

Data Evaluation Report on the acute toxicity of Thidiazuron Technical to aquatic vascular plants *Lemna gibba*

PMRA Submission #: {.....}

EPA MRID#: 46203506

Data Requirement:
PMRA Data Code: {.....}
EPA DP Barcode: D294536
OECD Data Point: {.....}
EPA MRID: 46203506
EPA Guideline: 123-2

Test material: Thidiazuron Technical (a.i.) **Purity:** 99.5% w/w/ a.i.
Common name: Thidiazuron
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: 51707-55-2
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/28/2004

QC Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation

Signature:
Date: 5/3/2004

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


Date: 11/16/04

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 7-Day Static-Renewal Toxicity Test with Duckweed (*Lemna gibba*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 149A-154. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Experimental start date April 1, 2003 and experimental termination date April 8, 2003. The final report issued May 6, 2003.



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Secondary Reviewer(s): {.....}
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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater aquatic vascular plant Duckweed, *Lemna gibba* G3, was exposed to Thidiazuron technical at nominal concentrations of 0 (negative control), 0.26, 0.64, 1.6, 4.0, 10, and 25 ppm a.i. under static renewal conditions. The mean-measured concentrations were <0.100 (<LOQ, negative control), 0.21, 0.55, 1.2, 3.2, 8.6, and 24 ppm a.i. Frond count was inhibited -12, -17, 7.6, 17, 27, and 46% at the 0.21, 0.55, 1.2, 3.2, 8.6, and 24 ppm a.i. treatment levels, respectively, compared to the negative control. Growth rate was inhibited -4.8, -6.3, 2.8, 7.1, 12, and 25% at the 0.21, 0.55, 1.2, 3.2, 8.6, and 24 ppm a.i. treatment levels, respectively. Chlorotic fronds (98-100%) were observed at the 24 ppm a.i. treatment level. Treatment-related effects based on the lack of plants breaking apart (aggregation) was observed at all treatment levels, except for the control. The NOEC based on aggregation was <0.21 ppm a.i., the lowest concentration tested. The NOEC and EC₅₀ values based on frond count and growth rate were 3.2 and >24 ppm a.i., respectively.

This study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an acute toxicity aquatic plant growth study, Tier I. Consequently, this study is classified as **Core**.

Results Synopsis

Test Organism: *Lemna gibba* G3
Test Type: Static Renewal

Frond Count:

NOEC: 3.2 ppm a.i.
LOEC: 8.6 ppm a.i.
EC₀₅: 0.57 ppm a.i. 95% C.I.: 0.062-5.3 ppm a.i.
EC₅₀/IC₅₀: >24 ppm a.i. 95% C.I.: N/A
Slope: 1.04

Growth Rate (0-7 Day):

NOEC: 3.2 ppm a.i.
LOEC: 8.6 ppm a.i.
EC₀₅: Not reported 95% C.I.: N/A
EC₅₀/IC₅₀: >24 ppm a.i. 95% C.I.: N/A
Slope: N/A

Most Sensitive Endpoint: Frond count, based on the reviewer determined EC₀₅ value

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 850.4400 (*Draft*); ASTM Standard Guide 1415-91 E, 1991, Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3; and OECD Proposal for a New Guideline 221: *Lemna sp. Growth Inhibition Test*. The following deviations from U.S. EPA Guideline 123-2 were noted:

1. The dilution water total organic carbon, particulate matter and residual chlorine concentrations were not reported.
2. The definitive study light intensity, 6700-8110 lux, was higher than recommended, 4,200-5,800 lux.
2. Replicate data were not provided for growth rate.

These deviations did not affect the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. 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); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17); and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999.

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% w/w/ a.i.

Stability of Compound

Under Test Conditions: The new test concentrations (days 0, 2, and 6) were 81.3-118% of nominal concentrations and the old test concentrations were (days 2 and 7) were 38.0-94.1% of nominal concentrations.

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

Storage conditions of test chemicals: Stored under ambient conditions.

