Data Evaluation Report on the acute toxicity of Thidiazuron on the Marine Diatom, *Skeletonema costatum*
PMRA Submission #:{.....} EPA MRID #: 46203505

Data Requirement:		
	PMRA DATA CODE	{.....}
	EPA DP Barcode	D294536
	OECD Data Point	{.....}
	EPA MRID	46203505
	EPA Guideline	123-2 (OPPTS 850.5400)

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

Purity: 99.5%

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/20/2004

QC Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/21/2004

Primary Reviewer:
{EPA/OECD/PMRA} William Evans

Date: {5/17/04}

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. Thidiazuron: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 149A-153. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Experimental start date April 21, 2003 and experimental termination date April 25, 2003. The final report issued June 5, 2003.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Skeletonema costatum* were exposed to Thidiazuron Technical under static conditions at nominal concentrations of 0 (negative control), 0.16, 0.31, 0.63, 1.3, and 2.5 ppm Thidiazuron Technical. The day 0 measured concentrations were <0.100 (<LOQ, negative control), 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical. The cell density percent inhibition was -22, 83, -8.4, 86, and 89% in the 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical treatment groups, respectively. The area under the growth curve/biomass (0 to 96 hours) percent inhibition was -23, 73, -9.5, 75, and 69% in the 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibition was -5.6, 51, -6.2, 50, and 48% in the 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical treatment groups, respectively. All endpoints were significantly inhibited at the 0.22 ppm treatment level, as well as at the 1.1 and 2.2 ppm treatment levels. Biomass was the most sensitive endpoint, with an EC_{50} of 0.86 ppm.

The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Skeletonema costatum*. This study is classified as **Core**.

Results Synopsis

Test Organism: *Skeletonema costatum*
Test Type: Static

Cell density:
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.31 ppm 95% C.I.: 0.038-2.6 ppm
EC_{50}/IC_{50}: 0.89 ppm 95% C.I.: 0.36-2.2 ppm
Slope: 3.63±2.26

Growth rates:
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.056 ppm 95% C.I.: 0.00017-18 ppm
EC_{50}/IC_{50}: 2.3 ppm 95% C.I.: 0.43-12 ppm
Slope: 1.02±0.745

Plant biomass (area under the growth curve):
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.071 ppm 95% C.I.: 0.00054-9.2 ppm
EC_{50}/IC_{50}: 0.86 ppm 95% C.I.: 0.16-4.5 ppm
Slope: 1.52±1.05

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines, OPPTS Number 850.5400, *Algal Toxicity, Tiers I and II*; 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.

This deviation did not affect the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the U.S. EPA 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 67.9-87.1% of the nominal concentrations and the 96-hour measured test concentrations were <LOQ and 28.3-45.7% 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 ambient conditions.

2. Test organism:

Name: *Skeletonema costatum*

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: CCMP 1332
Source: Originally from Provasoli-Guillard National Center for Culture of Marine Phytoplankton. Current in-house laboratory cultures.
Age of inoculum: ≥ 14 days
Method of cultivation: Saltwater 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 Saltwater 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	96 hours	*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.*

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):**	 Saltwater algal medium 8.0 7.7-8.2 Yes Stock nutrient solution 30 ppt	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)	Yes	See Appendix 2, p. 31
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 and artificial saltwater Filter -sterilized (0.22 µm) 8.1 30‰ pH adjusted with 10% HCl Not reported Not reported See Appendix 3, pp. 32-33 Not detected Not reported	 *EPA pH: Skeletonema costatum = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *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.*

Parameter	Details	Remarks *Criteria*
Number of replicates control: solvent control: treated ones:	 3 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.*
Test concentrations nominal: measured:	0 (negative control), 0.16, 0.31, 0.63, 1.3, and 2.5 ppm Thidiazuron Technical <0.100 (<LOQ, negative control), 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical	The measured concentrations were from day 0 samples. *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 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	 20.1-20.8°C 16 hours light/8 hours dark 3700-4000 lux, cool-white fluorescent light.	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*

Parameter	Details	Remarks *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).	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell counts using a electronic particle counter.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	

Parameters	Details	Remarks/Criteria
Indicate whether there was exponential growth in the control	Yes, dilution water control cell density at test termination was 82X greater than the dilution water control cell density at test initiation.	*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 inhibitions were -22, 83, -8.4, 86, and 89% in the 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical treatment groups, respectively. The area under the growth curve/biomass (0 to 96 hours) percent inhibitions were -23, 73, -9.5, 75, and 69% in the 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -5.6, 51, -6.2, 50, and 48% in the 0.11, 0.22, 0.51, 1.1, and 2.2 ppm Thidiazuron Technical treatment groups, respectively. The cell density, growth rates, and biomass were significantly different in the 0.22, 1.1 and 2.2 ppm Thidiazuron Technical treatment groups compared to the control. However, the 0.22 ppm Thidiazuron Technical effects were not considered to be treatment-related by the study authors due to the observed lack of concentration response.

