

US EPA ARCHIVE DOCUMENT

**Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical to Algae,
*Pseudokirchneriella subcapitata***

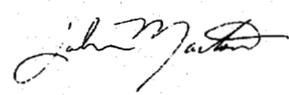
PMRA Submission Number 2006-2445

EPA MRID Number 468017-37

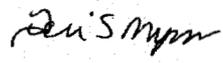
Data Requirement:	PMRA DATA CODE	9.8.2
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.4
	EPA MRID	468017-37
	EPA Guideline	850.5400 (123-2)

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: 5/17/06

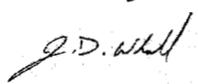
Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental, Inc.

Signature: 
Date: 5/25/06

Primary Reviewer: Melissa Panger
EPA

Date: 8/11/06 

Secondary Reviewer(s): J.D. Whall (Officer No. 1268)
PMRA

Date: 11/8/06 

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: 11-28-2006

CITATION: Kern, M.E. and C.V. Lam. 2004. Toxicity of AE 0317309 Technical to the Green Alga *Pseudokirchneriella subcapitata* (a.k.a. *Selenastrum capricornutum*). Unpublished study performed by Bayer CropScience, Research and Development Department, Stilwell, KS. Laboratory report number EBAIX005 (A9883501). 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



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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 green alga (*Pseudokirchneriella subcapitata*, SC-206) were exposed to AE 0317309 Technical at mean-measured concentrations of <0.19 (<LOQ; negative control), 2.6, 6.4, 16.4, 41.4 and 107 mg a.i./L under static conditions. The NOAEC and EC₅₀/IC₅₀ values based on cell density and biomass, endpoints with equal sensitivity, were 2.6 and 11 mg a.i./L, respectively. The % growth inhibition, based on cell density and biomass, in the treated algal culture as compared to the control ranged from 5 to 99%. The % growth inhibition, based on growth rate, in the treated algal culture as compared to the control ranged from 1 to 85%.

There was a major increase in pH (2.1 to 2.3 units) in the negative control and at the mean-measured 2.6 and 6.4 mg a.i./L 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 green algae *Pseudokirchneriella subcapitata*.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*, SC-206

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

Cell density:

EC₀₅: 3.2 mg a.i./L 95% C.I.: 2.1-4.9 mg a.i./L

EC₅₀: 11 mg a.i./L 95% C.I.: 9-13 mg a.i./L

NOAEC: 2.6 mg a.i./L

Probit Slope: 3.09±0.293

Growth rate (0-96 hours):

EC₀₅: 4.8 mg a.i./L 95% C.I.: 3.0-7.8 mg a.i./L

EC₅₀: 30 mg a.i./L 95% C.I.: 25-36 mg a.i./L

NOAEC: 2.6 mg a.i./L

Probit Slope: 2.08±0.173

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

EC₀₅: 2.7 mg a.i./L 95% C.I.: 1.8-3.9 mg a.i./L

EC₅₀: 11 mg a.i./L 95% C.I.: 9.4-13 mg a.i./L

NOAEC: 2.6 mg a.i./L

Probit Slope: 2.63±0.191

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

Most sensitive endpoint: Cell density and 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. The pretest health of the test organisms was not reported.
3. The recommended pH for this species is 8.1 ± 0.1 . The reported pH of the test solutions was below of the recommended range at 0-hours (5.3-7.9) and exceeded the range at 96-hours (5.4-10.0).
4. The results of a periodic screening analysis of the dilution water were not reported.

