Data Evaluation Report on the Acute Toxicity of Thidiazuron Technical to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number {.....} EPA MRID Number 46203503

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D294536
OECD Data Point	
EPA MRID	46203503
EPA Guideline	§72-2

Test material: Thidiazuron Technical
Common name: Thidiazuron
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: 51707-55-2
Synonyms: AE B049537

Purity: 99.5% (w:w)

Primary Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/04

QC Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/22/04

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Date: 5/26/04

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

Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 120301

Date Evaluation Completed:

CITATION: Blankinship, A.S., *et al.* 2003. Thidiazuron: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 149A-151. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Study initiated February 18, 2003 and completed May 7, 2003.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Thidiazuron Technical to the Cladoceran, *Daphnia magna*, was studied under static conditions. Neonate (<24-hour old) daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 0.31, 0.63, 1.3, 2.5, 5.0, or 10 ppm. Mean-measured concentrations were <0.201 (<LOQ, control), 0.30, 0.60, 1.2, 2.3, 4.7, and 9.5 ppm a.i.

By 48 hours, mortality/immobility was 5% in the control group, 0% in the 0.30 through 2.3 ppm a.i. groups, 25% in the 4.7 ppm a.i. group, and 100% in the 9.5 ppm a.i. group. The 48-hour LC_{50}/EC_{50} (with 95% C.I.) was 5.7 (4.7-9.5) ppm a.i., which categorizes Thidiazuron Technical as moderately toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. Lethargy was observed in 100% of surviving daphnids from the 9.5 ppm a.i. group after 24 hours, and in 5 and 40% of surviving daphnids from the 2.3 and 4.7 ppm a.i. groups, respectively, after 48 hours (100% mortality was observed at 9.5 ppm a.i. by 48 hours). The 48-hour NOEC and LOEC values were 1.2 and 2.3 ppm a.i., based on sub-lethal effects data (most sensitive endpoint).

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2). This study is classified as CORE.

Results Synopsis

Test Organism Age (eg. 1st instar): Neonates, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour
LC_{50}/EC_{50}: 5.7 ppm a.i. 95% C.I.: 4.7-9.5 ppm a.i.
NOEC: 1.2 ppm a.i. (based on sub-lethal effects)
LOEC: 2.3 ppm a.i.
Endpoints affected: Mortality/immobility and sub-lethal effects
Most sensitive endpoint: Sub-lethal effects

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OECD Guideline No. 202 (1984); the U.S. EPA OPPTS No. 850.1010 (Draft, 1996); and ASTM Standard E729-88a (1994). Deviations from U.S. EPA §72-2 included:

1. Pre-test mortality of the laboratory culture and/or brood was not described.
2. The biomass loading rate was not specified.
3. The water hardness (136 mg/L as $CaCO_3$) was nearly three times higher than recommended (40-48 mg/L as $CaCO_3$).
4. The pH range (8.4-8.7) was greater than recommended (7.2-7.6).
5. Aeration of the test vessels was not addressed.

These deviations did not affect the acceptability or validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160 and 192), OECD (ENV/MC/CHEM (98)17), and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999; p. 3).

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 stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination at 0 and 48 hours. Recoveries were 91.9-96.8% of nominal concentrations at 0 hours and 90.2-98.6% of nominal at 48 hours (Table 1, p. 17).

Storage conditions of test chemicals: Stored at room temperature.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. **Test organism:**

Species: *Daphnia magna*

Age at test initiation: Neonates, <24 hours old

Source: In-house laboratory cultures; neonates were obtained from five individual adult daphnids.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive nominal test concentrations were selected in consultation with the sponsor, and were based upon the results of an exploratory range-finding toxicity test. The results of the range-finding study were not reported (p. 9).

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous laboratory cultures were maintained (at least 13 days).	
Conditions: (same as test or not)	Same as test	
Feeding:	*Daphnia* cultures were fed a mixture of yeast, Cerophyll and trout chow with a suspension of the freshwater green alga, *Selenastrum capricornutum*.	*EPA requires 7 day minimum acclimation period.*
Health: (any mortality observed)	No signs of disease or stress.	
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through	Static	
Type of dilution system (for flow through method)	N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Renewal rate (for static renewal)	N/A	
Aeration, if any	Not reported.	
Test vessel Material: *(glass/stainless steel)*	Glass beakers	
Size: Fill volume:	250 mL 200 mL	*EPA requires: size 250 ml or 3.9 L fill 200 ml*

