Data Requirement:

	PMRA DATA CODE	{............}
	EPA DP Barcode	D294536
	OECD Data Point	{............}
	EPA MRID	46203504
	EPA Guideline	123-2 (OPPTS 850.5400)

Test material: Thidiazuron Technical   **Purity:** 99.5%
Common name: Thidiazuron
Chemical name: IUPAC: Not reported
              CAS name: Not reported
              CAS No.: 51707-55-2
              Synonyms: Not reported

Primary Reviewer: Rebecca Bryan   Signature: *Rebecca Bryan*
Staff Scientist, Dynamac Corporation   Date: 4/20/2004

QC Reviewer: Greg Hess   Signature: *[signature]*
Staff Scientist, Dynamac Corporation   Date: 4/22/2004

                                                      11/16/04
Primary Reviewer: William Evans   Date: {............}
{EPA/OECD/PMRA}   Biologist: EPA/ERB1

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

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. Thidiazuron: A 72-Hour Toxicity Test with the Freshwater Alga (*Anabaena flos-aquae*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 149A-152. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Experimental start date April 7, 2003 and experimental termination date April 10, 2003. The final report issued April 25, 2003.

EXECUTIVE SUMMARY:

In a 72-hour acute toxicity study, cultures of *Anabaena flos-aquae* were exposed to Thidiazuron Technical under static conditions at nominal concentrations of 0 (negative control), 0.80, 1.6, 3.1, 6.3, 13, and 25 ppm Thidiazuron Technical. The mean-measured concentrations were <0.400 (<LOQ, negative control), 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical. Cell density percent inhibition was -14, -29, 7, 61, 50, and 99% (reviewer-determined) in the 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical treatment groups, respectively. Biomass (area under the growth curve; 0 to 72 hours) percent inhibition was -7.9, -17, 17, 47, 69, and 97% in the 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical treatment groups, respectively. Growth rate (0 to 72 hours) percent inhibition was -3.5, -6.6, 2.3, 19, 30, and 93% in the 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical treatment groups, respectively. Growth rate was significantly reduced at the 11 and 19 ppm Thidiazuron Technical treatment level. Biomass was the most sensitive endpoint, with an EC_{50} value of 6.0 ppm. Cell density and biomass were reduced at the 5.4, 11 and 19 ppm Thidiazuron Technical treatment levels. Cell density and growth rate EC_{50} values were 8.0 and 13 ppm Thidiazuron Technical, respectively. The NOEC for Thidiazuron was 2.8 ppm Thidiazuron Technical (based on cell density and biomass).

The study is scientifically sound but does not satisfy the guideline requirements for a Tier II aquatic nonvascular plant study with *Anabaena flos-aquae*. 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 and is therefore classified as Supplemental due the shorter than recommended definitive test duration.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type: Static

Cell density:
NOEC: 2.8 ppm Thidiazuron Technical
EC_{05}: 3.0 ppm Thidiazuron Technical   95% C.I.: 0.85-10 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 8.0 ppm Thidiazuron Technical   95% C.I.: 4.7-14 ppm Thidiazuron Technical
Slope: 3.80

Growth rates:
NOEC: 5.4 ppm Thidiazuron Technical
EC_{05}: 8.2 ppm Thidiazuron Technical   95% C.I.: 5.8-11 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 13 ppm Thidiazuron Technical   95% C.I.: 11-15 ppm Thidiazuron Technical
Slope: 8.38

Biomass (area under the growth curve):
NOEC/EC_{05}: 2.8 ppm Thidiazuron Technical
EC_{05}: 1.8 ppm Thidiazuron Technical   95% C.I.: 0.52-5.9 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 6.0 ppm Thidiazuron Technical   95% C.I.: 3.6-10 ppm Thidiazuron Technical
Slope: 3.06

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

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 test duration was 72-hours rather than the recommended 96-120.

3. The growth medium pH (7.9-8.1) was higher than recommended (~7.5).

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 <u>No</u> 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   Thidiazuron technical

   Description: Powder

   Lot No./Batch No. :   107623-03 (Product code: AE B049537 00 1D99 0003)

   Purity: 99.5%

Stability of Compound
Under Test Conditions: The 0 hour measured test concentrations were 68.0-94.8% of the nominal concentrations and the 72 hour measured test concentrations were 73.4-87.2% 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 ambient conditions.