The deviations did not affect 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 100-107% 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

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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: Green Alga, *Psuedokirchneriella subcapitata*

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: SC-206

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:

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1. Experimental Conditions

a. Range-finding study: Two range-finding studies were conducted prior to the definitive test. The first range-finding test was conducted from July 29 to August 2, 2002 with nominal concentrations of 0 (negative control), 0.001, 0.01, 0.1, 1.0 and 10 mg a.i./L. Inhibition of cell density when compared to the negative control was 14, 35, 35, 30 and 37%, respectively. A second range-finding study was conducted from January 13 to January 16, 2003 with nominal concentrations of 0.01, 0.5, 1.0, 50 and 100 mg a.i./L. Inhibition of cell density when compared to the negative control was 26, 5, 5, 50 and 71%, respectively.

b. Definitive Study

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Parameter	Details	Remarks
Size: Fill volume:	250 mL 100 mL	<hr style="border-top: 1px dashed black;"/> <p><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></p>
<u>Details of growth medium name</u> pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	1xAAP 5.3-7.9 5.4-10.0 Yes NaHCO ₃ N/A	<hr style="border-top: 1px dashed black;"/> <p><i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i></p> <p><i>EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</i></p>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	

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Parameter	Details	Remarks
		----- <i>Criteria</i> -----
<p><u>Dilution water</u> source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:</p>	<p>Distilled water 7.5±1 N/A Cold-filter sterilized Not reported Not reported Not reported Not reported</p>	<p>----- <i>EPA pH: Skeletonema costatum</i> = ~8.0 <i>Others</i> = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water. <i>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</i></p>
<p>Indicate how the test material is added to the medium (added directly or used stock solution)</p>	<p>Stock solutions were prepared. The highest concentration was prepared first and then serially diluted to obtain stock solutions for all other treatment levels.</p>	
<p>Aeration or agitation</p>	<p>Agitation (approx. 100 revs./min.)</p>	
<p>Initial cells density</p>	<p>10,000 cells/mL (for each replicate)</p>	<p>----- <i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.</i> <i>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.</i></p>

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Parameter	Details	Remarks
Criteria		
<p><u>Number of replicates</u> Control: Solvent control: Buffer control: Treatments:</p>	<p>3 N/A 3 3</p>	<p>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.</p> <hr style="border-top: 1px dashed black;"/> <p><i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <u>Navicula</u> sp. tests should be conducted with four replicate.</i></p> <p><i>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.</i></p>
<p><u>Test concentrations</u> Nominal: Measured:</p>	<p>0 (negative control), 2.56, 6.40, 16, 40 and 100 mg a.i./L</p> <p><0.19 (<LOQ; negative control), 2.6, 6.4, 16.4, 41.4 and 107 mg a.i./L</p>	<p>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.</p> <hr style="border-top: 1px dashed black;"/> <p><i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i></p> <p><i>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.</i></p>

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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.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	24.1-24.8°C Continuous 4.3 klux	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: <i>Anabaena</i> : 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:	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.

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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.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i>
Observation intervals	24-, 48-, 72- and 96-hours	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes. Cell density in the control increased by a factor of 296.9 by test termination.	<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?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At test termination, cell density reductions were 5, 19, 70, 98 and 99% at the mean-measured 2.6, 6.4, 16.4, 41.4 and 107 mg a.i./L treatment levels, respectively, when compared to the negative control. Biomass was reduced 5, 23, 65, 96 and 99% and mean growth rate was reduced 1, 4, 21, 68 and 85% at the mean measured 2.6, 6.4, 16.4, 41.4 and 107 mg a.i./L treatment levels, respectively, relative to the negative control. The EC50 values for cell density, biomass and growth rate were 11.6, 12.0 and 29.8 mg a.i./L, respectively.

There was a major change in pH in the negative control and at the mean-measured 2.6 and 6.4 mg a.i./L treatment levels. The reduction of cell density, biomass and growth rate were the only phytotoxic effects reported.

