**Data Evaluation Report on the acute toxicity of AE C421200 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus***

PMRA Submission #:{.....} EPA MRID #: 46203513

**Data Requirement:**

| | |
|---|---|
| PMRA DATA CODE | {.....} |
| EPA DP Barcode | D294536 |
| OECD Data Point | {.....} |
| EPA MRID | 46203513 |
| EPA Guideline | 123-2 (OPPTS 850.5400) |

**Test material:** AE C421200 (Thidiazuron Metabolite) **Purity:** 98.4%
Common name: Thidiazuron Metabolite (1-cyano-3-phenylurea)
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

**Primary Reviewer**: Rebecca Bryan
Staff Scientist, Dynamac Corporation

**Signature:**
**Date:** 4/27/2004

**QC Reviewer:** Greg Hess
Staff Scientist, Dynamac Corporation

**Signature:**
**Date:** 4/29/2004

**Primary Reviewer:** William Evans
**{Biologist: EPA/ERB1**

**Date:** November 16, 2004

**Company Code** {.....} [For PMRA]
**Active Code** {.....} [For PMRA]
**EPA PC Code** 120301

**Date Evaluation Completed:** {dd-mmm-yyyy}

**CITATION:** Desjardins, D., Kendall, T., and Krueger, H. 2003. AE C421200: A 72-Hour Toxicity Test with the Freshwater Alga (*Scenedesmus subspicatus*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 149A-160. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Experimental start date May 9, 2003 and experimental termination date May 12, 2003. The final report issued June 5, 2003.

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**Primary Reviewer:**
**{EPA/OECD/PMRA}**

**Date:** {.....}

**Secondary Reviewer(s):**{.....}
**{EPA/OECD/PMRA}**

**Date:** {.....}

**Company Code** {.....} [For PMRA]
**Active Code** {.....} [For PMRA]
**EPA PC Code** 120301

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**CITATION:** Desjardins, D., Kendall, T., and Krueger, H. 2003. AE C4201200: A 72-Hour Toxicity Test with the Freshwater Alga (*Scenedesmus subspicatus*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 149A-160. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Experimental start date May 9, 2003 and experimental termination date May 12, 2003. The final report issued June 5, 2003.

**EXECUTIVE SUMMARY:**

In a 72-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to AE C421200 (Thidiazuron Metabolite) under static conditions at nominal concentrations of 0 (negative control), 6.3, 13, 25, 50, and 100 ppm AE C421200. The mean-measured concentrations were <3.00 (<LOQ, negative control), 5.7, 12, 24, 49, and 99 ppm AE C421200. Cell density percent inhibition was -8, 8, 27, 7, and 98% at the 5.7, 12, 24, 49, and 99 ppm AE C421200 treatment level, respectively. Biomass (area under the growth curve, 0 to 72 hours) percent inhibition was -9.0, 8.6, 28, 5.4, and 97% at the 5.7, 12, 24, 49, and 99 ppm AE C421200 treatment level, respectively. Growth rate (0 to 72 hours) percent inhibition was -1.6, 1.9, 8.7, 1.7, and 87% at the 5.7, 12, 24, 49, and 99 mg a.i./L treatment level, respectively. Cell density, growth rate and biomass were significantly reduced at the 99 ppm AE C421200 treatment level compared to the negative control. The 24 and 49 ppm AE C421200 statistically significant reductions were not considered treatment-related due to a lack of concentration-response. Cell density, growth rate and biomass $EC_{50}$ values were 70, 93 and 89 ppm AE C421200, respectively. The NOEC for AE C421200 was 49 ppm, based on cell density, growth rate and biomass.

The study is scientifically sound but does not satisfy the guideline requirements for a Tier II aquatic nonvascular plant study with *Scenedesmus subspicatus*. According to US EPA Memorandum (Oct. 21, 1994), Closure on Nontarget Plant Phytotoxicity Policy Issues, three day OECD studies will be reviewed as Tier I screening studies only, therefore this study is classified as Supplemental due the shorter than recommended definitive test duration.

