjuvant had toxic properties for the marine diatom, *Skeletonema costatum*. The study authors concluded that it is impossible to discern the effects of the adjuvant from the effects of the test material, Orthosulfamuron.

These deviations affected 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 99.4-108% of nominal at Hour 0 and 92.9-106% 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: Marine diatom *Skeletonema costatum*

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: CCMP 1332

Source: Current in-house laboratory cultures originally obtained from Provasoli
Guillard National Center of Marine

Age of inoculum: Two weeks old

Method of cultivation: Artificially Enriched Seawater (AES) 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 Artificially Enriched Seawater (AES) 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 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):	7.9-8.0 7.9-8.6 disodium EDTA None 30‰	<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	77,000	<p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>
<u>Number of replicates</u> Control: Solvent control: Treatments:	3 3 3	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula sp.</i> tests should be conducted with four replicate.</p> <p>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</p>
<u>Test concentrations</u> Nominal: Measured:	0.018, 0.041, 0.091, 0.20, 0.45 and 1.0 mg/L 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 mg/L	<p>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</p> <p>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.</p>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	At 0 and 96 hours samples were analyzed by HPLC	

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Parameter	Details	Remarks ----- Criteria
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	20.0-21.6°C 16h light: 8h dark 3720-4250 lux, cool white light	<i>EPA temperature: <u>Skeletonema</u>: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)</i> <i>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i>
<u>Reference chemical (if used) name:</u> 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	<i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i>
Measurement technique for cell density and other end points	Hemocytometer and microscope	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</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	

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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 -18, -16, 33, 26, 93 and 94% for the 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 mg/L treatment groups, respectively, compared to the control. By 96 hours, biomass (area under the curve) inhibitions were 4.4, 5.6, 51, 53, 100 and 100% for 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were -6.4, -5.7, 17, 12, 100 and 100% for the 0.019, 0.043, 0.089, 0.20, 0.43 and 0.97 mg/L treatment groups, respectively, compared to the control.

There were no compound related phytotoxic effects.

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Table 3: Effect of Orthosulfamuron on marine diatom *Skeletonema costatum*

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	77,000	224,667	783,333	1,053,333	---
Adjuvant control	77,000	61,333	63,333	53,667	95
0.018 (0.19)	77,000	226,000	591,667	1,246,667	-18
0.041 (0.43)	77,000	210,000	596,667	1,226,667	-16
0.091 (0.89)	77,000	175,667	346,667	703,333	33
0.20 (0.20)	77,000	176,000	287,333	783,333	26
0.45 (0.43)	77,000	77,333	69,000	73,000	93
1.0 (0.97)	77,000	52,000	65,667	63,333	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)	0.043	0.043	0.20
EC ₅₀ (mg/L)	0.19	0.088	0.26
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	0.19 (0.13-0.29)	0.088 (0.063-0.33)	0.26 (0.23-0.29)
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 using 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 biomass required a square-root transformation to satisfy the assumptions of ANOVA. For biomass and growth rate, the highest treatment group data were excluded from the analysis because response was "0" for each replicate. The NOAEC values were determined using ANOVA, followed by William'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. Because the test concentrations were not corrected for the purity of the test material, the reviewer additionally calculated values based on active ingredient by multiplying estimates by 49.96%.

Cell density (96 Hours):

EC₀₅: 0.046 mg/L (0.023 mg ai/L) 95% C.I.: 0.019-0.12 mg/L (0.009-0.06 mg ai/L)
EC₅₀: 0.19 mg/L (0.09 mg ai/L) 95% C.I.: 0.13-0.29 mg/L (0.06-0.14 mg ai/L)
NOAEC: 0.043 mg/L (0.021 mg ai/L)
Probit Slope: 2.68±0.518

Growth rate (0-96 hours):

EC₀₅: 0.18 mg/L (0.09 mg ai/L) 95% C.I.: 0.15-0.22 mg/L (0.07-0.11 mg ai/L)
EC₅₀: 0.26 mg/L (0.13 mg ai/L) 95% C.I.: 0.23-0.29 mg/L (0.11-0.14 mg ai/L)
NOAEC: 0.043 mg/L (0.021 mg ai/L)
Probit Slope: 11.5±1.51

Biomass (0-96 hours):

EC₀₅: 0.059 mg/L (0.029 mg ai/L) 95% C.I.: 0.027-0.13 mg/L (0.013-0.06 mg ai/L)
EC₅₀: 0.16 mg/L (0.08 mg ai/L) 95% C.I.: 0.11-0.22 mg/L (0.05-0.11 mg ai/L)
NOAEC: 0.043 mg/L (0.021 mg ai/L)
Probit Slope: 3.86±0.921

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 marine diatom, *Skeletonema costatum*. The study authors concluded that it is impossible to discern the effects of the adjuvant from the effects of the test material, Orthosulfamuron.