2. Test organism:
          Name: *Anabaena flos-aquae*

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: UTCC 67
          Source: Originally from University of Toronto. 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: culturing media and conditions: (same as test or not) health: (any toxicity observed)	≥ 14 days Freshwater algal medium; same as test Algal cells were actively growing.	*EPA recommends two week acclimation period.* *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 static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	72 hours	US EPA considers 72-hour OECD tests to be acceptable as Tier I tests. *EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass 250 mL (Erlenmeyer flask) 100 mL	Test vessels were plugged with foam stoppers. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	Freshwater algal medium 7.9-8.1 7.9-8.0 Yes NaHCO$_3$ N/A	See Appendix 2, p. 30. *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	

Parameter	Details	Remarks
		Criteria
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Well water with reagent grade chemicals Filter -sterilized (0.22 μm) 7.9 N/A None Not reported Not reported Not detected Not detected Not reported	See Appendix 3, pp. 31-32 *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.* *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.* *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.*
Number of replicates control: solvent control: treated ones:	6 N/A 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.* *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.*

Parameter	Details	Remarks
		Criteria
Test concentrations nominal: measured:	0 (negative control), 0.80, 1.6, 3.1, 6.3, 13, and 25 ppm Thidiazuron Technical <0.400 (<LOQ, negative control), 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *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 temperature: photoperiod: light intensity and quality:	23.7-24.0°C Continuous 1950-2300 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 (±25%), Others: 4 - 5 Klux (±25%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used} 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).	*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 hemacytometer and microscope.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *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	
Indicate whether there was exponential growth in the control	Yes, dilution water control cell density at test termination was 52X greater than the dilution water control cell density at test initiation.	Mean cell densities were reviewer-calculated. *EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *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 -14, -29, 7, 61, 50, and 99% (reviewer-determined) in the 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical mean-measured treatment groups, respectively. Biomass (area under the growth curve; 0 to 72 hours) percent inhibition was -7.9, -17, 17, 47, 69, and 97% in the 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical treatment groups, respectively. Biomass was significantly reduced in the 11 and 19 ppm

Thidiazuron Technical treatment groups, however observed effects (not statistically significant) in the 5.4 ppm Thidiazuron Technical treatment group were considered treatment-related by the study authors (p. 17). The growth rate (0 to 72 hours) percent inhibition was -3.5, -6.6, 2.3, 19, 30, and 93% in the 0.68, 1.2, 2.8, 5.4, 11, and 19 ppm Thidiazuron Technical treatment groups, respectively. Growth rate was significantly reduced in the 19 ppm Thidiazuron Technical treatment group, however, the study authors considered the observed inhibition at the 11 ppm Thidiazuron Technical level to be treatment related (p. 17).

Table 3: Effect of Thidiazuron Technical on Blue-Green Algae (*Anabaena flos-aquae*)

Treatment Mean-Measured and (Nominal Concentrations) ppm Thidiazuron Technical	Initial Cell Density (cells/mL)	Mean Cell Density (cells/mL) at		
		24-hours	72-hours	
		cell count[a]	cell count[a]	% inhibition[a]
Dilution water control	10,000	71333	519167	--
0.68 (0.80)	10,000	43333	594000	-14
1.2 (1.6)	10,000	24000	670333	-29
2.8 (3.1)	10,000	34667	485000	7
5.4 (6.3)	10,000	36000	317667	61*
11 (13)	10,000	29667	261333	50*
19 (25)	10,000	0	8000	99*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The cell density means and percent inhibition compared to the control were reviewer-determined based on the data provided in Appendix 5, p. 45. Negative percent inhibition indicates increased growth compared to the control.
* Considered to be treatment related by the reviewer; not analyzed statistically by the study author.

