Data Evaluation Report on the acute toxicity effects of 5-OH metabolite of pyroxsulam (XDE-742) on earthworms

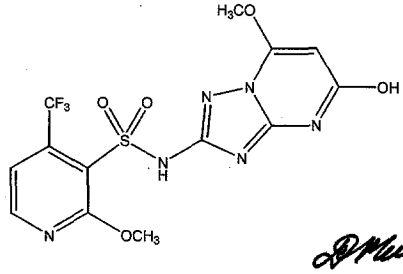
PMRA Submission Number 2006-4727; 1283180 EPA MRID Number 469084-xx 85704 **APVMA ATS 40362**

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: 5-hydroxy-pyroxsulam or 5-hydroxy-XDE-742 **Purity (%):** 97%

Common name:	5-OH Metabolite of XDE-742
Chemical name:	3-pyridinesulfonamide, N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
IUPAC:	N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:	N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.:	Not available
Synonyms:	X11250642

Chemical Structure:



D Murphy 22/02/07

Primary Reviewer: Daryl Murphy Date: 30 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland 12/2/08 Date: 30 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Ann Lee (#1639) Date: May 3, 2007
Environmental Assessment Directorate, PMRA Caroline Laurière for Ann Lee 05/03/08

Christopher Salice Date: 20 June 2007
Environmental Fate and Effects Division, U.S. Environmental Protection Agency 4/16/08

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

CITATION: Sindermann, A.B. Porch, J.R. and Krueger, H.O. 2006. 5-OH Metabolite of XDE-742: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate. Wildlife International, Ltd, 8598 Commerce Drive, Easton, MD 21601. Wildlife International, Ltd. Project Number 379-160 and Dow AgroSciences Study Number 050128. The Dow Chemical Company, Midland MI 48674, USA for Dow AgroSciences, LLC, Indianapolis IN, 46268 USA. February 13, 2006. Unpublished report.

Data Evaluation Report on the acute toxicity effects of 5-OH metabolite of pyroxsulam (XDE-742) on earthworms

PMRA Submission Number 2006-4727; 1283180 EPA MRID Number 469085-04 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: 5-hydroxy-pyroxsulam or 5-hydroxy-XDE-742 **Purity (%):** 97%

Common name: 5-OH Metabolite of XDE-742

Chemical name: 3-pyridinesulfonamide, N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)

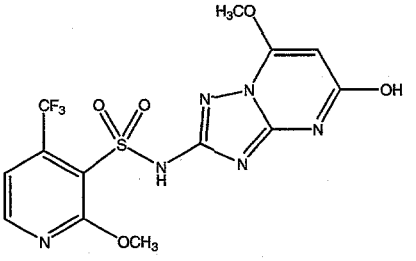
IUPAC: N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

CAS name: N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

CAS No.: Not available

Synonyms: XII250642

Chemical Structure:



Primary Reviewer: Daryl Murphy Date: 30 March 2007
Australian Government Department of the Environment and Water Resources

Secondary Reviewers: Jack Holland Date: 30 March 2007
Australian Government Department of the Environment and Water Resources

Ann Lee (#1639) Date: May 3, 2007
Environmental Assessment Directorate, PMRA

Christopher Salice Date: 20 June 2007
Environmental Fate and Effects Division, U.S. Environmental Protection Agency

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

CITATION: Sindermann, A.B. Porch, J.R. and Krueger, H.O. 2006. 5-OH Metabolite of XDE-742: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate. Wildlife International, Ltd, 8598 Commerce Drive, Easton, MD 21601. Wildlife International, Ltd. Project Number 379-160 and Dow AgroSciences Study Number 050128. The Dow Chemical Company, Midland MI 48674, USA for Dow AgroSciences, LLC, Indianapolis IN, 46268 USA. February 13, 2006. Unpublished report.

