

**DATA EVALUATION RECORD  
EARTHWORM SUBCHRONIC TOXICITY TEST  
OPPTS 850.6200**

1. **CHEMICAL:** Thidiazuron

PC Code No.: 120301

2. **TEST MATERIAL:** Thidiazuron, SC 500 G/L

Purity: 42.6%

3. **CITATION:**

Author: Nienstedt, K.M.

Title: Thidiazuron, SC 500 G/L: A 14-Day Acute Toxicity Test  
with the Earthworm *Eisenia fetida*

Study Completion Date: April 9, 2003

Laboratory: Springborn Laboratories (Europe) AG  
Seestrasse 21, CH-9326  
Horn, Switzerland

Sponsor: Bayer CropScience  
2 T.W. Alexander Drive  
Research Triangle Park, NC 27709

Laboratory Report ID: C031948

MRID No.: 46203522

DP Barcode: D294536

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

**Signature:**

**Date:** 5/3/04

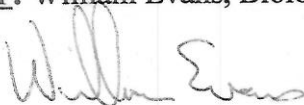
**APPROVED BY:** Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

**Signature:**

**Date:** 5/3/04

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

**Signature:**



**Date:** 11/016/04



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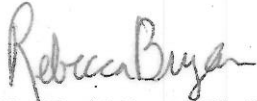
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**Signature:**

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## 6. STUDY PARAMETERS:

**Scientific Name of Test Organism:** *Eisenia fetida*  
**Age/Size of Test Organism:** Adult (5-6 months old), 301-598 mg  
**Type of Test Concentration:** Nominal  
**Definitive Study Duration:** 14 days

## 7. CONCLUSIONS:

The earthworm, *Eisenia fetida*, was exposed to Thiadiazuron, SC 500 g/L at nominal test concentrations of 62.5, 125, 250, 500, and 1000 mg/kg. By 14 days, there were no mortalities in the control or 62.5, 125, and 250 mg/kg treatment groups. There was 10.0 and 22.5% mortality in the 500 and 1000 mg/kg treatment groups, respectively. The mean day 14 weights were significantly different in the 250, 500, and 1000 treatment groups compared to the control. The weight differences by day 14 were significant in the 1000 mg a.i./kg treatment groups compared to the control. **The LC<sub>50</sub> was >1000 mg/kg; a NOEC value was estimated as 125 mg/kg (based on terminal body weight).** 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<sub>50</sub>: >1000 mg/kg    95% C.I.: N/A  
NOEC: 125 mg/kg    Probit Slope: N/A  
LOEC: 250 mg/kg

## 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* (1984).

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 1.5 liter glass containers. 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 (Thidiazuron, SC 500 G/L) to earthworms for the purpose of chemical reregistration.

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b>Species:</b> <i>Eisenia fetida andrei</i> (Bouche)	<i>Eisenia fetida</i>
<b>Weight:</b> 300-600 mg	301-598 mg
<b>Age:</b> Adult	5-6 months old
<b>Source:</b>	Laboratory cultures (original supplier BBA, Braunschweig, Germany).

**B. Test System**

Guideline Criteria	Reported Information
<b>Test Container:</b> Glass canning jars (1 pint capacity) or equivalent	Glass beakers covered by loose-fitting glass lid, 1.5 L volume
<b>Artificial Soil Medium:</b> <b>Dry weight mixture of:</b> 68% No. 70 mesh silica sand, 20% kaolin clay, 10% sphagnum peat moss, 2% calcium carbonate	70% industrial sand 20% kaolin clay 10% sphagnum peat
<b>Weight of Soil:</b> 270 g (wet soil)	750 ± 2 g
<b>Moisture Content of Soil:</b> 35%	36.82-38.60% (initiation); 34.86-36.12% (termination).

Guideline Criteria	Reported Information
<b>Temperature:</b> 22 ± 2°C	19.5-21.0°C
<b>Relative Humidity:</b> 85%	Not reported
<b>Light Intensity:</b> 400 lux	540-690 lux
<b>Photoperiod:</b> Continuous	Continuous
<b>pH:</b> 6.5 ± 0.5	6.04-6.11 (initiation); 5.97-6.03 (termination).