Table 4: Effect of Thidiazuron Technical on Blue-Green Algae (*Anabaena flos-aquae*)

Treatment Mean-Measured and (Nominal Concentrations) ppm Thidiazuron Technical	Initial Cell Density (cells/mL)	Mean Growth Rate per day	% Inhibition (Mean Growth Rate per day)[b]	Mean Biomass (Area Under Growth Curve)	% Inhibition Biomass (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	0.0542	--	10,906,000	--
0.68 (0.80)	10,000	0.0560	-3.5	11,768,000	-7.9
1.2 (1.6)	10,000	0.0577	-6.6	12,716,000	-17
2.8 (3.1)	10,000	0.0529	2.3	9,076,000	17
5.4 (6.3)	10,000	0.0438	19	5,780,000[d]	47
11 (13)	10,000	0.0381[d]	30	3,408,000[c]	69

Treatment Mean-Measured and (Nominal Concentrations) ppm Thidiazuron Technical	Initial Cell Density (cells/mL)	Mean Growth Rate per day	% Inhibition (Mean Growth Rate per day)[b]	Mean Biomass (Area Under Growth Curve)	% Inhibition Biomass (Mean Area Under Growth Curve)[b]
19 (25)	10,000	0.0041[c]	93	320,000[c]	97
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.
[c] Significantly reduced ($p<0.05$) from the control per Dunnett's test.
[d] Considered treatment related by the study authors.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC_{05} (ppm Thidiazuron Technical)	2.8	5.4	2.8*
EC_{50} (ppm Thidiazuron Technical)	6.0	13	Not Reported
IC_{50} or EC_{50} (ppm Thidiazuron Technical) (95% C.I.)	3.6-10	11-15	N/A
IC_{25}/EC_{25} (ppm Thidiazuron Technical) (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.

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/EC_{05}: 5.4 ppm Thidiazuron Technical
EC_{05}: Not reported   95% C.I.: N/A
EC_{50}/IC_{50}: 13 ppm Thidiazuron Technical   95% C.I.: 11-15 ppm Thidiazuron Technical
Slope: Not reported

Biomass (area under the growth curve):
NOEC/EC$_{05}$: 2.8 ppm Thidiazuron Technical
EC$_{05}$: Not reported   95% C.I.: N/A
EC$_{50}$/IC$_{50}$: 6.0 ppm Thidiazuron Technical   95% C.I.: 3.6-10 ppm Thidiazuron Technical
Slope: Not reported

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, biomass (area under the growth curve), and growth rate 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 EC$_{05}$ and EC$_{50}$ values for cell density, biomass and growth rate were determined using the Probit method via Nuthatch statistical software.

Cell density:
NOEC: 11 ppm Thidiazuron Technical
EC$_{05}$: 3.0 ppm Thidiazuron Technical   95% C.I.: 0.85-10 ppm Thidiazuron Technical
EC$_{50}$/IC$_{50}$: 8.0 ppm Thidiazuron Technical   95% C.I.: 4.7-14 ppm Thidiazuron Technical
Slope: 3.80

Growth rates:
NOEC: 5.4 ppm Thidiazuron Technical
EC$_{05}$: 8.2 ppm Thidiazuron Technical   95% C.I.: 5.8-11 ppm Thidiazuron Technical
EC$_{50}$/IC$_{50}$: 13 ppm Thidiazuron Technical   95% C.I.: 11-15 ppm Thidiazuron Technical
Slope: 8.38

Biomass (area under the growth curve):
NOEC: 5.4 ppm Thidiazuron Technical
EC$_{05}$: 1.8 ppm Thidiazuron Technical   95% C.I.: 0.52-5.9 ppm Thidiazuron Technical
EC$_{50}$/IC$_{50}$: 6.0 ppm Thidiazuron Technical   95% C.I.: 3.6-10 ppm Thidiazuron Technical
Slope: 3.06

