DATA EVALUATION RECORD
EARTHWORM SUBCHRONIC TOXICITY TEST
OPPTS 850.6200

1. **CHEMICAL:** Thidiazuron — PC Code No.: 120301

2. **TEST MATERIAL:** AE F132347 — Purity: 97.4%

3. **CITATION:**

Author: Sindermann, A.B., J.R. Porch, and H.O. Krueger

Title: AE F132347: An Acute Toxicity Study with the Earthworm *(Eisenia fetida)* in an Artificial Soil Substrate.

Study Completion Date: May 22, 2003

Laboratory: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601 U.S.A.

Sponsor: Bayer CropScience
Industriepark Höchst, D-65926
Frankfurt am Main, Germany

Laboratory Report ID: 149-191

MRID No.: 46203507

DP Barcode: D294536

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: **Date:** 5/3/04

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: **Date:** 5/3/04

5. **APPROVED BY:** William Evans, Biologist, EFED/ERB-1

Signature: **Date:** 11/16/04

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5. APPROVED BY:

Signature: **Date:**

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Eisenia fetida*

Age/Size of Test Organism: Adult (with clitellum), 480-570 mg

Type of Test Concentration: Nominal

Definitive Study Duration: 14 days

7. CONCLUSIONS:

The earthworm, *Eisenia fetida*, was exposed to AE F132347 (Thidiazuron) at nominal test concentrations of 62.5, 125, 250, 500, and 1000 mg a.i./kg soil. By 14 days, there were no mortalities in the control or 62.5, 125, 250, and 500 mg a.i./kg soil treatment groups. The 1000 mg a.i./kg soil treatment group had 3 total mortalities (7.5%). In the 500 and 1000 mg a.i./kg soil treatment groups, the clinical signs of reduced reaction to mechanical stimuli and thinness were observed. There were significant weight differences in the 500 and 1000 mg a.i./kg soil treatment groups compared to the control. **The LC_{50} was >1000 mg a.i./kg soil and the NOEC value was 250 mg a.i./kg soil.** This study is classified as Supplemental, because US EPA does not presently require subchronic toxicity testing with earthworms for pesticide registration, so SEP guidelines do not exist. The results of this study, however, are useful for risk assessment purposes.

Results Synopsis:

LC_{50}: >1000 mg a.i./kg soil 95% C.I.: N/A
NOEC: 250 mg a.i./kg soil Probit Slope: N/A
LOEC: 500 mg a.i./kg soil

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: US EPA does not presently require subchronic toxicity testing with earthworms for pesticide registration, so SEP guidelines do not exist. OPPTS guidelines exist for subchronic toxicity testing with earthworms and there were several deviations from these experimental protocol in this study.

C. Repairability: None. The results of this study are useful for risk assessment purposes.

9. GUIDELINE DEVIATIONS: This study was based on procedures of the OECD Guideline No. 207, *Guidelines for Testing of Chemicals, Earthworm, Acute Toxicity Tests.*

1. The study duration was 14 days. Under the Ecological Effects Test Guidelines, "The test duration is 28 days" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p.4, item 3(x)).

2. The weight of wet soil per replicate was 750 g. Guideline regulations specify that the wet soil weight per replicate shall be 270 g (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Medium preparation, item (A))

3. The test chambers for this study were 1 liter glass beakers. Guideline regulations specify that the tests chambers should be of a 1 pint capacity (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Test chambers, item (A)).

4. The temperature and pH (at initiation and termination) were reported for this study. Guideline regulations specify that temperature and pH measurements are to be reported "...at start of test and on days 7, 14, 21, and 28 of the test" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 10, item (vii)).

5. The reported concentrations of the test substance are assumed to be the initial concentrations at the beginning of the study. Guideline regulations specify that "the concentration of the test substance in artificial soil should be measured at a minimum in each chamber at the beginning (zero-hour, before earthworms are added) and every 7 days thereafter" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 5, item (A)).

6. Worms were counted on days 0, 7 and 14 and weighed on days 0 and 14. Guideline regulations specify that "each test and control chamber should be checked for dead or affected earthworms and observations recorded 7, 14, 21, and 28 days after the beginning of the test..." (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 4, Test Results, item (iii)).

7. The relative humidity was not reported. The guidelines specify that "relative humidity should be maintained above 85%" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Construction materials (beginning on p. 6), item (D)).

10. SUBMISSION PURPOSE: This study was submitted to provide data on the acute toxicity of Thidiazuron (AE F132347) to earthworms for the purpose of chemical reregistration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: ***Eisenia fetida* andrei (Bouche)**	*Eisenia fetida*
Weight: **300-600 mg**	480-570 mg
Age: **Adult**	Adult (with clitellum)
Source:	Laboratory cultures (original supplier was University of Maryland, Queenstown, Maryland).

B. Test System

Guideline Criteria	Reported Information
Test Container: **Glass canning jars (1 pint capacity) or equivalent**	1 L glass beakers covered with perforated plastic wrap.
Artificial Soil Medium: **Dry weight mixture of:** **68% No. 70 mesh silica sand,** **20% kaolin clay,** **10% sphagnum peat moss,** **2% calcium carbonate**	70% sand 20% kaolin clay 10% sphagnum peat pH-adjusted with $CaCO_3$
Weight of Soil: **270 g (wet soil)**	750 g
Moisture Content of Soil: **35%**	31.4-32.2% (initiation); 30.1-31.0% (termination).

Guideline Criteria	Reported Information
Temperature: **22 ± 2°C**	20-21°C
Relative Humidity: **85%**	Not reported
Light Intensity: **400 lux**	400-636 lux
Photoperiod: **Continuous**	Continuous
pH: **6.5 ± 0.5**	7.1-7.2 (initiation); 7.7-7.8 (termination).

