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# Data Evaluation Report on the Acute Toxicity Effects of Pyrasulfotole on Earthworms

PMRA Submission #: 2006-2445 EPA MRID #: 468017-41

| **Data Requirement:** | | |
|---|---|---|
| | PMRA Data Code | DACO 9.2.3.1 |
| | EPA DP Barcode | D328639 |
| | OECD Data Point | IIA 8.9.1 |
| | EPA MRID | 468017-41 |
| | EPA Guideline | OPPTS 850.6200 (OECD 207) |

**Test material:** Pyrasulfotole Purity: 95.7%
Common name: AE 0317309
Chemical name: IUPAC: (5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4-trifluoromethylphenyl)methanone
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

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**Date:** 5/16/06

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**Date:** 11/22/06 J.D. Whall
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**Reference/Submission No.:** {.....}

| | |
|---|---|
| **Company Code** | BCZ |
| **Active Code** | PSA |
| **Use Site Category:** | 13, 14 |
| **EPA PC Code** | 000692 |

**Date Evaluation Completed:** 11-29-2006

**CITATION:** Lechelt-Kunze, C. 2004. AE 0317309, substance, technical (Code: AE 0317309 00 1C96 0001): Acute Toxicity to Earthworms (*Eisenia fetida)* tested in Artificial Soil. Unpublished study performed by Bayer CropScience AG, Institute for Ecotoxicology, Monheim, Germany. Study No. E 310 2671-0. Study sponsored by Bayer CropScience AG, Monheim, Germany. The final report issued June 21, 2004.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to earthworms. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

US EPA ARCHIVE DOCUMENT

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## EXECUTIVE SUMMARY:

In an acute limit toxicity study, earthworms (*Eisenia fetida*) were exposed to Pyrasulfotole at 1000 mg a.i./kg dry weight of artificial soil substrate. The $LC_{50}$ and $EC_{50}$ were >1000 mg a.i./kg. The NOAEC was ≥1000 mg a.i./kg for all endpoints. The LOAEC was >1000 mg a.i./kg. Pyrasulfotole is considered to be non-toxic to earthworms up to a concentration of 1000 mg a.i./kg.

By 14 days, one mortality was observed in the 1000 mg a.i./kg treatment group. No other mortalities occurred during testing. The percent weight change was -2% in the 1000 mg a.i./kg treatment group, compared to 1% for the control. The NOAEC based on weight change was ≥1000 mg a.i./kg. No sublethal effects were observed during testing.

This study is classified as **SUPPLEMENTAL** to the **US EPA** [it is scientifically sound but deviates from guideline requirements for a subchronic toxicity study with earthworms (OPPTS 850.6200) (see below)] and **ACCEPTABLE** to the **PMRA** and **DEH**.

### Results Synopsis

**Test Organism Size/Age(Mean Wt or Length):** > 2 months old, 300-450 mg
**Test Type (Flow-through, Static, Static Renewal):** Not applicable; Artificial soil substrate

| | | |
|---|---|---|
| $LC_{50}/EC_{50}$: | >1000 mg a.i./kg | 95% C.I.: N/A |
| NOAEC: | ≥1000 mg a.i./kg | |
| Probit Slope: | Not calculable | 95% C.I.: N/A |
| Endpoint(s) Affected: | None | |

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** This study was based on procedures of the OECD Guideline No. 207, *Guidelines for Testing of Chemicals, Earthworm, Acute Toxicity Tests* (1984). The following deviations from U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.6200, *Earthworm subchronic toxicity test* were noted:

1. The study duration was 14 days instead of the recommended 28 days.
2. The temperature range of 20 ± 2°C was slightly lower than recommended (22 ± 2°C).
3. The light intensity range of 400-800 lux ranged higher than recommended (400 lux).
4. The relative humidity was not reported.
5. The acclimation period of 1 day was less than recommended (7 days).

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the US EPA-FIFRA Good Laboratory Practice (40 CFR Part 160).

