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**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to freshwater diatom *Navicula pelliculosa***

PMRA Submission Number {.....}

EPA MRID Number 465789-35

<b>Data Requirement:</b>	PMRA DATA CODE	{.....}
	EPA DP Barcode	D319377
	OECD Data Point	{.....}
	EPA MRID	465789-35
	EPA Guideline	123-2

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

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

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

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

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

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

**Date:** 6/30/06

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

**Date:** 7/31/06

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

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<b>EPA PC Code</b>	108209	

**Date Evaluation Completed:** 31-07-2006

**CITATION:** Desjardins, D., T.Z. Kendall and H.O. Krueger. 2003. IR5878 50WG: A 96 hour Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-115 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued April 11, 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 diatom, *Navicula pelliculosa* were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.96%) at nominal concentrations of 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L under static conditions. The measured (mean) concentrations were 0.28, 0.57, 1.1, 2.3 and 4.6 mg/L.

By 96 hours, cell density percent inhibitions were 3.5, 0.49, -1.6, 44 and 100% for the 0.28, 0.57, 1.1, 2.3 and 4.6 mg/L treatment groups, respectively, compared to the control. The cell density EC<sub>50</sub> was 2.4 mg/L and the NOAEC was 1.1 mg/L. By 96 hours, biomass (area under the curve) inhibitions were -14, -5.3, 9.7, 83 and 100% for the 0.28, 0.57, 1.1, 2.3 and 4.6 mg/L treatment groups, respectively, compared to the control. Biomass was the most sensitive endpoint, with an EC<sub>50</sub> of 1.6 mg/L and a NOAEC of 0.57 mg/L. By 96 hours growth rate inhibitions were 0.68, -0.009, -0.42, 13 and 100% for the 0.28, 0.57, 1.1, 2.3 and 4.6 mg/L treatment groups, respectively, compared to the control. The growth rate EC<sub>50</sub> was 3.3 mg/L and the NOAEC was 1.1 mg/L.

There were no compound related phytotoxic effects.

The study is scientifically sound, however, it does not satisfy the guideline requirement for an aquatic nonvascular plant study with *Navicula pelliculosa*. At 96 hours the cell density inhibition in the adjuvant control was 100%, showing that the adjuvant had toxic properties for the freshwater diatom *Navicula pelliculosa*. The study authors concluded that it is impossible to discern the effects of the adjuvant from the effects of the test material, Orthosulfamuron. Given the uncertainties associated with the potential toxicity of the adjuvant, this study is classified as SUPPLEMENTAL.

**Results Synopsis**

Test Organism: *Navicula pelliculosa*

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

**Cell density (96 Hours); reviewer-reported:**

EC <sub>05</sub> :	1.6 mg/L (0.8 mg ai/L)	95% C.I.: 1.5-1.8 mg/L (0.75-0.90 mg ai/L)
EC <sub>50</sub> :	2.4 mg/L (1.2 mg ai/L)	95% C.I.: 2.3-2.5 mg/L (1.1-1.2 mg ai/L)
NOAEC:	1.1 mg/L (0.55 mg ai/L)	
Probit Slope:	9.98±0.993	

**Growth rate (0-96 hours); study author-reported:**

EC <sub>05</sub> :	>1.1 mg/L (>0.55 mg ai/L)	95% C.I.: N/A
EC <sub>50</sub> :	3.3 mg/L (1.6 mg ai/L)	95% C.I.: 3.2-3.4 mg/L (1.6-1.7 mg ai/L)
NOAEC:	1.1 mg/L (0.55 mg ai/L)	
Probit Slope:	could not determine using this model	

**Biomass (0-96 hours); reviewer-reported:**

EC <sub>05</sub> :	0.87 mg/L (0.43 mg ai/L)	95% C.I.: 0.75-1.0 mg/L (0.37-0.50 mg ai/L)
EC <sub>50</sub> :	1.6 mg/L (0.8 mg ai/L)	95% C.I.: 1.5-1.7 mg/L (0.75-0.85 mg ai/L)
NOAEC:	0.57 mg/L (0.28 mg ai/L)	
Probit Slope:	6.26±0.493	

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 deviation from these guidelines is:

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

At 96 hours the cell density inhibition in the adjuvant control was 100%, showing that the adjuvant had toxic properties for the freshwater diatom *Navicula pelliculosa*. The study authors concluded that it is impossible to discern the effects of the adjuvant from the effects of the test material, Orthosulfamuron.