**Results Synopsis**

Test Organism: *Scenedesmus subspicatus*
Test Type: Static

**Cell density:**
NOEC/$EC_{05}$: 49 ppm AE C421200
$EC_{05}$: 53 ppm AE C421200 95% C.I.: 25-110 ppm AE C421200
$EC_{50}/IC_{50}$: 70 ppm AE C421200 95% C.I.: 47-110 ppm AE C421200
Slope: 13.2

**Growth rates:**
NOEC/$EC_{05}$: 49 ppm AE C421200
$EC_{05}$: Not reported 95% C.I.: N/A
$EC_{50}/IC_{50}$: 93 ppm AE C421200 95% C.I.: 92-93 ppm AE C421200
Slope: N/A

**Biomass (area under the growth curve):**
NOEC/$EC_{05}$: 49 ppm AE C421200
$EC_{05}$: Not reported 95% C.I.: N/A
$EC_{50}/IC_{50}$: 89 ppm AE C421200 95% C.I.: 86-91 ppm AE C421200
Slope: N/A

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

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The test was based on the following guidelines: OECD Guideline for Testing of Chemicals, 201: *Algal Growth Inhibition Test* and Official Journal of the European Communities No. L383, Method C.3: *Algal Growth Inhibition Test*. The following deviations from U.S. EPA Guideline, §123-2 were noted:

1. The dilution water total organic carbon, particulate matter and residual chlorine concentrations were not reported.
2. The growth medium pH ranged (5.2-9.0) more than recommended, ~7.5 from beginning to end of the test.
3. Light intensity (6500-9050 lux) was higher than recommended (~43000 lux) and the photoperiod was continuous rather than 14:10 as recommended.
4. The test duration was 72-hours rather than the recommended 96-120 hours.

The shorter than recommended test duration affected the acceptability of the study, consequently this Tier II test is acceptable as a Tier I test.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the U.S. CFR Title 40, parts 160 and 792 (August 17, 1989).

### A. MATERIALS:

**1. Test Material** AE C421200 (1-cyano-3-phenylurea; Thidiazuron Metabolite)

**Description:** White powder

**Lot No./Batch No. :** DSC1441/Product Code: AE C421200 00 1B98 0001

**Purity:** 98.4%

**Stability of Compound Under Test Conditions:** The 0-hour measured test concentrations were 91.4-101% of the nominal concentrations and the 72-hour measured test concentrations were 90.1-96.9% of the nominal concentrations (Table 1, p. 21).

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

**Storage conditions of test chemicals:** The test material was stored under frozen conditions.

**2. Test organism:**

**Name:** *Scenedesmus subspicatus*

*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*

**EXECUTIVE SUMMARY:**

In a 72-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to AE C421200 (Thidiazuron Metabolite) under static conditions at nominal concentrations of 0 (negative control), 6.3, 13, 25, 50, and 100 ppm AE C421200. The mean-measured concentrations were <3.00 (<LOQ, negative control), 5.7, 12, 24, 49, and 99 ppm AE C421200. Cell density percent inhibition was -8, 8, 27, 7, and 98% at the 5.7, 12, 24, 49, and 99 ppm AE C421200 treatment level, respectively. Biomass (area under the growth curve, 0 to 72 hours) percent inhibition was -9.0, 8.6, 28, 5.4, and 97% at the 5.7, 12, 24, 49, and 99 ppm AE C421200 treatment level, respectively. Growth rate (0 to 72 hours) percent inhibition was -1.6, 1.9, 8.7, 1.7, and 87% at the 5.7, 12, 24, 49, and 99 mg a.i./L treatment level, respectively. Cell density, growth rate and biomass were significantly reduced at the 99 ppm AE C421200 treatment level compared to the negative control. The 24 and 49 ppm AE C421200 statistically significant reductions were not considered treatment-related due to a lack of concentration-response. Cell density, growth rate and biomass $EC_{50}$ values were 70, 93 and 89 ppm AE C421200, respectively. The NOEC for AE C421200 was 49 ppm, based on cell density, growth rate and biomass.

