Data Evaluation Report on the Acute Toxicity of AE F132347 (Metabolite of Thidiazuron) to Freshwater Invertebrates - *Daphnia magna*
PMRA Submission Number{.....} EPA MRID Number 46203509

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

Test material: AE F132347 **Purity:** 97.4% (w:w)
Common name: Metabolite of thidiazuron
Chemical name: IUPAC: 1-Phenyl-3-(1,2,5-thiadiazol-3-yl)urea
CAS name: Not reported
CAS No.: Not reported
Synonyms: None reported

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Staff Scientist, Dynamac Corporation
Signature:
Date: 4/1/04

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Secondary Reviewer(s):
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Date:

Reference/Submission No.:

Company Code:
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Date Evaluation Completed:

CITATION: Blankinship, A.S., *et al.* 2003. AE F132347: 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 Unpublished study performed by Wildlife International, Ltd., Easton, MD.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-155. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Study initiated April 8, 2003 and completed June 30, 2003.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of AE F132347 (a metabolite of thidiazuron) 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 and solvent controls), 1.3, 2.5, 5.0, 10, or 20 ppm. Mean-measured concentrations were <0.600 (<LOQ, control), 1.3, 2.6, 5.1, 9.6, and 12 ppm a.i.

By 48 hours, mortality/immobility was 0% in both control groups and the 1.3 through 5.1 ppm a.i. groups, 10% in the 9.6 ppm a.i. group, and 5% in the 12 ppm a.i. group. The 48-hour EC_{50} was >12 ppm a.i., the highest reasonably attainable concentration, which categorizes AE F132347 as slightly toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. Lethargy was observed in 6 and 5% of surviving daphnids from the 9.6 and 12 ppm a.i. groups, respectively, after 48 hours. The 48-hour NOEC and LOEC values were 5.1 and 9.6 ppm a.i., based on mortality and sub-lethal effects data (same conclusions).

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}: >12 ppm a.i. 95% C.I.: N/A
NOEC: 5.1 ppm a.i.
LOEC: 9.6 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

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 (140 mg/L as $CaCO_3$) was three times higher than recommended (40-48 mg/L as $CaCO_3$).
4. The pH range (8.3-8.7) 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 AE F132347 (a metabolite of thidiazuron)

Description: Rust-colored powder

Lot No./Batch No.: GMT 216P (Product code: AE F132347 00 1B97 0001)

Purity: 97.4% (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. At the nominal 1.3 through 10 ppm test levels, recoveries were 97.6-107% of nominal concentrations at 0 hours and 93.8-104% of nominal at 48 hours (Table 1, p. 17). At the nominal 20 ppm test level, the recovery was 59.3% at 0 hours and 62.2-63.2% at 48 hours, indicating stability in solution.

Storage conditions of test chemicals: Stored frozen.

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 14 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	 140 mg/L as $CaCO_3$ 8.3-8.7 8.3-8.5 mg/L (≥92% saturation) 19.5-21.0°C <1.0 mg/L Not reported See Appendix 3, p. 27. <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. 26-27. *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:	 20 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:	 2 2 2	

Parameter	Details	Remarks *Criteria*
Treatment concentrations nominal: measured:	0 (negative and solvent controls), 1.3, 2.5, 5.0, 10, and 20 ppm <0.600 (<LOQ, controls), 1.3, 2.6, 5.1, 9.6, and 12 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)	Dimethyl formamide (DMF), 0.1 mL/L	*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. At the nominal 1.3 through 10 ppm test levels, recoveries were 93.8-107% of nominal concentrations, and at the nominal 20 ppm level, recoveries were 59.3-63.2% of the nominal concentration, with no evidence of instability (Table 1, p. 17).	Low recoveries were observed at the nominal 20 ppm level; however, the concentrations were consistent over time.
Recovery of chemical Level of Quantitation Level of Detection	97.6-100% of nominal 0.600 ppm a.i. Not reported	Based on quality control matrix spikes fortified at 1.00, 5.00, or 20.0 ppm and analyzed concurrently with the samples (Appendix 4.5, p. 33).
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 0% in both control groups and the 1.3 through 5.1 ppm a.i. groups, 10% in the 9.6 ppm a.i. group, and 5% in the 12 ppm a.i. group (Table 4, p. 20). The 48-hour EC_{50} was >12 ppm a.i. (Table 5, p. 21).