**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 95.1-113% of nominal at Hour 0 and 84.8-93.8% at 96 hours.

(*OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound*) Only the water solubility was reported.

**Storage conditions of test chemicals:**

The test material was stored under ambient conditions.

**Physicochemical properties of orthosulfamuron**

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

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**2. Test organism:**

**Name:** Freshwater diatom *Navicula pelliculosa*

*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:** UTEX 667

**Source:** Current in-house laboratory cultures originally obtained from Culture Collection of Algae at the University of Austin.

**Age of inoculum:** Two weeks old

**Method of cultivation:** Algal Assay Procedure (AAP) medium

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. A range-finding study was not reported.

b. Definitive Study

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**Table 1: Experimental Parameters**

Parameter	Details	Remarks ----- Criteria
Acclimation period:  Culturing media and conditions: (same as test or not)  Health: (any mortality observed)	Continuous  Algal Assay Procedure (AAP) medium; same as test.  Not reported	  <i>EPA recommends two week acclimation period.</i>  <i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i>
<u>Test system</u> Static/static renewal  Renewal rate for static renewal	Static	  <i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Environmental chamber	
Duration of the test	96 hours	  <i>EPA requires: 96-120 hours</i> <i>OECD: 72 hours</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Erlenmeyer flasks 250 mL 100 mL	  <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
<u>Details of growth medium name</u>		

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Parameter	Details	Remarks
		Criteria
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	7.6 7.6-7.8 disodium EDTA NaHCO <sub>3</sub> N/A	OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.  EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
<u>Dilution water</u> source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	well water Not reported  Not reported Not reported <LOD <LOD Not reported	EPA pH: <i>Skeletonema costatum</i> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.  OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 rpm	

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Parameter	Details	Remarks ----- Criteria
Initial cells density	10,000	<p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>
<u>Number of replicates</u> Control: Solvent control: Treatments:	4 4 4	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</p> <p>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</p>
<u>Test concentrations</u> Nominal:  Measured:	0.31, 0.63, 1.3, 2.5 and 5.0 mg/L  0.28, 0.57, 1.1, 2.3 and 4.6 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:	21.5-24.6°C continuous 3990-4650 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: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)  OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.
<u>Reference chemical (if used)</u> name: concentrations:	None	
Other parameters, if any	None	

**2. Observations:**

**Table 2: Observation parameters**

Parameters	Details	Remarks ----- Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density, biomass (area under the curve), growth rate	EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.
Measurement technique for cell density and other end points	Hemocytometer 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	



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Parameters	Details	Remarks ----- Criteria
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 percent inhibitions were 3.5, 0.49, -1.6, 44 and 100% for the 0.28, 0.57, 1.1, 2.3 and 4.6 mg/L treatment groups, respectively, compared to the control. By 96 hours, biomass (area under the curve) inhibitions were -14, -5.3, 9.7, 83 and 100% for the 0.28, 0.57, 1.1, 2.3 and 4.6 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were 0.68, -0.009, -0.42, 13 and 100% for the 0.28, 0.57, 1.1, 2.3 and 4.6 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 freshwater diatom *Navicula pelliculosa***

Treatment (record measured and nominal concentration (mg/L))	Initial cell density	Cell density at			
		24 hours	48 hours	96 hours	
				cell count	% inhibition
Negative control	10,000	33,750	350,000	1,027,500	---
Adjuvant control	10,000	2,500	2,500	1,000	100
0.31 (0.28)	10,000	35,000	489,250	991,250	3.5
0.63 (0.57)	10,000	32,750	431,000	1,022,500	0.49
1.3 (1.1)	10,000	13,750	252,250	1,043,750	-1.6
2.5 (2.3)	10,000	3,250	12,500	575,000	44
5.0 (4.6)	10,000	2,000	1,500	2,500	100
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

