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

PMRA Submission #: {.....} EPA MRID #: 468017-42

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D328639
	OECD Data Point	{.....}
	EPA MRID	468017-42
	EPA Guideline	OPPTS 850.6200 (OECD 207)

Test material:	Isoxaflutole	Purity: 989 g/kg
Common name:	RPA 203328	
Chemical name:	IUPAC: Not reported	
	CAS name: Not reported	
	CAS No.: Not reported	
	Synonyms: Not reported	

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Date: {.....}

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Company Code	{.....} [For PMRA]
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Date Evaluation Completed: 8-31-2006

CITATION: Odin-Feurtet, M. 1997. RPA 203328, Acute Toxicity (14-Day) to Earthworms (*Eisenia foetida*). Unpublished study performed by Rhone-Poulenc Agro, Centere de Recherche, Sophia Antipolis Cedex. Study No. SA 97408. Study sponsored by Rhone-Poulenc Agro, Lyon Cedex. The final report issued October 28, 1997.

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.

EXECUTIVE SUMMARY:

In an acute toxicity study, earthworms (*Eisenia foetida*) were exposed to Pyrasulfotole at 95, 171, 309, 556, and 1000 mg a.i./kg dry weight of artificial substrate. The LC_{50} and EC_{50} were >1000 mg a.i./kg. The NOAEC, based on sublethal effects was 556 mg a.i./kg. The LOAEC, based on sublethal effects 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, there were no mortalities in the control or treatment groups. The percent weight change was -6.17, -4.14, -2.76, -2.65, and -2.55% in the 95, 171, 309, 556, and 1000 mg a.i./kg treatment groups, respectively, compared to -7.62% for the control. The mean weight changes were significantly different in the ≥171 mg a.i./kg treatment groups, due to greater weight loss in the control than the treatment groups. The sublethal effect of reduced mobility was observed in the 1000 mg a.i./kg treatment group. No other sublethal effects were observed during testing.

This study is classified as **SUPPLEMENTAL**, is scientifically sound and does satisfy guideline requirements for an acute toxicity study with earthworms.

Results Synopsis

Test Organism Size/Age(Mean Wt or Length): At least 2 months old, 440-559 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: 556 mg a.i./kg
Probit Slope: Not calculable 95% C.I.: N/A
Endpoint(s) Affected: Sublethal effects (reduced mobility)

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 20.0°C was slightly lower than recommended (22 ± 2°C).
3. The light intensity of 586-640 lux was greater than recommended (400 lux).
4. The relative humidity was not reported.
5. The acclimation period of 24 hours 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 (RPA 203328)

Description: White powder

Lot No./Batch No. : GH705

Purity: 989 g/kg

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: Stored in the dark at room temperature.

Physicochemical properties of Pyrasulfotole.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Species: *Eisenia fetida*

(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: At least 2 months old with a clitellum.

Weight at study initiation: 440-559 mg

Source: SARL Moulin, Litz, France

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive test concentrations were based on previous toxicity information. No results were reported.

b. Definitive Study

1. Artificial soil was used; see properties below.

Table 1: Physicochemical Properties of Artificial Soil

Property	Value	Remarks *Criteria*
For natural soil: Texture: % sand % silt % clay Textural classification For artificial substrate (provide composition):	N/A (artificial soil used) 69.4% Industrial quartz sand 20.1% kaolinite clay 10.1% sphagnum moss 0.4% 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 (%)	18% organic material	
Moisture (%)	36.80-38.89% at test initiation 35.64-37.26 at test termination	

Table 2: Experimental Design

Parameter	Detail	Remarks *Criteria*
Acclimation: duration: conditions (state if same as the test conditions): health:	 24 hours same as test Not reported	The acclimation period of 24 hours was less than recommended (7 days). *Earthworms should be acclimated at test temperature for 7 days.*
Soil [fresh or stored]	Stored	
Test Container material size amount of soil/substrate	 Disposible plastic containers 1.5 L 500 g soil dry weight with 180 mL of deionized water.	
No. of replicates: per treatment group: per control:	 4 4	 *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: nominal: measured:	 95, 171, 309, 556, and 1000 mg/kg 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) name: concentration:	N/A	

