Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical (Pyrasulfotole) to Algae, *Navicula pelliculosa*

PMRA Submission Number 2006-2445 EPA MRID Number 468017-38

Data Requirement:	PMRA DATA CODE	9.8.2
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.4
	EPA MRID	468017-38
	EPA Guideline	850.5400 (123-2)

Test material: AE 0317309 Technical **Purity:** 95.4%
Common name: Pyrasulfotole
Chemical name: IUPAC: (5-Hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl) phenyl] methanone
CAS name: Not reported
CAS No.: 365400-11-9
Synonyms: None reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 5/17/06

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 5/25/06

Primary Reviewer: Melissa Panger **Date:** 9/29/06
EPA

Secondary Reviewer(s): J.D. Whall (Officer No. 1268) **Date:** 11/09/06
PMRA

Secondary Reviewer(s): David McAdam **Date:** 9 Nov 2006
Australian Government Department of the Environment and Heritage (DEH).

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

Company Code	BCZ
Active Code	PSA
Use Site Category:	13, 14
EPA PC Code	000692

Date Evaluation Completed: {28-11-2006}

CITATION: Kern, M.E. and C.V. Lam. 2004. Toxicity of AE 0317309 Technical to the Freshwater Diatom *Navicula pelliculosa*. Unpublished study performed by Bayer CropScience, Research and Development Department, Stilwell, KS. Laboratory report number EBAIX006 (A9883401). Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study completed April 5, 2004.

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 freshwater diatoms (*Navicula pelliculosa*, NP-90) were exposed to AE 0317309 Technical at mean-measured concentrations of <0.51 (<LOQ; negative control), 5.95, 12.4, 25.8, 51.4 and 105 mg a.i./L under static conditions. The NOAEC and EC_{50}/IC_{50} values based on biomass, the most sensitive endpoint, were 25.8 and 53 mg a.i./L, respectively. The % growth inhibition, based on cell density, in the treated algal culture as compared to the control ranged from -48 to 96%. The % growth inhibition, based on biomass, in the treated algal culture as compared to the control ranged from -10 to 94%. The % growth inhibition, based on growth rate, in the treated algal culture as compared to the control ranged from -10 to 79%.

With the exception of the mean-measured 105 mg a.i./L treatment level, there was a major increase in pH (0.4 to 1.2 units) at all treatment levels. The reduction of cell density, biomass and growth rate were the only phytotoxic effects reported.

This toxicity study is classified as **ACCEPTABLE**, is deemed scientifically sound, and does satisfy the guideline requirement for a nonvascular aquatic plant toxicity study with the freshwater diatom, *Navicula pelliculosa*.

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density:

EC_{05}:	41 mg a.i./L	95% C.I.: 31-55 mg a.i./L
EC_{50}:	64 mg a.i./L	95% C.I.: 55-75 mg a.i./L
NOAEC:	51.4 mg a.i./L	
Probit Slope:	8.60±1.47	

Growth rate (0-96 hours):

EC_{05}:	52 mg a.i./L	95% C.I.: 41-64 mg a.i./L
EC_{50}:	83 mg a.i./L	95% C.I.: 77-90 mg a.i./L
NOAEC:	51.4 mg a.i./L	
Probit Slope:	7.96±1.14	

Area under the growth curve (biomass, 0-96 hours):

EC_{05}:	26 mg a.i./L	95% C.I.: 20-34 mg a.i./L
EC_{50}:	53 mg a.i./L	95% C.I.: 47-60 mg a.i./L
NOAEC:	25.8 mg a.i./L	
Probit Slope:	5.53±0.617	

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

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was based on guidelines outline in ASTM Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae, ASTM Standard E1218; OECD Test Guideline 201, Alga Growth Inhibition Test; USEPA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation, Non-Target Plants, EPA-540/9/82-020; USEPA Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; USEPA Standard Evaluation Procedure, Non-Target Plants, Growth and Reproduction of Aquatic Plants, Tiers 1 and 2, EPA-540/9-86-134; USEPA Pesticide Reregistration Rejection Rate Analysis, EPA738-R94-035; and USEPA OPPTS 850.5400 *draft*, Algal Toxicity, Tiers 1 and 2. The following deviations were noted:

1. The physiochemical properties of the test material were not reported.
2. Pretest health of the test organisms was not reported.
3. The recommended pH for this species is 7.5±0.1. The reported pH of the test solutions was outside of the recommended range at 0-hours (5.1-7.9) and at 96-hours (5.1-8.7).
4. The results of a periodic screening analysis of the dilution water were not reported.
5. Only three replicates of *Navicula pelliculosa* were tested in each treatment level; given the inherently variable response of this species, four replicates are recommended in the study design.

