

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


William Evans

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 Unpublished study performed by Wildlife International, Ltd., Easton, Maryland.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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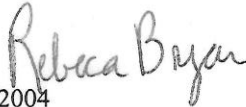
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Primary Reviewer:
{EPA/OECD/PMRA}

Date: {.....}

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

Date: {.....}

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

Date Evaluation Completed: {dd-mmm-yyyy}

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 Unpublished study performed by Wildlife International, Ltd., Easton, Maryland.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 EC₅₀ 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/EC₀₅: 49 ppm AE C421200

EC₀₅: 53 ppm AE C421200

EC₅₀/IC₅₀: 70 ppm AE C421200

Slope: 13.2

95% C.I.: 25-110 ppm AE C421200

95% C.I.: 47-110 ppm AE C421200

Growth rates:

NOEC/EC₀₅: 49 ppm AE C421200

EC₀₅: Not reported

EC₅₀/IC₅₀: 93 ppm AE C421200

Slope: N/A

95% C.I.: N/A

95% C.I.: 92-93 ppm AE C421200

Biomass (area under the growth curve):

NOEC/EC₀₅: 49 ppm AE C421200

EC₀₅: Not reported

EC₅₀/IC₅₀: 89 ppm AE C421200

Slope: N/A

95% C.I.: N/A

95% C.I.: 86-91 ppm AE C421200

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. capricornutum, and a freshwater diatom is tested

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

PMRA Submission #: {.....}

EPA MRID #: 46203513

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 EC₅₀ 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/EC₀₅: 49 ppm AE C421200
EC₀₅: 53 ppm AE C421200 95% C.I.: 25-110 ppm AE C421200
EC₅₀/IC₅₀: 70 ppm AE C421200 95% C.I.: 47-110 ppm AE C421200
Slope: 13.2

Growth rates:

NOEC/EC₀₅: 49 ppm AE C421200
EC₀₅: Not reported 95% C.I.: N/A
EC₅₀/IC₅₀: 93 ppm AE C421200 95% C.I.: 92-93 ppm AE C421200
Slope: N/A

Biomass (area under the growth curve):

NOEC/EC₀₅: 49 ppm AE C421200
EC₀₅: Not reported 95% C.I.: N/A
EC₅₀/IC₅₀: 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 C4201200 (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. 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

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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
		Criteria
Acclimation period:	≥ 14 days	
culturing media and conditions: (same as test or not)	Freshwater algal medium; same as test	<i>EPA recommends two week acclimation period.</i>
health: (any toxicity observed)	Algal cells were actively growing.	<i>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.</i>
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	72 hours	<i>EPA requires: 96 - 120 hours</i> <i>OECD: 72 hours</i>
Test vessel material: (glass/polystyrene) size: fill volume:	Glass 250 mL (Erlenmeyer flask) 100 mL	Test vessels were plugged with foam stoppers. <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>

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Parameter	Details	Remarks
		Criteria
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	Freshwater algal medium 5.2-7.9 6.0-9.0 Yes NaHCO ₃ N/A	See Appendix 2, p. 33. <hr/> OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used. <hr/> EPA recommends 20X-AAP medium.
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Well water, NANOpure® filtered with reagent grade chemicals added Filter -sterilized (0.22 µm) 8.1 ± 0.1 N/A pH adjusted with 10% HCl Not reported Not reported <LOD <LOD Not reported	<hr/> EPA pH: <i>Skeletonema costatum</i> = ~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. <hr/> 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.	<hr/> EPA recommends agitation only for <i>Selenastrum</i> at 100 cycles per min and <i>Skeletonema</i> at ~60 cycles per min. Aeration is not recommended.
Initial cells density	Approximately 10,000 cells/mL	<hr/> EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i> , cell counts on day 2 are not required. <hr/> OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i> . When other species are used the biomass should be comparable.