2. Test organism:

Name: Duckweed, *Lemna gibba* (EPA requires a vascular species: *Lemna gibba*)

Strain, if provided: G3

Source: Laboratory cultures (original supplier: United States Department of Agriculture)

Age of inoculum: ≥3 weeks old (p. 11)

Method of cultivation: 20X AAP culture 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

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Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	At least 3 weeks 20X AAP Medium; same as test. Actively growing.	
Test system static/static renewal/ renewal rate for static renewal:	Static Renewal Days 2, 4, and 6	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Environmental chamber	
Duration of the test	7 days	<i>EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.</i>
Test vessel material: (glass/polystyrene) size: fill volume:	Glass beakers 250 mL 100 mL	Test vessels covered with disposable petri dishes.
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP Medium 8.1-8.3 8.6-9.2 Yes NaHCO ₃	See Appendix 2, p. 30 <i>EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP.</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Well water, NANOpure [®] filtered, with reagent grade chemicals 7.5 Filter -sterilized (0.22 µm) and pH adjusted with 10% HCl Not reported Not reported <LOD <LOD Not reported	<i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>

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Parameter	Details	Remarks
		Criteria
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported.	
Sediment used (for rooted aquatic vascular plants) origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	3 N/A 3	
Number of plants/replicate	Four plants per replicate.	There were four plants for each treatment level. <i>EPA requires 5 plants.</i>
Number of fronds/plant	3 fronds per plant (12 total fronds per replicate)	<i>EPA requires 3 fronds per plant.</i>
Test concentrations nominal: measured:	0 (negative control), 0.26, 0.64, 1.6, 4.0, 10, and 25 ppm a.i. <0.100 (<LOQ, negative control), 0.21, 0.55, 1.2, 3.2, 8.6, and 24 ppm a.i.	<i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; Days 0, 2, and 6 (new solutions), and days 2 and 7 (old solutions).	
Test conditions temperature: photoperiod: light intensity and quality:	24.5-25.0°C continuous light 6700-8110 lux, warm-white fluorescent light	<i>EPA temperature: 25°C EPA photoperiod: continuous EPA light: 5.0 Klux (±15%)</i>
Reference chemical (if used) name: concentrations:	N/A	

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Parameter	Details	Remarks
		Criteria
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, growth rates, and toxicity symptoms.	
Measurement technique for frond number and other end points	Direct counts.	
Observation intervals	2, 4, 6, and 7 days.	
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes, frond number in the control on day 7 was approximately 12X the control frond number on day 0.	
Were raw data included?	Replicate data provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The percent inhibition of mean frond count was -12, -17, 7.6, 17, 27, and 46% at the mean-measured 0.21, 0.55, 1.2, 3.2, 8.6, and 24 ppm a.i. treatment levels, respectively, compared to the control. The percent inhibition of frond growth rate was -4.8, -6.3, 2.8, 7.1, 12, and 25% at the 0.21, 0.55, 1.2, 3.2, 8.6, and 24 ppm a.i. treatment levels, respectively. Chlorotic fronds (98-100%) were observed in the 24 ppm a.i. treatment level. There were treatment-related effects on the lack of plants breaking apart (aggregation) in all treatment groups, except for the control. The NOEC based on aggregation was <0.21 ppm a.i.. The frond count and growth rate NOEC values were 8.6 and 3.2 ppm a.i., respectively. The study authors reported that aggregation (lack of plants breaking apart) was observed at the 0.21, 0.55, 1.2 and 3.2 ppm a.i. treatment levels. Therefore, the 7-day ZNOEC based on the observations of plant aggregations was <0.21 ppm a.i., and the NOAEC was 3.2 ppm a.i.