**Table 4: Statistical endpoint values.**

Statistical Endpoint	biomass	growth rate	cell density
NOAEC or EC <sub>05</sub> (mg/L)	1.1	0.57	1.1
EC <sub>50</sub> (mg/L)	2.4	1.6	3.3
IC <sub>50</sub> or EC <sub>50</sub> (mg/L) (95% C.I.)	2.4 (2.3-2.5)	1.6 (1.5-1.7)	3.3 (3.2-3.4)
Other (IC <sub>25</sub> /EC <sub>25</sub> )	NR	NR	NR
Reference chemical, if used NOAEC IC <sub>50</sub> /EC <sub>50</sub>	N/A	N/A	NA

NR Not reported

**B. REPORTED STATISTICS:**

The 96-Hour treatment and control response data passed the tests for normality (Shapiro-Wilks) and homogeneity of variance (Levene's). The 96-Hour EC<sub>50</sub> value was determined by non-linear regression 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 required a square-root transformation 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<sub>x</sub> values were determined using non-linear regression via Nuthatch statistical software. Mean-measured concentrations were used to compute these estimates. Growth rate data did not fit the probit model, so the EC<sub>x</sub> values could not be determined for this endpoint.

### Cell density (96 Hours):

EC <sub>05</sub> :	1.6 mg/L (0.8 mg ai/L)	95% C.I.: 1.5-1.8 mg/L (0.75-0.90 mg ai/L)
EC <sub>50</sub> :	2.4 mg/L (1.2 mg ai/L)	95% C.I.: 2.3-2.5 mg/L (1.1-1.2 mg ai/L)
NOAEC:	1.1 mg/L (0.55 mg ai/L)	
Probit Slope:	9.98±0.993	

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

EC <sub>05</sub> :	>1.1 mg/L (>0.55 mg ai/L)	95% C.I.: N/A
EC <sub>50</sub> :	>2.3 mg/L (>1.1 mg ai/L)	95% C.I.: N/A
NOAEC:	1.1 mg/L (0.55 mg ai/L)	
Probit Slope:	could not determine using this model	

### Biomass (0-96 hours):

EC <sub>05</sub> :	0.87 mg/L (0.43 mg ai/L)	95% C.I.: 0.75-1.0 mg/L (0.37-0.50 mg ai/L)
EC <sub>50</sub> :	1.6 mg/L (0.8 mg ai/L)	95% C.I.: 1.5-1.7 mg/L (0.75-0.85 mg ai/L)
NOAEC:	0.57 mg/L (0.28 mg ai/L)	
Probit Slope:	6.26±0.493	

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 100%, showing that the adjuvant had toxic properties for the freshwater diatom *Navicula pelliculosa*. 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:

With the exception of the EC<sub>50</sub> for growth rate, the reviewer's conclusions were identical to the study authors'. Biomass was the most sensitive endpoint, with an EC<sub>50</sub> of 1.6 mg/L (0.8 mg ai/L). Growth rate data did not fit the probit model, so toxicity estimates could not be determined by the reviewer. In the Executive Summary and Conclusions sections, the reviewer's results are reported for cell density and biomass and the study authors' results are reported for growth rate.

The experimental start date was February 13, 2003 and the experimental termination date was February 24, 2003.

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**F. CONCLUSIONS:**

The study is scientifically sound, however, there are uncertainties associated with the toxicity of the adjuvant; therefore this study is classified SUPPLEMENTAL. Biomass was the most sensitive endpoint. The EC<sub>50</sub> was 1.6 mg/L (0.80 mg ai/L); the EC<sub>05</sub> and NOAEC values were 0.87 mg/L (0.43 mg ai/L) and 0.57 mg/L (0.28 mg ai/L), respectively.