### C. Test Design

Guideline Criteria	Reported Information
<b>Dose range:</b> ratio of 1.5 or 2.0 mg/kg	Approximately 2.0 mg/kg ratio
<b>Doses:</b> at least 5	62.5, 125, 250, 500, and 1000 mg/kg
<b>Controls:</b> at least 1	1 control (deionized water)
<b>Replicates per Dose:</b> 3	4
<b>Number of Worms per Replicate:</b> 10	10
<b>Test duration:</b> at least 28 days	14 days
<b>Observations made every 7 days after test initiation for dead or affected worms?</b>	Mortalities and health assessments were observed on days 7 and 14. Burrowing time was observed on Days 0 and 7. Weights were recorded at test initiation and on day 14.
<b>Maximum labeled rate:</b>	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/kg)	Mean Weight (mg) at Day: <sup>a</sup>				Weight Difference (mg)	# of Dead Worms at Day:				Mortality (%)
	0	7 <sup>NR</sup>	14	28 <sup>*</sup>		0	7	14	28 <sup>*</sup>	
Control	458.9	-	474.4	-	15.5	0	0	0	-	0
62.5	468.5	-	489.7	-	21.3	0	0	0	-	0
125	444.4	-	432.0	-	-12.5	0	0	0	-	0
250	449.7	-	420.4 <sub>a</sub>	-	-29.4	0	0	0	-	0
500	444.8	-	395.3 <sub>a</sub>	-	-46.7	0	0	4	-	10
1000	443.3	-	366.6 <sub>a</sub>	-	-87.5 <sup>a</sup>	0	0	9	-	22.5

<sup>a</sup> These values were significantly different from the control (ANOVA and Dunnett test).

NR = not reported

\* the test duration was 14 days, therefore, no results exist for day 28.

Statistical results:

Statistical Method: The day 0 and day 14 weights were analyzed using the Kolmogorov Smirnov's one sample test for normality and ANOVA tested for differences between treatments. The weight loss data was analyzed using ANOVA followed by a 1-sided Dunnett test. A linear regression was calculated with the weight loss data, since there

was a dose responsive effect. The  $LC_{50}$  was estimated based on mortality data. The NOEC and LOEC were visually determined using the significant effects data for weight. The statistical analyses were conducted using "SPSS for Windows", release 11.0.1, 2001 by SPSS Inc., Chicago IL, USA.

$LC_{50}$ : >1000 mg/kg    95% C.I.: N/A  
NOEC: 125 mg/kg    Probit Slope: N/A  
LOEC: 250 mg/kg

### **13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: The NOEC, LOEC, and  $LC_{50}$  were visually determined using the weight and mortality data; replicate treatment mean data were not provided, but individual worm weights were.

$LC_{50}$ : >1000 mg/kg    95% C.I.: N/A  
NOEC: 125 mg/kg    Probit Slope: N/A  
LOEC: 250 mg/kg

### **14. REVIEWER'S COMMENTS:**

The reviewer's conclusions were identical to the study author's. The  $LC_{50}$  was >1000 mg/kg; a NOEC value was estimated as 125 mg/kg (based on terminal body weight).

In order to validate the test system, the reference toxicant 2-chloroacetamide was tested (March 4-18, 2003). The  $LC_{50}$  for chloroacetamide was 27.1 mg/kg with 95% confidence interval of 25.4-29.0 mg/kg. The results of the reference toxicant test confirmed the validity of the definitive test.

The burrowing times for the earthworms were determined at test initiation and on Day 7 (Table 3, p. 21). No significant treatment effects on burrowing were observed.

This study was conducted in compliance with Good Laboratory Practices (GLP) Standards of the U.S. Environmental Protection Agency, 40 CFR 160; Swiss Ordinance, adopted February 2, 2000 [RS 813.016.5]; and OECD Council [C(97)186/Final]. A quality assurance statement was included.



**15. REFERENCES:**

Eidgenössisches Departement des Innern, Switzerland. March 2000. Swiss Ordinance relating to Good Laboratory Practice, adopted February 2, 2000 [RS 813.016.5]

European Commission Directive 96/12/EC of 8 March 1996. Official Journal of the European Communities.

European Commission Directive 91/414/EEC of 15 July 1991. Official Journal of the European Communities.

OECD. 1998. OECD Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles of Good Laboratory Practice (as revised in 1997). Environment Directorate. OECD. Paris. France. 41 pp.

OECD. 1984. OECD Guidelines for Testing of Chemicals, Guideline # 207. Earthworm, Acute Toxicity Tests.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3<sup>rd</sup> edition. W.H. Freeman Company. New York. pp. 887.