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**Purity:** 98.4%

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**Storage conditions of test chemicals:** The test material was stored under frozen conditions.

**2. Test organism:**

**Name:** *Scenedesmus subspicatus*

*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:** CCAP 276/22
**Source:** Originally from Culture Collection of Algae and Protozoa in the United Kingdom. Current in-house laboratory cultures.
**Age of inoculum:** ≥14 days
**Method of cultivation:** Freshwater algal medium

**B. STUDY DESIGN:**

a) Range-finding Study: A previous range-finding study was conducted in order to estimate the nominal concentration range for the definitive study. The results were not reported.

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 toxicity observed) | ≥14 days<br><br>Freshwater algal medium; same as test<br><br>Algal cells were actively growing. | *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.* |
| Test system<br>static/static renewal:<br>renewal rate for static renewal: | Static | |
| Incubation facility | Environmental chamber | |
| Duration of the test | 72 hours | *EPA requires: 96 - 120 hours*<br><br>*OECD: 72 hours* |
| Test vessel<br>material: (glass/polystyrene)<br>size:<br>fill volume: | Glass<br>250 mL (Erlenmeyer flask)<br>100 mL | Test vessels were plugged with foam stoppers.<br><br>*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.* |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Details of growth medium<br>name:<br>pH at test initiation:<br>pH at test termination:<br>Chelator used:<br>Carbon source:<br>Salinity **(for marine algae):** | <br>Freshwater algal medium<br>5.2-7.9<br>6.0-9.0<br>Yes<br>$NaHCO_3$<br>N/A | See Appendix 2, p. 33.<br>*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.*<br>*EPA recommends 20X-AAP medium.* |
| If non-standard nutrient medium was used, detailed composition provided (Yes/No) | N/A | |
| Dilution water<br>source:<br><br>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>Well water, NANOpure® filtered with reagent grade chemicals added<br>Filter -sterilized (0.22 µm)<br>8.1 ± 0.1<br>N/A<br>pH adjusted with 10% HCl<br>Not reported<br>Not reported<br><LOD<br><LOD<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>*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 | |
| Aeration or agitation | Agitation, 100 rpm. | *EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.* |
| Initial cells density | Approximately 10,000 cells/mL | *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>*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>treated ones: | <br>6<br>N/A<br>3 | *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicates.*<br><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 cultures should be included in the test.* |
| Test concentrations<br>nominal:<br><br>measured: | <br>0 (negative control), 6.3, 13, 25, 50, and 100 ppm AE C421200<br><br><3.00 (<LOQ, negative control), 5.7, 12, 24, 49, and 99 ppm AE C421200 | *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.*<br><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.* |
| Solvent (type, percentage, if used) | N/A | |
| Method and interval of analytical verification | HPLC; 0 and 72 hours. | |
| Test conditions<br>temperature:<br>photoperiod:<br>light intensity and quality: | <br>23.5-24.5°C<br>Continuous<br>6500-9050 lux, cool-white fluorescent light. | *EPA temperature: Skeletonema: 20°C, Others: 24-25°C; 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.* |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Reference chemical {if used)<br>name:<br>concentrations: | N/A | |
| 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 count (area under the growth curve and growth rates were calculated). | *EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.* |
| Measurement technique for cell density and other end points | Cell counts using a electronic particle counter. | *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 | Every 24 hours | *EPA and OECD: every 24 hours.* |
| Other observations, if any | None | |

| Parameters | Details | Remarks/Criteria |
|---|---|---|
| Indicate whether there was exponential growth in the control | Yes, dilution water control cell density at test termination was 75X greater than the dilution water control cell density at test initiation. | Mean cell densities were reviewer-calculated.<br><br>*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:

