

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the acute toxicity of AE F132347 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus*
PMRA Submission #: {.....}

EPA MRID #: 46203510

Data Requirement: PMRA DATA CODE {.....}
EPA DP Barcode D294536
OECD Data Point {.....}
EPA MRID 46203510
EPA Guideline 123-2 (OPPTS 850.5400)

Test material: AE F132347 (Thidiazuron Metabolite) **Purity:** 97.4%
Common name: Thidiazuron Metabolite (1-phenyl-3-(1,2,5-thiadiazol-3-yl)urea)
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: 4/20/2004

QC Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/23/2004

Primary Reviewer: William Evans, Biologist
{EPA/OPP/EFED/ERB-1

Date: 11/17/04



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 F132347: A 72-Hour Toxicity Test with the Freshwater Alga (*Scenedesmus subspicatus*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 149A-157. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Experimental start date May 12, 2003 and experimental termination date May 15, 2003. The final report issued June 19, 2003.



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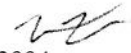
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{EPA/OECD/PMRA}

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EXECUTIVE SUMMARY:

In a 72-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to AE F132347 (Thidiazuron Metabolite) under static conditions at nominal concentrations of 0 (negative control), 0.25, 0.50, 1.0, 2.0, and 4.0 ppm AE F132347. The mean-measured concentrations were <0.100 (<LOQ, negative control), 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347. Cell density percent inhibition was 6, 15, 37, 92, and 94% (reviewer-calculated) in the 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups, respectively. Biomass (area under the growth curve, 0 to 72 hours) percent inhibition was 7.2, 14, 37, 80, and 83% in the 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups, respectively. Growth rate (0 to 72 hours) percent inhibition was 1.4, 3.3, 9.8, 53, and 61% in the 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups, respectively. Cell density, growth rate and biomass were significantly reduced in the 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups compared to the negative control. Cell density, growth rate and biomass EC₅₀ values were 0.98, 1.8, and 1.1 ppm AE F132347, respectively. The NOEC for AE F132347 was 0.22 ppm Thidiazuron Technical, 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: 0.22 ppm AE F132347
EC₀₅: 0.31 ppm AE F132347 95% C.I.: 0.20-0.48 ppm AE F132347
EC₅₀/IC₅₀: 0.98 ppm AE F132347 95% C.I.: 0.79-1.2 ppm AE F132347
Slope: 3.29

Growth rates:

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₀₅: 0.36 ppm AE F132347 95% C.I.: 0.20-0.65 ppm AE F132347
EC₅₀/IC₅₀: 1.8 ppm AE F132347 95% C.I.: 1.7-1.9 ppm AE F132347
Slope: 2.01

Biomass (area under the growth curve):

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₀₅: Not determined 95% C.I.: N/A
EC₅₀/IC₅₀: 1.1 ppm AE F132347 95% C.I.: 0.87-1.4 ppm AE F132347
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 deviation from U.S. EPA Guideline, §123-2 was noted:

1. The dilution water total organic carbon, particulate matter and residual chlorine concentrations were not reported.
2. The growth medium pH was higher (7.7-9.7) and ranged more than recommended, ~7.5 from beginning to end of the test.
3. Light intensity (6500-9000 lux) was higher than recommended (~43000 lux).
4. The test duration was 72-hours rather than the recommended 96-120.

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 F132347 (Thidiazuron Metabolite)

Description: Rust colored powder

Lot No./Batch No. : GMT216P

Purity: 97.4%

Stability of Compound

Under Test Conditions: The 0-hour measured test concentrations were 90.9-97.0% of the nominal concentrations and the 72-hour measured test concentrations were 84.9-92.7% of the nominal concentrations (Table 1, p. 20).

(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

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 7.7-7.8 8.2-9.7 Yes NaHCO ₃ N/A	See Appendix 2, p. 31. OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used. EPA recommends 20X-AAP 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 [®]) with reagent grade chemicals Filter-sterilized (0.22 µm) 8.1 ± 0.1 N/A pH adjusted with 10% HCl Not reported Not reported See Appendix 3, pp. 32-33 Not detected Not reported	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. 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 <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	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. 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</u> sp. 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), 0.25, 0.50, 1.0, 2.0, and 4.0 ppm AE F132347 <0.100 (<LOQ, negative control), 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347	<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:	23.8-24.0°C Continuous 6500-9000 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>

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 111X 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:

Cell density percent inhibition was 6, 15, 37, 92, and 94% (reviewer-calculated) in the 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups, respectively. Biomass (area under the growth curve, 0 to 72 hours) percent inhibition was 7.2, 14, 37, 80, and 83% in the 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups, respectively. Growth rate (0 to 72 hours) percent inhibition was 1.4, 3.3, 9.8, 53, and 61% in the 0.22, 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups, respectively. Growth rate was significantly reduced in the 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups compared to the negative control. Biomass was significantly reduced in the 0.46, 0.91, 1.9, and 3.7 ppm AE F132347 treatment groups compared to the control.