Parameter	Detail	Remarks *Criteria*
Test conditions: temperature Lighting conditions Moisture	 20.0°C Continuous, 586-640 lux Not reported (relative humidity)	The temperature range of 20.0°C was slightly lower than recommended (22 ± 2°C). The light intensity of 586-640 lux was greater than recommended (400 lux). The relative humidity was not reported. *Recommended temperature: 22 + 2°C* *Recommended lighting: Continuous illumination, with a light intensity of 400 lux* *Recommended relative humidity: above 85%*
Duration of the study	14 days	 *Recommended duration of study is 28 days.*

2. Observations:

Table 3: Observations

Parameters	Details	Remarks *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	

II. RESULTS AND DISCUSSIONS

A. MORTALITY:

By 14 days, there were no mortalities in the control or treatment groups. 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
95	0	0	0	0
171	0	0	0	0
309	0	0	0	0
556	0	0	0	0
1000	0	0	0	0
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 -6.17, -4.14, -2.76, -2.65, and -2.55% in the 95, 171, 309, 556, and 1000 mg a.i./kg treatment groups, respectively, compared to -7.62% for the control. The mean weight changes were significantly different in the ≥171 mg a.i./kg treatment groups, due to greater weight loss in the control than the treatment groups. The NOAEC based on weight change was ≥1000 mg a.i./kg. The sublethal effect of reduced mobility was observed in the 1000 mg a.i./kg treatment group. No other sublethal effects were observed during testing. The NOAEC based on sublethal effects was 556 mg a.i./kg.

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	532	492	-7.62
95	506	474	-6.17
171	507	486	-4.14*
309	462	449	-2.76*
556	509	496	-2.65*
1000	451	440	-2.55*
NOAEC	≥1000	≥1000	≥1000
LOAEC	>1000	>1000	>1000
EC_{50}	>1000	>1000	>1000
Reference chemical % mortality: LC_{50}	N/A	N/A	N/A

* The weight change was significantly different compared to the control (α=0.05). However, the control weights decreased more than the treatment group weights.

C. REPORTED STATISTICS:

The LC_{50}/EC_{50} values were estimated since there was no treatment group with mortality or weight effects greater than 50%. The body weight change data was analyzed for homogeneity of variance using Bartlett's test and ANOVA. The body weight changes of the treatment groups were compared to the control using the Mann-Whitney test. The NOAEC was determined based on sublethal effects. The statistical results were based on nominal concentrations.

D. <u>VERIFICATION OF STATISTICAL RESULTS</u>:

Statistical Method: Statistical methods were not necessary to verify the results of this study. There was no mortality and body weight change in treatment groups increased compared to the control group. The NOAEC was based on reduced immobility reported at the highest treatment level.

LC_{50}/EC_{50}: >1000 mg a.i./kg 95% C.I.: N/A
NOAEC: 556 mg a.i./kg
Probit Slope: Not calculable 95% C.I.: N/A
Endpoint(s) Affected: Sublethal effects

E. STUDY DEFICIENCIES:

The study deviated from U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.6200, *Earthworm subchronic toxicity test* in that the study duration was 14 days

instead of the recommended 28 days, and the acclimation period of 24 hours was less than recommended (7 days).

F. REVIEWER'S COMMENTS:

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

The earthworms were not fed during acclimation or testing.

The experimental start date was October 2, 1997 and the experimental termination date was October 17, 1997.

G. CONCLUSIONS:

This study is scientifically sound and is classified as **SUPPLEMENTAL**. The NOAEC was 556 mg a.i./kg based on sublethal effects (i.e., reduced mobility). 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: 556 mg a.i./kg
Probit Slope: Not calculable 95% C.I.: N/A
Endpoint(s) Affected: Sublethal effects (reduced mobility)

III. REFERENCES:

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