DATA EVALUATION RECORD
EARTHWORM SUBCHRONIC TOXICITY TEST
OPPTS 850.6200

1. CHEMICAL: Penoxsulam PC Code No.: 199031 119031

2. TEST MATERIAL: GF-443 Purity: 21.9%

3. CITATION:

Author: Boeri, R.L. and Ward, T.J.

Title: GF-443: Acute Toxicity to the Earthworm, *Eisenia foetida*

Study Completion Date: February 18, 2002

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Sponsor: Dow AgroSciences LLC
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Laboratory Report ID: T.R. Wilbury No. 2388-DO/ Dow No. 021050

MRID No.: 45830807

DP Barcode: D288160

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

Signature: Rebecca Bryan **Date:** 1/02/04

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

Signature: **Date:** 1/02/04

5. APPROVED BY:

James J. Goodyear, Ph.D.
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Signature: **Date:**

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Eisenia foetida*

Age/Size of Test Organism: Age not specified, 228.5-306.7 mg (mean replicate weights)

Type of Test Concentration: Nominal

Definitive Study Duration: 14 days

7. RESULTS and CONCLUSIONS:

The earthworm, *Eisenia foetida*, was exposed to GF-443, at a single, nominal test concentration of 10,000 mg TEP/kg or 2190mg a.i./kg. By 14 days, there was no mortality in the control or 2190 mg a.i./kg treatment group. Average reductions in body weight by day 14 were 5.1 and 1.6% in the control and 2,190 mg a.i./kg treatment groups. **The LC_{50} was >2,190 mg a.i./kg; a NOAEC value was estimated as 2,190 mg a.i./kg.** 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}: >2,190 mg a.i./kg 95% C.I.: N/A
NOAEC: 2,190 mg a.i./kg Probit Slope: N/A
LOAEC: >2,190 mg a.i./kg

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: The 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 the 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, "Earthworm, acute toxicity test." Deviations from the OPPTS 850.6200 guidelines include:

1. The test chemical was not technical grade (21.9% purity).

2. 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)).

3. The weight of wet soil per replicate was 1.0 kg. 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))

4. The test chambers for this study were 0.5 gallon glass jars. Guideline regulations specify that the test 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)).

5. The pH values were determined at test initiation and termination (day 14). 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)).

6. 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

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Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 5, item (A)).

7. 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)).

8. 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)).

9. The mean initial weights of the earthworm were 304.9-411.6 mg. The guidelines specify that "initial weights of the earthworm should be between 300 to 600 mg per container" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 2, Test Procedures, item (vi)).

10. SUBMISSION PURPOSE: This study was submitted to provide data on the subchronic toxicity of GF-443 to earthworms for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Eisenia foetida*	*Eisenia foetida*
Weight: 300-600 mg	304.9-411.6 mg (mean replicate weights)
Age: Adult	Adult (age not specified)

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Guideline Criteria	Reported Information
Source:	N_2 Worms, Vicksburg, Mississippi

B. Test System

Guideline Criteria	Reported Information
Test Container: **Glass canning jars (1 pint capacity) or equivalent**	0.5 gallon glass jars in tented water bath.
Artificial Soil Medium: **Dry weight mixture of:** **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 calcium carbonate (percentage not specified)
Weight of Soil: **270 g (wet soil)**	1.0 kg
Moisture Content of Soil: **35%**	25-27%
Temperature: **22 ± 2°C**	18.6-22.0°C
Relative Humidity: **≥85%**	Not reported
Light Intensity: **400 lux**	660 lux
Photoperiod: **Continuous**	Continuous
pH: **6.5 ± 0.5**	5.0-5.4

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C. Test Design

Guideline Criteria	Reported Information
Dose range: **ratio of 1.5 or 2.0 mg TEP / kg**	N/A; single dose concentration
Doses: **at least 5**	2,190 mg a.i./kg
Controls: **at least 1**	Negative control
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 sublethal effects were observed at test initiation and on days 7 and 14. The time required to burrow was determined 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?	Temperature data was reported (range was provided); pH values were reported at test initiation and termination.
	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)	Mean Weight (mg) at Day				Weight Change (%)	# of Dead Worms at Day				Mortality (%)
	0	7	14	28		0	7	14	28	
Control	351.1	-	333.4	-	5.1	0	0	0	-	0
2,190	377.5	-	372.3	-	1.6	0	0	0	-	0

NR = not reported
* the test duration was 14 days, therefore, no results exist for day 28.

Statistical results:

Statistical Method: The control weight data was compared to the 2,190 mg a.i./kg treatment group data using a t-test (Dunnett's). The LC_{50}, NOAEC, and LOAEC were visually determined using the weight and mortality data.

LC_{50}: >2,190 mg a.i./kg 95% C.I.: N/A
NOAEC: 2,190 mg a.i./kg Probit Slope: N/A
LOAEC: >2,190 mg a.i./kg

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50}, NOAEC, and LOAEC were visually determined using the weight and mortality data.

LC_{50}: >2,190 mg a.i./kg 95% C.I.: N/A
NOAEC: 2,190 mg a.i./kg Probit Slope: N/A
LOAEC: >2,190 mg a.i./kg

14. REVIEWER'S COMMENTS:

There were no significant effects of GF-443 on earthworm mortality or body weight.

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The study was reported in concentrations of "mg/kg." The study used a formulated product and the "mg/kg" refers to the concentration of the TEP. The study should have been reported as "mg a.i./kg."
In order to validate the test system, the reference toxicant 2-chloroacetamide was tested. The LC_{50} for 2-chloroacetamide was 17 mg/kg. The results of the reference toxicant test confirmed the validity of the definitive test.

A 14-day range finding test was conducted at nominal concentrations of 1.1, 10, 100, 1,000, and 2,190 mg a.i./kg with a negative control. There was no mortality in the control and treatment groups.

The U.S. EPA GLP Standards were followed with the following exceptions: the storage and test condition stability of the test substance was not verified; the calibration of a scale used to weigh artificial soil components and a balance used to measure calcium carbonate were not verifiable.

15. REFERENCES:

Edwards, C.A. and J.R. Lofty. 1977. Biology of Earthworms. John Wiley & Sons, New York.

Japan MAFF. 1984. Good Laboratory Practice Standard. 59 NohSan No. 3850.

OECD. 1997. OECD Principles of Good Laboratory Practice. [C(97) 186/ Final].

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 207, Earthworm, Acute Toxicity Test. Adopted April 11, 1984.

Stephan, C.E. 1983. Computer Program for Calculation of LC50 Values. U.S. EPA, Duluth, MN. Personal Communication.

U.S. EPA. 1993. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rule.

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