Parameter	Details	Remarks *Criteria*
Source of dilution water	The dilution water was freshwater obtained from an on-site laboratory well (40-m deep). The well water was sand filtered, UV irradiated and aerated prior to use.	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 136 mg/L as $CaCO_3$ 8.4-8.7 8.2-8.4 mg/L (≥93% saturation) 19.5-20.8°C <1.0 mg/L Not reported See Appendix 3, p. 26. <LOD Not reported	The hardness and pH were higher than recommended. Results of the analysis of the well water on July 31, 2002 for pesticides, organics, and metals are provided in Appendix 3, pp. 25-26. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 24 hr and ≥ 40% during 2nd 24 hr* *Flow-through: ≥60%*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 20 20	The biomass loading rate was not specified. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*
Number of replicates Solvent control: Negative control: Treatments:	 N/A 2 2	

Parameter	Details	Remarks *Criteria*
Treatment concentrations nominal: measured:	0 (negative control), 0.31, 0.63, 1.3, 2.5, 5.0 and 10 ppm <0.201 (<LOQ, control), 0.30, 0.60, 1.2, 2.3, 4.7 and 9.5 ppm a.i.	Mean-measured concentrations are provided in Table 1, p. 17. Concentrations were stable during the 48-hour study. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	N/A	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark with a 30-minute transition period.	Light intensity was approximately 203 lux at test initiation (p. 13). *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. Recoveries were 91.9-96.8% of nominal concentrations at 0 hours and 90.2-98.6% of nominal at 48 hours (Table 1, p. 17).	
Recovery of chemical Level of Quantitation Level of Detection	96.0-102% of nominal 0.201 ppm a.i. Not reported	Based on quality control matrix spikes fortified at 0.300, 1.50, or 10.0 ppm and analyzed concurrently with the samples (Appendix 4.5, p. 32).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects	Mortality/immobility and sub-lethal effects	
Observation intervals	After 2, 24, and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY

By 48 hours, mortality/immobility was 5% in the control group, 0% in the 0.30 through 2.3 ppm a.i. groups, 25% in the 4.7 ppm a.i. group, and 100% in the 9.5 ppm a.i. group (Table 4, p. 20). The 48-hour EC_{50} (with 95% C.I.) was 5.7 (4.7-9.5) ppm a.i. (Table 5, p. 21).

Table 3: Effects of Thidiazuron Technical on mortality/immobilization of *Daphnia magna*.

Treatment, ppm a.i. Measured and (nominal) concn.	No. of organisms	Observation period					
		2 Hours		24 Hours		48 Hours	
		No.	%	No.	%	No.	%
Negative Control	20	0	0	0	0	1	5
0.30 (0.31)	20	0	0	0	0	0	0
0.60 (0.63)	20	0	0	0	0	0	0
1.2 (1.3)	20	0	0	0	0	0	0
2.3 (2.5)	20	0	0	0	0	0	0
4.7 (5.0)	20	0	0	0	0	5	25
9.5 (10.0)	20	0	0	16	80	20	100
NOEC, ppm a.i.		Not reported		Not reported		Not reported	
LOEC, ppm a.i.		Not reported		Not reported		Not reported	
LC/EC_{50} (95% C.I.), ppm a.i.		Not reported		7.6 (4.7-9.5)		5.7 (4.7-9.5)	

B. SUB-LETHAL TOXICITY ENDPOINTS:

No signs of toxicity were observed after 2 hours of exposure at any test level (Table 4, p. 20). After 24 hours, all surviving daphnids from the 9.5 ppm a.i. group were lethargic. After 48 hours, 1/20 surviving daphnids from the 2.3 ppm a.i. group and 6/15 surviving daphnids from the 4.7 ppm a.i. group were lethargic, and all daphnids from the 9.5 ppm a.i. group were either dead or immobile.

Table 4: Sub-lethal Effects of Thidiazuron Technical on *Daphnia magna*.

Treatment, ppm a.i. Measured and (nominal) concn.	Observation period			
	24 hours		48 hours	
	endpoint	% affected[a]	endpoint	% affected[a]
Negative Control	Appear normal	0	Appear normal	0
0.30 (0.31)	Appear normal	0	Appear normal	0
0.60 (0.63)	Appear normal	0	Appear normal	0
1.2 (1.3)	Appear normal	0	Appear normal	0
2.3 (2.5)	Appear normal	0	Lethargic	5
4.7 (5.0)	Appear normal	0	Lethargic	40
9.5 (10.0)	Lethargic	100	—	—
NOEC, ppm a.i.	Not determined		Not determined	
LOEC, ppm a.i.	Not determined		Not determined	
EC_{50} (95% C.I.), ppm a.i.	Not determined		Not determined	

[a] The percent of affected daphnia was reviewer-calculated from number affected based on number of surviving daphnids.
— 100% Mortality/immobility

C. REPORTED STATISTICS:

The LC_{50}/EC_{50} values for mortality/immobility (with 95% C.I.) were calculated using the binomial probability method (Stephan, C.E., 1978) and mean-measured concentrations (p. 14). The study authors did not report NOEC or LOEC values, or an EC_{50} value based on sub-lethal effects.