Table 3: Effect of Thidiazuron on Marine Diatom (*Skeletonema costatum*)

Treatment day-0 mean-measured and nominal concentrations (ppm Thidiazuron Technical) [a]	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24-hours	96-hours	
		cell count	cell count	% inhibition[b]
Dilution water control	10,000	54302	821,218	--
0.11 (0.16)	10,000	61540	1,000,574	-22
0.22 (0.31)	10,000	48177	138,614*	83
0.51 (0.63)	10,000	66735	890,394	-8.4
1.1 (1.3)	10,000	49909	117,798**	86
2.2 (2.5)	10,000	55116	91,313**	89
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The percent inhibition was calculated by comparison of the treatment groups to the negative control. Negative percent inhibition indicates increased growth.
* Statistically significant, but not considered treatment-related by the study authors due to a lack of concentration response.
** Statistically significant difference ($p<0.05$) from the control using the Dunnett's test.

Table 4: Effect of Thidiazuron on Marine Diatom *(Skeletonema costatum)*

Treatment day-0 measured and Concentrations [a] (ppm Thidiazuron Technical)	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	0.0438	--	18,004,980	--
0.11 (0.16)	10,000	0.0463	-5.6	22,110,704	-23
0.22 (0.31)	10,000	0.0217*	51	4,922,528*	73
0.51 (0.63)	10,000	0.0466	-6.2	19,710,332	-9.5
1.1 (1.3)	10,000	0.0217**	50	4,560,672**	75
2.2 (2.5)	10,000	0.0228**	48	5,497,408**	69
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The percent inhibition was calculated by comparison of the treatment groups to the negative control. Negative percent inhibition indicates increased growth.
* Statistically significant, but not considered treatment-related by the study authors due to a lack of concentration response.
** 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 Thidiazuron Technical)	0.51	0.51	0.51
EC_{50} (ppm Thidiazuron Technical)	0.86	>2.2	0.90
IC_{50} or EC_{50} (ppm Thidiazuron Technical) (95% C.I.)	0.17->2.2	N/A	0.37-2.2
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.

B. REPORTED STATISTICS:

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

were determined using Dunnett's test. Non-linear regression was used to determine the 96-hour EC50. All toxicity values were determined via The SAS System for Windows statistical software and mean-measured day-0 treatment concentrations (pp. 16 and 18).

Cell density:
$NOEC/EC_{05}$: 0.51 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 0.90 ppm Thidiazuron Technical 95% C.I.: 0.37-2.2 ppm Thidiazuron Technical

Growth rates:
$NOEC/EC_{05}$: 0.51 ppm Thidiazuron Technical
EC_{50}/IC_{50}: >2.2 ppm Thidiazuron Technical 95% C.I.: N/A

Plant biomass (area under the growth curve):
$NOEC/EC_{05}$: 0.51 ppm Thidiazuron Technical
EC_{50}/IC_{50}: 0.86 ppm Thidiazuron Technical 95% C.I.: 0.17- >2.2 ppm Thidiazuron Technical

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, area under the growth curve (biomass), 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 via. The analyses described above were conducted using TOXSTAT statistical software and the day 0 measured concentrations were used for all calculations. The EC_{05} and EC_{50} values were determined using the Probit method via Nuthatch statistical software.

Cell density:
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.31 ppm 95% C.I.: 0.038-2.6 ppm
EC_{50}/IC_{50}: 0.89 ppm 95% C.I.: 0.36-2.2 ppm
Slope: 3.63±2.26

Growth rates:
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.056 ppm 95% C.I.: 0.00017-18 ppm
EC_{50}/IC_{50}: 2.3 ppm 95% C.I.: 0.43-12 ppm
Slope: 1.02±0.745

Plant biomass (area under the growth curve):
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.071 ppm 95% C.I.: 0.00054-9.2 ppm
EC_{50}/IC_{50}: 0.86 ppm 95% C.I.: 0.16-4.5 ppm
Slope: 1.52±1.05

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the EC_{50} values were identical to those of the study authors; however, the reviewer's NOEC estimate for all endpoints is lower than that of the study authors. The study authors dismissed the significant inhibition exhibited in the 0.22 treatment group because response at the next higher treatment level was

stimulated. The reviewer suspects that the inhibition at the 0.22 ppm treatment level may have been biologically significant (given the magnitude of the reduction and the similar inhibition exhibited by the two highest treatment levels). The reviewer-calculated NOEC values are reported for the purpose of risk assessment.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Skeletonema costatum*. This study is classified as Core.