Table 3: Effect of AE F132347 (Thidiazuron Metabolite) on Algae (*Scenedesmus subspicatus*)

Treatment mean measured and nominal concentrations ^a (ppm AE F132347)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours ^b	72 hours	
			cell count ^b	% inhibition ^b
Dilution water control	10,000	61,097	1,109,483	--
0.22 (0.25)	10,000	56,222	1,039,411	6
0.46 (0.50)	10,000	61,282	947,164	15
0.91 (1.0)	10,000	54,334	695,495	37
1.9 (2.0)	10,000	59,320	90,615	92
3.7 (4.0)	10,000	54,593	64,163	94
Reference chemical (if used)	N/A	N/A	N/A	N/A

^a Nominal test concentrations are in parentheses.

^b The cell density means and % inhibition compared to the control were reviewer-calculated based on data in Appendix 5, p. 46.

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Table 4: Effect of AE F132347 (Thidiazuron Metabolite) Algae (*Scenedesmus subspicatus*)

Treatment mean measured and Concentrations ^a (ppm AE F132347)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day) ^b	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve) ^b
Dilution water control	10,000	0.0653	--	19,535,358	--
0.22 (0.25)	10,000	0.0644	1.4	18,129,160	7.2
0.46 (0.50)	10,000	0.0632	3.3	16,816,176*	14
0.91 (1.0)	10,000	0.0589*	9.8	12,219,784*	37
1.9 (2.0)	10,000	0.0305*	53	3,954,968*	80
3.7 (4.0)	10,000	0.0258*	61	3,365,676*	83
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

^a Nominal test concentrations are in parentheses.

^b The percent inhibitions were calculated by comparison of the treatment groups to the control.

* Statistically significant difference (p<0.05) from the control using the Dunnett's test.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC ₀₅ (ppm AE F132347)	0.22	0.46	0.22*
EC ₅₀ (ppm AE F132347)	1.1	1.8	Not Reported
IC ₅₀ or EC ₅₀ (ppm AE F132347) (95% C.I.)	0.87-1.4	1.7-1.9	N/A
IC ₂₅ /EC ₂₅ (ppm AE F132347) (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.

* Reviewer determined reviewer-determined based on the data provided in Appendix 5, p. 45.6

B. REPORTED STATISTICS:

Statistical Method: The area under the growth curve and growth rate formulas are reported on pages 25-16 of the study report. Percent inhibition was determined for all endpoints. Data were evaluated for normality and homogeneity of variances using Shapiro-Wilk's test and Levene's test, respectively. NOEC and LOEC values were determined using Dunnett's test. 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 (pp. 15-16).

Cell density: Not reported; not calculated or statistically analyzed by the study authors.

Growth rates:

NOEC/EC₀₅: 0.46 ppm AE F132347
EC₅₀/IC₅₀: 1.8 ppm AE F132347 95% C.I.: 1.7-1.9 ppm AE F132347

Biomass (area under the growth curve):

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₅₀/IC₅₀: 1.1 ppm AE F132347 95% C.I.: 0.87-1.4 ppm AE F132347

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 satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). The NOEC and LOEC were determined using ANOVA and William's multiple comparison test. The analyses described above were conducted via TOXSTAT statistical software using mean-measured treatment concentrations for all toxicity calculations. The EC₀₅ EC₅₀ values were determined for cell density and growth rate using the Probit method via Nuthatch statistical software. EC values for biomass could not be calculated/verified using the probit method via Nuthatch statistical software, consequently, the study author-reported values for this endpoint were reported in the Executive Summary and Conclusion sections of this DER.