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

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 19 ppm Thidiazuron level, however, the observed 61 and 50% reduction at the 5.4 and 11 ppm Thidiazuron Technical, respectively, are also considered to be treatment-related. Reviewer-determined toxicity values based on growth rate data were identical to those of the study authors'. The reviewer-determined NOEC (5.4 ppm Thidiazuron Technical) based on biomass data was one treatment level higher than that of study authors' (2.8 Thidiazuron Technical). This difference in NOEC values (biomass) is presumably due to the different statistical methods used and the fact that the study authors considered the observed 47% inhibition at the 5.4 ppm Thidiazuron Technical level to be treatment related compared to the negative control; the reviewer agrees. Therefore, in an effort to report the most conservative toxicity values, the study authors' determined NOEC for biomass is reported in the Executive Summary and the Conclusion sections of this DER. The reviewer calculated EC_{05} values 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 *Anabaena flos-aquae*. 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: 2.8 ppm Thidiazuron Technical
EC_{05}: 3.0 ppm Thidiazuron Technical   95% C.I.: 0.85-10 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 8.0 ppm Thidiazuron Technical   95% C.I.: 4.7-14 ppm Thidiazuron Technical
Slope: 3.80

Growth rates:
NOEC: 5.4 ppm Thidiazuron Technical
EC_{05}: 8.2 ppm Thidiazuron Technical   95% C.I.: 5.8-11 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 13 ppm Thidiazuron Technical   95% C.I.: 11-15 ppm Thidiazuron Technical
Slope: 8.38

Biomass (area under the growth curve):
NOEC/EC_{05}: 2.8 ppm Thidiazuron Technical
EC_{05}: 1.8 ppm Thidiazuron Technical   95% C.I.: 0.52-5.9 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 6.0 ppm Thidiazuron Technical   95% C.I.: 3.6-10 ppm Thidiazuron Technical
Slope: 3.06

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

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*.

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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.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Thidiazuron Cell density
File: 3504cd   Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	966600500005.000	161100083334.250	3.750
Within (Error)	17	730226833329.000	42954519607.563	
Total	23	1696827333334.000		

```
Critical F value =   2.70  (0.05,6,17)
Since  F > Critical F  REJECT  Ho:All groups equal
```

Thidiazuron Cell density
File: 3504cd   Transform: NO TRANSFORM

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	519166.667	519166.667		
2	0.68	594000.000	594000.000	-0.511	
3	1.2	670333.333	670333.333	-1.031	
4	2.8	485000.000	485000.000	0.233	
5	5.4	317666.667	317666.667	1.375	
6	11	261333.333	261333.333	1.759	
7	19	8000.000	8000.000	3.488	*

Bonferroni T table value = 2.65   (1 Tailed Value, P=0.05,   df=17,6)

Thidiazuron Cell density
File: 3504cd   Transform: NO TRANSFORM

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	0.68	3	389093.487	74.9	-74833.333
3	1.2	3	389093.487	74.9	-151166.667
4	2.8	3	389093.487	74.9	34166.667
5	5.4	3	389093.487	74.9	201500.000
6	11	3	389093.487	74.9	257833.333
7	19	3	389093.487	74.9	511166.667

Thidiazuron Cell density
File: 3504cd   Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)   TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	6	519166.667	519166.667	575666.667
2	0.68	3	594000.000	594000.000	575666.667
3	1.2	3	670333.333	670333.333	575666.667
4	2.8	3	485000.000	485000.000	485000.000
5	5.4	3	317666.667	317666.667	317666.667
6	11	3	261333.333	261333.333	261333.333
7	19	3	8000.000	8000.000	8000.000

Thidiazuron Cell density
File: 3504cd   Transform: NO TRANSFORM

WILLIAMS TEST   (Isotonic regression model)   TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg Control	575666.667				
0.68	575666.667	0.386		1.74	k= 1, v=17
1.2	575666.667	0.386		1.82	k= 2, v=17
2.8	485000.000	0.233		1.85	k= 3, v=17
5.4	317666.667	1.375		1.87	k= 4, v=17
11	261333.333	1.759		1.87	k= 5, v=17
19	8000.000	3.488	*	1.88	k= 6, v=17

s = 207254.722
Note: df used for table values are approximate when v > 20.

Estimates of EC%
?[0m---

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	3.0	0.85	10.	0.26	0.29
EC10	3.7	1.3	11.	0.22	0.34
EC25	5.3	2.4	12.	0.17	0.45
EC50	8.0	4.7	14.	0.11	0.59

Slope =   3.80 Std.Err. =   1.52

Goodness of fit: p =   0.44 based on DF=   4.0   17.

