# 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

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| **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 **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:** 8/11/06
**EPA**

**Secondary Reviewer(s):** J.D. Whall (Officer No. 1268) **Date:** 11/8/06
**PMRA**

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

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

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|---|---|
| **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 Unpublished study performed by Bayer CropScience, Research and Development Department, Stilwell, KS.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

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 $EC_{50}$/$IC_{50}$ 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:**

| | | |
|---|---|---|
| $EC_{05}$: | 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

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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

**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<br>69.1 at pH 7<br>49.0 at pH 9 | Very soluble |
| Vapor Pressure/Volatility | $2.7 \times 10^{-7}$ Pa at 20°C<br>$6.8 \times 10^{-7}$ Pa at 25°C | Non-volatile |
| UV Absorption | water $\lambda_{max} = 264$<br>0.1M HCl $\lambda_{max} = 241$<br>0.1M NaOH $\lambda_{max} = 216$ | Not likely to undergo photolysis. |
| Pka | $4.2 \pm 0.15$ | |
| log $K_{ow}$ at 23°C | 0.276 at pH 4<br>-1.362 at pH 7<br>-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. 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:** 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:**

**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

**Table 1: Experimental Parameters**

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Acclimation period:<br><br>Culturing media and conditions: (same as test or not)<br><br>Health: (any mortality observed) | Continuous<br><br>1xAAP, same as test<br><br>Not reported | *EPA recommends two week acclimation period.*<br><br>*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><br>Static/static renewal<br><br>Renewal rate for static renewal | Static<br><br>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*<br>*OECD: 72 hours* |
| <u>Test vessel</u><br>Material: *(glass/stainless steel)* | Glass | |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Size:<br>Fill volume: | 250 mL<br>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<br>pH at test initiation:<br>pH at test termination:<br>Chelator used:<br>Carbon source:<br>Salinity (**for marine algae**): | 1xAAP<br>5.3-7.9<br>5.4-10.0<br>Yes<br>$NaHCO_3$<br>N/A | *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.*<br><br>*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<br>*Criteria* |
|---|---|---|
| Dilution water<br>source/type:<br>pH:<br>salinity (**for marine algae**):<br>water pretreatment (if any):<br>Total Organic Carbon:<br>particulate matter:<br>metals:<br>pesticides:<br>chlorine: | <br>Distilled water<br>7.5±1<br>N/A<br>Cold-filter sterilized<br>Not reported<br>Not reported<br>Not reported<br>Not reported<br>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.*<br><br>*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.*<br><br>*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<br>*Criteria* |
|---|---|---|
| Number of replicates<br>Control:<br>Solvent control:<br>Buffer control:<br>Treatments: | <br>3<br>N/A<br>3<br>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.<br>*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.*<br>*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<br>Nominal:<br>Measured: | 0 (negative control), 2.56, 6.40, 16, 40 and 100 mg a.i./L<br><br><0.19 (<LOQ; negative control), 2.6, 6.4, 16.4, 41.4 and 107 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.<br>*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.*<br>*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<br>*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<br>Temperature:<br>Photoperiod:<br>Light intensity and quality: | <br>24.1-24.8°C<br>Continuous<br>4.3 klux | <br><br>*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%)*<br><br>*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)<br>name:<br>concentrations: | <br>N/A<br>N/A | A reference chemical was not used. |
| Other parameters, if any | None | |

**2. Observations:**

**Table 2: Observation parameters**

| Parameters | Details | Remarks<br>**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<br>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*<br><br>*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 296.9 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.*<br><br>*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 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.

**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 $EC_{25}$ 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) | 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.

## 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' $EC_{50}$ estimates were associated with slightly narrower confidence intervals than the reviewers'. 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.

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.

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 $EC_{50}$ 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 |
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| 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

**III. REFERENCES:**

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

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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 5 4 3 2 1 |
|---|---|---|---|---|
| 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 Lower | 95% Bounds Upper | Std.Err. | Lower Bound /Estimate |
|---|---|---|---|---|---|
| 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 |
|---|---|---|---|---|---|---|

| | | 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 5 4 3 2 1 |
|---|---|---|---|---|
| 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 Lower | Upper | Std.Err. | Lower Bound /Estimate |
|---|---|---|---|---|---|
| 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

!!!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

| 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 Lower | 95% Bounds Upper | Std.Err. | Lower Bound /Estimate |
|---|---|---|---|---|---|
| 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

!!!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 |

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
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
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