**Cell density (96 Hours); reviewer-reported:**

EC<sub>05</sub>: 1.6 mg/L (0.8 mg ai/L) 95% C.I.: 1.5-1.8 mg/L (0.75-0.90 mg ai/L)  
EC<sub>50</sub>: 2.4 mg/L (1.2 mg ai/L) 95% C.I.: 2.3-2.5 mg/L (1.1-1.2 mg ai/L)  
NOAEC: 1.1 mg/L (0.55 mg ai/L)  
Probit Slope: 9.98±0.993

**Growth rate (0-96 hours); study author-reported:**

EC<sub>05</sub>: >1.1 mg/L (>0.55 mg ai/L) 95% C.I.: N/A  
EC<sub>50</sub>: 3.3 mg/L (1.6 mg ai/L) 95% C.I.: 3.2-3.4 mg/L (1.6-1.7 mg ai/L)  
NOAEC: 1.1 mg/L (0.55 mg ai/L)  
Probit Slope: could not determine using this model

**Biomass (0-96 hours); reviewer-reported:**

EC<sub>05</sub>: 0.87 mg/L (0.43 mg ai/L) 95% C.I.: 0.75-1.0 mg/L (0.37-0.50 mg ai/L)  
EC<sub>50</sub>: 1.6 mg/L (0.8 mg ai/L) 95% C.I.: 1.5-1.7 mg/L (0.75-0.85 mg ai/L)  
NOAEC: 0.57 mg/L (0.28 mg ai/L)  
Probit Slope: 6.26±0.493

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.

Cohen, J. 1977. *Statistical Power Analysis for the Behavioral Sciences*. Academic Press, New York.

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

cell density (96 h)

File: 8935c

Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2969.286	593.857	272.162
Within (Error)	18	39.278	2.182	
Total	23	3008.565		

Critical F value = 2.77 (0.05,5,18)  
Since F > Critical F REJECT Ho:All groups equal

cell density (96 h)

File: 8935c

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	neg control	32.011	1027.500		
2	0.28	31.478	991.250	0.510	
3	0.57	31.975	1022.500	0.034	
4	1.1	32.294	1043.750	-0.272	
5	2.3	24.124	587.500	7.551	*
6	4.6	1.537	2.500	29.176	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

cell density (96 h)

File: 8935c

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	neg control	4			
2	0.28	4	154.823	15.1	36.250
3	0.57	4	154.823	15.1	5.000
4	1.1	4	154.823	15.1	-16.250
5	2.3	4	154.823	15.1	440.000
6	4.6	4	154.823	15.1	1025.000

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to freshwater diatom *Navicula pelliculosa***

PMRA Submission Number {.....}

EPA MRID Number 465789-35

cell density (96 h)  
File: 8935c Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	1027.500	32.011	32.011
2	0.28	4	991.250	31.478	31.916
3	0.57	4	1022.500	31.975	31.916
4	1.1	4	1043.750	32.294	31.916
5	2.3	4	587.500	24.124	24.124
6	4.6	4	2.500	1.537	1.537

cell density (96 h)  
File: 8935c Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	32.011				
0.28	31.916	0.091		1.73	k= 1, v=18
0.57	31.916	0.091		1.82	k= 2, v=18
1.1	31.916	0.091		1.85	k= 3, v=18
2.3	24.124	7.551	*	1.86	k= 4, v=18
4.6	1.537	29.175	*	1.87	k= 5, v=18

s = 1.477

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	1.6	1.5	1.8	0.019	0.91
EC10	1.8	1.7	1.9	0.016	0.93
EC25	2.1	1.9	2.2	0.012	0.95
EC50	2.4	2.3	2.5	0.0087	0.96

Slope = 9.98 Std.Err. = 0.993

Goodness of fit: p = 0.88 based on DF= 3.0 18.

8935C : cell density (96 h)

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to freshwater diatom *Navicula pelliculosa***

PMRA Submission Number {.....}

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Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. - Pred.	Pred. %Control	%Change
0.00	4.00	1.03e+03	1.02e+03	6.18	100.	0.00
0.280	4.00	991.	1.02e+03	-30.1	100.	2.23e-14
0.570	4.00	1.02e+03	1.02e+03	1.18	100.	2.22e-08
1.10	4.00	1.04e+03	1.02e+03	22.8	100.	0.0352
2.30	4.00	588.	588.	-0.0888	57.5	42.5
4.60	4.00	2.50	2.49	0.0100	0.244	99.8

biomass

File: 8635b

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	4441995748.797	1110498937.199	323.629
Within (Error)	15	51470856.000	3431390.400	
Total	19	4493466604.797		

