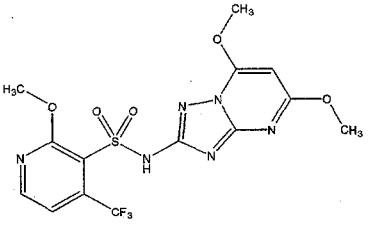
Data Evaluation Report on the acute toxicity effects of pyroxsulam (XDE-742) on earthworms

PMRA Submission Number 2006-4727; ID 1283187 **EPA MRID Number 469085-05 169084-xx APVMA ATS 40362**

Data Requirement:	PMRA DATA CODE:	9.2.3.1
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.9.1
	EPA Guideline:	Non-guideline study

Test material: Pyroxsulam or XR-742 **Purity (%):** 98%
Common name: XDE-742
Chemical name: 3-pyridinesulfonamide,N,-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl).
IUPAC: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.: 422556-08-9
Synonyms: XR-742, X666742

Chemical Structure:



Primary Reviewer: Daryl Murphy 22/02/08 **Date:** 29 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland 22/2/08 **Date:** 29 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Ann Lee (#1639) **Date:** May 8, 2007
Environmental Assessment Directorate, PMRA for Ann Lee 05/03/08

Christopher Salice **Date:** 20 June 2007
Environmental Fate and Effects Division, U.S. Environmental Protection Agency 4/14/08

Company Code: DWE
Active Code: JUA
Use Site Category: 13, 14
EPA PC Code: 108702

CITATION: Ward, T. J. and Boeri, R. L. 2004. XR-742: 14 Day Soil Exposure Acute Toxicity to the Earthworm, *Eisenia foetida*. T.R. Wilbury Laboratories, Inc. 40 Doaks Lane, Marblehead, Massachusetts 01945. T.R. Wilbury Study Number 2731-DO and Dow Study Number 040029. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana 46268. June 7, 2004. Unpublished report.

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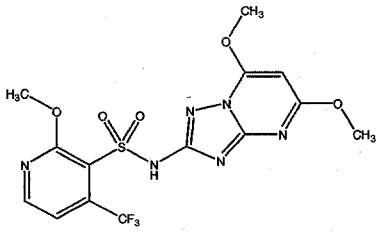

Primary Reviewer: Daryl Murphy **Date:** 29 March 2007
Australian Government Department of the Environment and Water Resources (DEW)

Secondary Reviewers: Jack Holland **Date:** 29 March 2007
Australian Government Department of the Environment and Water Resources

Ann Lee (#1639) **Date:** May 8, 2007
Environmental Assessment Directorate, PMRA

Christopher Salice **Date:** 20 June 2007
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EXECUTIVE SUMMARY:

Note: The PMRA suggests using 'transformation product' instead of 'metabolite' throughout the DER. The US EPA has commented, with respect to this proposal that, " ... some reviewers prefer particular terminology. Thus, whether the term metabolite, degradate or transformation product is used, depends on the individual reviewer. It is my understanding that metabolite is typically used to refer to a product of biotic degradation, where degradate can apply to either abiotic or biotic processes. The term transformation product is ... where the compound may not be "degraded" but simply conjugated with say a glucuronide. It is up to the team to decide which term to use; however, ideally, it should be used consistently throughout all of the DERs." DEW supports this approach which would mean that "metabolite" is retained for the pyroxsulam ecotoxicity studies and, that in future work share projects, a decision is made at an early stage whether to refer to metabolites or transformation degradation products.

In a 14 day acute toxicity study, earthworms (*Eisenia foetida)* were exposed to pyroxsulam at 0 and 10,000 mg pyroxsulam/kg dry weight of artificial soil. The reference chemical used was 2-chloracetamide with the concentration in the soil not reported. The experiment was carried out in accordance with OECD 207, Earthworm, Acute Toxicity Tests. Mortalities and sublethal effects apart from weight change were not observed in the either the control or pyroxsulam-exposed earthworms over the 14 day exposure period. Weight change in the control earthworms ranged from -12 to +9.4% while in the pyroxsulam-exposed earthworms, the weight change range was -32 to -4.2%. The 14 day LC_{50} was >10,000 mg pyroxsulam/kg dw of artificial soil. The 14 day EC_{50} for sublethal effects apart from weight loss was >10,000 mg pyroxsulam/kg dw of artificial soil.The 14 day NOEC, based on mortality and sublethal effects other than weight loss was 10,000 mg pyroxsulam/kg dw of artificial soil. Based on weight loss, the 14 day NOEC was <10,000 mg pyroxsulam/kg dw of artificial soil.

The mortality and sublethal effects (including loss of weight) result in technical grade pyroxsulam being considered as very slightly toxic to the earthworm, *Eisenia foetida* (14 day LC50 and EC50 both >10,000 mg/kg soil, dry weight) based on a nominal concentration of pyroxsulam based on the Australian DEW classification.

The study significantly deviated from OECD 207 with respect to soil moisture content (the soil moisture content was approximately 25% at the beginning of the test and approximately 26% at the end of the test whereas OECD 207 refers to overall moisture content of about 35 per cent of the dry weight.) and use of a limit test (not part of OECD 207). As a result, the study is classified as supplemental for the APVMA.

While not required by OECD 207, the absence of pyroxsulam determinations in the treated soil to confirm that the nominal test dose of 10,000 mg/kg soil, dry weight was achieved and that the chemical was stable in the test medium are considered study deficiencies. Additionally, a definitive NOEC value for weight loss could not be determined.

The PMRA does not have share the same study acceptability classification scheme as the APVMA or the US EPA. This study is classified acceptable to the PMRA as it contains useful information for risk assessment purposes.