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Table 3: Effect of AE 0317309 on algal growth (Green Alga, *Pseudokirchneriella subcapitata*)

Treatment (record 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	5.34	28.3	296.9	--
2.6 (2.56)	10,000	5.94	31.7	283.4	5
6.4 (6.40)	10,000	5.33	25.3	239.1	19
16.4 (16)	10,000	5.13	17.0	87.8	70
41.4 (40)	10,000	3.51	5.21	6.27	98
107 (100)	10,000	2.46	2.28	2.39	99
Reference chemical (if used)		N/A	N/A	N/A	N/A

Table 4: Effect of AE 0317309 Technical on algal growth (Green Alga, *Pseudokirchneriella subcapitata*)

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	0.059302	--	7530.5	--
2.6 (2.56)	10,000	0.058808	1	7142.2	5
6.4 (6.40)	10,000	0.057044	4	5761.7	23
16.4 (16)	10,000	0.046594	21	2633.7	65
41.4 (40)	10,000	0.019112	68	320.1	96
107 (100)	10,000	0.008885	85	110.6	99

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 and cumulative biomass data passed the criteria for normality and homogeneity of variance. Therefore, parametric analyses were conducted on these endpoints. Raw data for growth rate did not pass the criteria for homogeneity of variance and therefore, were analyzed using a non-parametric analysis. The 96-hour EC₂₅ and EC₅₀ 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

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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)	2.6 ^a	6.4 ^a	2.6 ^a
EC ₅₀ (mg a.i./L)	11.6	29.8	12.0
IC ₅₀ or EC ₅₀ (mg a.i./L) (95% C.I.)	11.1-12.1	26.2-33.5	11.2-12.7
Other (IC ₂₅ /EC ₂₅)	7.6 (7.0-8.2)	15.2 (12.0-18.5)	7.2 (6.4-8.0)
Reference chemical, if used NOAEC IC ₅₀ /EC ₅₀	N/A	N/A	N/A

^a Represents the NOAEC value.

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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 :	3.2 mg a.i./L	95% C.I.: 2.1-4.9 mg a.i./L
EC ₅₀ :	11 mg a.i./L	95% C.I.: 9-13 mg a.i./L
NOAEC:	2.6 mg a.i./L	
Probit Slope:	3.09±0.293	

Growth rate (0-96 hours):

EC ₀₅ :	4.8 mg a.i./L	95% C.I.: 3.0-7.8 mg a.i./L
EC ₅₀ :	30 mg a.i./L	95% C.I.: 25-36 mg a.i./L
NOAEC:	2.6 mg a.i./L	
Probit Slope:	2.08±0.173	

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

EC ₀₅ :	2.7 mg a.i./L	95% C.I.: 1.8-3.9 mg a.i./L
EC ₅₀ :	11 mg a.i./L	95% C.I.: 9.4-13 mg a.i./L
NOAEC:	2.6 mg a.i./L	
Probit Slope:	2.63±0.191	

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

Most sensitive endpoint: Cell density and 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' EC₅₀ estimates were associated with slightly narrower confidence intervals than the reviewers'. However, because the reviewers were able to provide EC₀₅ values and probit slopes, the reviewers' results are reported in the Executive Summary and Conclusions sections of this DER.

The reviewers' analyses for cell density and biomass were conducted using non-parametric tests, as the data did not meet the assumptions of ANOVA (normality and homogeneity). As such, the test did not detect significant differences at the mean-measured 6.4-41.4 mg a.i./L treatment levels for cell density and biomass, despite the cell density reductions of 19-98% and biomass reductions of 23-96%, relative to the negative control. The reviewers felt that these reductions were biologically significant, and, therefore, visually determined the NOAEC value for both cell density and biomass to be 2.6 mg a.i./L.

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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. In the nominal 100 mg a.i./L (buffered media) treatment group, cell densities were comparable to those of the mean-measured 107 mg a.i./L (distilled water media). Due to the similarity, it appears that the pH shift caused by AE 0317309 Technical had little effect on relative toxicity. 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 April 21 and April 25, 2003.

F. CONCLUSIONS:

This study is scientifically sound and is classified as **ACCEPTABLE**. The most sensitive endpoints were cell density and biomass with NOAEC and EC₅₀ values of 2.6 and 11 mg a.i./L, respectively.

