Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical (Pyrasulfotole) to Algae, *Anabaena flos-aquae*

PMRA Submission Number 2006-2445 EPA MRID Number 468017-39

Data Requirement:		
	PMRA DATA CODE	9.8.2
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.4
	EPA MRID	468017-39
	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
Staff Scientist, Cambridge Environmental, Inc.
Signature:
Date: 5/16/06

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

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

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

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

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

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

Date Evaluation Completed: 11-28-2006

CITATION: Kern, M.E., C.S. Banman and C.V. Lam. 2004. Toxicity of AE 0317309 Technical to the Blue-Green Algae *Anabaena flos-aquaea*. Unpublished study performed by Bayer CropScience, Research and Development Department, Stilwell, KS. Laboratory report number EBAAIX008 (A9883801). Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study completed January 22, 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.

Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical (Pyrasulfotole) to Algae, *Anabaena flos-aquae*

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of blue-green algae, *Anabaena flos-aquae* (AF-71), were exposed to AE 0317309 technical at mean-measured concentrations of <0.19 (<LOQ; negative control), 2.4, 6.3, 16.3, 10.1 and 100.4 mg a.i./L under static conditions. The NOAEC and EC_{50}/IC_{50} values based on growth rate, the most sensitive endpoint, were 40.1 and 45.71 mg a.i/L, respectively. The % growth inhibition, based on cell density, in the treated algal culture as compared to the control ranged from 18 to 100%. The % growth inhibition, based on biomass, in the treated algal culture as compared to the control ranged from 20 to 101%. The % growth inhibition, based on growth rate, in the treated algal culture as compared to the control ranged from 4 to 145%.

There was a major change in pH at the mean-measured 6.3 mg a.i./L treatment level (7.2 at Day 0 to 8.0 at Day 4), 16.3 mg a.i./L treatment level (6.7 at Day 0 to 7.9 at Day 4) and at the 40.1 mg a.i./L treatment level (6.4 at Day 0 to 8.4 at Day 4). The reduction of cell density, biomass and growth rate were the only phytotoxic effects reported.

The **EPA** classifies this toxicity study as **SUPPLEMENTAL** because a NOAEC for biomass could not be determined due to significant inhibition at all treatment levels. The **PMRA** and **DEH** classify this study as **ACCEPTABLE**. The study does satisfy the EPA, PMRA, and DEH guideline requirement for a nonvascular aquatic plant toxicity study with the blue-green algae, *Anabaena flos-aquae.*

Results Synopsis

Test Organism: *Anabaena flos-aquae* (AF-71)
Test Type (Flow-through, Static, Static Renewal): Static

Cell density:
EC_{05}: 32 mg a.i./L 95% C.I.: 21-47 mg a.i./L
EC_{50}: 50 mg a.i./L 95% C.I.: 39-65 mg a.i./L
NOAEC: 6.3 mg a.i./L
Probit Slope: 8.3±2.25

Growth rate (0-96 hours):
EC_{05}: 38.02 mg a.i./L 95% C.I.: Not determined
EC_{50}: 45.71 mg a.i./L 95% C.I.: Not determined
NOAEC: 40.1 mg a.i./L
Probit Slope: Not determined

Area under the growth curve (biomass, 0-96 hours):
EC_{05}: 44.412 mg a.i./L 95% C.I.: Not determined
EC_{50}: 47.17 mg a.i./L 95% C.I.: Not determined
NOAEC: <2.4 mg a.i./L
Probit Slope: Not determined

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

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 species was not reported.
3. The results of a periodic screening analysis of the dilution water were not reported.
4. The recommended pH for this species is 7.5±0.1. The reported pH of the test solutions exceeded the recommended range at 0-hours (7.4-8.1) and at 96-hours (7.9-8.8).
5. The NOAEC and EC_{05} values for biomass could not be determined (<2.4 mg a.i./L) because of significant inhibition at all treatment levels, including 20% at the lowest level; the reviewer was unable to determine an EC_{10} for this endpoint, as well.

The deviations do 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 94-104% 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 Technical.

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: Blue-Green Algae, *Anabaena flos-aquae*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: AF-71
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: The study authors report that the definitive nominal concentrations were based on previous AE 0317309 studies done with other algal species.