### A. MATERIALS:

**1. Test Material** Pyrasulfotole (AE 0317309)

**Description:** Light brown crystalline powder

**Lot No./Batch No. :** Op. 1-4

**Purity:** 95.7%

**Stability of compound under test conditions:** Not determined.
*(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)*

**Storage conditions of test chemicals:** Not reported.



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**Physicochemical properties of Pyrasulfotole.**

| Parameter | Value | Comment |
|---|---|---|
| Molecular weight | 362.3 g/mol | |
| Water Solubility (g/L) at 20°C | 4.2 at pH 4<br>69.1 at pH 7<br>49.0 at pH 9 | Very soluble |
| Vapor Pressure/Volatility | $2.7 \times 10^{-7}$ Pa at 20°C<br>$6.8 \times 10^{-7}$ Pa at 25°C | Non-volatile |
| UV Absorption | water $\lambda_{max} = 264$<br>0.1M HCl $\lambda_{max} = 241$<br>0.1M NaOH $\lambda_{max} = 216$ | Not likely to undergo photolysis. |
| Pka | $4.2 \pm 0.15$ | |
| log $K_{ow}$ at 23°C | 0.276 at pH 4<br>-1.362 at pH 7<br>-1.58 at pH 9 | Not likely to bioaccumulate |
| Stability of compound at room temperature, if provided | | No significant degradation over 12 months at ambient temperatures. |

Data obtained from pyrasulfatole chemistry review of Submission 2006-2445.

**2. Test organism:**

**Species:** *Eisenia fetida andrei*
*(EPA and OECD recommend Eisenia fetida andrei (Bouche). The earthworms should weigh 300-600 mg at the beginning of the test.)*

**Age at test initiation:** >2 months old.

**Weight at study initiation:** 300-450 mg

**Source:** Laboratory cultures (originally obtained from Prof Graff, Forschungsanstalt fur Landwirtschaft, Braunschweig, Germany).

**B. STUDY DESIGN:**

**1. Experimental Conditions**

**a. Range-finding Study:** The definitive test concentrations were based on a non-GLP Range finding test. The nominal range-finding concentrations were 0.1, 1, 10, 100, and 1000 mg a.i./kg dry weight soil. The $LC_{50}$ was >1000 mg/kg and the NOAEC was 1000 mg/kg.

**b. Definitive Study**

1. Artificial soil was used; see properties below.

**Table 1: Physicochemical Properties of Artificial Soil**



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| Property | Value | Remarks<br>*Criteria* |
|---|---|---|
| For natural soil:<br>Texture: % sand<br>% silt<br>% clay<br>Textural classification<br><br>For artificial substrate (provide composition): | N/A (artificial soil used)<br><br><br><br><br>69% Industrial quartz sand<br>20 % kaolinite clay<br>10% sphagnum peat<br>0.2-1% calcium carbonate | *Recommended testing medium is artificial soil consisting of a mixture of 68% of No. 70 mesh silica sand, 20% kaolin clay, 10 sphagnum peat moss, and 2% calcium carbonate, mixed and moistened to 35% by weight with deionized/distilled water.* |
| pH (__:__ soil:water) | 6.0 ± 0.5 | |
| Organic carbon (%) | Not reported | |
| Moisture (%) | 24.4-24.9% at test initiation<br>23.9-24.1% at test termination | |

**Table 2: Experimental Design**

| Parameter | Detail | Remarks<br>*Criteria* |
|---|---|---|
| Acclimation:<br><br>duration:<br>conditions (state if same as the test conditions):<br>health: | <br><br>1 day<br>Same as test.<br>Only healthy animals were used for testing. | The acclimation period of 1 day was less than recommended (7 days).<br>*Earthworms should be acclimated at test temperature for 7 days.* |
| Soil [fresh or stored] | Stored | |
| Test Container<br><br>material<br>size<br>amount of soil/substrate | <br><br>Preserving jars<br>1.5 L<br>500 g soil dry weight (625 g wet weight). | |
| No. of replicates: | | |