Table 3: Effects of AE F132347 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	0	0
Solvent Control	20	0	0	0	0	0	0
1.3 (1.3)	20	0	0	0	0	0	0
2.6 (2.5)	20	0	0	0	0	0	0
5.1 (5.0)	20	0	0	0	0	0	0
9.6 (10)	20	0	0	0	0	2	10
12 (20)	20	0	0	0	0	1	5
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		>12		>12	

B. SUB-LETHAL TOXICITY ENDPOINTS:

No signs of toxicity were observed up through 24 hours of exposure at any test level (Table 4, p. 20). After 48 hours, lethargy was observed in 1/18 surviving daphnids from the 9.6 ppm a.i. group and in 1/19 surviving daphnids from the 12 ppm a.i. group.

Table 4: Sub-lethal Effects of AE F132347 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
Solvent Control	Appear normal	0	Appear normal	0
1.3 (1.3)	Appear normal	0	Appear normal	0
2.6 (2.5)	Appear normal	0	Appear normal	0
5.1 (5.0)	Appear normal	0	Appear normal	0
9.6 (10)	Appear normal	0	Lethargic	6
12 (20)	Appear normal	0	Lethargic	5
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.

C. REPORTED STATISTICS:

Due to a lack of 50% mortality or immobility at any treatment level by 48-hours, the LC_{50}/EC_{50} value was empirically estimated to be grater than the highest treatment level (p. 14). The no-observed-effect-concentration (NOEC) was determined by visual interpretation of the mortality, immobility and sub-lethal effects data. All toxicity values were reported in terms of the mean-measured treatment concentrations.

48-Hour
LC_{50}/EC_{50}: >12 ppm a.i. 95% C.I.: N/A
NOEC: 5.1 ppm a.i.
LOEC: 9.6 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

D. VERIFICATION OF STATISTICAL RESULTS:

The 48-hour LC_{50}/EC_{50} was determined visually due to a lack of 50% mortality/immobility at any treatment level. The NOEC was visually determined as the highest concentration which exhibited no significant (<10%) mortality/immobility and sub-lethal effects. All toxicity values were determined in terms of the reported mean-measured treatment concentrations.

48-Hour
LC_{50}/EC_{50}: >12 ppm a.i. 95% C.I.: N/A
NOEC: 5.1 ppm a.i.
LOEC: 9.6 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

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.

The nominal 1.3 and 2.5 ppm test solutions appeared clear and colorless at test initiation and termination (p. 12). The 5.0 ppm test solution was clear and colorless with some white particles on the surface at test initiation, and was clear and colorless at test termination. At test initiation, the 10 and 20 ppm test solutions were colorless with rust colored particles throughout, increasing in amount with increasing concentration. By test termination, the solutions were clear and colorless, with particles on the bottom of the test chambers, increasing in amount with increasing concentration. The study authors also noted that the nominal 20 ppm (mean-measured 12 ppm a.i.) treatment concentration was the highest tested concentration due to the limit of solubility of the test material (p. 8). Based on the above statements, the reviewer concludes that the definitive test was performed as a "best effort" at or above the limit of test material solubility.

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, AE F132347 (a metabolite of thidiazuron) is categorized as slightly toxic to the Cladoceran, *Daphnia magna*, on an acute toxicity basis. The 48-hour NOEC and LOEC values were 5.1 and 9.6 ppm a.i., respectively, based on both mortality and sub-lethal effects data (same conclusions).

48-Hour
LC_{50}/EC_{50}: >12 ppm a.i. 95% C.I.: N/A
NOEC: 5.1 ppm a.i.
LOEC: 9.6 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

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.