Thidiazuron Biomass
File: 3504bd   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	418159176003584.000	69693196000576.000	4.503
Within (Error)	17	263107608004608.000	15476918117920.000	
Total	23	681266784008192.000		

Critical F value =   2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

```
Thidiazuron Biomass
File: 3504bd        Transform: NO TRANSFORMATION

        BONFERRONI T-TEST    -    TABLE 1 OF 2          Ho:Control<Treatment
-------------------------------------------------------------------------------
                            TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION        MEAN        ORIGINAL UNITS    T STAT  SIG
-----    --------------     -----------    -----------------  ------  ---
  1      Neg Control10906000.000          10906000.000
  2          0.6811768000.000             11768000.000       -0.310
  3          1.212716000.000              12716000.000       -0.651
  4          2.8 9076000.000               9076000.000        0.658
  5          5.4 5780000.000               5780000.000        1.843
  6           11 3408000.000               3408000.000        2.695   *
  7           19  320000.000                320000.000        3.805   *
-------------------------------------------------------------------------------
Bonferroni T table value =  2.65     (1 Tailed Value, P=0.05,  df=17,6)
```

```
Thidiazuron Biomass
File: 3504bd        Transform: NO TRANSFORMATION

        BONFERRONI T-TEST    -    TABLE 2 OF 2          Ho:Control<Treatment
-------------------------------------------------------------------------------
                       NUM OF   Minimum Sig Diff  % of      DIFFERENCE
GROUP    IDENTIFICATION  REPS   (IN ORIG. UNITS)  CONTROL   FROM CONTROL
-----    --------------  ----   ----------------  -------   ------------
  1      Neg Control      6
  2          0.68         3      7385701.820       67.7   -862000.000
  3          1.2          3      7385701.820       67.7  -1810000.000
  4          2.8          3      7385701.820       67.7   1830000.000
  5          5.4          3      7385701.820       67.7   5126000.000
  6           11          3      7385701.820       67.7   7498000.000
  7           19          3      7385701.820       67.7  10586000.000
-------------------------------------------------------------------------------
```

```
Thidiazuron Biomass
File: 3504bd        Transform: NO TRANSFORMATION

        WILLIAMS TEST   (Isotonic regression model)    TABLE 1 OF 2
-------------------------------------------------------------------------------
GROUP                           ORIGINAL    TRANSFORMED    ISOTONIZED
         IDENTIFICATION    N      MEAN         MEAN          MEAN
------   --------------   ---  -----------  -----------    -----------
   1      Neg Control    610906000.000     10906000.000   11574000.000
   2          0.68       311768000.000     11768000.000   11574000.000
   3          1.2        312716000.000     12716000.000   11574000.000
   4          2.8        3 9076000.000      9076000.000    9076000.000
   5          5.4        3 5780000.000      5780000.000    5780000.000
   6           11        3 3408000.000      3408000.000    3408000.000
   7           19        3  320000.000       320000.000     320000.000
-------------------------------------------------------------------------------
```

```
Thidiazuron Biomass
File: 3504bd        Transform: NO TRANSFORMATION

        WILLIAMS TEST   (Isotonic regression model)    TABLE 2 OF 2
```

