Data Evaluation Report on the acute toxicity of AE F132345 (Metabolite of Thidiazuron) to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number {.....} EPA MRID Number 46203515

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D294536
OECD Data Point	
EPA MRID	46203515
EPA Guideline	§72-1c

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 **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 4/21/04

QC Reviewer: Christie E. Padova **Signature:** C. E. Padova
Staff Scientist, Dynamac Corporation **Date:** 4/26/04

Primary Reviewer: Bill Evans, Biologist
OPP/EFED/ERB - I **Date:** 11/16/04

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

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 120301

Date Evaluation Completed:

CITATION: Palmer, S.J., *et al.* 2003. AE F132345: A 96-Hour Static Acute Toxicity Test with the Rainbow Torut (*Oncorhynchus mykiss*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 149A-161. Study sponsored by Bayer CropScience, Frankfurt am Main, Germany. Study initiated April 8, 2003 and completed August 25, 2003.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow Trout (*Oncorhynchus mykiss*) were exposed under static conditions to AE F132345 (a metabolite of thidiazuron) at nominal concentrations of 0 (negative control), 6.3, 13, 25, 50, or 100 ppm. Mean-measured concentrations were <4.00 (<LOQ, control), 6.5, 13, 25, 51, and 101 ppm a.i.

No mortality or sub-lethal effects were observed in any test group following 96 hours of exposure. The 96-hour LC_{50} was >101 ppm a.i., which categorizes AE F132345 (a metabolite of thidiazuron) as practically non-toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOEC and LOEC, based on both mortality and sub-lethal effects, were 101 and >101 ppm a.i., respectively.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with Rainbow Trout (§72-1c). This study is classified as CORE.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	10.5 weeks old (reviewer-calculated); mean of 1.1 g (wet) and 5.0 cm (mean of 10 negative control fish at test termination)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}: >101 ppm a.i.
NOEC: 101 ppm a.i.
LOEC: >101 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OECD Guideline No. 203 (1993); the U.S. EPA OPPTS No. 850.1075 (Draft, 1996); and ASTM Standard E729-88a (1994). Deviations from U.S. EPA §72-1 included:

1. Aeration of the test vessels was not addressed.
2. The water hardness (112 mg/L as $CaCO_3$) was nearly three times higher than recommended (40-48 mg/L as $CaCO_3$).
3. The pH range (8.2-8.7) was greater than recommended (7.2-7.6).
4. The total organic carbon (TOC), particulate matter, and residual chlorine concentrations in the dilution water were not reported.

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

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160), OECD, and Japan MAFF (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 verified by analytical determination at 0, 48, and 96 hours (Table 1, p. 17). Recoveries were 105-114% of nominal concentrations at 0 hours, 99.1-101% of nominal at 48 hours, and 98.3-101% of nominal at 96 hours.

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: Rainbow Trout (*Oncorhynchus mykiss*)

Age at test initiation: 10.5 weeks old (reviewer-calculated, hatched on May 15, 2003).

Weight at study initiation: Not provided; the blotted wet weight of 10 negative control fish measured at test termination averaged 1.1 g (range of 0.84-1.5 g).

Length at study initiation: Not provided; the length of 10 negative control fish measured at test termination averaged 5.0 cm (range of 4.8-5.3 cm).

Source: Thomas Fish Company, Anderson, CA.

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: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	At least 14 days prior to testing Same as test Fed commercially-prepared diet supplied by Zeigler Brothers Inc., Gardners, PA. Fish were not fed two days prior to and during testing. During the 48 hours prior to testing, fish showed no signs of disease, stress or mortality.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96-hour	*EPA/OECD requires: 96 hour*
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Static N/A N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Not reported	*EPA requires: no aeration; OECD permits aeration*
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	 Stainless steal aquaria with stainless steal cover 38 L 20 L (15.1-cm depth)	*EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
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, aerated, then filtered (0.45 µm) again prior to use.	*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

Parameter	Details	Remarks Criteria
Water parameters: Hardness	112 mg $CaCO_3$/L	The hardness and pH were higher than recommended.
pH	8.2-8.7	Total alkalinity was 185 mg/L as $CaCO_3$.
Dissolved oxygen	7.5-9.5 mg/L (≥69% saturation)	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.
Total Organic Carbon	Not reported	
Particulate Matter	Not reported	
Metals	See Appendix 3, p. 27.	
Pesticides	<LOD	
Chlorine	Not reported	***Hardness and pH*** *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 12°C for coldwater species and 17-22°C for warmwater species. OECD requires range of 21 - 25°C for bluegill and 13-17°C for rainbow trout.* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Temperature	11.9 to 13.0°C	
Intervals of water quality measurement	The DO, pH and temperature were measured in both replicate aquaria at 0-, 24-, 48-, 72- and 96-hours. Temperature was also measured continuously in one negative control aquaria.	