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.*
<u>Test system</u> 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*

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 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)**:	 4.7-8.0 4.7-8.4 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), 2.56, 6.4, 16, 40 and 100 mg a.i./L <0.19 (<LOQ; negative control), 2.4, 6.3, 16.3, 40.1 and 104 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:	24.5-24.8°C Continuous Light 2.2 klux	*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 light microscope and an Improved Nebauer meocytometer. 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 57.8 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 percent reductions were 18, 30, 36, 37 and 100%, when compared to the negative control, at the mean-measured 2.4, 6.3, 16.3, 40.1 and 104 mg a.i./L treatment levels, respectively. Biomass was reduced 20, 30, 36, 22 and 101% and mean growth rate was reduced 4, 8, 10, 10 and 145%, at the mean-measured 2.4, 6.3, 16.6, 40.1 and 104 mg a.i./L treatment levels, respectively. The EC_{50} values for cell density, biomass and growth rate were 43.5, 45.6 and 48.8 mg a.i./L, respectively.

There was a major change in pH at the mean-measured 6.3 mg a.i./L treatment level (7.2 at Day 0 to 8.0 at Day 4), 16.3 mg a.i./L treatment level (6.7 at Day 0 to 7.9 at Day 4) and at the 40.1 mg a.i./L treatment level (6.4 at Day 0 to 8.4 at Day 4). The reduction of cell density, biomass and growth rate were the only phytotoxic effects reported.

Table 3: Effect of AE 0317309 Technical on algal growth, Blue-Green Algae (*Anabaena flos-aquae*)

Treatment Measured and (nominal) concentration (mg a.i./L)	Initial cell density	Cell density at			
		24 hours	48 hours	x_n hours	
				cell count ($x10^4$)	% inhibition
Negative control	10,000	1.7	0.6	57.8	--
2.4 (2.56)	10,000	1.1	2.6	47.6	18
6.3 (6.4)	10,000	1.2	1.6	40.5	30
16.3 (16)	10,000	0.2	2.1	37.2	36
40.1 (40)	10,000	1.3	2.8	36.3	37
104 (100)	10,000	0.8	0.6	0.2	100
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Effect of AE 0317309 Technical on algal growth Blue-Green Algae (*Anabaena flos-aquae*)

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	0.04176	--	1052.4	--
2.4 (2.56)	10,000	0.04005	4	838.4	20
6.3 (6.4)	10,000	0.03852	8	732.0	30
16.3 (16)	10,000	0.03766	10	672.8	36
40.1 (40)	10,000	0.03738	10	825.2	22
104 (100)	10,000	-0.01878	145	-12.0	101

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 cumulative biomass data and growth rate data passed the criteria for normality and homogeneity of variance. Therefore, parametric analyses were conducted on these endpoints. Statistical analysis of the raw and transformed 96-hour data for cell density did not pass the criteria for homogeneity of variance. Therefore, a non-parametric analysis was performed for this endpoint. 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. All toxicity values were determined using the mean-measured concentrations.

Table 5: Statistical endpoint values reported by study authors.

Statistical Endpoint	Cell Density	Growth Rate	Biomass
NOAEC or EC_{05} (mg a.i./L)	6.3[a]	40.1[a]	<2.4
EC_{50} (mg a.i./L)	43.5	48.8	45.6
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	40.0-46.9	11.8-85.7	44.2-46.9
Other (IC_{25}/EC_{25})	36.6 (33.3-39.9)	43.7 (10.6-76.8)	40.8 (39.6-42.0)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Values represent the NOAEC.
Toxicity values were determined 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. Nuthatch is unable to handle negative numbers; therefore, for growth rate and biomass, the ECx values were determined using the Bruce and Versteeg (1992) method using SAS statistical software. All toxicity values were determined using the mean-measured concentrations. The replicate values for growth rate were multiplied by 1000 to avoid mean values of 0.

