DP Barcode: D328639 MRID: 468017-43

DATA EVALUATION RECORD
EARTHWORM TOXICITY TEST
Non-guideline Reproduction and Growth Study

1. **CHEMICAL:** Pyrasulfotole PC Code No.: 000692

2. **TEST MATERIAL:** Isoxaflutole-RPA 203328 (AE B197555) Purity: 99.6%

3. **CITATION**

Authors: Moser, Th. and Scheffczyk, A.

Title: Isoxaflutole-RPA 203328 (AE B197555): Reproduction toxicity to the earthworm *Eisenia fetida* in artificial soil

Study Completion Date: April 26, 2004

Laboratory: ECT Oekotoxikologie GmbH
Bottgerstr. 2-14
Florsheim, Germany

Sponsor: Bayer CropScience AG
Ecotoxicology
Monheim, Germany

Laboratory Report ID: P15RR

MRID No.: 468017-43

DP Barcode: D328639

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

Signature: **Date:** 5/17/06

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: **Date:** 5/23/06

5. **APPROVED BY:** Melissa Panger, OPP/EFED/ERB4

Signature:  **Date:** 10/04/06

6. 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 following chronic (growth and reproduction) exposure. 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.

7. STUDY PARAMETERS:

Scientific Name of Test Organism: *Eisenia fetida*

Age/Size of Test Organism: Adult (>2 months old), 303-585 mg

Type of Test Concentration: Nominal

Definitive Study Duration: 56 days

8. CONCLUSIONS:

The earthworm, *Eisenia fetida*, was exposed to Pyrasulfotole for 56 days at nominal test concentrations of 10.0, 31.6, 100.0, 316.0, and 1000.0 mg a.i./kg dry soil with a negative control. By 28 days, there were no mortalities in the control or treatment groups. No effects on body weights at 28 days or reproduction at 56 days were observed in any treatment group. No effects on food consumption or behavior were observed during testing. The LC_{50} was >1000mg a.i./kg, the EC_{50} was >1000mg a.i./kg, and the NOEC was ≥1000mg a.i./kg.

This study is classified as **SUPPLEMENTAL** (it is a non-guideline study) and is scientifically sound for a chronic (growth and reproduction) toxicity study with earthworms.

Results Synopsis:

LC_{50}: >1000mg a.i./kg 95% C.I.: N/A
NOAEC: ≥1000mg a.i./kg
LOAEC: >1000mg a.i./kg
EC_{50}: >1000mg a.i./kg

Endpoints Affected: None.

9. ADEQUACY OF THE STUDY:

A. Classification: SUPPLEMENTAL

B. Rationale: This study is scientifically sound but is non-guideline.

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: This study was based on procedures of the ISO Guideline 11268-2 "Soil quality-Effects of pollutants on earthworms (*Eisenia fetida*) Part 2: Determination of effects on reproduction" (July 1998).

11. SUBMISSION PURPOSE: This study was submitted to provide data on the chronic (growth and reproduction) toxicity of Pyrasulfotole to earthworms for the purpose of chemical registration.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Eisenia fetida* **andrei (Bouche)**	*Eisenia fetida*
Acclimation Period:	At least 24 hours.
Feeding during acclimation:	Not reported.
Weight: **300-600 mg**	303-585 mg
Age: **Adult**	Adult (>2 months old)
Source:	In-house laboratory cultures

B. Test System

Guideline Criteria	Reported Information
Test Container: **Glass canning jars (1 pint capacity) or equivalent**	Bellaplast container (11 x 15.5 cm area, height of 6 cm) with transparent lids.
Artificial Soil Medium: **Dry weight mixture of:** **68% No. 70 mesh silica sand,** **20% kaolin clay,** **10% sphagnum peat moss,** **2% calcium carbonate**	69.6% quartz sand 20% kaolin clay 10% sphagnum peat 0.4% calcium carbonate pH: 6 ± 0.5 Moisture: 50 ± 10 % WHC_{max} (deionized water added) The test substance was dissolved in deionized water, and mixed into the artificial soil for 10 minutes.
Feeding	5 grams of finely ground cow manure was added to each test vessel weekly.
Weight of Soil: **270 g (wet soil)**	500 g (dry weight).
Moisture Content of Soil: **35%**	40.3-43.1% (study start); 45.1-50.0% (end of study)
Temperature: **22 ∀ 2EC**	18-22EC
Relative Humidity: **∃85%**	Not reported
Light Intensity: **400 lux**	494-574 lux
Photoperiod: **Continuous**	16 hours light/8 hours dark

Guideline Criteria	Reported Information
pH: **6.5 ∀ 0.5**	5.5-5.7 (study start); 5.8-5.9(end of study).