Parameter	Details	Remarks Criteria
Concentration of test material: nominal: measured:	0 (negative control), 6.3, 13, 25, 50, and 100 ppm <4.00 (<LOQ, control), 6.5, 13, 25, 51, and 101 ppm a.i.	Mean-measured concentrations are provided in Table 1, p. 17. Concentrations were stable during the 96-hour study. Stock solutions were adjusted for purity of the test material (p. 11). *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	N/A	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent , exceed 100 mg/L.*
Number of fish/replicates: negative control: solvent control: treated:	20 fish, divided into two replicates containing 10 fish each N/A 20 fish, divided into two replicates containing 10 fish each	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.54 g fish/L	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark, with a 30-minute transition period.	Light intensity of 206 lux at the water surface during daylight hours. *EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*

'Parameter	Details	Remarks Criteria
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	99.4-104% 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/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	at 4 hours and every 24 hours thereafter	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality occurred in any group during the 96-hour study (Table 4, p. 20). The 96-hour LC_{50} was >101 ppm a.i., the highest concentration tested (Table 5, p. 21). The NOEC based on mortality data was 101 ppm a.i.

Table 3: Effect of AE F132345 on mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. Measured and (nominal) concn.	No. of fish at start of study	Observation Period					
		0-48 Hours		72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	20	0	0	0	0	0	0
6.5 (6.3)	20	0	0	0	0	0	0
13 (13)	20	0	0	0	0	0	0
25 (25)	20	0	0	0	0	0	0
51 (50)	20	0	0	0	0	0	0
101 (100)	20	0	0	0	0	0	0
NOEC (mortality)	101 ppm a.i.						
LC_{50} (95% C.I.)	>101 ppm a.i.						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. NON-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in any group during the 96-hour study (Table 4, p. 20). The NOEC based on sub-lethal effects was 101 ppm a.i.

Table 4. Sub-lethal effects of AE F132345 on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. Measured and (nominal) concn.	Observation Period			
	endpoint at 5-24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected	% affected	% affected	% affected
Negative control	AN	AN	AN	AN
6.5 (6.3)	AN	AN	AN	AN
13 (13)	AN	AN	AN	AN
25 (25)	AN	AN	AN	AN
51 (50)	AN	AN	AN	AN
101 (100)	AN	AN	AN	AN
NOEC (sub-lethal)	101 ppm a.i.			
LOEC (sub-lethal)	>101 ppm a.i.			
EC_{50}	Not determined			
Positive control, if used % sub-lethal effect: EC_{50}:	N/A	N/A	N/A	N/A

AN - All surviving fish appeared normal.

C. REPORTED STATISTICS:

Due to a lack of mortality or sub-lethal effects at any treatment level by 96 hours, the 96-hour LC_{50}, NOEC, and LOEC values were determined by visual observation, and were based on mean-measured treatment concentrations (p. 14).

96-Hour
LC_{50}: >101 ppm a.i.
NOEC: 101 ppm a.i.
LOEC: >101 ppm a.i.
Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50} was determined visually due to a lack of mortality at any treatment level by 96 hours. The NOEC was visually determined as the highest concentration which exhibited no significant mortality or sub-lethal effects. All toxicity values were determined in terms of the reported mean-measured treatment concentrations.

96-Hour
LC_{50}: >101 ppm a.i.
NOEC: 101 ppm a.i.
LOEC: >101 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-1c that affected the acceptability or validity of this study.

F. REVIEWER'S COMMENTS:

Results of the reviewer's statistical verification were identical to those of the study author.

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

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish, cold water species (§72-1c). This study is classified as CORE. The 96-hour LC_{50} was >101 ppm a.i., which classifies AE F132345 (a metabolite of thidiazuron) as practically non-toxic to Rainbow Trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOEC (for mortality and sub-lethal effects) was 101 ppm a.i., the highest concentration tested.

96-Hour
LC_{50}: >101 ppm a.i.
NOEC: 101 ppm a.i.
LOEC: >101 ppm a.i.
Endpoints affected: None

III. REFERENCES:

Organization for Economic Co-Operation and Development (OECD). 1993. Guideline for the Testing of Chemicals. *Guideline 203: Fish Acute Toxicity Test*, Adopted by Council on 12 July 1992.

U.S. Environmental Protection Agency. 1996. *Fish Acute Toxicity Test, Freshwater and Marine*. Series 850 - Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1075.

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.