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| Parameter | Detail | Remarks<br>*Criteria* |
|---|---|---|
| per treatment group:<br>per control: | | *Recommended number of replicates include at least 3 and a control.* |
| No. of earthworms per treatment | 40 earthworms per treatment (10 per replicate container). | *Recommended number of earthworms per treatment include a minimum of 30 plus a control; 10 per each of three replicates and a control.* |
| Solvents used or not (if yes report the name and concentration) | N/A | |
| Rates of application:<br>nominal:<br>measured: | 1000 mg a.i./kg<br>Not determined. | *Earthworms should be exposed to at least five test concentrations, in geometric series, in which the ratio is between 1.5 and 2.0 mg of test chemical per kg (air-dry weight) of artificial soil.* |
| Reference chemical (if used)<br>name:<br>concentration: | Chloroacetamide<br>2.6, 10, 18, 24, and 32 mg a.i./kg | The 14-day $LC_{50}$ was 16 mg a.i./kg.<br>The reference test was non-GLP and conducted prior to this definitive test. |
| Test conditions:<br>temperature<br>Lighting conditions<br>Moisture | 20 ± 2°C<br>Continuous, 400-800 lux<br>Not reported (relative humidity) | The temperature range of 20 ± 2°C was slightly lower than recommended (22 ± 2°C). The light intensity range of 400-800 lux ranged higher than recommended (400 lux). The relative humidity was not reported.<br>*Recommended temperature: 22 + 2°C*<br>*Recommended lighting: Continuous illumination, with a light intensity of 400 lux*<br>*Recommended relative humidity: above 85%* |
| Duration of the study | 14 days | *Recommended duration of study is 28 days.* |

**2. Observations:**



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**Table 3: Observations**

| Parameters | Details | Remarks<br>*Criteria* |
|---|---|---|
| Observation intervals | 7 and 14 days | *Recommended observation intervals are days 7, 14, 21, and 28.* |
| Parameters measured including the sublethal effects/toxicity symptoms | Mortality, bodyweights, and sublethal effects. | *The test is usually not acceptable if more than 20% of control earthworms die or the total mean weight of control earthworms lose 20% or more of body weight.* |
| Were raw data included? | Yes | |
| Other observations, if any | None | |

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## II. RESULTS AND DISCUSSIONS

### A. MORTALITY:

By 14 days, one mortality was observed in the 1000 mg a.i./kg treatment group. No other mortalities occurred during testing. The NOAEC based on mortality was ≥1000 mg a.i./kg.

**Table 4: Effect of Pyrasulfotole on Mortality of *Eisenia fetida***

| Treatment (mg ai/kg soil) [nominal conc.] | Observation period | | | |
|---|---|---|---|---|
| | Day 7 | | Day 14 | |
| | No Dead | % mortality | No Dead | % mortality |
| Control | 0 | 0 | 0 | 0 |
| 1000 | 1 | 3 | 1 | 3 |
| NOAEC | ≥1000 | | ≥1000 | |
| LOAEC | >1000 | | >1000 | |
| $LC_{50}$ | >1000 | | >1000 | |
| Reference chemical % mortality: $LC_{50}$ | N/A | N/A | N/A | N/A |

### B. SUB-LETHAL TOXICITY ENDPOINTS:

The percent weight change was -2% in the 1000 mg a.i./kg treatment group, compared to 1% for the control. The NOAEC based on weight change was ≥1000 mg a.i./kg. No sublethal effects were observed during testing.



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**Table 5: Sub-lethal Effect of Pyrasulfotole on *Eisenia fetida*.**

| Treatment (mg ai/kg soil) [nominal conc.] | Observation period | | |
|---|---|---|---|
| | Day 0 | Day 14 | |
| | Mean weight (mg) | Mean weight (mg) | % change |
| Control | 330 | 333 | 1 |
| 1000 | 330 | 323 | -2 |
| NOAEC | ≥1000 | ≥1000 | ≥1000 |
| LOAEC | >1000 | >1000 | >1000 |
| $EC_{50}$ | >1000 | >1000 | >1000 |
| Reference chemical % mortality: $LC_{50}$ | N/A | N/A | N/A |

**C. REPORTED STATISTICS:**

The $LC_{50}/EC_{50}$ values were estimated since there were no mortality or weight effects greater than 50%. The body weight change of the treatment group was compared to the control using the Mann-Whitney U-test. The NOAEC was determined based on body weight effects. The statistical results were based on nominal concentrations.

**D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Percent body weight gain was calculated and analyzed using a t-test assuming unequal variances. Additionally, the reviewer compared survival data using a t-test assuming equal variances. These analyses are provided in Appendix I of this DER.