Cell density:

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₀₅: 0.31 ppm AE F132347 95% C.I.: 0.20-0.48 ppm AE F132347
EC₅₀/IC₅₀: 0.98 ppm AE F132347 95% C.I.: 0.79-1.2 ppm AE F132347
Slope: 3.29

Growth rates:

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₀₅: 0.36 ppm AE F132347 95% C.I.: 0.20-0.65 ppm AE F132347
EC₅₀/IC₅₀: 2.4 ppm AE F132347 95% C.I.: 2.0-2.8 ppm AE F132347
Slope: 2.01

Biomass (area under the growth curve):

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₀₅: Not determined 95% C.I.: N/A
EC₅₀/IC₅₀: Not determined 95% C.I.: N/A
Slope: N/A

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

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 characterized 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 0.46, 0.91, 1.9 and 3.7 ppm AE F132347 treatment level. Reviewer determined NOEC value based on biomass data was identical to that of the study authors'. The reviewer determined NOEC (0.22 ppm AE F132347) based on growth rate data was one treatment level lower than that of study authors' (0.46 ppm AE F132347). However, the reviewer determined EC₅₀ value (2.4 ppm AE F132347) was higher than that of the study authors' (1.8 ppm AE F132347). These differences are presumably due to the different statistical methods used. Therefore, in an effort to report the most conservative toxicity values, the study authors' EC₅₀ for growth rate and the reviewer's NOEC are reported in the Executive Summary and the Conclusion sections of this DER. The reviewer calculated EC₀₅ values , with the exception of an EC₀₅ value based on biomass, are reported for all endpoints for the purpose of risk assessment.

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: 0.22 ppm AE F132347
EC₀₅: 0.31 ppm AE F132347 95% C.I.: 0.20-0.48 ppm AE F132347
EC₅₀/IC₅₀: 0.98 ppm AE F132347 95% C.I.: 0.79-1.2 ppm AE F132347
Slope: 3.29

Growth rates:

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₀₅: 0.36 ppm AE F132347 95% C.I.: 0.20-0.65 ppm AE F132347
EC₅₀/IC₅₀: 1.8 ppm AE F132347 95% C.I.: 1.7-1.9 ppm AE F132347
Slope: 2.01

Biomass (area under the growth curve):

NOEC/EC₀₅: 0.22 ppm AE F132347
EC₀₅: Not determined 95% C.I.: N/A
EC₅₀/IC₅₀: 1.1 ppm AE F132347 95% C.I.: 0.87-1.4 ppm AE F132347
Slope: N/A

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*.

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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.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

AE F132347 Cell Density

File: 3510cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3851498265556.000	770299653111.000	104.542
Within (Error)	15	110524810464.000	7368320697.602	
Total	20	3962023076020.000		

Critical F value = 2.90 (0.05,5,15)
 Since F > Critical F REJECT Ho:All groups equal

AE F132347 Cell Density

File: 3510cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	1109482.500	1109482.500		
2	0.22	1039410.667	1039410.667	1.154	
3	0.46	947164.000	947164.000	2.674	*
4	0.91	695494.667	695494.667	6.821	*
5	1.9	90614.667	90614.667	16.786	*
6	3.7	64163.000	64163.000	17.222	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

AE F132347 Cell Density

File: 3510cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	6			
2	0.22	3	157995.032	14.2	70071.833
3	0.46	3	157995.032	14.2	162318.500
4	0.91	3	157995.032	14.2	413987.833
5	1.9	3	157995.032	14.2	1018867.833
6	3.7	3	157995.032	14.2	1045319.500

AE F132347 Cell Density

File: 3510cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED
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Data Evaluation Report on the acute toxicity of AE F132347 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus*

PMRA Submission #:{.....}

EPA MRID #: 46203510

IDENTIFICATION	N	MEAN	MEAN	MEAN
1	Neg control	6	1109482.500	1109482.500
2	0.22	3	1039410.667	1039410.667
3	0.46	3	947164.000	947164.000
4	0.91	3	695494.667	695494.667
5	1.9	3	90614.667	90614.667
6	3.7	3	64163.000	64163.000

AE F132347 Cell Density

File: 3510cd

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	1109482.500				
0.22	1039410.667	1.154		1.75	k= 1, v=15
0.46	947164.000	2.674	*	1.84	k= 2, v=15
0.91	695494.667	6.821	*	1.87	k= 3, v=15
1.9	90614.667	16.786	*	1.88	k= 4, v=15
3.7	64163.000	17.222	*	1.89	k= 5, v=15

s = 85838.923

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

Estimates of EC%

?[0m

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.31	0.20	0.48	0.093	0.64
EC10	0.40	0.27	0.59	0.081	0.68
EC25	0.61	0.45	0.82	0.062	0.74
EC50	0.98	0.79	1.2	0.043	0.81

Slope = 3.29 Std.Err. = 0.384

!!!Poor fit: p < 0.001 based on DF= 3.00 15.0

AE F132347 Biomass

File: 3510bd

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	901803522167808.000	180360704433664.000	113.544
Within (Error)	15	23827071877120.000	1588471458474.000	
Total	20	925630594044928.000		