Data Evaluation Report on the acute toxicity effects of 5-OH metabolite of pyroxsulam (XDE-742) on earthworms

PMRA Submission Number 2006-4727; 1283180 EPA MRID Number 469085-04 APVMA ATS 40362

EXECUTIVE SUMMARY:

In a 14 day acute toxicity study, earthworms (*Eisenia foetida*) were exposed to the 5-OH metabolite of pyroxsulam at 0, 62.5, 125, 250, 500 and 1000 mg 5-OH metabolite of pyroxsulam/kg dry weight of artificial soil substrate. Reference toxicity tests with the toxicant 2-chloracetamide were conducted periodically under similar test conditions and with earthworms from the same source, to monitor the techniques used and sensitivity of the test population. The experiment was conducted in accordance with OECD 207 "Earthworm, Acute Toxicity Test". Test concentrations in soil were not verified.

There were no mortalities in the control group or any treatment groups during the 14-day exposure period. All earthworms in the control group and treatment groups were normal in appearance and behaviour throughout the exposure period. Earthworms in both the control and treatment groups exhibited no aversion to the soil when observed on days 0 and 7 for burrowing behavior. Body weight reduction among earthworms in the treatment groups was not statistically significant (Dunnett's 2-tailed test, $p>0.05$) at any concentration tested when compared to the control group. The 14-day NOEC based on mortality and sublethal effects was 1000 mg 5-OH-XDE-742 /kg dry weight of soil. The 14-day LOEC, based on based on mortality and sublethal effects (including weight change), was >1000 mg 5-OH metabolite of pyroxsulam/kg dry weight (dw) of soil substrate. The 14-day LC_{50} and EC_{50} based sublethal effects was > 1000 mg 5-OH-XDE-742 /kg dry weight of soil.

The 5-OH metabolite of pyroxsulam is considered to be very slightly toxic to earthworms above a concentration of 1000 mg 5-OH metabolite of pyroxsulam/kg dw of soil substrate based on the classification system used by DEW.

This study is classified as supplemental by DEW and satisfies the guideline requirements for an acute toxicity study for 5-OH metabolite of pyroxsulam with earthworms.

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

The EPA secondary reviewer advised that the study is scientifically sound but classified as supplemental since there are no current EPA guideline requirements for an earthworm toxicity test. In addition, the lack of measured concentrations indicates some uncertainty regarding the toxicity estimates.

Results Synopsis

Test Organism:	Earthworm, *Eisenia foetida*
Size:	Control earthworms had an initial mean weight of 0.42 g/earthworm. Earthworms exposed to the 5-OH metabolite of pyroxsulam had initial mean weights of 0.40 to 0.44 g/earthworm.
Age:	Adults with clitella
Test Type:	Acute toxicity
14 day LC_{50}:	>1000 mg 5-OH metabolite of pyroxsulam/kg dw soil
95% C.I.:	Not determined
14 day NOEC:	1000 mg 5-OH metabolite of pyroxsulam/kg dw soil (for mortality and sublethal effects)
Probit Slope:	Not determined
95% C.I.:	Not determined
14 day EC_{50}:	>1000 mg 5-OH metabolite of pyroxsulam/kg dw soil
95% C.I.:	Not determined
14 day LOEC:	>1000 mg 7-OH metabolite of pyroxsulam/kg dw soil (for mortality and sublethal effects)
Endpoint(s) Effected:	There were no compound related effects on mortality and sublethal effects.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
The study was conducted based upon the procedures outlined in the Wildlife International, Ltd. protocol, "XDE-742 (5-OH Metabolite): An Acute Toxicity Study With The Earthworm In An Artificial Soil Substrate." The protocol was based upon procedures outlined in the Organization for Economic Cooperation and Development (OECD) Guideline No. 207, Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests

There were a number of deviations from the Guideline and other deficiencies identified (see page Table 11, page 26 of this DER).

COMPLIANCE:
This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Parts 160 and 792, 17 August 1989, OECD Principles of Good Laboratory Practice, ENV/MC/CHEM (98) 17, Paris, 1998 and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999.

The following exceptions were reported in the Good Laboratory Practice Compliance Statement:

> Verification of the test concentrations, stability and homogeneity of the test substance in the soil were not determined. Periodic analyses of soil and well water for potential contaminants were not conducted according to Good Laboratory Practice Standards. However, periodic analyses of soil and water were performed using a certified laboratory and standard US EPA analytical methods.