The cell density percent inhibition was -8, 8, 27, 7, and 98% (reviewer-calculated from raw data, p. 48 ) at the mean-measured 5.7, 12, 24, 49, and 99 ppm AE C421200 treatment level, respectively. Biomass (area under the growth curve) (0 to 72 hours) percent inhibition was -9.0, 8.6, 28, 5.4, and 97% at the 5.7, 12, 24, 49, and 99 ppm AE C421200 treatment level, respectively. Growth rate (0 to 72 hours) percent inhibition was -1.6, 1.9, 8.7, 1.7, and 87% at the 5.7, 12, 24, 49, and 99 ppm AE C421200 treatment level, respectively. Growth rate and biomass were significantly reduced at the 99 ppm AE C421200 treatment levels compared to the negative control. The statistically significant reductions observed at the 24 and 49 ppm AE C421200 treatment levels were not considered treatment-related due to a lack of concentration-response.

**Table 3: Effect of AE C421200 (Thidiazuron Metabolite) on Algae (*Scenedesmus subspicatus*)**

| Treatment Mean-Measured and (Nominal Concn.); ppm AE C421200 | Initial Cell Density (cells/mL) | Observation Period | | |
|---|---|---|---|---|
| | | 24-Hours | 72-Hours | |
| | | | Cell Count[a] | % Inhibition[a] |
| Dilution water control | 10,000 | 28310 | 754616 | -- |
| 5.7 (6.3) | 10,000 | 30274 | 814479 | -8 |
| 12 (13) | 10,000 | 28792 | 694611 | 8 |
| 24 (25) | 10,000 | 23838 | 548458 | 27 |
| 49 (50) | 10,000 | 30246 | 700249 | 7 |
| 99 (100) | 10,000 | 16489 | 17955 | 98 |
| Reference chemical (if used) | N/A | N/A | N/A | N/A |

[a] The cell density means and % inhibition compared to the control were reviewer-calculated based on data provided in Appendix 5, p. 46. Negative percent inhibition indicates increased growth.

**Table 4: Effect of AE C421200 (Thidiazuron Metabolite) on Algae *(Scenedesmus subspicatus)***

| Treatment Mean-Measured and (Nominal Concn.); ppm AE C421200 | Initial Cell Density (cells/mL) | Mean Growth Rate per Day | % Inhibition, Mean Growth Rate per Day | Mean Area Under Growth Curve (Biomass) | % Inhibition, Biomass |
|---|---|---|---|---|---|
| Dilution water control | 10,000 | 0.0600 | -- | 12388640 | -- |
| 5.7 (6.3) | 10,000 | 0.0610 | -1.6 | 13506180 | -9.0 |
| 12 (13) | 10,000 | 0.0589 | 1.9 | 11325624 | 8.6 |
| 24 (25) | 10,000 | 0.0548* | 8.7 | 8911348* | 28 |
| 49 (50) | 10,000 | 0.0590* | 1.7 | 11724804* | 5.4 |
| 99 (100) | 10,000 | 0.0081* | 87 | 312864* | 97 |
| Reference chemical (if used) | N/A | N/A | N/A | N/A | N/A |

* Statistically significant difference ($p<0.05$) from the control using the Kruskal-Wallis and Jonkheere-Terpstra tests. The 24 and 49 mg a.i./L effects were not considered treatment-related by the study authors due to a lack of concentration-response.

**Table 5: Statistical endpoint values.**

| Statistical Endpoint | Biomass | Growth rate | Cell density |
|---|---|---|---|
| NOEC or $EC_{05}$ (mg a.i./L) | 49 | 49 | Not Reported |
| $EC_{50}$ (mg a.i./L) | 89 | 93 | Not Reported |
| $IC_{50}$ or $EC_{50}$ (mg a.i./L) (95% C.I.) | 86-91 | 92-93 | N/A |
| other $IC_{25}$/$EC_{25}$ (mg a.i./L) (95% C.I.) | Not Reported | Not Reported | Not Reported |
| Reference chemical, if used NOAEC $IC_{25}$/$EC_{25}$ | N/A | N/A | N/A |

N/A = Not applicable.