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Table 3: Effect of Thidiazuron Technical on frond number of Duckweed, *Lemna gibba*

Treatment Mean-Measured (Nominal) Concentrations ppm a.i.	Initial Frond Number/Test Solution	Mean Live Frond Number at:				Day 7 Growth Rate	
		2 days	4 days	7 days	% Inhibition at 7 days ^a	Mean	% Inhibition
Negative control (dilution water)	12	17	42	144	---	0.354	--
0.21 (0.26)	12	21	43	162	-12	0.371	-4.8
0.55 (0.64)	12	22	48	169	-17	0.376	-6.3
1.2 (1.6)	12	20	41	133	7.6	0.344	2.8
3.2 (4.0)	12	16	37	120	17	0.328	7.1
8.6 (10)	12	18	36	106	27	0.311*	12
24 (25)	12	17	36	77*	46	0.266*	25
Reference chemical (if used)	N/A						

^a The treatment groups were compared to the control for day-7 number of live fronds and growth rates.

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

Table 4: Statistical endpoint values.

Statistical Endpoint ^a	Frond Count	Growth rate
NOEC or EC ₀₅ (ppm a.i.)	8.6	3.2
LOEC (ppm a.i.)	24	8.6
EC ₅₀ (ppm a.i.) (95% C.I.)	>24	>24
EC ₂₅ (ppm a.i.) (95% C.I.)	Not reported	Not reported
Reference chemical NOAEC IC ₂₅ /EC ₂₅	Not applicable	Not applicable

^a Results are based on mean-measured test concentrations.

B. REPORTED STATISTICS:

The growth rate formula is found on page 15. Percent inhibition was determined for all endpoints (frond number and growth rate). Data were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. The 7-day NOEC, and LOEC values were determined using ANOVA and Dunnett's test. The 7-day EC50 value was empirically determined for both endpoints points because a 50% reduction in frond count or growth rate was not observed at any treatment level by 7-days. All toxicity values were determined using the mean-measured treatment concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

After confirming normality and homogeneity of variances, NOEC and LOEC values were determined based on day-7 live frond count data using ANOVA and William's multiple comparison test via TOXSTAT statistical software. An EC₀₅ value based on frond count data was determined using the Probit method via Nuthatch statistical software. The EC₅₀ value based on frond count data was empirically-determined due to a lack of 50% reduction by 7-days at any treatment level. The reviewer was unable to statistically determine/verify EC₀₅, EC₅₀, NOEC and LOEC values for the growth rate endpoint because replicate data were not provided. Consequently, the study authors' reported toxicity values for growth rate are reported in the Executive Summary and Conclusion sections of this DER.

FronD Count:

NOEC: 3.2 ppm a.i.
LOEC: 8.6 ppm a.i.
EC₀₅: 0.57 ppm a.i. 95% C.I.: 0.062-5.3 ppm a.i.
EC₅₀/IC₅₀: > 24 ppm a.i. 95% C.I.: N/A
Slope: 1.04

Growth Rate (0-7 Day):

NOEC: Not determined
LOEC: Not determined
EC₀₅: Not determined 95% C.I.: Not determined
EC₅₀/IC₅₀: Not determined 95% C.I.: Not determined
Slope: Not determined

Most Sensitive Endpoint: Frond count

D. STUDY DEFICIENCIES:

All deficiencies were considered minor and did not affect the acceptability or validity of the definitive study.

E. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification were similar to those of the study authors'. The reviewer determined the NOEC and LOEC values (3.2 and 8.6 ppm a.i., respectively) based on frond count data to be one treatment level lower than those of the study authors' (8.6 and 24 ppm a.i., respectively), presumably due to the statistical methods used. The reviewer determined NOEC, LOEC and EC₀₅ values based on frond count data is reported in the Executive Summary and Conclusion sections of this DER because they are a more conservative estimate of the toxicity of Thidiazuron Technical to *Lemna gibba*. The reviewer's and study authors' EC₅₀ value based on frond count data were identical. The reviewer was unable to statistically determine/verify EC₀₅, EC₅₀, NOEC and LOEC values for the growth rate endpoint because replicate data were not provided. Consequently, the study authors' reported toxicity values for growth rate are reported in the Executive Summary and Conclusion sections of this DER.

F. CONCLUSIONS:

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an acute aquatic plant growth study, Tier II. Consequently, this study is classified as **Core**.