Cell density:
EC_{05}: 32 mg a.i./L 95% C.I.: 21-47 mg a.i./L
EC_{50}: 50 mg a.i./L 95% C.I.: 39-65 mg a.i./L
NOAEC: 6.3 mg a.i./L
Probit Slope: 8.3±2.25

Growth rate (0-96 hours):
EC_{05}: 38.02 mg a.i./L 95% C.I.: Not determined
EC_{50}: 45.71 mg a.i./L 95% C.I.: Not determined
NOAEC: 40.1 mg a.i./L
Probit Slope: Not determined

Area under the growth curve (biomass, 0-96 hours):
EC_{05}: 44.412 mg a.i./L 95% C.I.: Not determined
EC_{50}: 47.17 mg a.i./L 95% C.I.: Not determined
NOAEC: <2.4 mg a.i./L
Probit Slope: Not determined

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

D. STUDY DEFICIENCIES:

A NOAEC could not be determined for biomass because inhibition at the lowest treatment level was 20%.

E. REVIEWERS' COMMENTS:

The reviewers were unable to derive NOAEC for the biomass endpoint, therefore, this study does not meet the necessary criteria for the EPA to classify the study as 'acceptable'. The EPA finds the data from the study adequate for use in risk assessments, however, and, therefore, classifies the study as 'supplemental'. The lack of a definitive NOAEC for the biomass endpoint does not affect the acceptability of the study for the PMRA or DEH, therefore, the study is considered 'acceptable' to the PMRA and DEH.

During preliminary work, it was determined that when added to 1xAAP media prepared in 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 104 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. The buffered data were used for empirical comparison and were not included in the statistical analysis.

The in-life portion of the definitive algal toxicity test was conducted between June 23 and June 27, 2003.

F. CONCLUSIONS:

The EPA classifies this study as **SUPPLEMENTAL**. Growth rate was the most sensitive endpoint with EC_{50} and NOAEC values of 45.7 and 40.1 mg a.i./L, respectively. A definitive NOAEC value for biomass could not be determined (<2.4 mg a.i./L) because of significant inhibition at all treatment levels, including 20% at the lowest level. The **PMRA** and **DEH** classify this study as **ACCEPTABLE**. The study does satisfy the EPA, PMRA, and DEH guideline requirement for a nonvascular aquatic plant toxicity study with the blue-green algae, *Anabaena flos-aquae*.

Cell density:
EC_{05}: 32 mg a.i./L 95% C.I.: 21-47 mg a.i./L
EC_{50}: 50 mg a.i./L 95% C.I.: 39-65 mg a.i./L
NOAEC: 6.3 mg a.i./L
Probit Slope: 8.3±2.25

Growth rate (0-96 hours):
EC_{05}: 38.02 mg a.i./L 95% C.I.: Not determined
EC_{50}: 45.71 mg a.i./L 95% C.I.: Not determined
NOAEC: 40.1 mg a.i./L
Probit Slope: Not determined

Area under the growth curve (biomass, 0-96 hours):
EC_{05}: 44.412 mg a.i./L 95% C.I.: Not determined
EC_{50}: 47.17 mg a.i./L 95% C.I.: Not determined
NOAEC: <2.4 mg a.i./L
Probit Slope: Not determined

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

III. REFERENCES:

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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:

Blue green algae, cell density, mg a.i./L; 96-hours
File: 1739cd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	57.833	57.833	47.000
2	2.4	47.600	47.600	40.000
3	6.3	40.467	40.467	32.000
4	16.3	37.200	37.200	24.000
5	40.1	36.300	36.300	22.000
6	104	0.200	0.200	6.000

Calculated H Value = 12.345 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Blue green algae, cell density, mg a.i./L; 96-hours
File: 1739cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 6	5	4	3	2	1
6	104	0.200	0.200	\					
5	40.1	36.300	36.300	.	\				
4	16.3	37.200	37.200	.	.	\			
3	6.3	40.467	40.467	.	.	.	\		
2	2.4	47.600	47.600	.	.	.	.	\	
1	neg control	57.833	57.833	*	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 4.359

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	32.	21.	47.	0.080	0.67
EC10	35.	25.	50.	0.072	0.70
EC25	42.	31.	56.	0.060	0.75
EC50	50.	39.	65.	0.053	0.77