**B. REPORTED STATISTICS:**

**Statistical Method:** Biomass (area under the growth curve) and growth rate formulas are found on pages 15-16. Percent inhibition was determined for biomass and growth rate. Data were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Levene's test. The data failed the assumptions for normality and homogeneity of variances, therefore the Kruskal-Wallis and Jonkheere-Terpstra tests were used to determine NOEC values for biomass and growth rate endpoints. Non-linear regression or linear interpolation were used to determine the 72-hour EC50. All toxicity values were determined via The SAS System for Windows statistical software using mean-measured treatment concentrations (p. 20).

**Cell density:** Not reported

**Growth rates:**
$NOEC/EC_{05}$: 49 mg a.i./L
$EC_{50}/IC_{50}$: 93 mg a.i./L 95% C.I.: 92-93 mg a.i./L

**Biomass (area under the growth curve):**
$NOEC/EC_{05}$: 49 mg a.i./L
$EC_{50}/IC_{50}$: 89 mg a.i./L 95% C.I.: 86-91 mg a.i./L

Endpoint(s) Affected: Biomass and growth rates

## C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, biomass (area under the growth curve), and dry weight data did not satisfy the assumptions of ANOVA (i.e., normality and homogeneity of variances). Therefore, the NOEC and LOEC values for all three endpoints were determined using the non-parametric Kruskal-Wallis test. The analyses described above were conducted via TOXSTAT statistical software using mean-measured treatment concentrations for all toxicity calculations. The $EC_{05}$ and $EC_{50}$ values based on cell density data were determined using the Probit method via Nuthatch statistical software. $EC_{50}$ values based on biomass and growth rate data could not be calculated/verified by the reviewer using the probit method via Nuthatch statistical software, consequently, the study authors' reported values for these endpoints are reported in the Executive Summary and Conclusion sections of this DER. $EC_5$ values were not reported by the study authors and could not be determined by the reviewer for biomass and growth rate endpoints due to the software limitation mentioned above.

**Cell density:**
$NOEC/EC_{05}$: 49 ppm AE C421200
$EC_{05}$: 53 ppm AE C421200 95% C.I.: 25-110 ppm AE C421200
$EC_{50}/IC_{50}$: 70 ppm AE C421200 95% C.I.: 47-110 ppm AE C421200
Slope: 13.2

**Growth rates:**
$NOEC/EC_{05}$: 49 ppm AE C421200
$EC_{05}$: Not determined 95% C.I.: Not determined
$EC_{50}/IC_{50}$: Not determined 95% C.I.: Not determined
Slope: Not determined

**Biomass (area under the growth curve):**
$NOEC/EC_{05}$: 49 ppm AE C421200
$EC_{05}$: Not determined 95% C.I.: Not determined
$EC_{50}/IC_{50}$: Not determined 95% C.I.: Not determined
Slope: Not determined

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

**D. STUDY DEFICIENCIES:**

The duration of the definitive study affected the acceptability of this study as a Tier II Aquatic Plant Growth Study. According to US EPA Memorandum (Oct. 21, 1994), Closure on Nontarget Plant Phytotoxicity Policy Issues:

> "Aquatic Plant Growth Studies (122-2, 123-2), 1.) Four or 5 day algal studies will be accepted for review by the agency. Three day OECD studies will be reviewed as Tier I screening studies only. (This is a harmonization issue)."

Consequently, this study is classified as SUPPLEMENTAL.