Cell density:
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.31 ppm 95% C.I.: 0.038-2.6 ppm
EC_{50}/IC_{50}: 0.89 ppm 95% C.I.: 0.36-2.2 ppm
Slope: 3.63±2.26

Growth rates:
NOEC: 0.11 ppm Thidiazuron Technical
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EC_{50}/IC_{50}: 2.3 ppm 95% C.I.: 0.43-12 ppm
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Plant biomass (area under the growth curve):
NOEC: 0.11 ppm Thidiazuron Technical
EC_{05}: 0.071 ppm 95% C.I.: 0.00054-9.2 ppm
EC_{50}/IC_{50}: 0.86 ppm 95% C.I.: 0.16-4.5 ppm
Slope: 1.52±1.05

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

III. REFERENCES:

U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400, *Algal Toxicity, Tiers I and II.*

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.*

ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static 96-hour Toxicity Tests with Microalgae.* American Society for Testing and Materials. Philadelphia, Pennsylvania.

U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure, Acute Toxicity Test for Aquatic Plants. Hazard Evaluation Division. Office of Pesticide Programs, EPA 540/9-85-006. Washington, D.C.

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

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

U.S. Environmental Protection Agency. 1994. Pesticide Reregistration Rejection Rate Analysis. Ecological Effects. EPA 738-R-94-035.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density Thidiazuron:
File: 3505cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2847802606372.000	569560521274.000	5.249
Within (Error)	12	1302132366928.000	108511030577.375	
Total	17	4149934973300.000		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Cell density Thidiazuron
File: 3505cd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	821218.333	821218.333		
2	0.11	1000574.000	1000574.000	-0.667	
3	0.22	138614.000	138614.000	2.538	*
4	0.51	890393.667	890393.667	-0.257	
5	1.1	117798.000	117798.000	2.615	*
6	2.2	91313.333	91313.333	2.714	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Cell density Thidiazuron
File: 3505cd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.11	3	672405.602	81.9	-179355.667
3	0.22	3	672405.602	81.9	682604.333
4	0.51	3	672405.602	81.9	-69175.333
5	1.1	3	672405.602	81.9	703420.333
6	2.2	3	672405.602	81.9	729905.000

Cell density Thidiazuron
File: 3505cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED

	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	Neg control	3	821218.333	821218.333	910896.167
2	0.11	3	1000574.000	1000574.000	910896.167
3	0.22	3	138614.000	138614.000	514503.833
4	0.51	3	890393.667	890393.667	514503.833
5	1.1	3	117798.000	117798.000	117798.000
6	2.2	3	91313.333	91313.333	91313.333

Cell density Thidiazuron
File: 3505cd 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	910896.167				
0.11	910896.167	0.333		1.78	k= 1, v=12
0.22	514503.833	1.140		1.87	k= 2, v=12
0.51	514503.833	1.140		1.90	k= 3, v=12
1.1	117798.000	2.615	*	1.92	k= 4, v=12
2.2	91313.333	2.714	*	1.93	k= 5, v=12

s = 329410.125
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.31	0.038	2.6	0.43	0.12
EC10	0.39	0.064	2.4	0.37	0.16
EC25	0.58	0.15	2.2	0.28	0.26
EC50	0.89	0.36	2.2	0.18	0.41

Slope = 3.63 Std.Err. = 2.26

?[7m
!!!Poor fit: p = 0.015 based on DF= 3.0 12.
?[0m

Biomass Thidiazuron:
File: 3505bd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	10324211912048 64.000206484238241024.000		5.854
Within (Error)	12	423266452905984.00035272204408832.000		
Total	17	145568764411084 8.000		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Biomass Thidiazuron
File: 3505bd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	18004980.000	18004980.000		
2	0.11	22110704.000	22110704.000	-0.847	
3	0.22	4922528.000	4922528.000	2.698	*
4	0.51	19710332.000	19710332.000	-0.352	
5	1.1	4560672.000	4560672.000	2.772	*
6	2.2	5497408.000	5497408.000	2.579	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Biomass Thidiazuron
File: 3505bd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.11	3	12123016.059	67.3	-4105724.000
3	0.22	3	12123016.059	67.3	13082452.000
4	0.51	3	12123016.059	67.3	-1705352.000
5	1.1	3	12123016.059	67.3	13444308.000
6	2.2	3	12123016.059	67.3	12507572.000