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

A signed and dated Quality Assurance statement was provided.

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

A. MATERIALS:

1. Test Material	5-OH metabolite of XDE-742 (referred to as the 5-OH metabolite of pyroxsulam in this DER).
Description:	Solid
Lot No./Batch No. :	XN8-33938-95
TSN No.:	TSN105419
Purity:	97% of active ingredient.
Stability of Compound Under Test Conditions:	Not determined (as stated in the GLP compliance statement). See "Stability and homogeneity of test material in the medium" in Table 11, page 26 of this DER.

With respect to the stability of the 5-OH metabolite of pyroxsulam in soil, the aerobic soil degradation study of radiolabelled pyroxsulam in four European soils (Yoder *et al.*, 2006) reported that the DT50 for the 5-OH metabolite of pyroxsulam formed from pyroxsulam degradation was 3.4 days (DT90 11.2 days) in one soil and 2.7 days (DT90 9.1 days) in a second soil with conversion to "other" degradates. DT50 values for the other two soils not reported. Such results indicate that the 5-OH metabolite of pyroxsulam could be expected to have undergone significant degradation in the 14 days of the earthworm exposure study.

Storage conditions of test chemicals: Stored under ambient conditions.

Physicochemical properties of 5-OH metabolite of pyroxsulam.

Parameter	Values	Comments
Water solubility at 20°C	Not available	Stated in the Study Profile Template (Sindermann *et al.*, 2006a) as not available at the time of publication of the Study Profile Template.
Vapour pressure	Not available	
UV absorption	Not available	
pKa	Not available	
Kow	Not available	

2. Test organism:

Species: Earthworms (*Eisenia foetida)*
Age at test initiation: Adults (with clitella)
Weight at study initiation:

Average weights of earthworms at test initiation, as average earthworm body weights in grams (10 earthworms/replicate), 4 replicates/control and test concentration.						
	Controls	mg 5-OH metabolite of pyroxsulam/kg soil dry weight				
		62.5	125	250	500	1000
Mean:	**0.42**	**0.43**	**0.44**	**0.43**	**0.40**	**0.41**
Range:	**0.41 to 0.45**	**0.38 to 0.46**	**0.40 to 0.47**	**0.38 to 0.45**	**0.36 to 0.45**	**0.37 to 0.45**

Source: Earthworms for the test were from in-house cultures started with worms obtained from the University of Maryland, Wye Research & Education Center, Queenstown, Maryland.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A range finding study was not conducted.

A reference toxicity test was conducted under a separate protocol to determine the LC50 value for earthworms exposed to the reference toxicant, chloroacetamide (referred to as 2-chloracetamide in the study profile template (Sindermann *et al.*, 2006a)), in artificial soil. The test was conducted under conditions reported as similar to those used in this test, and with earthworms from the same source, to monitor the techniques used and sensitivity of the test population. The earthworms were exposed to chloroacetamide in the soil at nominal concentrations of 13, 25, 50 mg/kg dry soil.

The 14-day LC50 value for the most current reference toxicity test was approximately 24.5 mg 2-chloroacetamide/kg dry soil with a 95% confidence interval of 13 and 50 mg 2-chloroacetamide/kg dry soil. These results were reported as consistent with those observed in previous studies, and verify the adequacy and consistency of the methods used in this study with the 5-OH metabolite of pyroxsulam.

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.

b. Definitive Study

The in-life portion of this test was conducted from 17 to 31 January, 2006. An artificial soil was pre-mixed with the 5-OH metabolite of pyroxsulam to give nominal concentrations of, 0, 62.5, 125, 250, 500 and 1,000 mg 5-OH metabolite of pyroxsulam/kg dry soil. Earthworms (*Eisenia fetida*) were exposed to these concentrations of 5-OH metabolite of pyroxsulam in the soil. The objective of the study was to evaluate the acute effects of 5-OH metabolite of pyroxsulam on earthworms during a 14-day exposure period in the artificial soil substrate.