The EPA secondary reviewer noted that this study is not required under current EPA guidelines and is classified as Supplemental and may be useful for risk assessment purposes. However, the lack of pyroxsulam measurements in soil and the lower than specified soil moisture content are uncertainties.

Results Synopsis

14 day LC_{50}:	>10,000 mg pyroxsulam/kg dry weight (dw) artificial soil
95% C.I.:	Not determined

14 day NOEC: (mortality and sublethal effects other than weight loss):	10,000 mg pyroxsulam/kg dw artificial soil
14 day NOEC (weight loss):	<10,000 mg pyroxsulam/kg dw artificial soil
Probit Slope and 95% C.I.:	Not reported
14 day EC_{50} (including weight loss):	>10,000 mg pyroxsulam/kg dw artificial soil
95% C.I.:	Not reported
Endpoint(s) affected:	There were no compound-related effects (survival or sublethal) apart from weight loss noted during this study.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

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

The test was conducted for Dow AgroSciences LLC, according to the protocol developed for T.R. Wilbury Study Number 2731-DO.

Deficiencies and deviations from guidelines are summarised on Table 10, page 19 of this draft DER.

COMPLIANCE:

The study was conducted in compliance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency - FIFRA GLPs Title 40 CFR Part 160- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule;

Japan Ministry of Agriculture, Forestry and Fisheries (MAFF) Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals; and

Organization for Economic Cooperation and Development (OECD) The OECD Principles for Good Laboratory Practice [C(97) 186 / Final].

Stability of the test substance under storage and test conditions was not verified. The calibration of the scale used to weigh artificial soil components was not verifiable. Characterization of the reference toxicant, 2-chloroacetamide, was not performed in compliance with applicable GLP rules.

A signed and dated Statement of No Data Confidentiality Claims was provided.

A signed and dated Compliance with Good Laboratory Practice Standards statement was provided.

A signed and dated Quality Assurance Statement was provided.

A. MATERIALS:

1. Test Material XR-742, referred to as pyroxsulam in this draft DER.

Description: An off-white powder.

Lot No./Batch No.: TSN103826 (Study profile template (Mercer, 2006) refers to Lot No./Batch No. as E0952-52-01).

Purity: 98% pyroxsulam

Stability of Compound under Test Conditions: Stability of the test substance under storage and test conditions was assumed but not verified by the analysis of samples during the definitive toxicity test (See Table 10 Summary of deficiencies/deviations from guidelines, page 19 of this draft DER).

With respect to the assumption of pyroxsulam stability in soil, the study reports for hydrolytic stability (Yoder, 2004), aerobic soil degradation studies (Yoder *et al.*, 2006a and b) and a field dissipation in Canada (Roberts *et al.*, 2006) identify pyroxsulam as being hydrolytically stable but having DT50 values ranging from 2 to 10 days (Yoder *et al.*, 2006a) or 1 to 17 days with a mean of 4.6 days (Yoder *et al.*, 2006b), or in the Canadian field dissipation study, a mean field half-life of 13 days with a range of 5-29 days (Roberts *et al.*, 2006). The soil dissipation half-lives are indicative of a potential degradation of pyroxsulam in the artificial soil used in the earthworm study.

Storage conditions of test chemicals: Prior to use, the pyroxsulam was stored at room temperature in the dark.

Physicochemical properties of pyroxsulam:

Parameter	Values	Comments
Water solubility at 20°C		
pH 4	0.0164 g/L	Turner, 2004 (a)
pH 6	0.0626 g/L	Turner, 2004 (a)
pH 7	3.2 g/L	Turner, 2004 (a)
Vapour pressure	<1E-7	Madsen 2003
UV absorption	Not available	
pKa	4.670	Cathie, 2004
Kow		
pH 4	12.1 (log Pow = 1.08)	Turner, 2004 (b)
pH 7	0.097 (log Pow = -1.01)	Turner, 2004 (b)
pH 9	0.024 (log Pow = -1.60)	Turner, 2004 (b)

The physicochemical properties are taken from the Study Profile Template (Mercer, 2006) which noted that the UV data were unavailable at the time of publication of the Study Profile Template. **Note that the Kow values shown in the study profile template were misordered. The correct values (confirmed by examination of Turner (2004b) in Madsen (2006)) are shown above in the physicochemical properties of pyroxsulam table.**

2. Test organism:

Species:	Earthworms, *Eisenia foetida*
Age at test initiation:	Not reported but the earthworms had well developed clitella.
Weight at study initiation:	Control earthworms Mean 0.305 g/earthworm, range 0.3001-0.3118 g/earthworm Earthworms exposed to pyroxsulam Mean 0.300 g/earthworm, range 0.3000-0.3008 g/earthworm
Source:	A commercial supplier (Paul Cosmides, 432 Lawton Street, San Francisco, California).

All worms were in good condition at the beginning of the study.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study:
A range-finding test was conducted for 14 days from 15 to 29 April 2004. Nominal concentrations of test substance were 0 (control), 1.1, 10, 100, 1,000, and 10,000 mg pyroxsulam/kg (dry weight) soil. The test substance was added to dry soil, and the soil was then mixed well and hydrated with deionised water. After 14 days of exposure during the range-finding toxicity test there was 100% survival in the control and at 1.1, 10, 100, 1,000, and 10,000 mg pyroxsulam/kg.