Cell density:

EC ₀₅ :	3.2 mg a.i./L	95% C.I.: 2.1-4.9 mg a.i./L
EC ₅₀ :	11 mg a.i./L	95% C.I.: 9-13 mg a.i./L
NOAEC:	2.6 mg a.i./L	
Probit Slope:	3.09±0.293	

Growth rate (0-96 hours):

EC ₀₅ :	4.8 mg a.i./L	95% C.I.: 3.0-7.8 mg a.i./L
EC ₅₀ :	30 mg a.i./L	95% C.I.: 25-36 mg a.i./L
NOAEC:	2.6 mg a.i./L	
Probit Slope:	2.08±0.173	

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

EC ₀₅ :	2.7 mg a.i./L	95% C.I.: 1.8-3.9 mg a.i./L
EC ₅₀ :	11 mg a.i./L	95% C.I.: 9.4-13 mg a.i./L
NOAEC:	2.6 mg a.i./L	
Probit Slope:	2.63±0.191	

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

Most sensitive endpoint: Cell density and biomass

**Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical to Algae,
*Pseudokirchneriella subcapitata***

PMRA Submission Number 2006-2445

EPA MRID Number 468017-37

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Green algae, cell density, mg a.i./L; 96-hours
File: 1737cd 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	296.940	296.940	48.000
2	2.6	283.447	283.447	45.000
3	6.4	239.140	239.140	33.000
4	16.4	87.810	87.810	24.000
5	41.4	6.270	6.270	15.000
6	107	2.387	2.387	6.000

Calculated H Value = 16.158 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho: All groups are equal.

Green algae, cell density, mg a.i./L; 96-hours
File: 1737cd 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	107	2.387	2.387	\						
5	41.4	6.270	6.270	. \						
4	16.4	87.810	87.810	. . \						
3	6.4	239.140	239.140	. . . \						
2	2.6	283.447	283.447	* . . . \						
1	neg control	296.940	296.940	* \						

* = 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		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	3.2	2.1	4.9	0.084	0.66
EC10	4.2	2.9	6.1	0.074	0.70
EC25	6.6	5.0	8.8	0.057	0.76
EC50	11.	9.0	13.	0.040	0.82

Slope = 3.09 Std.Err. = 0.293

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

1737CD : Green algae, cell density, mg a.i./L; 96-hours

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs.	Pred.	Obs.	Pred.	%Change
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US EPA ARCHIVE DOCUMENT

**Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical to Algae,
Pseudokirchneriella subcapitata**

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		Mean	Mean	-Pred.	%Control	
0.00	3.00	297.	298.	-1.27	100.	0.00
2.60	3.00	283.	290.	-6.75	97.3	2.69
6.40	3.00	239.	228.	11.1	76.5	23.5
16.4	3.00	87.8	88.0	-0.154	29.5	70.5
41.4	3.00	6.27	11.2	-4.93	3.76	96.2
107.	3.00	2.39	0.339	2.05	0.114	99.9

Green algae, cell biomass, mg a.i./L; 96-hours
File: 1737cb 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	7530.480	7530.480	49.000
2	2.6	7142.240	7142.240	44.000
3	6.4	5761.680	5761.680	33.000
4	16.4	2633.720	2633.720	24.000
5	41.4	320.120	320.120	15.000
6	107	110.560	110.560	6.000

Calculated H Value = 16.251 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho: All groups are equal.

Green algae, cell biomass, mg a.i./L; 96-hours
File: 1737cb 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	107	110.560	110.560	\						
5	41.4	320.120	320.120	. \						
4	16.4	2633.720	2633.720	. . \						
3	6.4	5761.680	5761.680	. . . \						
2	2.6	7142.240	7142.240 \						
1	neg control	7530.480	7530.480	* \						

* = 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		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	2.7	1.8	3.9	0.077	0.69
EC10	3.7	2.6	5.1	0.067	0.72
EC25	6.2	4.8	8.0	0.052	0.77
EC50	11.	9.4	13.	0.037	0.83