1. Soil

An artificial soil was prepared in bulk by blending approximately 70% sand, 20% kaolin clay and 10% sphagnum peat in a soil mixer for approximately 20 minutes. The pH of the bulk soil prior to hydration was adjusted to 5.6 using calcium carbonate (1% of soil mass).

The soil's moisture content was brought to ~33% by addition of deionised water to the appropriate amount of bulk soil taking into account the estimated 4% moisture content present in the bulk soil stored at ambient temperatures. The bulk artificial soil was stored in a sealed container under ambient conditions until used to prepare the test soils.

Test soil was prepared by premixing the appropriate amount of test substance with an aliquot of dry artificial soil. Sufficient water was added to the dry artificial soil to achieve a moisture content of approximately 33% by weight. Test soil components were mixed for a total of 25 minutes in order to achieve a homogeneous mixture. Negative control soil was prepared in the same manner as the treated soil but with only the addition of water and a mixing time of 20 minutes. Seven hundred fifty grams of prepared soil were added to each of four test chambers for each of the treatment and control group. The test concentrations were adjusted for the purity of the test substance, therefore, test concentrations and the LC50 value are reported as milligrams of test substance active ingredient per kilogram of soil on a dry weight basis (mg 5-OH metabolite of pyroxsulam/kg soil, dry weight).

Soil moisture content was determined by measuring the initial weight of the soil sample then weighing the soil sample after it was dried at approximately 105°C.

The study report provided the weights of 5-OH metabolite of pyroxsulam added to the weights of soil. Based on these data, the reviewer calculated test concentrations were, after correction for the estimated 4% moisture content of the soil mix and the 97% purity of the 5-OH metabolite of pyroxsulam, 62.5, 125, 250, 500 and 1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight, i.e. as given in the test report.

Analyses of the treated soil to confirm that dosing had been correct and that the mixing procedure had evenly distributed the 5-OH metabolite of pyroxsulam throughout the treated soils were not conducted (See Table 11, Summary of deficiencies/deviations from guidelines, page 26 of this DER).

Note that in Table 1, Table 2 and Table 3 (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 substrate (provide composition)	Quartz sand 63.0 kg Kaolin clay 18.0 kg Sphagnum peat 9.0 kg Calcium carbonate 0.9 kg As percentages (wt/wt):	Requirement considered met. *EPA/OECD require that the testing medium be artificial soil consisting of a mixture of 68% of No. 70 mesh*

	69.3% quartz sand 19.8% kaolin clay 9.9% sphagnum peat 0.99% calcium carbonate	*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) The study profile template (Sindermann *et al.*, 2006a) refers to a 1:1 soil water ratio but this information was not located in the study report.	The pH of the bulk soil prior to hydration was adjusted to 5.6 using calcium carbonate. The soil pH ranged from 7.2 to 7.3 in the control and test soils at test initiation and from 7.4 to 7.6 in the controls and test soils at test termination.	See Table 11, Summary of deficiencies/deviations from guidelines, page 26 of this DER. OECD 207 refers to the pH of the artificial soil being 6.0 ± 0.5.
Organic carbon (%)	Not reported.	Not provided but not required by OECD 207. Therefore not considered a deviation from that guideline.
Moisture (%)	Soil moisture content was reported as 34% in each test group at test initiation and between 29 to 32% at test termination. Tabulated data reported the initial moisture content as ranging from 33.6 to 34.2% and, at test termination, 29.2 to 32%.	Requirement considered met. OECD 207 refers to the soil having an overall content of about 35% moisture content based on the dry weight. Reviewer calculations of soil data provided in the study report for the preparation of the test soils, indicates the reported values are on a soil dry weight basis.