Additionally, a 14-day soil toxicity test was conducted from 4 to 18 December 2003 with the reference toxicant, 2-chloracetamide, resulting in an LC50 of 16 mg/kg, wet weight. This value was reported as within the range normally obtained in the performing laboratory. OECD 207 recommends that the test report should include, *inter alia*, results for mortality of the test and reference substances and LC50 results and the data used to calculate such values. As the OECD wording of "should" is used, this is taken as a non-binding requirement and the failure to present the reference material toxicity data is not considered a deviation from the OECD guideline.

The results from the range finding test were used to determine the conditions for the definitive study by conducting that study as a limit test using nominal concentrations of 0 (control) and 10,000 mg pyroxsulam/kg soil.

b. Definitive Study

The study was conducted according to T.R. Wilbury Study Protocol 2731-DO (XR-742: 14 Day Soil Exposure Acute Toxicity Study with the Earthworm, *Eisenia foetida*), which was signed by the study director on 27 February 2004. It is based on OECD Guideline No. 207 (1984) and procedures of the U.S. FDA (1987). A copy of the study protocol was provided in the study report.

The experimental start date was 15 April 2004 and the experimental termination date, 24 May 2004 with the definitive test conducted from 7 to 21 May 2004.

The earthworms were exposed to only one test concentration (10,000 mg pyroxsulam/kg soil, dry weight), i.e. a limit test. It is noted that US EPA OPPTS 850.6200 Earthworm Subchronic Toxicity Test refers to that situation where, if a range finding study shows the 28 day LC50 value is >1,000 mg test substance (100 percent active ingredient) per kilogram dry weight of artificial soil, the definitive test does not have to be done (OPPTS 850.6200 (2) Range-finding test (iii) refers), which is the situation here. However, OECD 207 does not refer to use of a limit test. Instead it states that five concentrations in a geometric series are used and notes that one concentration resulting in 0 mortality and one resulting in total mortality should be used.

Consequently, this deviation from OECD 207 is considered a deficiency (see Table 10, Summary of deficiencies/deviations from guidelines, page 19 of this draft DER).

1. **Soil**

   Soil (Lot # 040704) was formulated by combining industrial sand, kaolin clay, and finely ground sphagnum peat in a 70:20:10 ratio by weight. At the beginning of the test the control soil had a moisture content of 25% and a pH of 6.5. At the end of the test the soil moisture content was 26% and the pH was 6.3. A representative sample of artificial soil was characterized for pesticides and PCBs. That soil sample contained <0.167 mg/kg organophosphorus pesticides, PCBs, and organochlorine pesticides. Water used to hydrate the soil during the toxicity test was deionised water prepared at T.R. Wilbury Laboratories in Marblehead, Massachusetts.

Note that in **Error! Reference source not found.**, **Error! Reference source not found.** and **Error! Reference source not found.** (and elsewhere where relevant), the template has references to EPA/OECD requirements. The PMRA has provided advice for other ecotoxicity DERs that these template requirements are outdated and reference is now made to current guidelines. As a result, while the template requirements with respect to the EPA/OECD requirements are still shown in the tables, compliance of the study is judged against the current relevant US EPA, OECD etc. requirements.

Table 1. Physicochemical properties of soil.

Property	Value	Remarks *Criteria*
For artificial soil substrate Composition	Artificial soil: Industrial sand 70% Kaolin clay 20% Sphagnum peat 10% % values are by weight and the sphagnum peat was finely ground.	See Table 10, Summary of deficiencies/deviations from guidelines, page 19 of this draft DER with respect to pH and the moisture content. *EPA/OECD require that the testing medium be 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 Reported in the Study Profile Template (Mercer, 2006) as 1:1 soil:water.	At the beginning of the test the control soil had a pH of 6.5 and, at the end of the test, 6.3. No reference was made to the use of calcium carbonate being used for pH adjustment of the soil prior to hydration.	No experimental details were provided. OECD 207 states that the pH of the artificial soil is adjusted to 6.0 ± 0.5 by addition of calcium carbonate.
Organic carbon (%)	Not reported.	No provided but not required by

		OECD 207. Therefore not considered a deviation from that guideline.
Moisture (%)	At the beginning of the test the control soil had a moisture content of 25% and, at the end of the test, 26%.	*EPA/OECD requires the soil be moistened to 35% by weight with deionized/distilled water.* Assumed to be on a dry weight basis based on information in study report.

Table 2. Experimental Design.

Parameter	Value	Remarks *Criteria*
Acclimation:		See Table 10, Summary of deficiencies/deviations from guidelines, page 19 of this draft DER with respect to the acclimatisation period.
Duration:	Worms were acclimatised in artificial soil at T.R. Wilbury Laboratories for two days before the toxicity test. Temperature during acclimation ranged from 21.2 to 21.5°C.	*EPA/OECD require that earthworms be acclimated at test temperature for 7 days.* OECD 207 refers to earthworms which have been conditioned for 24 hours in an artificial soil.
Conditions (state if same as the test conditions):	Not specified but the study protocol stated that the earthworms would be maintained in artificial soil during acclimatisation. The temperature during acclimatisation ranged from 21.2 to 21.5°C. In the exposure period, the temperature range was 19.9 to 21.1°C. The earthworms were not fed during acclimation or testing.	Parameter considered met. As noted above, OECD 207 states that the earthworms be conditioned in an artificial soil.
Health:	During acclimation worms were not treated for disease and they were free of apparent disease, injuries, and abnormalities at the beginning of the test. No mortality was observed during the acclimation period.	Parameter considered met. No specific reference to the earthworms' health identified in OECD 207.