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Parameter	Details	Remarks
		Criteria
Number of replicates control: solvent control: treated ones:	6 N/A 3	<i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <u>Navicula sp.</u> tests should be conducted with four replicates.</i> <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 cultures should be included in the test.</i>
Test concentrations nominal: measured:	0 (negative control), 6.3, 13, 25, 50, and 100 ppm AE C421200 <3.00 (<LOQ, negative control), 5.7, 12, 24, 49, and 99 ppm AE C421200	<i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i> <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>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; 0 and 72 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	23.5-24.5°C Continuous 6500-9050 lux, cool-white fluorescent light.	<i>EPA temperature: <u>Skeletonema</u>: 20°C, Others: 24-25°C; EPA photoperiod: <u>S. costatum</u> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <u>Anabaena</u>: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)</i> <i>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.</i>

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Parameter	Details	Remarks
		Criteria
Reference chemical {if used} name: 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).	<i>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.</i>
Measurement technique for cell density and other end points	Cell counts using a electronic particle counter.	<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	Every 24 hours	<i>EPA and OECD: every 24 hours.</i>
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. <i>EPA requires control cell count at termination to be $\geq 2X$ 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:

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 Conc.); 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.

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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/EC₀₅: 49 mg a.i./L
EC₅₀/IC₅₀: 93 mg a.i./L 95% C.I.: 92-93 mg a.i./L

Biomass (area under the growth curve):

NOEC/EC₀₅: 49 mg a.i./L
EC₅₀/IC₅₀: 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 EC₀₅ and EC₅₀ values based on cell density data were determined using the Probit method via Nuthatch statistical software. EC₅₀ 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. EC₅ 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/EC₀₅: 49 ppm AE C421200
EC₀₅: 53 ppm AE C421200 95% C.I.: 25-110 ppm AE C421200
EC₅₀/IC₅₀: 70 ppm AE C421200 95% C.I.: 47-110 ppm AE C421200
Slope: 13.2

Growth rates:

NOEC/EC₀₅: 49 ppm AE C421200
EC₀₅: Not determined 95% C.I.: Not determined
EC₅₀/IC₅₀: Not determined 95% C.I.: Not determined
Slope: Not determined

Biomass (area under the growth curve):

NOEC/EC₀₅: 49 ppm AE C421200
EC₀₅: Not determined 95% C.I.: Not determined
EC₅₀/IC₅₀: 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'. EC₅₀ 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. EC₅ 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/EC₀₅: 49 ppm AE C421200

EC₀₅: 53 ppm AE C421200

EC₅₀/IC₅₀: 70 ppm AE C421200

Slope: 13.2

95% C.I.: 25-110 ppm AE C421200

95% C.I.: 47-110 ppm AE C421200

Growth rates:

NOEC/EC₀₅: 49 ppm AE C421200

EC₀₅: Not reported

EC₅₀/IC₅₀: 93 ppm AE C421200

Slope: N/A

95% C.I.: N/A

95% C.I.: 92-93 ppm AE C421200

Biomass (area under the growth curve):

NOEC/EC₀₅: 49 ppm AE C421200

EC₀₅: Not reported

EC₅₀/IC₅₀: 89 ppm AE C421200

Slope: N/A

95% C.I.: N/A

95% C.I.: 86-91 ppm AE C421200

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

PMRA Submission #: {.....}

EPA MRID #: 46203513

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

III. REFERENCES:

Organisation for Economic Cooperation and Development. 1984. OECD Guideline for Testing of Chemicals, 201: *Alga, Growth Inhibition Test*.

Official Journal of the European Communities. 1992. No. L383. Method C.3.: *Algal Inhibition Test*.

The SAS System for Windows. 1996. Release 8.02, TS Level 0020. SAS Institute, Inc. Cary, North Carolina.

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 (IC_p) Approach*. Version 2.0. U.S. Environmental Protection Agency. National Effluent Toxicity Assessment Center. Duluth, Minnesota. Technical Report 03-93.

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

PMRA Submission #:{.....}

EPA MRID #: 46203513

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	0	0	0	0	0	
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)
 Table q value (0.05,6) = 2.936

. = no significant difference
 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
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
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Data Evaluation Report on the acute toxicity of AE C421200 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus*

PMRA Submission #:{.....}

EPA MRID #: 46203513

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		5.713506180.000	13506180.000	52.000
3		1211325624.000	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
6		99 312864.000	312864.000	\
4		24 8911348.000	8911348.000	. \
3		1211325624.000	11325624.000	. . \
5		4911724804.000	11724804.000	. . . \
1	Neg Control	12388640.000	12388640.000	* . . . \
2		5.713506180.000	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	50.500
3		12	0.589	30.500
4		24	0.548	18.000

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

PMRA Submission #:{.....}

EPA MRID #: 46203513

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	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.