C. Test Design

Guideline Criteria	Reported Information
Dose range: **ratio of 1.5 or 2.0 mg a.i./kg soil**	2 mg a.i./kg soil ratio
Doses: **at least 5**	62.5, 125, 250, 500, and 1000 mg a.i./kg soil
Controls: **at least 1**	Negative control (water only)
Replicates per Dose: **3**	4
Number of Worms per Replicate: **10**	10
Test duration: **at least 28 days**	14 days
Observations made every 7 days after test initiation for dead or affected worms?	Mortalities and clinical signs were observed on days 0, 7, and 14. Weights were recorded at test initiation and on day 14.
Maximum labeled rate:	Not reported.

12. REPORTED RESULTS:

Guideline Criteria		Reported Information
Initial and 7-, 14-, 21-, and 28-day:	**worm weight reported?**	Initial and day 14 worm weights were reported.
	temperature and pH reported?	Initial and day 14 temperature and pH data were reported.
	chemical concentrations reported?	Mean measured concentrations were not reported.
Raw data included?		Raw data were reported.

Dose Response

Nominal Concentration in Soil (mg a.i./kg soil)	Mean Weight (mg) at Day:[a]				Weight Change (mg)	# of Dead Worms at Day:				Mortality (%)
	0	7[NR]	14	28*		0	7	14	28*	
Control	530	-	430	-	-100	0	0	0	-	0
62.5	530	-	420	-	-110	0	0	0	-	0
125	540	-	430	-	-120	0	0	0	-	0
250	550	-	430	-	-120	0	0	0	-	0
500	540	-	360	-	-180[a]	0	0	0	-	0
1000	530	-	350	-	-170[a]	0	2	3	-	7.5

[a] The body weight change was significantly different than the control (Dunnett's test, $p<0.05$).
NR = not reported
* the test duration was 14 days, therefore, no results exist for day 28.

Statistical results:

Statistical Method: The mean treatment group body weights were compared to the control body weights with Dunnett's test ($\propto$=0.05) using SAS Version 8. The data were tested for homogeneity of variance and normal distribution prior to the Dunnett's test. The NOEC, LOEC, and LC_{50} were visually determined using the weight and mortality data.

LC_{50}: >1000 mg a.i./kg soil 95% C.I.: N/A
NOEC: 250 mg a.i./kg soil Probit Slope: N/A

LOEC: 500 mg a.i./kg soil

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} was visually determined because mortality did not exceed 50%. Body weight change data were analyzed using ANOVA, followed by William's test via TOXSTAT statistical software.

LC_{50}: >1000 mg a.i./kg soil 95% C.I.: N/A
NOEC: 250 mg a.i./kg soil Probit Slope: N/A
LOEC: 500 mg a.i./kg soil

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'; the LC_{50} was >1000 mg a.i./kg soil and the NOEC value was 250 mg a.i./kg soil (based on reductions in body weight change and clinical effects, including thinness).

In order to validate the test system, the reference toxicant chloroacetamide was tested at concentrations of 7.5, 15, and 30 mg a.i./kg soil. The LC_{50} for chloroacetamide was 26.2 mg a.i./kg soil with 95% confidence interval of 15 and 30 mg a.i./kg soil. The results of the reference toxicant test confirmed the validity of the definitive test.

This study was conducted in compliance with Good Laboratory Practices (GLP) Standards of the U.S. Environmental Protection Agency, 40 CFR, Parts 160 and 792, 17 August 1989; OECD, ENV/MC/CHEM (98) 17, Paris, 1998; and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999, with the following exception: the verification of test concentration, stability, and homogeneity of the test substance in the soil was not determined. A quality assurance statement was included.

15. REFERENCES:

OECD Guideline No. 207. 1984. *Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests*. Organization for Economic Cooperation and Development.

Wildlife International, Ltd. 2002. Chloroacetamide: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate. Project No. 100R-106.

SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8, Cary, NC, SAS Institute, Inc.

Stephan, C.E. 1977. Methods for Calculating an LC50. *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Pub. No. STP 634:65-84.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

35-07 body weight
File: 35-07bw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0257	0.0051	10.200
Within (Error)	18	0.0096	0.0005	
Total	23	0.0354		

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

35-07 body weight
File: 35-07bw 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	-0.098	-0.098		
2	62.5	-0.110	-0.110	0.791	
3	125	-0.115	-0.115	1.107	
4	250	-0.123	-0.123	1.581	
5	500	-0.183	-0.183	5.376	*
6	1000	-0.175	-0.175	4.902	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

35-07 body weight
File: 35-07bw 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	4			
2	62.5	4	0.038	-39.1	0.012
3	125	4	0.038	-39.1	0.017
4	250	4	0.038	-39.1	0.025
5	500	4	0.038	-39.1	0.085
6	1000	4	0.038	-39.1	0.078

35-07 body weight
File: 35-07bw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	-0.098	-0.098	-0.098
2	62.5	4	-0.110	-0.110	-0.110
3	125	4	-0.115	-0.115	-0.115
4	250	4	-0.123	-0.123	-0.123
5	500	4	-0.183	-0.183	-0.179
6	1000	4	-0.175	-0.175	-0.179

35-07 body weight
File: 35-07bw 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	-0.098				
62.5	-0.110	0.768		1.73	k= 1, v=18
125	-0.115	1.075		1.82	k= 2, v=18
250	-0.123	1.536		1.85	k= 3, v=18
500	-0.179	4.991	*	1.86	k= 4, v=18
1000	-0.179	4.991	*	1.87	k= 5, v=18

s = 0.023
Note: df used for table values are approximate when v > 20.