Parameter	Value	Remarks *Criteria*
Soil [fresh or stored]	Not stated in the study report but indicated as made up for the study.	
Test Container Material: Size: Amount of soil or substrate:	Glass jars Half gallon (~1.9 L) 1.0 kg of hydrated artificial soil.	Parameter considered met.
No. of replicates Per treatment group: Per control:	4 4	Parameter considered met. *EPA/OECD requires 3 replicates and a control.*
No. of earthworms per treatment	10 per treatment or control replicate (A total of 40 earthworms for the control replicates and of 40 for the pyroxsulam treatment replicates). The study protocol noted that the loading rate was 25 g earthworms/kg soil.	Parameter considered met as OECD 207 refers to use of four control replicates and four replicates for each test concentration. Each replicate to contain 10 earthworms. *EPA/OECD requires a minimum of 30 earthworms per treatment and a control, 10 per each of three replicates and the control.*
Co-solvents used or not (if yes report the name and concentration)	No co-solvents were reported used.	Parameter met.
Rates of application Nominal:	Nominal concentrations of the test substance, determined on a dry weight basis, were 0 mg pyroxsulam/kg (control) and 10,000 mg pyroxsulam/kg.	With respect to use of a limit test, see Table 10, Summary of deficiencies/deviations from guidelines, page 19 of this draft DER.

Parameter	Value	Remarks *Criteria*
Measured:	Not measured. The study protocol reported that the measured concentration of pyroxsulam in the test media would not be determined analytically nor would samples be collected from the exposure vessels.	*EPA/OECD require exposure 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.* Appropriate amounts of test substance were added to dry soil in each test vessel and the soil was then hydrated with deionised water and thoroughly mixed. OECD 207 does not require that test concentrations be analytically determined.
Stability and homogeneity of test material in the medium?	Stability of the test substance under the test conditions was assumed but not verified by the analysis of samples during the definitive toxicity test.	See Table 10, Summary of deficiencies/deviations from guidelines, page 19 of this draft DER with respect to stability in the test medium. OECD 207 does not refer to homogeneity of the test material in the medium.
Test conditions:		
Temperature:	Temperature during the definitive toxicity test ranged from 19.9 to 21.0 in the controls and 19.9 to 21.1°C in the pyroxsulam test vessels over 14 days.	Parameter considered met as OECD 207 indicates a temperature of 20 ± 2°C as acceptable. *EPA requirements:* *Temperature: 22 ± 2°C.*
Lighting conditions:	A 24 hour light and 0 hour dark photoperiod was maintained throughout the test at an average light intensity of approximately 780 lux.	*Lighting: Continuous illumination, with a light intensity of 400 lux* Parameter considered met as OECD 207 refers to a light intensity of 400 to 800 lux.
Moisture:	At the beginning of the test the control soil had a moisture content of 25% and, at the end of the test, 26%. Relative humidity was not reported.	*Relative humidity: above 85%* See Table 10, Summary of deficiencies/ deviations from guidelines, page 19 of this draft DER with respect to moisture content and relative humidity.

Parameter	Value	Remarks *Criteria*
Duration of the study	14 days	Requirement considered met with respect to the OECD 207 requirement that the test be conducted for 14 days. *EPA/OECD require a 28-day test.*
Reference chemical, if used Name: Concentration:	2-chloracetamide as the reference toxicant. Not reported.	Requirement considered met. Although OECD 207 states the test report should give results on the mortalities seen in the reference substance exposure, this is not mandatory. Consequently, while the absence of such data is a deficiency, it is not considered a deviation from the OECD guideline.

2. Observations:

Table 3. Observations

Parameters	Details	Remarks *Criteria*
Observation intervals	Number of surviving earthworms and occurrence of sublethal effects (excluding weight loss) were recorded at days 0 (start), 7 and 14. Burrowing time was stated to have been recorded after 0, 7, and 14 days (after placing the earthworms on the soil surface). Average wet weight of surviving worms was determined at the beginning of the test and after 14 days (weight loss is considered a sublethal effect determined by calculation to distinguish it from sublethal effects determined by observation.) Soil temperature was measured and recorded daily in each test vessel. pH and moisture content of the soil were determined at the beginning and end of the test.	Requirement considered met with respect to the OECD 207's requiring determination of mortality at day 0 an 7. Although a 14 day burrowing time is referred to, this is considered an error.
Parameters measured including the sublethal effects/toxicity symptoms	Mortality at days 7 and 14. Burrowing time at days 0, 7 and 14. Occurrence of sublethal effects including average wet weight change over 14 days.	Requirement considered met. A worm was considered alive if there was a response to gentle prodding of its anterior end. The study protocol identified sublethal effects as presence (or absence) of cocoons, lethargy, softness, colour change etc. Before weighing, the earthworms were gently rinsed and blotted. *EPA/OECD require that the test be found unacceptable 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?	The raw data and final report are archived at Dow AgroSciences LLC	Requirement considered met as OECD 207 does not refer to the need to supply

	Indianapolis, Indiana. Tabulated mortality and average weights for controls and pyroxsulam-exposed earthworms were presented. The mortality and sublethal effects data were the number of dead or affected (including burrowing time) earthworms in each of the control and 10,000 mg pyroxsulam/kg soil replicates (in all cases, the value recorded as zero). The tabulated average weight data gave the mean weights of the ten worms in each control and 10,000 mg pyroxsulam/kg soil replicate at day 0 and day 14. Daily temperature records were also presented. Data on the burrowing times were not submitted but it was reported that all earthworms had burrowed into the soil within 10 minutes on days 0, 7 and 14.	raw data. The tabulated data presented were sufficient to allow statistical verification of the study's results and, consequently, the absence of raw data is not considered to have adversely affected the reviewer's assessment of the study. The US EPA advised elsewhere that tabular data are usually considered "raw data" with the guiding principle being whether the data presented allowed repeating of the statistical analyses. This is considered to support the decision that the raw data absence was not of significance on this occasion.
Other observations, if any	The continuously recorded temperature was 20 ± 2°C rather than 20 ± 1°C during the first seven days of the test, apparently as the result of an improper calibration of the recorder (temperatures in each test vessel were measured directly once each day and these temperatures were all 20 ± 1°C).	This protocol deviation is not considered a deficiency as OECD 207 allows for a temperature range of 20 ± 2°C, which was the continuously recorded test temperature range.