E. REVIEWER'S COMMENTS:

The reviewer's analysis detected a lower NOAEC value for growth rate than the study authors' analysis. Additionally, the study authors' analysis identified a lower EC₅₀ value for biomass with a wider 95% confidence interval than that estimated by the reviewer. Aside from these differences, the reviewer's conclusions were identical to the study authors'. The reviewer's more conservative NOAEC for growth rate and the reviewer's more sound EC₅₀ value for biomass are reported in the Executive Summary and Conclusions sections. Given the toxicity of the adjuvant, it is impossible to attribute inhibition in the treated groups to Orthosulfamuron alone.

The experimental start date was March 28, 2003 and the experimental termination date was April 11, 2003.

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F. CONCLUSIONS:

The study is scientifically sound, however, there are uncertainties associated with the toxicity of the adjuvant; this study is classified SUPPLEMENTAL. Biomass was the most sensitive endpoint. The EC₅₀ was 0.16 mg/L (0.08 mg ai/L); the EC₀₅ and NOAEC values were 0.059 mg/L (0.029 mg ai/L) and 0.043 mg/L (0.021 mg ai/L), respectively.

Cell density (96 Hours):

EC₀₅: 0.046 mg/L (0.023 mg ai/L) 95% C.I.: 0.019-0.12 mg/L (0.009-0.06 mg ai/L)
EC₅₀: 0.19 mg/L (0.09 mg ai/L) 95% C.I.: 0.13-0.29 mg/L (0.06-0.14 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:

- 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.
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- 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.
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- 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: 8936c

Transform: NATURAL LOG(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	33.364	5.561	106.942
Within (Error)	14	0.730	0.052	
Total	20	34.094		

Critical F value = 2.85 (0.05,6,14)

Since F > Critical F REJECT Ho:All groups equal

cell density (96h)

File: 8936c

Transform: NATURAL LOG(Y)

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	6.959	1053.333		
2	0.019	7.126	1246.667	-0.897	
3	0.043	7.108	1226.667	-0.801	
4	0.089	6.509	703.333	2.415	
5	0.2	6.655	783.333	1.635	
6	0.43	4.094	63.000	15.387	*
7	0.97	4.146	63.333	15.107	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

cell density (96h)

File: 8936c

Transform: NATURAL LOG(Y)

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	0.019	3	395.426	37.5	-193.333
3	0.043	3	395.426	37.5	-173.333
4	0.089	3	395.426	37.5	350.000
5	0.2	3	395.426	37.5	270.000
6	0.43	3	395.426	37.5	990.333

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Marine Diatom, *Skeletonema costatum*

PMRA Submission Number {.....}

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7 0.97 3 395.426 37.5 990.000

cell density (96h)
File: 8936c Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	1053.333	6.959	7.064
2	0.019	3	1246.667	7.126	7.064
3	0.043	3	1226.667	7.108	7.064
4	0.089	3	703.333	6.509	6.582
5	0.2	3	783.333	6.655	6.582
6	0.43	3	63.000	4.094	4.120
7	0.97	3	63.333	4.146	4.120

cell density (96h)
File: 8936c 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	7.064				
0.019	7.064	0.565		1.76	k= 1, v=14
0.043	7.064	0.565		1.85	k= 2, v=14
0.089	6.582	2.022	*	1.88	k= 3, v=14
0.2	6.582	2.022	*	1.89	k= 4, v=14
0.43	4.120	15.228	*	1.90	k= 5, v=14
0.97	4.120	15.228	*	1.91	k= 6, v=14

s = 0.228

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	0.046	0.019	0.12	0.19	0.40
EC10	0.063	0.029	0.14	0.16	0.45
EC25	0.11	0.058	0.19	0.12	0.55
EC50	0.19	0.13	0.29	0.086	0.66

Slope = 2.68 Std.Err. = 0.518

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Marine Diatom, *Skeletonema costatum*

PMRA Submission Number {.....}

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!!!Poor fit: p < 0.001 based on DF= 4.00 14.0

8936C : cell density (96h)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. - Pred.	Pred. %Control	%Change
0.00	3.00	1.05e+03	1.16e+03	-103.	100.	0.00
0.0190	3.00	1.25e+03	1.15e+03	94.9	99.6	0.361
0.0430	3.00	1.23e+03	1.11e+03	119.	95.9	4.14
0.0890	3.00	703.	939.	-236.	81.2	18.8
0.200	3.00	783.	552.	232.	47.7	52.3
0.430	3.00	63.0	198.	-135.	17.1	82.9
0.970	3.00	63.3	33.4	29.9	2.89	97.1

biomass (0-96)

File: 8936b

Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	116124.751	23224.950	58.488
Within (Error)	12	4765.099	397.092	
Total	17	120889.850		

Critical F value = 3.11 (0.05,5,12)

Since F > Critical F REJECT Ho:All groups equal

biomass (0-96)

File: 8936b

Transform: SQUARE ROOT(Y)