The deviations did not impact the acceptability of the study.

COMPLIANCE: Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with 40 CFR Part 160.

A. MATERIALS:

1. Test material AE 0317309 Technical

Description: Light Brown Powder

Lot No./Batch No. : Op. 1-4

Purity: 95.4%

Stability of compound under test conditions: Analytical verification of the test material was conducted at 0- and 96-hours. Mean recoveries were 95-106% of nominal

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored under ambient laboratory conditions

Physicochemical properties of AE 0317309.

Parameter	Value	Comment
Molecular weight	362.3 g/mol	
Water Solubility (g/L) at 20°C	4.2 at pH 4 69.1 at pH 7 49.0 at pH 9	Very soluble
Vapor Pressure/Volatility	2.7×10^{-7} Pa at 20°C 6.8×10^{-7} Pa at 25°C	Non-volatile
UV Absorption	water $\lambda_{max} = 264$ 0.1M HCl $\lambda_{max} = 241$ 0.1M NaOH $\lambda_{max} = 216$	Not likely to undergo photolysis.
Pka	4.2 ± 0.15	
log K_{ow} at 23°C	0.276 at pH 4 -1.362 at pH 7 -1.58 at pH 9	Not likely to bioaccumulate
Stability of compound at room temperature, if provided		No significant degradation over 12 months at ambient temperatures.

Data obtained from pyrasulfatole chemistry review of Submission 2006-2445.

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. 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: NP-90
Source: In-house laboratory cultures
Age of inoculum: 4-Days
Method of cultivation: Grown under test conditions (1xAAP) in an environmental chamber

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Initial concentrations for the range-finding test were 0 (negative control), 0.1, 1.0, 10.0, 50.0 and 80.0 mg a.i./L. No growth effects were noted at any test level, except 80 mg a.i./L, based on cell counts. Inhibition was nearly 100% at this level. It was noticed that at 80 mg a.i./L, there was an effect on pH. Therefore, additional preliminary work was done at 80 and 100 mg a.i./L and with controls prepared with and without buffered media. Cell counts in buffered media appeared to be slightly higher than in test solutions prepared in distilled water.

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 1xAAP, same as test Pretest health was 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 N/A	*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	
Duration of the test	96-hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)*	Glass	

Parameter	Details	Remarks *Criteria*
Size: Fill volume:	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**):	1xAAP 5.1-7.9 5.1-8.7 Yes $NaHCO_3$ 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)	Yes	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Distilled water 7.5±1 N/A Cold-filter sterilized Not reported Not reported Not reported Not reported 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 solutions were prepared. The highest concentration was prepared first and then serially diluted to obtain stock solutions for all other treatment levels.	
Aeration or agitation	Agitation (approx. 100 revs./min.)	
Initial cells density	10,000 cells/mL (for each replicate)	*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.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Buffer control: Treatments:	 3 N/A 3 3	A solvent control was not used. A buffer control was used as the highest test concentration (100 mg a.i. /L) required a buffered solution to maintain target pH range. *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 (negative control), 6.25, 12.5, 25.0, 50.0 and 100 mg a.i./L <0.51 (<LOQ, negative control), 5.95, 12.4, 25.8, 51.4 and 105 mg a.i./L	An additional control and nominal 100 mg a.i./L treatment level were prepared with buffered water as opposed to distilled water. During preliminary work, it was determined that when added to 1xAAP media prepared it distilled water, AE 0317309 significantly reduced the pH at the nominal 100 mg a.i./L treatment level. Additional vessels were prepared in buffered water to assess the effects of this pH shift on relative toxicity. *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.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A; a solvent was not used	
Method and interval of analytical verification	Test solutions were analyzed for the presence of AE 0317309 Technical at 0- and 96-hours using HPLC.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 24.1-25.5°C Continuous illumination Approximately 4.3klux	*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:	 N/A N/A	A reference chemical was not used.
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 and 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.*

Parameters	Details	Remarks Criteria
Measurement technique for cell density and other end points	Cell density was determined using a Z1 Beckman Coulter® particle counter. Growth rate was determined by comparing the change in cell density from Day 0 to Day 4. The cumulative biomass, was determined by plotting the daily cell density and determining the area under the curve.	*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. Cell density in the control increased by a factor of 66.6 by test termination.	*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?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At test termination, cell density reductions were -4, -5, -48, 9 and 96% at the mean-measured 5.95, 12.4, 25.8, 51.4 and 105 mg a.i./L treatment levels, respectively, relative to the negative control. Biomass was reduced 2, 16, -10, 52 and 94% and mean growth rate was reduced -1, -1, -10, 2 and 79% at the mean-measured 5.95, 12.4, 25.8, 51.4 and 105 mg a.i./L treatment levels, respectively, relative to the negative control. The reported EC_{50} values for cell density, biomass and growth rate were 68.9, 50.8 and 87.8 mg a.i./L, respectively.