II. RESULTS AND DISCUSSIONS

A. MORTALITY:

Forty earthworms (10 per replicate) were exposed to a control and forty to 10,000 mg pyroxsulam/kg soil, on a dry weight basis. There were no mortalities in any of the control or pyroxsulam treated replicates. Exposure of worms to pyroxsulam resulted in 7-day and 14-day median lethal concentrations (LC50s) greater than 10,000 mg pyroxsulam/kg, based on survival. The reference toxicant, 2-chloroacetamide, was reported as having an LC50 of 16 mg/kg, wet weight, a value stated as within the range normally obtained in the performing laboratory.

The OECD 207 validity requirement that mortality in the controls no exceed 10% at the end of the soil test was met by the study.

The absence of mortality in the controls and 10,000 mg pyroxsulam/kg soil dry weight is demonstrated by the data shown in Table 4.

Table 4. Effect of pyroxsulam on mortality of the earthworm, *Eisenia foetida*.

Treatment (mg pyroxsulam/kg soil) [record measured and nominal conc. used]	Observation period					
	Day 0		Day 7		Day 14	
	No. Dead	% mortality	No. Dead	% mortality	No. Dead	% mortality
Control						
Replicate 1	0	0	0	0	0	0
Replicate 2	0	0	0	0	0	0
Replicate 3	0	0	0	0	0	0
Replicate 4	0	0	0	0	0	0
10,000 mg pyroxsulam/kg soil, dry weight						
Replicate 1	0	0	0	0	0	0
Replicate 2	0	0	0	0	0	0
Replicate 3	0	0	0	0	0	0
Replicate 4	0	0	0	0	0	0

NOEC (mortality at 7 and 14 days)		10,000 mg pyroxsulam/kg soil, dry weight
LOEC (mortality at 7 and 14 days)		Not reported
LC_{50} (mortality at 7 and 14 days)		>10,000 mg pyroxsulam/kg soil, dry weight
Reference chemical (2-chloroacetamide)	% mortality:	Not reported
	LC_{50}:	14 day LC50 reported as 16 mg/kg, wet weight.

B. SUB-LETHAL TOXICITY ENDPOINTS:

All worms exposed to the control and 10,000 mg pyroxsulam/kg burrowed into the soil within 10 minutes on days 0 and 7 (burrowing time data were not provided). No sublethal effects (excluding weight loss) were observed in the control exposure. Average weight loss of control worms was 1.9% and the average weight loss of the worms exposed to 10,000 mg pyroxsulam/kg was 18%.

The 14-day no observed effect concentration (NOEC) was 10,000 mg pyroxsulam/kg, based on time to burrow at days 0 and 7, survival, and sublethal effects other than weight loss. The NOEC based on weight loss was <10,000 mg pyroxsulam/kg.

The average earthworm weights at days 0 and 14 in the control and pyroxsulam-exposed earthworms and the percentage weight changes at the end of the exposure period are shown in Table 5.

Table 5. Sub-lethal effect of pyroxsulam, as weight change over 14 days on the earthworm, *Eisenia foetida*.

Treatment (mg pyroxsulam/kg soil) (nominal concentration)		Observation period			
		Number of earthworms alive on day 14	Average weight/worm (grams)		
			Day 0	Day 14	% change
Control					
Replicate 1		10	0.3001	0.2892	-3.6%
Replicate 2		10	0.3118	0.3412	+9.4%
Replicate 3		10	0.3030	0.2678	-12%
Replicate 4		10	0.3062	0.3022	-1.3%
10,000 mg pyroxsulam/kg soil, dry weight					
Replicate 1		10	0.3000	0.2420	-19%
Replicate 2		10	0.3001	0.2049	-32%
Replicate 3		10	0.3001	0.2262	-25%
Replicate 4		10	0.3008	0.2881	-4.2
NOEC (weight loss)		<10,000 mg pyroxsulam/kg soil, dry weight			
NOEC (burrowing time, survival and sublethal effects other than weight loss)		10,000 mg pyroxsulam/kg soil, dry weight			
LOEC		Not reported			
EC_{50}		Not reported			
Reference chemical	% mortality:	Not reported			
	LC_{50}:	16 mg 2-chloracetamide/kg, wet weight			

Note: The earthworms were not fed during the acclimatisation or exposure periods.

C. REPORTED STATISTICS:

The 7-day and 14-day LC50 values were not calculated using the moving average method (Stephan, 1978. This reference was not in the study report's references section) because 100% survival occurred at the only tested concentration. The NOEC was determined at 14 days using survival/sublethal effects data (but not weight loss), and again using weight loss data. Because 100% survival occurred at the single tested concentration with no sublethal effects visually determinable, these data did not require statistical analysis. Weight loss data (which are considered a calculated sublethal effect to distinguish it from visually determined sublethal effects) were statistically analysed using a parametric "t" test. Tabulated mortality and sublethal effects data were provided in the study report.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Because of the study's results (absence of mortality and sub-lethal effects apart from weight loss), the statistical evaluation of the biological data was not attempted. Because mortality and sublethal effects (apart from weight loss) were less than 50% at the concentration tested, statistical determination of LC50 and EC50 values could not be determined. Consequently, the 7 and 14 day LC50 and EC50 values were all empirically determined to be greater than the nominal concentration tested i.e. >10,000 mg pyroxsulam/kg soil, dry weight basis. The day 7 and day 14 NOECs were determined as the nominal concentration tested that exhibited no mortality or sub-lethal effects apart from weight loss.