$LC_{50}/EC_{50}$: >1000 mg a.i./kg 95% C.I.: N/A
NOAEC: ≥1000 mg a.i./kg
Probit Slope: Not calculable 95% C.I.: N/A
Endpoint(s) Affected: None

**E. STUDY DEFICIENCIES:**

There were no study deficiencies.

**F. REVIEWERS' COMMENTS:**

Results of the reviewers' statistical verification based were similar to those of the study author.

The earthworms were not fed during testing.

The experimental start date was April 19, 2004 and the experimental termination date was May 4, 2004.



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**G. CONCLUSIONS:**

This study is scientifically sound and is classified as **SUPPLEMENTAL** to the **US EPA** [it deviates from OPPTS testing guidelines for a subchronic toxicity test with earthworms (OPPTS 850.6200) primarily because the test duratiuon was 14 days instead of the required 28 days], and is **ACCEPTABLE** to the **PMRA** and **DEH**, as it fulfills the data requirement for an acute earthworm study. The NOAEC was ≥1000 mg a.i./kg. The $LC_{50}$ and $EC_{50}$ were >1000 mg a.i./kg, the highest treatment group.

$LC_{50}/EC_{50}$: >1000 mg a.i./kg 95% C.I.: N/A
NOAEC: ≥1000 mg a.i./kg
Probit Slope: Not calculable 95% C.I.: N/A
Endpoint(s) Affected: None

## III. REFERENCES:

ECO 85, = UPEC 15, " Test guideline for the assessment of toxicity to earthworms (*Eisenia fetida* SAV.) Laboratory test Draft", BBA AP-3000 b and 2600, February 1981

EPPO (2002): Environmental risk assessment scheme for plant protection products. Chapter 8, Soil organisms and functions. WPPO Bull, in prep.

Finney, D.J.: "Statistical Methods in Biological Assays." Griffin, Weycombe, UK, 1978.

OECD-Guideline No. 207 "OECD- Guideline for Testing Chemicals," "Earthworm, Acute Toxicity Tests", Adopted April 4, 1984.

Ratte, H.T.: "ToxRatPro, Version 2.06 @"

Ratte, H.T.: "ToxRatPro, Version 2.09 @"

Sachs, L.: Angewandte Statistik, Springer Verlag 1978.



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**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

| survival | control | 1000 | t-test |
|---|---|---|---|
| | 10 | 10 | **0.177959** |
| | 10 | 10 | |
| | 10 | 9 | |
| | 10 | 10 | |
| **mean** | **10** | **9.75** | |
| **var** | **0** | **0.25** | |

| weight change | Control | | %wt gain | t-test |
|---|---|---|---|---|
| | day 0 | day 14 | | **0.262369** |
| | 0.32 | 0.31 | -3.125 | |
| | 0.34 | 0.35 | 2.941176 | |
| | 0.33 | 0.34 | 3.030303 | |
| | 0.33 | 0.33 | 0 | |
| | | **mean** | **0.71162** | |
| | | **var** | **8.524423** | |
| | 1000 | | | |
| | 0.32 | 0.31 | -3.125 | |
| | 0.34 | 0.32 | -5.88235 | |
| | 0.33 | 0.32 | -3.0303 | |
| | 0.33 | 0.34 | 3.030303 | |
| | | mean | **-2.25184** | |
| | | var | **14.15002** | |



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| survival | control | 1000 | t-test |
|---|---|---|---|
| | 10 | 10 | **0.177959** |
| | 10 | 10 | |
| | 10 | 9 | |
| | 10 | 10 | |
| **mean** | **10** | **9.75** | |
| **var** | **0** | **0.25** | |

| weight change | Control | | %wt gain | t-test |
|---|---|---|---|---|
| | day 0 | day 14 | | **0.262369** |
| | 0.32 | 0.31 | -3.125 | |
| | 0.34 | 0.35 | 2.941176 | |
| | 0.33 | 0.34 | 3.030303 | |
| | 0.33 | 0.33 | 0 | |
| | | **mean** | **0.71162** | |
| | | **var** | **8.524423** | |
| | 1000 | | | |
| | 0.32 | 0.31 | -3.125 | |
| | 0.34 | 0.32 | -5.882353 | |
| | 0.33 | 0.32 | -3.030303 | |
| | 0.33 | 0.34 | 3.030303 | |
| | | mean | **-2.251838** | |
| | | var | **14.15002** | |

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