There was a major change in pH at all treatment levels with the exception of the mean-measured 105 mg a.i./L treatment level. The reduction of cell density, biomass and growth rate were the only phytotoxic effects reported.

Table 3: Effect of AE 0317309 on algal growth Freshwater Diatom, *Navicula pelliculosa.*

Treatment Measured and (nominal) concentration (mg a.i./L)	Initial cell density	Cell density at			
		24 hours	48 hours	96-hours	
				cell count ($x10^4$)	% inhibition
Negative control	10,000	3.46	3.28	66.6	--
5.95 (6.25)	10,000	2.28	3.16	69.1	-4
12.4 (12.5)	10,000	2.12	2.79	69.7	-5
25.8 (25.0)	10,000	2.94	3.30	98.8	-48
51.4 (50.0)	10,000	3.39	2.81	60.3	9
105 (100)	10,000	2.74	2.61	2.48	96
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Effect of AE 0317309 Technical on algal growth Freshwater Diatom, *Navicula pelliculosa*

Treatment measured and (nominal) concentrations (mg a.i./L)	Initial cell density	Mean Growth Rate		Mean Area Under the Growth Curve	
		0-96 hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	10,000	0.043563	--	1915.7	--
5.95 (6.25)	10,000	0.04402	-1	1873.8	2
12.4 (12.5)	10,000	0.044201	-1	1618.7	16
25.8 (25.0)	10,000	0.047791	-10	2123.0	-10
51.4 (50.0)	10,000	0.042688	2	916.3	52
105 (100)	10,000	0.009335	79	123.0	94

B. REPORTED STATISTICS:

Statistical analysis was performed for the endpoints of cell density, growth rate and cumulative biomass (area under the growth curve). Statistical analysis of the raw or transformed 96-hour cell density, cumulative biomass data and growth rate data passed the criteria for normality and homogeneity of variance. Therefore, parametric analyses were conducted on these endpoints. The 96-hour EC_{25} and EC_{50} values were determined using regression analysis. All analyses were conducted using PC-based computer programs (SAS version 8). The additional replicates of the

control and nominal 100 mg a.i./L treatment level which were prepared with buffered water were not included in the statistical analysis of the data. Mean measured concentrations were used to derive all toxicity estimations.

Table 5: Statistical endpoint values reported by study authors.

Statistical Endpoint	Cell Density	Growth Rate	Biomass
NOAEC or EC_{05} (mg a.i./L)	51.4[a]	51.4[a]	5.95[a]
EC_{50} (mg a.i./L)	68.9	87.8	50.8
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	66.6-71.2	64.7-110.9	48.6-53.0
Other (IC_{25}/EC_{25})	59.7 (57.3-62.2)	75.5 (38.8-112.1)	38.6 (35.3-41.9)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Represent NOAEC values.
Toxicity values based on mean-measured concentrations

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Replicate data for cell density, biomass (area under the growth curve), and growth rate were tested for normality and homogeneity. If these assumptions of ANOVA were met, the NOAEC value was determined using the parametric Dunnett's and William's Test. If the assumptions were not met, the NOAEC value was determined using the non-parametric Kruskal-Wallis Test. All NOAEC values were determined using Toxstat Statistical Software. ECx values (with 95% C.I.) and probit slopes were determined using probit analyses via Nuthatch Statistical Software. All toxicity values were determined using the 96-hour mean-measured concentrations. The replicate values for growth rate were multiplied by 1000 to avoid mean values of 0.