Verification of weight loss statistics

The mean earthworm weight data reported were analysed by the TidePool Scientific Software program, ToxCalc (v5.0.23A).

The following comparisons were made:

- Comparison of control and pyroxsulam-exposed mean earthworm weights at day 0;
- Comparison of control earthworm mean weights at day 0 compared to day 14;
- Comparison of the mean weights of earthworms exposed to 10,000 mg pyroxsulam/kg soil, dry weight at days 0 and 14; and
- Comparison of mean day 14 weights of the control earthworms and the earthworms exposed to 10,000 mg pyroxsulam/kg soil, dry weight.

Comparison of control and pyroxsulam-exposed mean earthworm weights at day 0

To determine if the mean weights of the control and pyroxsulam-exposed worms were statistically significantly different at the start of the exposure period, the mean control and exposed earthworms weights at day 0 were compared. The respective mean weights at day 0 are shown in Table 6.

Table 6. Mean weights of control and pyroxsulam-exposed earthworms at day 0.

Replicate number	Mean earthworm weights (mg) based on 10 earthworms/replicate at day 0	
	Control	10,000 mg pyroxsulam/kg
1	0.3001	0.3
2	0.3118	0.3001
3	0.303	0.3001
4	0.3062	0.3008

The ToxCalc analysis of the data gave the following results:

Conc-ppm	1	2	3	4								
Control	0.3001	0.3118	0.3030	0.3062								
10000	0.3000	0.3001	0.3001	0.3008								
			Transform: Untransformed						1-Tailed			
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
Control	0.3053	1.0000	0.3053	0.3001	0.3118	1.642	4					
10000	0.3003	0.9835	0.3003	0.3000	0.3008	0.123	4	1.999	2.353	0.0059		
Auxiliary Tests							Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.8942		0.749		0.68627	2.57852
F-Test indicates unequal variances (p = 1.35E-03)							183.872		47.4672			
Hypothesis Test (1-tail, 0.05)							MSDu	MSDp	MSB	MSE	F-Prob	df
Heteroscedastic t Test indicates no significant differences Treatments vs B-Control							0.00591	0.01937	5.1E-05	1.3E-05	0.0925	1, 6

As the t score (1.999) is less than the critical one tailed t value of 2.353, no statistically significant difference in the mean earthworm weights at days 0 for the control and pyroxsulam-exposed earthworms is indicated.

Comparison of control earthworm mean weights at day 0 compared to day 14

To see if the mean weights of the control earthworms differed significantly between day 0 and day 14, the mean control weights at those times were compared. The mean control weights at days 0 and 14 are shown in Table 7.

Table 7. Mean weights of the control earthworms at days 0 and 14.

	Mean earthworm weights (mg) based on 10 earthworms/replicate	
Control	Day 0	Day 14
Replicate 1	0.3001	0.2892
Replicate 2	0.3118	0.3412
Replicate 3	0.303	0.2678
Replicate 4	0.3062	0.3022

The ToxCalc analysis of this control data gave the following results:

Conc-ppm	1	2	3	4								
Day 0	0.3001	0.3118	0.3030	0.3062								
Day 14	0.2892	0.3412	0.2678	0.3022								
			Transform: Untransformed						1-Tailed			
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
Day 0	0.3053	1.0000	0.3053	0.3001	0.3118	1.642	4					
Day 14	0.3001	0.9830	0.3001	0.2678	0.3412	10.281	4	0.331	1.943	0.0304		
Auxiliary Tests							Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.90074		0.749		0.76851	2.84078
F-Test indicates equal variances (p = 0.01)							37.8806		47.4672			
Hypothesis Test (1-tail, 0.05)							MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences Treatments vs B-Control							0.03037	0.09948	5.4E-05	0.00049	0.75181	1, 6

As the t score (0.33) is less than the critical one tailed t value of 1.94, no statistically significant difference in the mean earthworm weights at days 0 and 14 is indicated in the controls.

Comparison of the mean weights of earthworms exposed to 10,000 mg pyroxsulam/kg soil, dry weight at days 0 and 14

To determine if the mean weights of the pyroxsulam-exposed earthworms at days 0 and 14 were statistically significantly different, the mean weights of the exposed earthworms at those times were compared. The respective mean weights are shown in Table 8.

Table 8. Mean weights of pyroxsulam-exposed earthworms at day 0 and day 14.

10,000 mg pyroxsulam/kg soil, dry weight	Mean earthworm weights (mg) based on 10 earthworms/replicate	
	Day 0	Day 14
Replicate 1	0.3	0.242
Replicate 2	0.3001	0.2049
Replicate 3	0.3001	0.2262
Replicate 4	0.3008	0.2881

The ToxCalc analysis of the data gave the following results:

Conc-ppm	1	2	3	4							
Day 0	0.3000	0.3001	0.3001	0.3008							
Day 14	0.2420	0.2049	0.2262	0.2881							
			Transform: Untransformed						1-Tailed		
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	
Day 0	0.3003	1.0000	0.3003	0.3000	0.3008	0.123	4				
*Day 14	0.2403	0.8003	0.2403	0.2049	0.2881	14.693	4	3.396	2.353	0.0415	
Auxiliary Tests							Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.82331		0.749		0.95706	3.30331
F-Test indicates unequal variances (p = 3.90E-06)							9121.22		47.4672			
Hypothesis Test (1-tail, 0.05)							MSDu	MSDp	MSB	MSE	F-Prob	df
Heteroscedastic t Test indicates significant differences Treatments vs B-Control							0.04155	0.13837	0.00719	0.00062	0.01457	1, 6

As the t score (3.396) is greater than the critical one tail t value of 2.35, a statistically significant decrease in the mean earthworm weights at days 0 and 14 is indicated in the earthworms exposed to pyroxsulam.