Table 2. Experimental Design

Parameter	Value	Remarks *Criteria*
Acclimation: Duration:	Approximately 24 hours.	Requirement considered met. OECD 207 refers to earthworms which have been conditioned for 24 hours in an artificial soil. *EPA/OECD require that earthworms be acclimated at test temperature for 7 days.*
Conditions (state if same as the test conditions):	Acclimatisation took place in the prepared artificial soil adjusted to a moisture content of ~33% by weight. While the earthworms were not fed during testing, the study report did not state if feed was withheld during the acclimatisation period.	Requirement considered met. As noted above, OECD 207 states that the earthworms be conditioned in an artificial soil. Given the acclimatisation period was 24 hours, it is expected that feeding was withheld in that period.
Health:	All surviving earthworms in the control group and treatment groups were normal in appearance and behaviour throughout the test period. Sindermann *et al.* (2006a), in the Study Profile Template referred to the earthworms being normal in appearance and behaviour at the completion of acclimation.	Requirement considered met. No specific reference to the earthworms' health identified in OECD 207.
Soil [fresh or stored]	The bulk artificial soil was stored in a sealed container under ambient conditions until used to prepare the test soils.	Requirement considered met.
Test Container Material:	Glass beakers covered with plastic wrap that was perforated for air exchange. Test chambers were held in an environmental chamber during testing.	Requirement considered met.

Parameter	Value	Remarks *Criteria*
Size: Amount of soil or substrate:	1 Litre Seven hundred fifty grams of prepared, hydrated soil/test chamber.	
No. of replicates Per treatment group: Per control:	Four test chambers for each of the treatment groups. Four test chambers for the control group.	Requirement met. *EPA/OECD requires 3 replicates and a control.* OECD 207 recommends 4 replicates/treatment.
No. of earthworms per treatment	Forty earthworms per treatment and control (Each test and control chamber contained 10 earthworms).	Requirement met. *EPA/OECD requires a minimum of 30 earthworms per treatment and a control, 10 per each of three replicates and the control.* OECD 207 specifies 10 earthworms/container.
Co-solvents used or not (if yes report the name and concentration)	No solvent used. Test soils were prepared by premixing (25 minutes) the appropriate amount of 5-OH metabolite of pyroxsulam with the dry artificial soil followed by adjustment to ~33% moisture content by addition of deionised water.	Requirement met.
Rates of application Nominal:	Nominal concentrations of 0 (control), 62.5, 125, 250, 500 and 1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight. The test concentrations were adjusted for the purity of the test substance and test concentrations are reported as milligrams of test substance active constituent per kilogram of soil on a dry weight basis (mg 5-OH metabolite of pyroxsulam/kg).	Requirement considered met. The weights of 5-OH-pyroxsulam added to known soil weights were provided in the study report and calculated by the reviewer to be equivalent to the nominal concentrations reported used.

Parameter	Value	Remarks *Criteria*
Measured:	Verification of test concentrations of the 5-OH metabolite of pyroxsulam in the soil was not conducted (GLP compliance statement).	OECD 207 does not require that test concentrations be analytically determined. *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.*
Stability and homogeneity of test material in the medium?	Verification of the stability and homogeneity of the 5-OH metabolite of pyroxsulam in the soil was not conducted (GLP compliance statement). Information on the stability of the 5-OH metabolite of pyroxsulam shows there is potential for degradation during	See Table 11, Summary of deficiencies/deviations from guidelines, page 26 of this DER. OECD 207 indicates chemical stability in water, soil and light are known OECD 207 does not refer to homogeneity of the test material in the medium.
Test conditions: Temperature:	During the test, the worms were maintained in an environmental chamber set to maintain a temperature of approximately 20 ± 2°C. Air temperature was measured at least once daily in the environmental chamber. Soil temperature was measured in one replicate of each treatment and control group at test initiation and termination using a hand-held digital thermometer. The measured soil temperature was 20.0-20.5°C in each test group at test initiation and also at test termination.	Requirements considered met. *EPA requirements:* *Temperature: 22 + 2°C*