Cell density:

EC_{05}:	41 mg a.i./L	95% C.I.: 31-55 mg a.i./L
EC_{50}:	64 mg a.i./L	95% C.I.: 55-75 mg a.i./L
NOAEC:	51.4 mg a.i./L	
Probit Slope:	8.60±1.47	

Growth rate (0-96 hours):

EC_{05}:	52 mg a.i./L	95% C.I.: 41-64 mg a.i./L
EC_{50}:	83 mg a.i./L	95% C.I.: 77-90 mg a.i./L
NOAEC:	51.4 mg a.i./L	
Probit Slope:	7.96±1.14	

Area under the growth curve (biomass, 0-96 hours):

EC_{05}:	26 mg a.i./L	95% C.I.: 20-34 mg a.i./L
EC_{50}:	53 mg a.i./L	95% C.I.: 47-60 mg a.i./L
NOAEC:	25.8 mg a.i./L	
Probit Slope:	5.53±0.617	

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

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWERS' COMMENTS:

In general, the reviewers' results were similar to the study authors'. The study authors' EC_{50} estimates for cell density and biomass were associated with narrower confidence intervals than the reviewers', while the reviewers' results for growth rate were more reliable. However, because the reviewers were able to provide EC_{05} values and probit slopes, the reviewers' results are reported in the Executive Summary and Conclusions sections of this DER.

Only three replicates of *Navicula pelliculosa* were tested in each treatment level; given the inherently variable response of this species, four replicates are recommended in the study design. Algal response in this study was not linear, but it did appear to be dose-dependent, particularly at higher concentrations. Given that reliable toxicity values could be derived from this study, this deviation is not considered to have measurably affected the results.

During preliminary work, it was determined that when added to 1xAAP media prepared it distilled water, AE 0317309 significantly reduced the pH at the nominal 100 mg a.i./L treatment level. Additional control and 100 mg a.i./L vessels were prepared in buffered water to assess the effects of this pH shift on relative toxicity. The daily cell density data generated for the nominal 100 mg a.i./L (buffered media) and negative control (buffered media) was used to determine the potential impacts of the shift in pH that occurred in the mean-measured 104 mg a.i./L test concentration. At the mean-measured 105 mg a.i./L treatment level, growth was severely inhibited, while growth was good in the buffered media at the nominal 100 mg a.i./L treatment level, suggesting that growth inhibition observed was due to the pH shift, and, thus the observed endpoints may not be representative of actual effects of pyrasulfotole on the diatom.. The buffered data were used for empirical comparison and were not included in the statistical analysis.

Additionally, the study reports after 96 hours that at the 106 mg ac/L (buffered) level cell density was significantly higher, 77 X 10^4 cells/mL, than in the buffered control, 37.6 X 10^4 cells/mL, but similar to that in the blank control (66.6 X 10^4 cells/mL). Yet after 72 h, the cell density was 43.3 and 46.9 X 10^4 cells/mL for blank and buffer controls respectively with 37.5 X 10^4 cells/mL for 106 mg ac/L (buffer). Therefore the reviewers believe that the 96-hour results for buffered control might have been incorrectly reported as more cells would be expected after 96 h. This, however, does not affect the reported results.

The in-life portion of the definitive algal toxicity test was conducted between April 7 and April 11, 2003.

F. CONCLUSIONS:

This study is scientifically sound and is classified as **ACCEPTABLE**. The most sensitive endpoint was biomass with NOAEC and EC_{50} values of 25.8 and 53 mg a.i./L, respectively.

Cell density:

EC_{05}:	41 mg a.i./L	95% C.I.: 31-55 mg a.i./L
EC_{50}:	64 mg a.i./L	95% C.I.: 55-75 mg a.i./L
NOAEC:	51.4 mg a.i./L	
Probit Slope:	8.60±1.47	

Growth rate (0-96 hours):

EC_{05}:	52 mg a.i./L	95% C.I.: 41-64 mg a.i./L
EC_{50}:	83 mg a.i./L	95% C.I.: 77-90 mg a.i./L
NOAEC:	51.4 mg a.i./L	
Probit Slope:	7.96±1.14	

Area under the growth curve (biomass, 0-96 hours):

EC_{05}:	26 mg a.i./L	95% C.I.: 20-34 mg a.i./L
EC_{50}:	53 mg a.i./L	95% C.I.: 47-60 mg a.i./L
NOAEC:	25.8 mg a.i./L	
Probit Slope:	5.53±0.617	

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

III. REFERENCES:

American Public Health Association (APHA). 1989. Standard Methods for the Examination of Water and Wastewater, 17th Edition. Washington, D.C.

American Society for Testing and Materials (ASTM). 1997. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM Standard E1218, Philadelphia, PA.