C. Test Design

Guideline Criteria	Reported Information
Dose range: **ratio of 1.5 or 2.0 mg/kg**	Approximately 3.2 mg a.i./kg
Doses: **at least 5**	10.0, 31.6, 100.0, 316.0, and 1000.0 mg a.i./kg dry weight of soil
Controls: **at least 1**	Negative (untreated) control
Replicates per Dose: **3**	4
Number of Worms per Replicate: **10**	10
Test duration: **at least 28 days**	56 days (28 day with adults and 28 days with juveniles)
Observations made every 7 days after test initiation for dead or affected worms?	The mortality of adult earthworms was determined after 28 days. The adult body weights were determined on days 0 and 28. At test termination (56 days), juveniles were counted for reproduction data.
Maximum labeled rate:	Not reported

13. REPORTED RESULTS:

Guideline Criteria		Reported Information
Initial and 7-, 14-, 21-, and 28-day:	**worm weight reported?**	Initial and day 28 adult 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 determined.
Raw data included?		Raw data were reported.

Dose Response

Adult earthworm body weights and mortality

Nominal Concentration in Soil (mg a.i./kg)	Mean Weight (mg) at Day:				% of initial weight (28 days)	# of Dead Worms at Day:				Mortality (%)
	0	7 NR	14 NR	28		0	7 NR	14 NR	28	
Negative Control	417.1	-	-	551.1	132.1	0	-	-	0	0
10.0	411.3	-	-	545.8	132.7	0	-	-	0	0
31.6	407.2	-	-	542.3	133.2	0	-	-	0	0
100.0	406.3	-	-	538.1	132.4	0	-	-	0	0
316.0	405.2	-	-	548.4	135.3	0	-	-	0	0
1000.0	407.8	-	-	583.3	143.0	0	-	-	0	0

NR = not reported

Observations: By 28 days, there were no mortalities in the control or treatment groups. No effects on body weights were observed in any treatment group. The NOAEC based on mortality and body weights was ≥1000.0 mg a.i./kg.

Reproduction

Nominal Concentration in Soil (mg a.i./kg)	Day 56 Number of Juveniles	
	Mean (± stan. dev.)	% of control
Negative Control	285.8 ± 9.5	--
10.0	317.3 ± 64.0	111.0
31.6	270.0 ± 25.4	94.5
100.0	320.8 ± 44.9	112.2
316.0	251.3 ± 90.4	87.9
1000.0	289.0 ± 46.8	101.1

Observations: By 56 days, the mean number juveniles were 317.3, 270.0, 320.8, 251.3, and 289.0 in the 10.0, 31.6, 100.0, 316.0, and 1000.0 mg a.i./kg treatment groups, respectively, compared to 285.8 juveniles in the control. No reproduction effects were observed during testing. The NOAEC based on reproduction was ≥1000.0 mg a.i./kg.

No effects on food consumption or behavior were observed during testing.

Statistical results:

Statistical Method: The data were tested for homogeneity using the Cochran's test and normality using the R/s test procedure. ANOVA and Dunnett's test ($p \leq 0.05$) were used to analyze the body weight and reproduction (number of juveniles) data. The body weight and reproduction data were compared to the control. The LC_{50} and EC_{50} (reproduction) were estimated, since no effects were greater than 50%. The statistical analyses were conducted using the computer software, ToxRat Professional 2.09.

LC_{50}: >1000mg a.i./kg 95% C.I.: N/A
NOAEC: ≥1000mg a.i./kg
LOAEC: >1000mg a.i./kg
EC_{50}: >1000mg a.i./kg

Endpoints Affected: None.

Most sensitive endpoint: Reproduction

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOAEC, LOAEC, and LC_{50} values were visually determined for weight and mortality data, as there were no reductions from control. Reproduction data satisfied the assumptions of normality and homogeneity of variances, so the NOAEC and LOAEC values were determined using ANOVA.