Biomass Thidiazuron
File: 3505bd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	18004980.000	18004980.000	20057842.000
2	0.11	3	22110704.000	22110704.000	20057842.000
3	0.22	3	4922528.000	4922528.000	12316430.000
4	0.51	3	19710332.000	19710332.000	12316430.000
5	1.1	3	4560672.000	4560672.000	5029040.000
6	2.2	3	5497408.000	5497408.000	5029040.000

Biomass Thidiazuron
File: 3505bd 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	20057842.000				

```
                0.1120057842.000      0.423              1.78       k= 1, v=12
                0.2212316430.000      1.173              1.87       k= 2, v=12
                0.5112316430.000      1.173              1.90       k= 3, v=12
                 1.15029040.000       2.676      *       1.92       k= 4, v=12
                 2.25029040.000       2.676      *       1.93       k= 5, v=12
-------------------------------------------------------------------------------
s = 5939040.698
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            0.071   0.00054        9.2       0.99     0.0076
EC10           0.12    0.0020         7.4       0.83     0.017
EC25           0.31    0.018          5.3       0.58     0.058
EC50           0.86    0.16           4.5       0.34     0.19

         Slope =     1.52  Std.Err. =     1.05

?[7m
!!!Poor fit: p =    0.0055  based on DF=       3.0       12.
?[0m
```

Growth rate Thidiazuron:

```
File: 3505gd        Transform: NO TRANSFORMATION

                                ANOVA TABLE
-------------------------------------------------------------------------------

SOURCE              DF              SS                MS              F
-------------------------------------------------------------------------------
Between              5              0.0025          0.0005          5.000

Within (Error)      12              0.0009          0.0001
-------------------------------------------------------------------------------
Total               17              0.0034
-------------------------------------------------------------------------------

  Critical F value =   3.11  (0.05,5,12)
  Since  F > Critical F  REJECT  Ho:All groups equal


Growth rate Thidiazuron
File: 3505gd        Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2            Ho:Control<Treatment
-------------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  SIG
----- --------------------    ----------    ------------------  ------  ---
  1           Neg control       0.044             0.044
  2                  0.11       0.046             0.046        -0.302
  3                  0.22       0.022             0.022         2.711  *
  4                  0.51       0.047             0.047        -0.331
  5                   1.1       0.022             0.022         2.707  *
  6                   2.2       0.023             0.023         2.580  *
-------------------------------------------------------------------------------
Dunnett table value =  2.50     (1 Tailed Value, P=0.05,  df=12,5)


Growth rate Thidiazuron
File: 3505gd        Transform: NO TRANSFORMATION
```

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.11	3	0.020	46.6	-0.002
3	0.22	3	0.020	46.6	0.022
4	0.51	3	0.020	46.6	-0.003
5	1.1	3	0.020	46.6	0.022
6	2.2	3	0.020	46.6	0.021

Growth rate Thidiazuron
File: 3505gd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	0.044	0.044	0.045
2	0.11	3	0.046	0.046	0.045
3	0.22	3	0.022	0.022	0.034
4	0.51	3	0.047	0.047	0.034
5	1.1	3	0.022	0.022	0.022
6	2.2	3	0.023	0.023	0.022

Growth rate Thidiazuron
File: 3505gd 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	0.045				
0.11	0.045	0.181		1.78	k= 1, v=12
0.22	0.034	1.422		1.87	k= 2, v=12
0.51	0.034	1.422		1.90	k= 3, v=12
1.1	0.022	3.159	*	1.92	k= 4, v=12
2.2	0.022	3.159	*	1.93	k= 5, v=12

s = 0.008
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	0.056	0.00017	18.	1.2	0.0031
EC10	0.13	0.0013	12.	0.94	0.010
EC25	0.50	0.033	7.6	0.55	0.066
EC50	2.3	0.43	12.	0.34	0.19

Slope = 1.02 Std.Err. = 0.745

?[7m
!!!Poor fit: p = 0.0061 based on DF= 3.0 12. [0m