**E. REVIEWER'S COMMENTS:**

The reviewer's conclusions differed from those of the study authors'. The study authors did not report toxicity values based on cell density data, only toxicity values based on growth rate and biomass. Therefore, the reviewer determined toxicity values for cell density are reported in the Executive Summary and Conclusion sections of this DER. Cell density was reduced significantly at the mean-measured 99 ppm AE C421200 treatment level. The study authors detected statistically significant reductions in biomass and growth rate at the mean-measured 24, 49 and 99 ppm AE C421200 treatment levels, however, the differences at the 24 and 49 ppm AE C421200 were not considered to be treatment related due to a lack of concentration-response. The reviewer agrees with these conclusions. Therefore, reviewer-determined NOEC values based on biomass and growth rate data were identical to those of the study authors'. $EC_{50}$ values based on biomass and growth rate data could not be calculated/verified by the reviewer using the probit method via Nuthatch statistical software, consequently, the study authors' reported values for these endpoints are reported in the Executive Summary and Conclusion sections of this DER. $EC_5$ values were not reported by the study author's and could not be determined by the reviewer for biomass and growth rate endpoints due to the software limitations.

**F. CONCLUSIONS:**

The study is scientifically sound but does not satisfy the guideline requirements for a Tier II aquatic nonvascular plant study with *Scenedesmus subspicatus*. According to US EPA Memorandum (Oct. 21, 1994), Closure on Nontarget Plant Phytotoxicity Policy Issues, three day OECD studies will be reviewed as Tier I screening studies only, therefore this study is classified as Supplemental due the shorter than recommended definitive test duration.

**Cell density:**
NOEC/$EC_{05}$: 49 ppm AE C421200
$EC_{05}$: 53 ppm AE C421200 95% C.I.: 25-110 ppm AE C421200
$EC_{50}/IC_{50}$: 70 ppm AE C421200 95% C.I.: 47-110 ppm AE C421200
Slope: 13.2

**Growth rates:**
NOEC/$EC_{05}$: 49 ppm AE C421200
$EC_{05}$: Not reported 95% C.I.: N/A
$EC_{50}/IC_{50}$: 93 ppm AE C421200 95% C.I.: 92-93 ppm AE C421200
Slope: N/A

**Biomass (area under the growth curve):**
NOEC/$EC_{05}$: 49 ppm AE C421200
$EC_{05}$: Not reported 95% C.I.: N/A
$EC_{50}/IC_{50}$: 89 ppm AE C421200 95% C.I.: 86-91 ppm AE C421200
Slope: N/A

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

## III. REFERENCES:

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West, Inc. and Gulley, D.D. 1996. TOXSTAT Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry*. 11: 1485-1494.

Norberg-King, T. J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach.* Version 2.0. U.S. Environmental Protection Agency. National Effluent Toxicity Assessment Center. Duluth, Minnesota. Technical Report 03-93.

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

**Cell Density**
File: 3513cd 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 | 754616.667 | 754616.667 | 96.000 |
| 2 | 5.7 | 814479.000 | 814479.000 | 50.000 |
| 3 | 12 | 694610.667 | 694610.667 | 32.000 |
| 4 | 24 | 548458.333 | 548458.333 | 18.000 |
| **5** | **49** | **700249.000** | **700249.000** | **29.000** |
| 6 | 99 | 17955.333 | 17955.333 | 6.000 |

Calculated H Value = 14.805 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Cell Density
File: 3513cd Transform: NO TRANSFORMATION

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

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | ORIGINAL MEAN | GROUP 0 6 | 0 4 | 0 3 | 0 5 | 0 1 | 0 2 |
|---|---|---|---|---|---|---|---|---|---|
| 6 | 99 | 17955.333 | 17955.333 | \ | | | | | |
| 4 | 24 | 548458.333 | 548458.333 | . | \ | | | | |
| 3 | 12 | 694610.667 | 694610.667 | . | . | \ | | | |
| 5 | 49 | 700249.000 | 700249.000 | . | . | . | \ | | |
| 1 | Neg Control | 754616.667 | 754616.667 | * | . | . | . | \ | |
| 2 | 5.7 | 814479.000 | 814479.000 | . | . | . | . | . | \ |

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

**Estimates of EC%**

| Parameter | Estimate | 95% Bounds Lower | Upper | Std.Err. | Lower Bound /Estimate |
|---|---|---|---|---|---|
| EC5 | 53. | 25. | 1.1E+02 | 0.16 | 0.47 |
| EC10 | 56. | 29. | 1.1E+02 | 0.14 | 0.51 |
| EC25 | 63. | 36. | 1.1E+02 | 0.11 | 0.58 |
| EC50 | 70. | 47. | 1.1E+02 | 0.085 | 0.66 |

Slope = 13.2 Std.Err. = 7.58

!!!Poor fit: p = 0.016 based on DF= 3.0 15.