Parameter	Value	Remarks *Criteria*
Lighting conditions: Moisture:	The photoperiod during the test was 24 hours of continuous light per day provided by overhead fluorescent bulbs. The target light intensity during the test was approximately 400 to 800 lux, and was verified on day 2 of the test. An average light intensity of 563 ± 40.3 lux, with a reported range over the surface of the test chambers of 524 to 622 lux. Soil moisture ranged from 33.6% to 34.2% at day 0. At test termination, the moisture content ranged from 29.2 to 32.0%. Relative humidity was not reported.	Requirement met. *Lighting: Continuous illumination, with a light intensity of 400 lux* OECD 207 refers to an illuminated cabinet or chamber controllable to ± 2°C with a light intensity of 400 to 800 lux. Requirement considered met. *Relative humidity: above 85%* OECD 207 does not specify a relative humidity requirement.
Duration of the study	14 days	Requirement considered met. *EPA/OECD require a 28-day test.* OECD 207 refers to a 14 day test duration.
Reference chemical, if used Name: Concentration:	2-Chloracetamide 13, 25 and 50 mg 2-chloroacetamide/kg dry soil (nominal concentrations).	Requirement considered met. At Wildlife International, Ltd., reference toxicity tests with a reference toxicant, 2-chloracetamide, were conducted periodically to assess the sensitivity of the test species and test procedures. These studies are conducted under separate protocols, as independent studies. 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	Observations of mortality and clinical signs were conducted on days 7 and 14. Observations of burrowing behaviour were conducted once at test initiation and after observations for signs of toxicity on day 7. Weights were measured made at day 0 and 14. Air temperature was measured at least once daily in the environmental chamber, except for two days on which the temperature was not recorded. Soil temperature, moisture content and pH were determined at test commencement and termination.	Requirement considered met with respect to the OECD 207's requiring determination of mortality at day 7 and 14, weight change, temperature, pH and moisture content. *EPA/OECD require that observations be made on days 7, 14, 21, and 28.*
Parameters measured including the sublethal effects/toxicity symptoms	Burrowing behaviour, mortality, behavioural or pathological abnormalities and body weight.	Requirement considered met. While the e study protocol refers to a mechanical stimulus being applied to the earthworms and the reactions recorded, the study report did not specifically indicate that this was done. The study protocol referred to observation of behavioural or pathological signs. Group earthworm weights were determined at day 0. Before group weighing at day 14, the earthworms were gently rinsed and blotted. Group weights were measured for each replicate and average individual body weights were calculated.

		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?	Tabulated soil moisture, pH and temperature data were presented as were mortality, effects and weight change data. Copies of the raw data are filed in archives at the Wildlife International, Ltd. site.	Requirement considered met as OECD 207 does not refer to the need to supply 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	None.	

II. RESULTS AND DISCUSSIONS

A. MORTALITY:

Forty earthworms (10 per replicate) were exposed to a control and forty to each pyroxsulam containing test soil. Observations of mortality were conducted on days 7 and 14. There were no mortalities in the control group or any of the treatment groups during the 14-day test. Because mortality was less than 50% in the highest concentration, the LC50 could not be statistically defined and was judged to be greater than the highest concentration tested. The no-observed-effect-concentration was determined by visual examination of the mortality data.

No dose response relationship was observed with respect to mortality.

The mortality results are summarised in Table 4, page 15 of this DER. The following endpoints were reported:

14-day LC50:	>1000 mg 5-OH metabolite of pyroxsulam/kg dry soil
No Observed Effect Concentration (mortality):	1000 mg 5-OH metabolite of pyroxsulam/kg dry soil

Table 4. Effect of 5-OH metabolite of pyroxsulam on mortality of the earthworm (*Eisenia foetida*).

Treatment - mg 5-OH metabolite of pyroxsulam/kg soil, dry weight (nominal)	Replicate (with 10 earth-worms/replicate)	Observation period			
		Day 7		Day 14	
		No. dead	% mortality	No. dead	% mortality
Negative control (0)	A	0/10	0	0/10	0
	B	0/10	0	0/10	0
	C	0/10	0	0/10	0
	D	0/10	0	0/10	0
Total number dead and total % mortality in the controls:		**0/40**	**0**	**0/40**	**0**
62.5	A	0/10	0	0/10	0
	B	0/10	0	0/10	0
	C	0/10	0	0/10	0
	D	0/10	0	0/10	0
	Total:	**0/40**	**0**	**0/40**	**0**
125	A	0/10	0	0/10	0
	B	0/10	0	0/10	0
	C	0/10	0	0/10	0
	D	0/10	10	1/10	10
	Total:	**0/40**	**0**	**0**	**0**
250	A	0/10	0	0/10	0
	B	0/10	0	0/10	0
	C	0/10	0	0/10	0
	D	0/10	0	0/10	0
	Total:	**0/40**	**0**	**0/40**	**0**
500	A	0/10	0	0/10	0
	B	0/10	0	0/10	0
	C	0/10	0	0/10	0
	D	0/10	0	0/10	0
	Total:	**0/40**	**0**	**0/40**	**0**
1000	A	0/10	0	0/10	0
	B	0/10	0	0/10	0
	C	0/10	0	0/10	0
	D	0/10	0	0/10	0
	Total:	**0/40**	**0**	**0/40**	**0**
Total number dead and total % mortality in exposed earthworms		**0/200**	**0%**	**0/200**	**0%**
14 day NOEC (mortality)		1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight			