Critical F value = 3.06 (0.05,4,15)

Since F > Critical F REJECT Ho:All groups equal

biomass

File: 8635b

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	42630.000	42630.000		
2	0.28	48777.000	48777.000	-4.693	
3	0.57	44880.000	44880.000	-1.718	
4	1.1	38505.000	38505.000	3.149	*
5	2.3	7380.000	7380.000	26.912	*

Dunnnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

biomass

File: 8635b

Transform: NO TRANSFORMATION



**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to freshwater diatom *Navicula pelliculosa***

PMRA Submission Number {.....}

EPA MRID Number 465789-35

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.28	4	3091.235	7.3	-6147.000
3	0.57	4	3091.235	7.3	-2250.000
4	1.1	4	3091.235	7.3	4125.000
5	2.3	4	3091.235	7.3	35250.000

biomass  
File: 8635b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	42630.000	42630.000	45703.500
2	0.28	4	48777.000	48777.000	45703.500
3	0.57	4	44880.000	44880.000	44880.000
4	1.1	4	38505.000	38505.000	38505.000
5	2.3	4	7380.000	7380.000	7380.000

biomass  
File: 8635b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	45703.500				
0.28	45703.500	2.346	*	1.75	k= 1, v=15
0.57	44880.000	1.718		1.84	k= 2, v=15
1.1	38505.000	3.149	*	1.87	k= 3, v=15
2.3	7380.000	26.912	*	1.88	k= 4, v=15

s = 1852.401

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	0.87	0.75	1.0	0.032	0.86
EC10	1.0	0.87	1.1	0.028	0.87

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to freshwater diatom *Navicula pelliculosa***

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EC25	1.2	1.1	1.4	0.021	0.90
EC50	1.6	1.5	1.7	0.013	0.94

Slope = 6.26 Std.Err. = 0.493

!!!Poor fit: p = 0.0096 based on DF= 2.0 15.

8635B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. - Pred.	Pred. %Control	%Change
0.00	4.00	4.26e+04	4.55e+04	-2.84e+03	100.	0.00
0.280	4.00	4.88e+04	4.55e+04	3.30e+03	100.	0.000107
0.570	4.00	4.49e+04	4.54e+04	-481.	99.8	0.250
1.10	4.00	3.85e+04	3.85e+04	25.1	84.6	15.4
2.30	4.00	7.38e+03	7.38e+03	-2.18	16.2	83.8

growth rate  
File: 8935g

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	1.226	0.306	18.000
Within (Error)	15	0.251	0.017	
Total	19	1.477		

Critical F value = 3.06 (0.05,4,15)  
Since F > Critical F REJECT Ho:All groups equal

growth rate  
File: 8935g

Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	4.823	4.823		
2	0.28	4.787	4.787	0.380	
3	0.57	4.820	4.820	0.027	
4	1.1	4.840	4.840	-0.190	
5	2.3	4.200	4.200	6.752	*

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to freshwater diatom *Navicula pelliculosa***

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Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

growth rate  
File: 8935g Transform: NO TRANSFORMATION

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

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GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.28	4	0.218	4.5	0.035
3	0.57	4	0.218	4.5	0.003
4	1.1	4	0.218	4.5	-0.018
5	2.3	4	0.218	4.5	0.622

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growth rate  
File: 8935g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

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GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	4.823	4.823	4.823
2	0.28	4	4.787	4.787	4.816
3	0.57	4	4.820	4.820	4.816
4	1.1	4	4.840	4.840	4.816
5	2.3	4	4.200	4.200	4.200

---

growth rate  
File: 8935g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

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IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	4.823				
0.28	4.816	0.073		1.75	k= 1, v=15
0.57	4.816	0.073		1.84	k= 2, v=15
1.1	4.816	0.073		1.87	k= 3, v=15
2.3	4.200	6.806	*	1.88	k= 4, v=15

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s = 0.129

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

**Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to freshwater diatom  
*Navicula pelliculosa***

PMRA Submission Number {.....}

EPA MRID Number 465789-35

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