3513CD : Cell Density

Observed vs. Predicted Treatment Group Means

| Dose | #Reps. | Obs. Mean | Pred. Mean | Obs. -Pred. | Pred. %Control | %Change |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.00 | 6.00 | 7.55e+05 | 7.13e+05 | 4.13e+04 | 100. | 0.00 |
| 5.70 | 3.00 | 8.14e+05 | 7.13e+05 | 1.01e+05 | 100. | 1.63e-14 |
| 12.0 | 3.00 | 6.95e+05 | 7.13e+05 | -1.87e+04 | 100. | 1.63e-14 |
| 24.0 | 3.00 | 5.48e+05 | 7.13e+05 | -1.65e+05 | 100. | 2.93e-08 |
| 49.0 | 3.00 | 7.00e+05 | 7.00e+05 | 0.209 | 98.2 | 1.84 |
| 99.0 | 3.00 | 1.80e+04 | 1.80e+04 | -0.000278 | 2.52 | 97.5 |

**Biomass**
File: 3513bd 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 | 12388640.000 | 12388640.000 | 91.000 |
| 2 | 5.7 | 13506180.000 | 13506180.000 | 52.000 |
| 3 | 12 | 11325624.000 | 11325624.000 | 29.000 |
| 4 | 24 | 8911348.000 | 8911348.000 | 15.000 |
| **5** | | **4911724804.000** | **11724804.000** | **38.000** |
| 6 | 99 | 312864.000 | 312864.000 | 6.000 |

Calculated H Value = 15.303 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Biomass
File: 3513bd 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 4 3 5 1 2 |
|---|---|---|---|---|
| 6 | 99 | 312864.000 | 312864.000 | \ |
| 4 | 24 | 8911348.000 | 8911348.000 | . \ |
| 3 | 12 | 11325624.000 | 11325624.000 | . . \ |
| 5 | | 4911724804.000 | 11724804.000 | . . . \ |
| 1 | Neg Control | 12388640.000 | 12388640.000 | * . . . \ |
| 2 | 5.7 | 13506180.000 | 13506180.000 | * . . . . \ |

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

**Estimates of EC%**
!!!Failure#1: near-singular matrix, model possibly unsuitable.

**Growth Rate**
File: 3513gd 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 | 0.601 | 0.601 | 96.500 |
| 2 | 5.7 | 0.610 | 0.610 | 50.500 |
| 3 | 12 | 0.589 | 0.589 | 30.500 |
| 4 | 24 | 0.548 | 0.548 | 18.000 |

| | | | | |
|---|---|---|---|---|
| **5** | **49** | **0.590** | **0.590** | **29.500** |
| 6 | 99 | 0.081 | 0.081 | 6.000 |

Calculated H Value = 15.128 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Growth Rate
File: 3513gd 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 4 3 5 1 2 |
|---|---|---|---|---|
| 6 | 99 | 0.081 | 0.081 | \ |
| 4 | 24 | 0.548 | 0.548 | . \ |
| 3 | 12 | 0.589 | 0.589 | . . \ |
| 5 | 49 | 0.590 | 0.590 | . . . \ |
| 1 | Neg Control | 0.601 | 0.601 | * . . . \ |
| 2 | 5.7 | 0.610 | 0.610 | . . . . . \ |

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

**Estimates of EC%**
!!!Failure#1: near-singular matrix, model possibly unsuitable.