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USEPA. 1996. OPPTS 850.5400 *draft*: Algal Toxicity, Tiers I and II.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Freshwater diatom, cell denstiy, mg a.i./L; 96-hours
File: 1738cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	15073.315	3014.663	36.291
Within (Error)	12	996.840	83.070	
Total	17	16070.155		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Freshwater diatom, cell denstiy, mg a.i./L; 96-hours
File: 1738cd 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	66.583	66.583		
2	5.95	69.127	69.127	-0.342	
3	12.4	69.737	69.737	-0.424	
4	25.8	98.767	98.767	-4.325	
5	51.4	60.273	60.273	0.848	
6	105	2.483	2.483	8.614	*

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

Freshwater diatom, cell denstiy, mg a.i./L; 96-hours
File: 1738cd 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	neg control	3			
2	5.95	3	18.604	27.9	-2.543
3	12.4	3	18.604	27.9	-3.153
4	25.8	3	18.604	27.9	-32.183
5	51.4	3	18.604	27.9	6.310
6	105	3	18.604	27.9	64.100

Freshwater diatom, cell denstiy, mg a.i./L; 96-hours
File: 1738cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	66.583	66.583	76.053
2	5.95	3	69.127	69.127	76.053
3	12.4	3	69.737	69.737	76.053
4	25.8	3	98.767	98.767	76.053
5	51.4	3	60.273	60.273	60.273
6	105	3	2.483	2.483	2.483

Freshwater diatom, cell denstiy, mg a.i./L; 96-hours
File: 1738cd 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	76.053				
5.95	76.053	1.273		1.78	k= 1, v=12
12.4	76.053	1.273		1.87	k= 2, v=12
25.8	76.053	1.273		1.90	k= 3, v=12
51.4	60.273	0.848		1.92	k= 4, v=12
105	2.483	8.614	*	1.93	k= 5, v=12

s = 9.114
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	41.	31.	55.	0.058	0.75
EC10	45.	35.	58.	0.051	0.78
EC25	53.	44.	66.	0.041	0.82
EC50	64.	55.	75.	0.032	0.85

Slope = 8.60 Std.Err. = 1.47

!!!Poor fit: p = 0.0031 based on DF= 3.0 12.

1738CD : Freshwater diatom, cell denstiy, mg a.i./L; 96-hours

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	66.6	76.0	-9.44	100.	0.00
5.95	3.00	69.1	76.0	-6.90	100.	1.87e-14
12.4	3.00	69.7	76.0	-6.29	100.	4.25e-08
25.8	3.00	98.8	76.0	22.8	100.	0.0339
51.4	3.00	60.3	60.4	-0.155	79.5	20.5

105.	3.00	2.48	2.47	0.0121	3.25	96.7

Freshwater diatom, cell biomass, mg a.i./L; 96-hours
File: 1738cb Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	8762557.812	1752511.562	136.161
Within (Error)	12	154450.819	12870.902	
Total	17	8917008.631		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Freshwater diatom, cell biomass, mg a.i./L; 96-hours
File: 1738cb 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	1915.720	1915.720		
2	5.95	1873.760	1873.760	0.453	
3	12.4	1618.680	1618.680	3.207	*
4	25.8	2123.040	2123.040	-2.238	
5	51.4	916.320	916.320	10.789	*
6	105	123.000	123.000	19.353	*

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

Freshwater diatom, cell biomass, mg a.i./L; 96-hours
File: 1738cb 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	neg control	3			
2	5.95	3	231.579	12.1	41.960
3	12.4	3	231.579	12.1	297.040
4	25.8	3	231.579	12.1	-207.320
5	51.4	3	231.579	12.1	999.400
6	105	3	231.579	12.1	1792.720

Freshwater diatom, cell biomass, mg a.i./L; 96-hours

File: 1738cb Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	1915.720	1915.720	1915.720
2	5.95	3	1873.760	1873.760	1873.760
3	12.4	3	1618.680	1618.680	1870.860
4	25.8	3	2123.040	2123.040	1870.860
5	51.4	3	916.320	916.320	916.320
6	105	3	123.000	123.000	123.000

Freshwater diatom, cell biomass, mg a.i./L; 96-hours
File: 1738cb 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	1915.720				
5.95	1873.760	0.453		1.78	k= 1, v=12
12.4	1870.860	0.484		1.87	k= 2, v=12
25.8	1870.860	0.484		1.90	k= 3, v=12
51.4	916.320	10.789	*	1.92	k= 4, v=12
105	123.000	19.353	*	1.93	k= 5, v=12

s = 113.450
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	26.	20.	34.	0.054	0.77
EC10	31.	24.	39.	0.047	0.79
EC25	40.	33.	47.	0.036	0.84
EC50	53.	47.	60.	0.025	0.89