LOEC		Not reported
14 day LC_{50}		>1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight
Reference chemical	**% mortality:**	Not reported
	14 day LC_{50}:	~24.5 mg chloracetamide/kg dry soil, 95% confidence interval of 13 and 50 mg chloracetamide/kg dry soil. These results were reported as consistent with those of previous studies, and verified the adequacy and consistency of the methods used in the present study.

B. SUB-LETHAL TOXICITY ENDPOINTS:

Observations of clinical signs were conducted on days 7 and 14. Observations of burrowing behaviour were conducted at test initiation and after observations for signs of toxicity on day 7.

The EC50 and no-observed-effect-concentration were determined by visual examination of the clinical observation data.

The no-observed-effect-concentration was determined by visually inspecting the clinical observation data. Body weights, and change in body weights, were statistically compared with Dunnett's test (α=0.05) using SAS Version 8. Prior to conducting Dunnett's test, the data were tested for homogeneity of variance and normal distribution.

All earthworms in the control group and treatment groups were reported as normal in appearance and behaviour throughout the test period. A summary of these results is provided in Table 5, page 17 of this DER.

Earthworms in both the control and treatment groups exhibited no aversion to the soil during observations of burrowing behaviour on days 0 and 7 (relevant data were not provided in the study report).

No dose response relationship was observed with respect to sublethal effects.

The data support the following endpoints:

14 day EC50:	>1000 mg 5-OH metabolite of pyroxsulam/kg dry soil
No Observed Effect Concentration: (sublethal effects, based on clinical observation data)	1000 mg 5-OH metabolite of pyroxsulam/kg dry soil

The sublethal effects (apart from changes in weight) results are summarised in Table 5, page 17 of this DER.

Table 5. Sublethal effect (not weight loss) of 5-OH metabolite of pyroxsulam on the earthworm (*Eisenia foetida*).

Treatment - mg 5-OH metabolite of pyroxsulam/kg soil, dry weight (nominal)	Replicate (10 earthworms/replicate)	Observation period	
		Day 7	Day 14
		Observed effects	Observed effects
Negative control (0)	A	10 appeared normal*	10 appeared normal
	B	10 appeared normal	10 appeared normal
	C	10 appeared normal	10 appeared normal
	D	10 appeared normal	10 appeared normal
Total number abnormal and total % abnormality in the controls:		0/40, 0%	0/40, 0%
62.5	A	10 appeared normal	10 appeared normal
	B	10 appeared normal	10 appeared normal
	C	10 appeared normal	10 appeared normal
	D	10 appeared normal	10 appeared normal
	Total:	0/40, 0%	0/40, 0%
125	A	10 appeared normal	10 appeared normal
	B	10 appeared normal	10 appeared normal
	C	10 appeared normal	10 appeared normal
	D	10 appeared normal	10 appeared normal
	Total:	0/40, 0%	0/40, 0%
250	A	10 appeared normal	10 appeared normal
	B	10 appeared normal	10 appeared normal
	C	10 appeared normal	10 appeared normal
	D	10 appeared normal	10 appeared normal
	Total:	0/40, 0%	0/40, 0%
500	A	10 appeared normal	10 appeared normal
	B	10 appeared normal	10 appeared normal
	C	10 appeared normal	10 appeared normal
	D	10 appeared normal	10 appeared normal
	Total:	0/40, 0%	0/40, 0%
1000	A	10 appeared normal	10 appeared normal
	B	10 appeared normal	10 appeared normal
	C	10 appeared normal	10 appeared normal
	D	10 appeared normal	10 appeared normal
	Total:	0/40, 0%	0/40, 0%
Total number dead and total % mortality in exposed earthworms		0/200, 0%	0/200, 0%