LC_{50}: >1000mg a.i./kg 95% C.I.: N/A
NOAEC: ≥1000mg a.i./kg
LOAEC: >1000mg a.i./kg
EC_{50}: >1000mg a.i./kg

Endpoints Affected: None.

15. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with those of the study author.

In order to validate the test system, the reference toxicant Carbendazim was tested. The results indicated a 100.0% reduction in earthworm reproduction at 3 and 5 mg a.i./kg, compared to the control.

This study was conducted in compliance with Standards of the OECD Good Laboratory Practices (GLP). The quality assurance and no data confidentiality statements were included.

The experimental start date was February 12, 2004 and the experimental termination date was April 8, 2004.

16. REFERENCES:

BBA (1994): Auswirkungen von Pflanzenschutzmitteln auf die Reproduktion und das Wachstum von *Eisenia fetida/Eisenia andrei*. Richtlinien der Biologischen Bundesanstalt fur Land- und Forstwirtschaft fur die Prufung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil VI, 2-2.

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

EU (1991): Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal of the European Communities L230, Vol 34.

Gesetz zum Schutz vor gefahrlichen Stoffen (Chemikaliengesetz-ChemG) vom 25. Juli 1994 (BGBI. I S. 1703) zuletzt geandert am 09. September 2001 (BGBI. I S. 2331) Anhaug 1 (zu §19a Abs. 1) Grundsatze der Guten Laborpraxis (GLP); BGBI. I 2001, 844-854.

Gesetz zur Forderung der Kreislaufwirtschaft und Sicherung der umweltvertraglichen Beseitigung von Abfallen (Kreislaufwirtschafts- und Abfallgesetz-KrW-/AbfG), vom 27.September 1994, Verkundet als Art. 1 G zur Vermeidung, Verwertung und Beseitigung von Abfallen v. 27.9.1994 (BGBI. S. 2705); zuletzt geand. durch Art. 4 G v. 25.8.1998 (BGBI. IS 2455).

ISO (International Organization for Standardization) (1998) Soil Quality-Effects of Pollutants on Earthworms (*Eisenia fetida*). Part 2: Determination of Effects on Reproduction. International Standard ISO 11268-2.

OECD (1984): Guideline for Testing of Chemicals No. 207 "Earthworm, Acute Toxicity Test", Paris.

Sachs L. (1982). Statistische Methoden (5th edition), Springer Verlag, Berlin, Heidelberg, New York.

The OECD Principles of Good Laboratory Practice (1998). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1. Organization for Economic Cooperation and Development, Paris 1998.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

number of juveniles
File: 1743r Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	14411.000	2882.200	1.005
Within (Error)	18	51613.000	2867.389	
Total	23	66024.000		

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

number of juveniles
File: 1743r 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	control	285.750	285.750		
2	10	317.250	317.250	-0.832	
3	31.6	270.000	270.000	0.416	
4	100	320.750	320.750	-0.924	
5	316	251.250	251.250	0.911	
6	1000	289.000	289.000	-0.086	

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

number of juveniles
File: 1743r 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	control	4			
2	10	4	91.253	31.9	-31.500
3	31.6	4	91.253	31.9	15.750
4	100	4	91.253	31.9	-35.000
5	316	4	91.253	31.9	34.500
6	1000	4	91.253	31.9	-3.250

number of juveniles
File: 1743r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	285.750	285.750	285.750
2	10	4	317.250	317.250	289.650
3	31.6	4	270.000	270.000	289.650
4	100	4	320.750	320.750	289.650
5	316	4	251.250	251.250	289.650
6	1000	4	289.000	289.000	289.650

number of juveniles
File: 1743r 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
control	285.750				
10	289.650	0.103		1.73	k= 1, v=18
31.6	289.650	0.103		1.82	k= 2, v=18
100	289.650	0.103		1.85	k= 3, v=18
316	289.650	0.103		1.86	k= 4, v=18
1000	289.650	0.103		1.87	k= 5, v=18

s = 53.548
Note: df used for table values are approximate when v > 20.

number of juveniles
6
4
4
4
4
4
4
control
273
286
296
288
10
238
293
374
364
31.6
293
249
247
291
100
305
304
287
387
316
314
119
268
304
1000
219
314
316
307