48-Hour
LC_{50}/EC_{50}: 5.7 ppm a.i. 95% C.I.: 4.7-9.5 ppm a.i.
NOEC: Not determined
LOEC: Not determined

D. VERIFICATION OF STATISTICAL RESULTS:

The 48-hour LC_{50} was determined for dead/immobile daphnids using the binomial probability method via TOXANAL statistical software, as this method provided a sound 95% confidence interval, compared to the probit and moving average methods. A NOEC was determined using Fisher's Exact Test via TOXSTAT statistical software based on mortality/immobility data. A NOEC was also visually determined as the highest concentration which exhibited no sub-lethal effects, the more sensitive endpoint. All toxicity values were determined in terms of the reported mean-measured treatment concentrations.

48-Hour
LC_{50}/EC_{50}: 5.7 ppm a.i. 95% C.I.: 4.7-9.5 ppm a.i.
NOEC: 1.2 ppm a.i. (based on sub-lethal effects)
LOEC: 2.3 ppm a.i.
Endpoints affected: Mortality/immobility and sub-lethal effects
Most sensitive endpoint: Sub-lethal effects

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-2 that affected the acceptability or validity of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those reported by the study authors. However, the study authors did not report a NOEC or LOEC value based on mortality/immobility or sub-lethal effects, therefore the reviewer determined these values using appropriate statistical methods. Consequently, the reviewer determined NOEC and LOEC values reported in the Conclusion and Executive Summary sections of this DER are based on the reviewer's statistical verification and the reported sub-lethal effects data.

The test solutions appeared clear and colorless at test initiation and termination (p. 11).

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-2, and is classified as CORE. Based on the results of this study, Thidiazuron Technical is categorized as moderately toxic to the Cladoceran, *Daphnia magna*, on an acute toxicity basis. The 48-hour NOEC and LOEC values were 1.2 and 2.3 ppm a.i., respectively, based on sub-lethal effects data , the most sensitive endpoint.

48-Hour
LC_{50}/EC_{50}: 5.7 ppm a.i. 95% C.I.: 4.7-9.5 ppm a.i.
NOEC: 1.2 ppm a.i. (based on sub-lethal effects)
LOEC: 2.3 ppm a.i.
Endpoints affected: Mortality/immobility and sub-lethal effects
Most sensitive endpoint: Sub-lethal effects

III. REFERENCES:

Organization for Economic Cooperation and Development. 1984. Guideline 202: *Daphnia sp. Acute Immobilisation Test and Reproduction Test*. OECD Guideline for Testing of Chemicals. Updated Guideline,

adopted April, 1984.

U.S. Environmental Protection Agency. 1996. *Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids.* Series 850 - Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1010.

ASTM Standard E729-88a. 1994. *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*. American Society for Testing and Materials.

APHA, AWWA, WPCF. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

Stephan, C.E. 1978. U.S. EPA. Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

Thompson, W.R. 1947. *Bacteriological Reviews*. Vol. II, No. 2. Pp. 115-145.

Finney, D.J. 1971. *Statistical Methods in Biological Assay*. Second edition. Griffin Press, London.

Stephan, C.E. 1977. Methods for Calculating and LC50, *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

TOXANAL RESULTS: Calculated using the reported mean measured concentrations (Table 3, p. 19).

LC50:

9.5	20	20	100	9.536742E-05
4.7	20	5	25	2.069473
2.3	20	0	0	9.536742E-05
1.2	20	0	0	9.536742E-05
.6	20	0	0	9.536742E-05
.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT **4.7 AND 9.5** CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE **LC50** FOR THIS SET OF DATA IS **5.679563**

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

TOXSTAT RESULTS: NOEC Determination based on mortality/immobilization data via Fisher's Exact Test.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	1	
1	0.3	20	0	
2	0.6	20	0	
3	1.2	20	0	
4	2.3	20	0	
5	**4.7**	**20**	**5**	
6	9.5	20	20	*