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

1. **CHEMICAL**: Penoxsulam — PC Code No.: ~~199031~~ 119031

2. **TEST MATERIAL**: XDE-638 — Purity: 97.5%

3. **CITATION**:

| | |
|---|---|
| Author: | Ward, T.J., Magazu J.P., and Boeri, R.L. |
| Title: | XDE-638: Acute Toxicity to the Earthworm, *Eisenia foetida* |
| Study Completion Date: | December 1, 1999 |
| Laboratory: | T.R. Wilbury Laboratories, Inc.<br>40 Doaks Lane<br>Marblehead, Massachusetts 01945 |
| Sponsor: | Dow AgroSciences LLC<br>9330 Zionsville Road<br>Indianapolis, Indiana 46268 |
| Laboratory Report ID: | T.R. Wilbury No. 1848-DO/ Dow No. 990124 |
| MRID No.: | 45830806 |
| 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:** Teri S Myers **Date:** 1/02/04

5. **APPROVED BY**:

James J. Goodyear, Ph.D.
Ecological Effects Biologist
Office of Pesticide Programs
703-305-7726

**Signature:** JGoodyear **Date:**

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1

## 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 XDE-638, at a single, nominal test concentration of 1000 mg/kg. By 14 days, there was no mortality in the control or 1000 mg/kg treatment group. Average reductions in body weight by day 14 were 9.6 and 5.1% in the control and1000 mg/kg treatment group. **The $LC_{50}$ was >1000 mg/kg; a NOAEC value was estimated as 1000 mg/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**:

$LC_{50}$: >1000 mg/kg
NOAEC: 1000 mg/kg
LOAEC: >1000 mg/kg

95% C.I.: N/A
Probit Slope: N/A

## 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.



(2)

**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 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 1000 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 3.8 L glass jars. 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 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)).
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)).
8. The mean initial weights of the earthworm were 228.5-306.7 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 XDE-638 to earthworms for the purpose of chemical registration.

## 11. MATERIALS AND METHODS:

### A. Test Organisms

| Guideline Criteria | Reported Information |
|---|---|
| **Species:**<br>***Eisenia foetida*** | *Eisenia foetida* |
| **Weight:**<br>**300-600 mg** | 228.5-306.7 mg (mean replicate weights) |
| **Age:**<br>**Adult** | Adult (age not specified) |
| **Source:** | Willingham Worm Farm, Butler, Georgia |

### B. Test System

| Guideline Criteria | Reported Information |
|---|---|
| **Test Container:**<br>**Glass canning jars (1 pint capacity) or equivalent** | 3.8 L glass jars in tented water bath. |
| **Artificial Soil Medium:**<br>**Dry weight mixture of:**<br>**68% No. 70 mesh silica sand,**<br>**20% kaolin clay,**<br>**10% sphagnum peat moss,**<br>**2% calcium carbonate** | 70% industrial sand<br>20% kaolin clay<br>10% sphagnum peat<br>calcium carbonate (percentage not specified) |
| **Weight of Soil:**<br>**270 g (wet soil)** | 1000 g |



4

| Guideline Criteria | Reported Information |
|---|---|
| **Moisture Content of Soil: 35%** | 25.5% (test termination) |
| **Temperature: 22 ± 2°C** | 20.1-21.9°C |
| **Relative Humidity: ≥85%** | Not reported |
| **Light Intensity: 400 lux** | 330 lux |
| **Photoperiod: Continuous** | Continuous |
| **pH: 6.5 ± 0.5** | 5.1-5.9 |

**C. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Dose range: ratio of 1.5 or 2.0 mg/kg** | N/A; single dose concentration |
| **Doses: at least 5** | 1000 mg/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 day 14. |
| **Maximum labeled rate:** | Not reported. |



5

## 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/kg) | Mean Weight (mg) | | | | Weight Difference (%) | # Dead Worms at Day | | | | Mortality (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| Control | 257.5 | - | 230.0 | - | 9.6 | 0 | 0 | 0 | - | 0 |
| 1000 | 267.6 | - | 253.8 | - | 5.1 | 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 10,000 mg/kg treatment group data using a t-test. The $LC_{50}$, NOAEC, and LOAEC were visually determined using the weight and mortality data.

$LC_{50}$: >1000 mg/kg
NOAEC: 1000 mg/kg
LOAEC: >1000 mg/kg

95% C.I.: N/A
Probit Slope: N/A

## 13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The $LC_{50}$, NOAEC, and LOAEC were visually determined using the



6

weight and mortality data.

$LC_{50}$: >1000 mg/kg
NOAEC: 1000 mg/kg
LOAEC: >1000 mg/kg

95% C.I.: N/A
Probit Slope: N/A

**14. REVIEWER'S COMMENTS:**

The reviewer's conclusions were identical to those of the study authors; there were no significant effects of XDE-638 on earthworm mortality or body weight.

In order to validate the test system, the reference toxicant 2-chloroacetamide was tested June 25-July 9, 1999. The $LC_{50}$ for 2-chloroacetamide was 16 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 10, 100, 500, and 1000 mg/kg with a negative control. After 14 days, there was 10% control mortality and 0% mortality in all 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 balance used to prepare pH samples could not be verified ;and the recalibration of pH meter used on day 14 was not dated and initialed.

**15. REFERENCES:**

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

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. FDA. 1987. Earthworm Subacute Toxicity. FDA Environmental Assessment Technical Guide No. 4.12.

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