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```
                    ISOTONIZED   CALC.      SIG      TABLE       DEGREES OF
    IDENTIFICATION     MEAN      WILLIAMS   P=.05    WILLIAMS    FREEDOM
----------------------------------------------------------------------------
        Neg Control11574000.000
           0.6811574000.000      0.240               1.74       k= 1, v=17
           1.211574000.000       0.240               1.82       k= 2, v=17
           2.89076000.000        0.658               1.85       k= 3, v=17
           5.45780000.000        1.843               1.87       k= 4, v=17
           113408000.000         2.695       *       1.87       k= 5, v=17
           19 320000.000         3.805       *       1.88       k= 6, v=17
----------------------------------------------------------------------------
```

s = 3934071.443
Note: df used for table values are approximate when v > 20.

Estimates of EC%

?[0m--
```
Parameter   Estimate    95% Bounds        Std.Err.   Lower Bound
                      Lower   Upper                  /Estimate
EC5          1.8      0.52     5.9         0.25        0.30
EC10         2.3      0.81     6.5         0.22        0.35
EC25         3.6      1.7      7.9         0.16        0.46
EC50         6.0      3.6      10.         0.11        0.60
```

            Slope =   3.06 Std.Err. =   0.941

Goodness of fit: p =   0.80 based on DF=   4.0   17.

Thidiazuron Growth rate
File: 3504gd   Transform: NO TRANSFORMATION

                              ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.0067	0.0011	11.000
Within (Error)	17	0.0017	0.0001	
Total	23	0.0084		

   Critical F value =   2.70 (0.05,6,17)
   Since F > Critical F REJECT Ho:All groups equal

Thidiazuron Growth rate
File: 3504gd   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	0.054	0.054		
2	0.68	0.056	0.056	-0.271	
3	1.2	0.058	0.058	-0.507	
4	2.8	0.053	0.053	0.172	
5	5.4	0.044	0.044	1.468	
6	11	0.038	0.038	2.275	
7	19	0.004	0.004	7.083	*

Bonferroni T table value = 2.65   (1 Tailed Value, P=0.05, df=17,6)

Thidiazuron Growth rate
File: 3504gd   Transform: NO TRANSFORMATION

         BONFERRONI T-TEST   -   TABLE 2 OF 2   Ho:Control<Treatment
----
                           NUM OF   Minimum Sig Diff % of   DIFFERENCE
GROUP   IDENTIFICATION   REPS   (IN ORIG. UNITS) CONTROL   FROM CONTROL
-----   --------------   ------   ---------------- -------   ------------
  1   Neg Control   6
  2   0.68   3   0.019   34.7   -0.002
  3   1.2   3   0.019   34.7   -0.004
  4   2.8   3   0.019   34.7   0.001
  5   5.4   3   0.019   34.7   0.010
  6   11   3   0.019   34.7   0.016
  7   19   3   0.019   34.7   0.050

Thidiazuron Growth rate
File: 3504gd   Transform: NO TRANSFORMATION

         WILLIAMS TEST   (Isotonic regression model)   TABLE 1 OF 2

GROUP   ORIGINAL   TRANSFORMED   ISOTONIZED
        IDENTIFICATION   N   MEAN   MEAN   MEAN
------ --------------   ---   --------   -----------   ----------
  1   Neg Control   6   0.054   0.054   0.056
  2   0.68   3   0.056   0.056   0.056
  3   1.2   3   0.058   0.058   0.056
  4   2.8   3   0.053   0.053   0.053
  5   5.4   3   0.044   0.044   0.044
  6   11   3   0.038   0.038   0.038
  7   19   3   0.004   0.004   0.004

Thidiazuron Growth rate
File: 3504gd   Transform: NO TRANSFORMATION

         WILLIAMS TEST   (Isotonic regression model)   TABLE 2 OF 2

                 ISOTONIZED   CALC.   SIG   TABLE   DEGREES OF
    IDENTIFICATION   MEAN   WILLIAMS   P=.05   WILLIAMS   FREEDOM
    -------------- -------- --------   -----   --------   ----------
      Neg Control   0.056
             0.68   0.056   0.194   1.74   k= 1, v=17
             1.2   0.056   0.194   1.82   k= 2, v=17
             2.8   0.053   0.172   1.85   k= 3, v=17
             5.4   0.044   1.468   1.87   k= 4, v=17
             11   0.038   2.275   *   1.87   k= 5, v=17
             19   0.004   7.083   *   1.88   k= 6, v=17
----
s =   0.010
Note: df used for table values are approximate when v > 20.

Estimates of EC%
?[0m---
Parameter   Estimate   95% Bounds   Std.Err.   Lower Bound
                      Lower   Upper   /Estimate

EC5	8.2	5.8	11.	0.070	0.71
EC10	9.0	6.7	12.	0.061	0.75
EC25	11.	8.5	13.	0.047	0.80
EC50	13.	11.	15.	0.034	0.85

Slope =   8.38 Std.Err. =   1.92

Goodness of fit: p =   0.76 based on DF=   4.0   17.