Slope = 8.30 Std.Err. = 2.25

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

1739CD : Blue green algae, cell density, 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	57.8	45.8	12.1	100.	0.00
2.40	3.00	47.6	45.8	1.82	100.	1.55e-14
6.30	3.00	40.5	45.8	-5.31	100.	3.49e-12
16.3	3.00	37.2	45.8	-8.58	100.	0.00244
40.1	3.00	36.3	36.3	0.00536	79.3	20.7
104.	3.00	0.200	0.202	-0.00173	0.441	99.6

Blue green algae, biomass, mg a.i./L; 96-hours
File: 1739cb Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1999010.880	399802.176	37.085
Within (Error)	12	129366.720	10780.560	
Total	17	2128377.600		

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

Blue green algae, biomass, mg a.i./L; 96-hours
File: 1739cb Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	1052.400	1052.400		
2	2.4	838.400	838.400	2.524	
3	6.3	732.000	732.000	3.779	*
4	16.3	672.800	672.800	4.478	*
5	40.1	825.200	825.200	2.680	
6	104	-12.000	-12.000	12.555	*

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=12,5)

Blue green algae, biomass, mg a.i./L; 96-hours
File: 1739cb Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	2.4	3	227.286	21.6	214.000
3	6.3	3	227.286	21.6	320.400

4	16.3	3	227.286	21.6	379.600
5	40.1	3	227.286	21.6	227.200
6	104	3	227.286	21.6	1064.400

Blue green algae, biomass, mg a.i./L; 96-hours
File: 1739cb 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	1052.400	1052.400	1052.400
2	2.4	3	838.400	838.400	838.400
3	6.3	3	732.000	732.000	743.333
4	16.3	3	672.800	672.800	743.333
5	40.1	3	825.200	825.200	743.333
6	104	3	-12.000	-12.000	-12.000

Blue green algae, biomass, mg a.i./L; 96-hours
File: 1739cb 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	1052.400				
2.4	838.400	2.524	*	1.78	k= 1, v=12
6.3	743.333	3.646	*	1.87	k= 2, v=12
16.3	743.333	3.646	*	1.90	k= 3, v=12
40.1	743.333	3.646	*	1.92	k= 4, v=12
104	-12.000	12.555	*	1.93	k= 5, v=12

s = 103.829
Note: df used for table values are approximate when v > 20.

Blue green algae, growth rate (x1000), mg ai/L; 96-hrs
File: 1739gr Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	41.760	41.760	47.000
2	2.4	40.047	40.047	40.000
3	6.3	38.517	38.517	32.000
4	16.3	37.657	37.657	24.000
5	40.1	37.377	37.377	22.000
6	104	-18.783	-18.783	6.000

Calculated H Value = 12.345 Critical H Value Table = 11.070

Since Calc H > Crit H REJECT Ho:All groups are equal.

Blue green algae, growth rate (x1000), mg ai/L; 96-hrs
File: 1739gr Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
6	104	-18.783	-18.783	\
5	40.1	37.377	37.377	. \
4	16.3	37.657	37.657	. . \
3	6.3	38.517	38.517	. . . \
2	2.4	40.047	40.047	. . . . \
1	neg control	41.760	41.760	* . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 4.359

Blue green algae, biomass, mg a.i./L; 96-hours
6
3
3
3
3
3
3
neg control
1245.6
1065.6
846
2.4
768
750
997.2
6.3
722.4
752.4
721.2
16.3
681.6
661.2
675.6
40.1
892.8
778.8
804
104
19.2
-57.6
2.4

Blue green algae, cell density, mg a.i./L; 96-hours
6
3
3
3
3
3
3
neg control
84.4
46.4
42.7
2.4
38.4
44.1
60.3
6.3
38.0
44.7
38.7
16.3
38.8
38.3
34.5
40.1
39.8
32.3
36.8
104
0.4
0.2
0

Blue green algae, growth rate (x1000), mg ai/L; 96-hrs
6
3
3
3
3
3
3
neg control
46.20
39.97
39.11
2.4
38.00
39.44
42.70
6.3
37.89
39.58
38.08
16.3
38.11
37.97
36.89
40.1
38.37
36.20
37.56
104
-9.55
-16.77
-30.03