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	234.353	54924.000		
2	0.019	229.130	52516.000	0.321	
3	0.043	227.592	51852.000	0.416	
4	0.089	159.149	26748.000	4.622	*
5	0.2	160.832	25932.000	4.519	*
6	0.43	4.000	48.000	14.158	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Marine Diatom, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 465789-36

biomass (0-96)

File: 8936b

Transform: SQUARE ROOT(Y)

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	0.019	3	17410.627	31.7	2408.000
3	0.043	3	17410.627	31.7	3072.000
4	0.089	3	17410.627	31.7	28176.000
5	0.2	3	17410.627	31.7	28992.000
6	0.43	3	17410.627	31.7	54876.000

biomass (0-96)

File: 8936b

Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	54924.000	234.353	234.353
2	0.019	3	52516.000	229.130	229.130
3	0.043	3	51852.000	227.592	227.592
4	0.089	3	26748.000	159.149	159.991
5	0.2	3	25932.000	160.832	159.991
6	0.43	3	48.000	4.000	4.000

biomass (0-96)

File: 8936b

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
control	234.353				
0.019	229.130	0.321		1.78	k= 1, v=12
0.043	227.592	0.416		1.87	k= 2, v=12
0.089	159.991	4.570	*	1.90	k= 3, v=12
0.2	159.991	4.570	*	1.92	k= 4, v=12
0.43	4.000	14.158	*	1.93	k= 5, v=12

s = 19.927

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

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Marine Diatom, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 465789-36

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.059	0.027	0.13	0.16	0.46
EC10	0.073	0.037	0.14	0.14	0.51
EC25	0.11	0.063	0.18	0.10	0.60
EC50	0.16	0.11	0.22	0.071	0.71

Slope = 3.86 Std.Err. = 0.921

!!!Poor fit: p < 0.001 based on DF= 3.00 12.0

8936B : biomass (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	5.49e+04	5.04e+04	4.48e+03	100.	0.00
0.0190	3.00	5.25e+04	5.04e+04	2.08e+03	100.	0.0198
0.0430	3.00	5.19e+04	4.97e+04	2.16e+03	98.5	1.49
0.0890	3.00	2.67e+04	4.19e+04	-1.51e+04	83.0	17.0
0.200	3.00	2.59e+04	1.73e+04	8.65e+03	34.3	65.7
0.430	3.00	48.0	2.30e+03	-2.25e+03	4.56	95.4

growth rate (0-96)

File: 8936g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	18.147	3.629	100.806
Within (Error)	12	0.433	0.036	
Total	17	18.580		

Critical F value = 3.11 (0.05,5,12)

Since F > Critical F REJECT Ho:All groups equal

growth rate (0-96)

File: 8936g Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
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Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Marine Diatom, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 465789-36

1	control	2.723	2.723	
2	0.019	2.897	2.897	-1.119
3	0.043	2.880	2.880	-1.011
4	0.089	2.257	2.257	3.012 *
5	0.2	2.407	2.407	2.044
6	0.43	0.013	0.013	17.493 *

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

growth rate (0-96)

File: 8936g

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	0.019	3	0.387	14.2	-0.173
3	0.043	3	0.387	14.2	-0.157
4	0.089	3	0.387	14.2	0.467
5	0.2	3	0.387	14.2	0.317
6	0.43	3	0.387	14.2	2.710

growth rate (0-96)

File: 8936g

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	2.723	2.723	2.833
2	0.019	3	2.897	2.897	2.833
3	0.043	3	2.880	2.880	2.833
4	0.089	3	2.257	2.257	2.332
5	0.2	3	2.407	2.407	2.332
6	0.43	3	0.013	0.013	0.013

growth rate (0-96)

File: 8936g

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
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**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Marine Diatom,
*Skeletonema costatum***

PMRA Submission Number {.....}

EPA MRID Number 465789-36

	control	2.833				
	0.019	2.833	0.709		1.78	k= 1, v=12
	0.043	2.833	0.709		1.87	k= 2, v=12
	0.089	2.332	2.526	*	1.90	k= 3, v=12
	0.2	2.332	2.526	*	1.92	k= 4, v=12
	0.43	0.013	17.478	*	1.93	k= 5, v=12

s = 0.190

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	0.18	0.15	0.22	0.037	0.83
EC10	0.20	0.17	0.23	0.034	0.85
EC25	0.22	0.19	0.26	0.029	0.87
EC50	0.26	0.23	0.29	0.024	0.89

Slope = 11.5 Std.Err. = 1.51

!!!Poor fit: p = 0.019 based on DF= 3.0 12.

8936G : growth rate (0-96)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	2.72	2.69	0.0342	100.	0.00
0.0190	3.00	2.90	2.69	0.207	100.	1.65e-14
0.0430	3.00	2.88	2.69	0.191	100.	1.65e-14
0.0890	3.00	2.26	2.69	-0.433	100.	5.58e-06
0.200	3.00	2.41	2.41	1.44e-06	89.5	10.5
0.430	3.00	0.0133	0.0133	-4.85e-08	0.496	99.5