FronD Count:

NOEC: 3.2 ppm a.i.
LOEC: 8.6 ppm a.i.

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EC₀₅: 0.57 ppm a.i. 95% C.I.: 0.062-5.3 ppm a.i.
EC₅₀/IC₅₀: > 24 ppm a.i. 95% C.I.: N/A
Slope: 1.04

Growth Rate (0-7 Day):

NOEC: 3.2 ppm a.i.
LOEC: 8.6 ppm a.i.
EC₀₅: Not reported 95% C.I.: N/A
EC₅₀/IC₅₀: >24 ppm a.i. 95% C.I.: N/A
Slope: N/A

Most Sensitive Endpoint: Frond count, based on the reviewer determined EC₀₅ value.

III. REFERENCES:

- U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (*draft*). OPPTS Number: *Aquatic Plant Toxicity Test Using Lemna spp., Tiers I and II*. Washington, D.C.
- ASTM Standard Guide 1415-91E. 1991. *Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3*. American Society for Testing and Materials. Philadelphia, Pennsylvania.
- Organization for Economic Cooperation and Development. October 2000. *Lemna* sp. Growth Inhibition Test. Proposal for a new Guideline 221.
- Microsoft Corporation. Microsoft Excel 2000. Copyright 1985-1999.
- West, Inc. and Gulley, D.D. 1996. TOXSTAT Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

FronD count (live)

File: 3506fd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	18575.905	3095.984	8.295
Within (Error)	14	5225.333	373.238	
Total	20	23801.238		

Critical F value = 2.85 (0.05,6,14)
 Since F > Critical F REJECT Ho:All groups equal

FronD count (live)

File: 3506fd 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	144.333	144.333		
2	0.21	162.000	162.000	-1.120	
3	0.55	168.667	168.667	-1.543	
4	1.2	133.333	133.333	0.697	
5	3.2	119.667	119.667	1.564	
6	8.6	106.000	106.000	2.430	
7	24	77.333	77.333	4.247	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

FronD count (live)

File: 3506fd 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	0.21	3	39.909	27.7	-17.667
3	0.55	3	39.909	27.7	-24.333
4	1.2	3	39.909	27.7	11.000
5	3.2	3	39.909	27.7	24.667
6	8.6	3	39.909	27.7	38.333
7	24	3	39.909	27.7	67.000

FronD count (live)

File: 3506fd Transform: NO TRANSFORMATION

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	144.333	144.333	158.333
2	0.21	3	162.000	162.000	158.333
3	0.55	3	168.667	168.667	158.333
4	1.2	3	133.333	133.333	133.333
5	3.2	3	119.667	119.667	119.667
6	8.6	3	106.000	106.000	106.000
7	24	3	77.333	77.333	77.333

FronD count (live)
File: 3506fd 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	158.333				
0.21	158.333	0.888		1.76	k= 1, v=14
0.55	158.333	0.888		1.85	k= 2, v=14
1.2	133.333	0.697		1.88	k= 3, v=14
3.2	119.667	1.564		1.89	k= 4, v=14
8.6	106.000	2.430	*	1.90	k= 5, v=14
24	77.333	4.247	*	1.91	k= 6, v=14

s = 19.319

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	0.57	0.062	5.3	0.46	0.11
EC10	1.3	0.22	7.5	0.36	0.17
EC25	4.9	1.8	14.	0.21	0.36
EC50	22.	12.	40.	0.12	0.55

Slope = 1.04 Std.Err. = 0.298

Goodness of fit: p = 0.27 based on DF= 4.0 14.

3506FD : FronD count (live)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	144.	157.	-12.9	100.	0.00
0.210	3.00	162.	154.	7.57	98.2	1.80
0.550	3.00	169.	150.	19.0	95.2	4.81
1.20	3.00	133.	142.	-9.02	90.5	9.48

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3.20	3.00	120.	127.	-7.35	80.8	19.2
8.60	3.00	106.	104.	1.58	66.4	33.6
24.0	3.00	77.3	76.2	1.17	48.4	51.6