Slope = 5.35 Std.Err. = 0.617

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

1738CB : Freshwater diatom, cell biomass, mg a.i./L; 96-hours

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	1.92e+03	1.89e+03	28.5	100.	0.00
5.95	3.00	1.87e+03	1.89e+03	-13.4	100.	1.77e-05
12.4	3.00	1.62e+03	1.89e+03	-268.	100.	0.0356
25.8	3.00	2.12e+03	1.80e+03	323.	95.4	4.64

51.4	3.00	916.	1.00e+03	-86.4	53.1	46.9
105.	3.00	123.	107.	15.8	5.68	94.3

Freshwater diatom, growth rate, mg a.i./L; 96-hours
File: 1738gr Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3129.352	625.870	245.825
Within (Error)	12	30.555	2.546	
Total	17	3159.907		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Freshwater diatom, growth rate, mg a.i./L; 96-hours
File: 1738gr Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	43.563	43.563		
2	5.95	44.032	44.032	-0.360	
3	12.4	44.201	44.201	-0.490	
4	25.8	47.791	47.791	-3.245	
5	51.4	42.688	42.688	0.672	
6	105	9.335	9.335	26.272	*

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

Freshwater diatom, growth rate, mg a.i./L; 96-hours
File: 1738gr Transform: NO TRANSFORM

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	3			
2	5.95	3	3.257	7.5	-0.469
3	12.4	3	3.257	7.5	-0.638
4	25.8	3	3.257	7.5	-4.228
5	51.4	3	3.257	7.5	0.875
6	105	3	3.257	7.5	34.228

Freshwater diatom, growth rate, mg a.i./L; 96-hours
File: 1738gr Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	43.563	43.563	44.897
2	5.95	3	44.032	44.032	44.897
3	12.4	3	44.201	44.201	44.897
4	25.8	3	47.791	47.791	44.897
5	51.4	3	42.688	42.688	42.688
6	105	3	9.335	9.335	9.335

Freshwater diatom, growth rate, mg a.i./L; 96-hours
File: 1738gr Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	44.897				
5.95	44.897	1.024		1.78	k= 1, v=12
12.4	44.897	1.024		1.87	k= 2, v=12
25.8	44.897	1.024		1.90	k= 3, v=12
51.4	42.688	0.672		1.92	k= 4, v=12
105	9.335	26.271	*	1.93	k= 5, v=12

s = 1.596
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	52.	41.	64.	0.044	0.80
EC10	57.	48.	69.	0.038	0.83
EC25	68.	60.	78.	0.027	0.87
EC50	83.	77.	90.	0.016	0.93

Slope = 7.96 Std.Err. = 1.14

Goodness of fit: p = 0.15 based on DF= 3.0 12.

1738GR : Freshwater diatom, growth rate, mg a.i./L; 96-hours

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	43.6	44.9	-1.33	100.	0.00
5.95	3.00	44.0	44.9	-0.863	100.	1.58e-14
12.4	3.00	44.2	44.9	-0.694	100.	2.57e-09

25.8	3.00	47.8	44.9	2.90	100.	0.00272
51.4	3.00	42.7	42.7	-0.00618	95.1	4.90
105.	3.00	9.34	9.34	0.000235	20.8	79.2

Freshwater diatom, cell biomass, mg a.i./L; 96-hours
6
3
3
3
3
3
3
neg control
1922.88
2083.08
1741.2
5.95
1806.24
1815.48
1999.56
12.4
1630.32
1513.2
1712.52
25.8
2286.48
1987.2
2095.44
51.4
925.2
864.84
958.92
105
105.24
108
155.76

Freshwater diatom, cell denstiy, mg a.i./L; 96-hours
6
3
3
3
3
3
3
neg control
50.64
71.43
77.68
5.95
59.72
65.79
81.87
12.4
65
73.88
70.33
25.8
108.98
85.96
101.36
51.4
58.54
58.49
63.79
105
2.67
1.94
2.84

Freshwater diatom, growth rate, mg a.i./L; 96-hours
6
3
3
3
3
3
3
neg control
40.8830000
44.466
45.3400000
5.95
42.6010000
43.6090000
45.8870000
12.4
43.4830000
44.8170000
44.3040000
25.8
48.8660000
46.3950000
48.1110000
51.4
42.3930000
42.3840000
43.2870000
105
10.2300000
6.90300000
10.8730000