Critical F value = 2.90 (0.05,5,15)

Since F > Critical F REJECT Ho:All groups equal

Data Evaluation Report on the acute toxicity of AE F132347 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus*
 PMRA Submission #:{.....}

EPA MRID #: 46203510

AE F132347 Biomass
 File: 3510bd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg Contyrol	19535358.000	19535358.000		
2	0.22	18129160.000	18129160.000	1.578	
3	0.46	16816176.000	16816176.000	3.051	*
4	0.91	12219784.000	12219784.000	8.209	*
5	1.9	3954968.000	3954968.000	17.483	*
6	3.7	3365676.000	3365676.000	18.144	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

AE F132347 Biomass
 File: 3510bd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg Contyrol	6			
2	0.22	3	2319791.102	11.9	1406198.000
3	0.46	3	2319791.102	11.9	2719182.000
4	0.91	3	2319791.102	11.9	7315574.000
5	1.9	3	2319791.102	11.9	15580390.000
6	3.7	3	2319791.102	11.9	16169682.000

AE F132347 Biomass
 File: 3510bd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Contyrol	6	19535358.000	19535358.000	19535358.000
2	0.22	3	18129160.000	18129160.000	18129160.000
3	0.46	3	16816176.000	16816176.000	16816176.000
4	0.91	3	12219784.000	12219784.000	12219784.000
5	1.9	3	3954968.000	3954968.000	3954968.000
6	3.7	3	3365676.000	3365676.000	3365676.000

AE F132347 Biomass
 File: 3510bd 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

Data Evaluation Report on the acute toxicity of AE F132347 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus*

PMRA Submission #:{.....}

EPA MRID #: 46203510

Neg Contyroll	19535358.000				
	0.2218129160.000	1.578		1.75	k= 1, v=15
	0.4616816176.000	3.051	*	1.84	k= 2, v=15
	0.9112219784.000	8.209	*	1.87	k= 3, v=15
	1.93954968.000	17.483	*	1.88	k= 4, v=15
	3.73365676.000	18.144	*	1.89	k= 5, v=15

s = 1260345.769

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

EC Estimate:

Starting Values:

?[7mControl Mean = 1.95e+07 logEC50 = 0.0402 SIGMA = 0.453

?[0m

!!!Failure#1: near-singular matrix, model possibly unsuitable.

AE F132347 Growth Rate

File: 3510gd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.5461	0.1092	546.000
Within (Error)	15	0.0032	0.0002	
Total	20	0.5492		

Critical F value = 2.90 (0.05,5,15)

Since F > Critical F REJECT Ho:All groups equal

AE F132347

File: 3510gd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg Control	0.653	0.653		
2	0.22	0.644	0.644	0.900	
3	0.46	0.632	0.632	2.167	
4	0.91	0.589	0.589	6.433	*
5	1.9	0.305	0.305	34.833	*
6	3.7	0.258	0.258	39.567	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

AE F132347

File: 3510gd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
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Data Evaluation Report on the acute toxicity of AE F132347 (Thidiazuron Metabolite) on the Algae, *Scenedesmus subspicatus*

PMRA Submission #:{.....}

EPA MRID #: 46203510

1	Neg Control	6			
2	0.22	3	0.026	4.0	0.009
3	0.46	3	0.026	4.0	0.022
4	0.91	3	0.026	4.0	0.064
5	1.9	3	0.026	4.0	0.348
6	3.7	3	0.026	4.0	0.396

AE F132347

File: 3510gd

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	6	0.653	0.653	0.653
2	0.22	3	0.644	0.644	0.644
3	0.46	3	0.632	0.632	0.632
4	0.91	3	0.589	0.589	0.589
5	1.9	3	0.305	0.305	0.305
6	3.7	3	0.258	0.258	0.258

AE F132347

File: 3510gd

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	0.653				
0.22	0.644	0.878		1.75	k= 1, v=15
0.46	0.632	2.114	*	1.84	k= 2, v=15
0.91	0.589	6.278	*	1.87	k= 3, v=15
1.9	0.305	33.994	*	1.88	k= 4, v=15
3.7	0.258	38.613	*	1.89	k= 5, v=15

s = 0.014

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.36	0.20	0.65	0.12	0.55
EC10	0.55	0.34	0.88	0.10	0.62
EC25	1.1	0.80	1.5	0.064	0.73
EC50	2.4	2.0	2.8	0.036	0.84

Slope = 2.01 Std.Err. = 0.262

??[7m

!!!Poor fit: p < 0.001 based on DF= 3.00 15.0