Comparison of mean day 14 weights of the control earthworms and the earthworms exposed to 10,000 mg pyroxsulam/kg soil, dry weight

The day 14 mean weights of the control earthworms were compared to the day 14 mean weights of the pyroxsulam exposed earthworms using data shown in Table 9.

Table 9. Mean weights of control and pyroxsulam-exposed earthworms at day 14.

Replicate number	Mean day 14 earthworm weights (mg) based on 10 earthworms/replicate	
	Control	Exposed
1	0.2892	0.242
2	0.3412	0.2049
3	0.2678	0.2262
4	0.3022	0.2881

The ToxCalc analysis of the data gave the following results:

Conc-ppm	1	2	3	4
Control	0.2892	0.3412	0.2678	0.3022
10000	0.2420	0.2049	0.2262	0.2881

			Transform: Untransformed						1-Tailed	
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.3001	1.0000	0.3001	0.2678	0.3412	10.281	4			
*10000	0.2403	0.8007	0.2403	0.2049	0.2881	14.693	4	2.551	1.943	0.0456

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90146	0.749	0.63554	-0.7342
F-Test indicates equal variances (p = 0.83)	1.30955	47.4672		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates significant differences Treatments vs B-Control	0.04556	0.1518	0.00715	0.0011	0.04345	1, 6

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs B-Control	<10000	10000			0.04548	0.15155	0.00715	0.0011	0.04345	1, 6

Note: The "B-Control" refers to control earthworm weights at day 14 and the "10000" to the pyroxsulam-exposed earthworm weights at day 14. "ppm" refers to mg pyroxsulam/kg of soil, dry weight.

As the t score (2.55) is greater than the critical one tail t value of 1.94, a statistically significant difference in the mean weights of the pyroxsulam-exposed earthworm at day 14 compared to the control earthworms is indicated to have occurred. Dunnett's Test sets the NOEC as <10,000 mg pyroxsulam/kg soil, dry weight.

Confirmation of average weight changes

In the controls, the average weight loss after 14 days was determined as 1.9%, i.e. the same value as that reported in the study. In the pyroxsulam-exposed earthworms, the recalculated weight loss after 14 days was 20% (cf. the reported 18% loss).

Conclusion

The statistical analysis of the mean earthworm data presented has confirmed that earthworms exposed to 10,000 mg pyroxsulam/kg soil, dry weight at 14 days had mean weights which were statistically significantly less than the control earthworms at 14 days. The statistical analysis also indicated that the mean weights of the pyroxsulam-exposed earthworms at day 14 were statistically significantly less than their starting weights at day 0. This latter result contrasts with the weight decrease seen in the control worms not being statistically significantly different between day 0 and day 14.

Statistical Method:

A statistically significant weight loss in earthworms exposed to pyroxsulam was determined by comparison of mean earthworm weights in the pyroxsulam-exposed earthworms at day 0 and day 14 with testing for normality of distribution and equality of variances of the data being compared and use of Dunnett's test used to identify the statistically significant difference. Using the same statistical procedures, a comparison of the mean weight of the control earthworms at day 14 with the mean weight of the earthworms exposed to pyroxsulam for 14 days also indicated a statistically significant weight loss in the exposed earthworms compared to the controls.

14 day NOEC (weight loss):	<10,000 mg pyroxsulam/kg soil, dry weight
14 day NOEC (mortality and sublethal effects apart from weight loss):	10,000 pyroxsulam/kg soil, dry weight
14 day LOEC (weight loss):	Not reported
14 day LOEC (mortality and	

sublethal effects apart from weight loss):	Not reported
7 and 14 day LC50 and EC50 values:	>10,000 mg pyroxsulam/kg soil, dry weight
Probit Slope:	Not reported
95% C.I.:	Not reported
Endpoint(s) affected:	Mortality, sublethal effects including weight loss after 14 days exposure to pyroxsulam containing soil.

E. STUDY DEFICIENCIES/DEVIATIONS FROM GUIDELINE:

The deviations from guidelines or deficiencies noted are described in Table 10 but are considered to be of such a nature as to not to have significantly affected the study's conduct or outcome. Reference to US EPA OPPTS 850.6200 is not made in the table because, as previously noted in this draft DER, that guideline refers to a subchronic toxicity test conducted over 28 days compared to the 14 days specified in OECD 207. Similarly, reference to the template's US/OECD requirements has not been made as PMRA advice provided for other ecotoxicity DERs has noted that these template requirements are outdated and reference is now made to current guidelines.

Table 10. Summary of deficiencies/deviations from OECD Guideline 207.