Slope = 2.63 Std.Err. = 0.191

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

1737CB : Green algae, 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	7.53e+03	7.58e+03	-51.3	100.	0.00
2.60	3.00	7.14e+03	7.22e+03	-79.8	95.3	4.75
6.40	3.00	5.76e+03	5.61e+03	152.	74.0	26.0
16.4	3.00	2.63e+03	2.53e+03	105.	33.3	66.7
41.4	3.00	320.	520.	-200.	6.86	93.1
107.	3.00	111.	38.6	71.9	0.510	99.5

Green algae, growth rate (x1000), mg a.i./L; 96-hours
File: 1737gr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	7346.839	1469.368	1134.647
Within (Error)	12	15.540	1.295	
Total	17	7362.380		

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

Green algae, growth rate (x1000), mg a.i./L; 96-hours
File: 1737gr 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	59.302	59.302		
2	2.6	58.808	58.808	0.531	
3	6.4	57.044	57.044	2.429	
4	16.4	46.594	46.594	13.677	*
5	41.4	19.112	19.112	43.254	*
6	107	8.885	8.885	54.261	*

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

Green algae, growth rate (x1000), mg a.i./L; 96-hours
File: 1737gr 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	3			
2	2.6	3	2.323	3.9	0.494
3	6.4	3	2.323	3.9	2.257
4	16.4	3	2.323	3.9	12.708
5	41.4	3	2.323	3.9	40.190
6	107	3	2.323	3.9	50.417

Green algae, growth rate (x1000), mg a.i./L; 96-hours
File: 1737gr 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	59.302	59.302	59.302
2	2.6	3	58.808	58.808	58.808
3	6.4	3	57.044	57.044	57.044
4	16.4	3	46.594	46.594	46.594
5	41.4	3	19.112	19.112	19.112
6	107	3	8.885	8.885	8.885

Green algae, growth rate (x1000), mg a.i./L; 96-hours
File: 1737gr 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	59.302				
2.6	58.808	0.531		1.78	k= 1, v=12
6.4	57.044	2.429	*	1.87	k= 2, v=12
16.4	46.594	13.676	*	1.90	k= 3, v=12
41.4	19.112	43.254	*	1.92	k= 4, v=12
107	8.885	54.260	*	1.93	k= 5, v=12

s = 1.138

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	4.8	3.0	7.8	0.097	0.62
EC10	7.2	4.8	11.	0.083	0.67
EC25	14.	11.	19.	0.061	0.74
EC50	30.	25.	36.	0.039	0.83

Slope = 2.08 Std.Err. = 0.173

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!!!Poor fit: p = 0.0012 based on DF= 3.0 12.

1737GR : Green algae, growth rate (x1000), 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	59.3	60.6	-1.30	100.	0.00
2.60	3.00	58.8	59.8	-0.965	98.6	1.37
6.40	3.00	57.0	55.6	1.40	91.8	8.18
16.4	3.00	46.6	42.8	3.77	70.7	29.3
41.4	3.00	19.1	23.3	-4.22	38.5	61.5
107.	3.00	8.88	7.58	1.30	12.5	87.5

US EPA ARCHIVE DOCUMENT

Green algae, cell biomass, mg a.i./L; 96-hours

6
3
3
3
3
3
3

neg control

6966
7766.16
7859.28
2.6
7162.32
7553.04
6711.36
6.4
5993.04
5760
5532
16.4
2693.76
2341.8
2865.6
41.4
318.96
328.56
312.84
107
93.6
107.4
130.68

Green algae, cell density, mg a.i./L; 96-hours

6
3
3
3
3
3
3
3

neg control

284.42
309.04
297.36
2.6
288.76
298.78
262.80
6.4
238.82
227.64
250.96
16.4
91.04
79.83
92.56
41.4
6.08
6.68
6.05
107
1.80
2.75
2.61

Green algae, growth rate (x1000), mg a.i./L; 96-hours

6
3
3
3
3
3
3
3

neg control

58.859
59.724
59.322
2.6
59.017
59.372
58.035
6.4
57.039
56.539
57.555
16.4
46.993
45.624
47.165
41.4
18.802
19.782
18.751
107
6.123
10.538
9.993