Data Evaluation Report on the Acute Toxicity of AE F132345 (Metabolite of Thidiazuron) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 46203516

Data Requirement:	PMRA DATA CODE	
	EPA DP Barcode	D294536
	OECD Data Point	
	EPA MRID	46203516
	EPA Guideline	§72-2

Test material:	AE F132345	**Purity:** 91% (w:w)
Common name:	Metabolite of thidiazuron	
Chemical name:	IUPAC: 1,2,3-Thiadiazol-5-ylurea	
	CAS name: Not reported	
	CAS No.: Not reported	
	Synonyms: None reported	

Primary Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 4/1/04

QC Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature:
Date: 4/23/04

Primary Reviewer: Bill Evans, Biologist
OPP/EFED/ERB - I
Date: 11/17/04

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

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 120301

Date Evaluation Completed:

CITATION: Palmer, S.J., *et al.* 2003. AE F132345: 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 Unpublished study performed by Wildlife International, Ltd., Easton, MD.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-149. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Study initiated February 18, 2003 and completed October 6, 2003.

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EXECUTIVE SUMMARY:

The 48-hour acute toxicity of AE F132345 (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 control), 6.3, 13, 25, 50, or 100 ppm. Mean-measured concentrations were <4.00 (<LOQ, control), 5.9, 12, 24, 49, and 98 ppm a.i.

By 48 hours, mortality/immobility was 0% in the control group and the 5.9 and 12 ppm a.i. groups, 15% in the 24 ppm a.i. group, 35% in the 49 ppm a.i. group, and 45% in the 98 ppm a.i. group. The 48-hour EC_{50} was >98 ppm a.i., which categorizes AE F132345 as slightly toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. Lethargy was observed in 15, 30, and 25% of surviving daphnids from the 24, 49, and 98 ppm a.i. groups, respectively, after 24 hours, and in 12 and 18% of surviving daphnids from the 24 and 98 ppm a.i. groups, respectively, after 48 hours. The 48-hour NOEC and LOEC values were 12 and 24 ppm a.i., based on mortality/immobility 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}: >98 ppm a.i. 95% C.I.: N/A
NOEC: 12 ppm a.i.
LOEC: 24 ppm a.i.
Endpoints affected: Mortality/immobility 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 (124 mg/L as $CaCO_3$) was nearly three times higher than recommended (40-48 mg/L as $CaCO_3$).
4. The pH range (8.4-8.6) 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 F132345 (a metabolite of thidiazuron)

Description: Light yellow powder

Lot No./Batch No.: JV0585+JV0585A (Product code: AE F132345 00 1C91 0001)

Purity: 91% (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 96.4-99.7% of nominal concentrations at 0 hours and 91.9-98.1% of nominal at 48 hours (Table 1, p. 17).

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 four 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	 124 mg/L as $CaCO_3$ 8.4-8.6 8.0-8.3 mg/L (≥89% saturation) 19.5-20.6°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:	 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), 6.3, 13, 25, 50, and 100 ppm <4.00 (<LOQ, control), 5.9, 12, 24, 49, and 98 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 96.4-99.7% of nominal concentrations at 0 hours and 91.9-98.1% of nominal at 48 hours (Table 1, p. 17).	
Recovery of chemical Level of Quantitation Level of Detection	99.6-100% of nominal 4.00 ppm a.i. Not reported	Based on quality control matrix spikes fortified at 6.00, 25.0, or 100 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 4, 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 the control group and the 5.9 and 12 ppm a.i. groups, 15% in the 24 ppm a.i. group, 35% in the 49 ppm a.i. group, and 45% in the 98 ppm a.i. group (Table 4, p. 20). The 48-hour EC_{50} was >98 ppm a.i. (Table 5, p. 21).

Table 3: Effects of AE F132345 on mortality/immobilization of *Daphnia magna*.

Treatment, ppm a.i. Measured and (nominal) concn.	No. of organisms	Observation period					
		3.5 Hours		24 Hours		48 Hours	
		No.	%	No.	%	No.	%
Negative Control	20	0	0	0	0	0	0
5.9 (6.3)	20	0	0	0	0	0	0
12 (13)	20	0	0	0	0	0	0
24 (25)	20	0	0	0	0	3	15
49 (50)	20	0	0	0	0	7	35
98 (100)	20	0	0	0	0	9	45
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		>98		>98	

B. SUB-LETHAL TOXICITY ENDPOINTS:

No signs of toxicity were observed after 3.5 hours of exposure at any test level (Table 4, p. 20). After 24 hours, following lethargic effects were observed: 3/20 daphnids (24 ppm ai); 6/20 daphnids (49 ppm ai); and 5/20 daphnids (98 ppm a.i). After 48 hours, 1/20 daphnids (49 ppm a.i), 2/17daphnids (49 ppm a.i.), and 2/11 daphnids (98 ppm a.i.) were lethargic.

Table 4: Sub-lethal Effects of AE F132345 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
5.9 (6.3)	Appear normal	0	Lethargic	5
12 (13)	Appear normal	0	Appear normal	0
24 (25)	Lethargic	15	Lethargic	12
49 (50)	Lethargic	30	Appear normal	0
98 (100)	Lethargic	25	Lethargic	18
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}: >98 ppm a.i. 95% C.I.: N/A
NOEC: 12 ppm a.i.
LOEC: 24 ppm a.i.
Endpoints affected: Mortality/immobility 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. 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 significant (≤10%) 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}: >98 ppm a.i. 95% C.I.: N/A
NOEC: 12 ppm a.i.
LOEC: 24 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 differed slightly compared to those of the study authors'. The reviewer's NOEC based on mortality/immobility data (24 ppm a.i.) was one treatment level higher than the study authors' reported NOEC (12 ppm a.i.; based on mortality/immobility and sub-lethal effects), presumably due to the different statistical methods used and the different endpoints included in the analysis. The reviewer also determined a NOEC based on sub-lethal effects, which was identical to the study authors' reported NOEC. The study authors' noted that the 5.9 and 12 ppm AE F132345 treatment groups appeared healthy and normal, with the exception of one lethargic daphnid in the 5.9 ppm AE F132345 treatment group. The study authors' concluded that since there were no effects observed among daphnids in the 12 ppm AE F132345 treatment group, this occurrence was not considered to be treatment-related (pp. 14-15). The reviewer concurs with the above statement, therefore, the study authors' NOEC, a more conservative estimate of the acute toxicity of AE F132345 to *Daphnia magna*, is reported in the Executive Summary and Conclusion sections of this DER.

The test solutions appeared clear and colorless (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, AE F132345 (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 24 and 50 ppm a.i., respectively, based on sub-lethal effects data (only endpoint affected).

48-Hour
LC_{50}/EC_{50}: >98 ppm a.i. 95% C.I.: N/A
NOEC: 12 ppm a.i.
LOEC: 24 ppm a.i.
Endpoints affected: Mortality/immobility 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.