Parameter	Study report result	OECD Guideline for Testing of Chemicals, "Earthworm, Acute Toxicity Tests", 207, adopted 4 April 1984
Stability of compound under Test Conditions	Stability of the test substance under test (and storage) conditions was assumed but not verified by the analysis of samples during the definitive toxicity test. Environmental fate studies indicate potential degradation.	Chemical stability in water, soil and light is identified in OECD 207 as guidance information.
Use of a limit test	The study was conducted at one test concentration only, 10,000 mg pyroxsulam/kg soil, dry weight.	For the definitive test, five concentrations in a geometric series are used.
pH	Reported in the Study Profile Template (Mercer, 2006) as 1:1 soil:water but not as such in the study report. No reference was made to use of calcium carbonate to adjust the pH but the reported pH range of 6.3 to 6.5 indicates that a pH	No reference

	adjustment to 6.0 ± 0.5, as required by OECD 207 could have been made.	
Soil moisture content	The soil's moisture content was 25% at day 0 and 26% at day 14.	Refers to an overall moisture content of about 35% of the dry weight.
Acclimation: **Duration:**	Worms were acclimatised in artificial soil for two days before the toxicity test.	Refers to earthworms, which have been conditioned for 24 hours in an artificial soil.
Moisture:	Relative humidity not referred to.	No reference identified. OECD 207 does not specify a relative humidity requirement.

With respect to OECD 207, the significant deviations are considered to be the ~26% soil moisture content compared to the 35% referred to in the Guideline, the use of a limit test which is not specifically allowed by OECD 207 and the lack of information of the stability of pyroxsulam under the test conditions. While the absence of results confirming the test dose of 10,000 mg pyroxsulam/kg soil, dry weight and that the pyroxsulam was uniformly distributed in the test soil are considered omissions, OECD 207 does not require confirmation of either the test concentrations or of uniform distribution.

F. REVIEWER'S COMMENTS:

This study was conducted as a limit test with a nominal concentration of 10,000 mg technical grade material/kg soil, dry weight, but with no actual measurement of the pyroxsulam's concentration in the treated soil.

The mortality and sublethal effects (apart from loss of weight) result in technical grade pyroxsulam being considered as, very slightly toxic to the earthworm, *Eisenia foetida* (14 day LC50 and EC50 both >10,000 mg pyroxsulam/kg soil, dry weight) based on a nominal concentration of pyroxsulam, based on the toxicity classification scheme of the Australian Government Department of the Environment and Water Resources (LC50 > 1,000 mg/kg soil, dry weight).

The demonstration of a significant weight loss after 14 days in the pyroxsulam-exposed earthworms resulted in a NOEC of <10,000 mg pyroxsulam/kg soil, dry weight.

The study report, the data it provided and the internal consistency of the study results are considered to show the study was conducted satisfactorily and that its results are sound. However, there were a number of significant deviations from OECD 207 (a lower soil moisture content than recommended by the OECD standard, use of a limit test and assumption of pyroxsulam stability in the soil). Consequently, the study is rates as supplemental although its results are considered usable.

With respect to assumption of pyroxsulam stability in the soil, the study reports for hydrolytic stability, aerobic soil degradation and field dissipation in Canada identify pyroxsulam as being hydrolytically stable (Yoder, 2004) but having DT50 values ranging from 2 to 10 days (Yoder *et al.*, 2006a) or 1 to 17 days (Yoder *et al.*, 2006b), or in a Canadian field dissipation study, a mean field half-life of 13 days with a range of 5-29 days (Roberts *et al.*, 2006). The soil dissipation half-lives are indicative of a potential degradation of the pyroxsulam in the artificial soil used in the earthworm study.

The validity criterion for OECD 207 (adopted 4 April 1984) with respect to control mortality being less than 10% at the end of the study was met (there was no mortality in the controls).

PMRA Comment: The PMRA does not have share the same study acceptability classification scheme as the APVMA or the US EPA. For the PMRA, a study is either acceptable or invalid. Despite the noted deficiencies, this study contains useful information for risk assessment purposes and is therefore classified acceptable to the PMRA.

G. CONCLUSIONS:

The study is scientifically sound; however, it failed to establish a NOEC for the loss of weight in earthworms exposed to pyroxsulam and had several significant deviations from the OECD 207 guideline (see below).

For mortality, the 14 day NOEC and LC50 were, respectively, 10,000 and >10,000 mg pyroxsulam/kg soil, dry weight. These values are based on a nominal concentration of pyroxsulam (98% purity). The same values apply to the 7 day mortality NOEC and EC50 parameters.

For sublethal effects other than weight loss, the 14 day NOEC and EC50 were, respectively, also 10,000 and >10,000 mg pyroxsulam/kg soil, dry weight. These values are based on a nominal concentration of pyroxsulam (98% purity). The same values apply to the 7 day NOEC and EC50 values for these parameters.

For loss of weight over 14 days, the NOEC was <10,000 mg pyroxsulam/kg soil, dry weight.

LOEC values were not determined by the study.

Pyroxsulam is considered to be very slightly toxic to the earthworm, *Eisenia foetida*, with respect to mortality and sublethal effects apart from weight loss, based on the toxicity classification scheme of the Australian Government Department of the Environment and Water Resources.

The study significantly deviated from OECD 207 with respect to use of a limit test, lack of information of the stability of pyroxsulam in the test medium, soil moisture content and absence of pyroxsulam determinations in the treated soil to confirm that the nominal test dose of 10,000 mg/kg soil, dry weight was achieved and that the pyroxsulam was uniformly distributed in the soil. Additionally, a definitive NOEC value for weight loss could not be determined. As a result, the study is classified as supplemental to the APVMA.

The PMRA does not have share the same study acceptability classification scheme as the APVMA or the US EPA. This study is classified acceptable to the PMRA.

The US EPA reviewer identified that the study is not required under current EPA guidelines and is classified as Supplemental. The study may be useful for risk assessment purposes. However, the lack of pyroxsulam measurements in soil and the lower than specified soil moisture content are uncertainties.

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