14 day NOEC (sublethal effects apart from weight change)		1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight
14 day LOEC (sublethal effects)		Not reported
14 day EC50 (sublethal effects apart from weight change)		>1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight
Reference chemical Chlor-acetamide	**% mortality:**	Not reported for sublethal effects
	14 day EC_{50}:	Not reported for sublethal effects

*** With respect to appearance and behaviour.**

Body Weights

Average individual body weights at test initiation and termination, and the change in body weight from test initiation to test termination, were calculated from the day 0 and day 14 replicate measurements (Table 6, page 19 of this DER). A slight loss in body weight from test initiation to test termination was noted in both the control and treatment groups and was not unexpected since the earthworms were not fed during the test.

. Final body weights and the change in body weight (initial - final) among earthworms in the treatment groups were not statistically significant (p>0.05) at any concentration tested when compared to the control group. The final body weights and change values were normally distributed and all data analysed had homogeneous variances.

The weight change results are summarised in Table 6, page 19 of this DER.

The weight change data support the following endpoints:
14 day EC50: > 1000 mg 5-OH metabolite of pyroxsulam/kg dry soil

No Observed Effect Concentration: 1000 mg 5-OH metabolite of pyroxsulam/kg dry soil
(weight loss)

Sub-lethal effects on the weights of earthworms exposed to the 5-OH metabolite of pyroxsulam in an artificial soil matrix are shown in Table 6, page 19 of this DER.

Table 6. Sub-lethal effect on the weights of earthworms, *Eisenia foetida* exposed to 5-OH metabolite of pyroxsulam in an artificial soil matrix. There were 10 earthworms/replicate.

Treatment (mg 5-OH metabolite of pyroxsulam/kg soil, dry weight, nominal)		Average Earthworm Body Concentration Weights (g)		
		Day 0[1]	Day 14[1]	Total change in weight[1]
Control (0)	Replicate	weight	weight	grams
	A	0.45	0.36	-0.09
	B	0.41	0.38	-0.03
	C	0.42	0.37	-0.05
	D	0.41	0.39	-0.02
Mean and standard deviation		0.42 ± 0.019	0.38 ± 0.013	-0.05 ± 0.031
62.5	A	0.44	0.40	-0.04
	B	0.42	0.36	-0.06
	C	0.38	0.36	-0.02
	D	0.46	0.42	-0.04
Mean and standard deviation		0.43 ± 0.034	0.39 ± 0.030	-0.04 ± 0.016
125	A	0.40	0.38	-0.02
	B	0.41	0.37	-0.04
	C	0.47	0.41	-0.06
	D	0.46	0.39	-0.07
Mean and standard deviation		0.44 ± 0.035	0.39 ± 0.017	-0.05 ± 0.022
250	A	0.45	0.42	-0.03
	B	0.45	0.41	-0.04
	C	0.43	0.39	-0.04
	D	0.38	0.36	-0.02
Mean and standard deviation		0.43 ± 0.033	0.40 ± 0.026	-0.03 ± 0.010
500	A	0.45	0.40	-0.05
	B	0.40	0.38	-0.02
	C	0.36	0.32	-0.04
	D	0.39	0.36	-0.03
Mean and standard deviation		0.40 ± 0.037	0.37 ± 0.034	-0.04 ± 0.013
1000	A	0.45	0.39	-0.06
	B	0.40	0.36	-0.04
	C	0.40	0.35	-0.05
	D	0.37	0.35	-0.02
Mean and standard deviation		0.41± 0.033	0.36 ± 0.019	-0.04 ± 0.017
14 day NOEC (weight loss)		1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight		
LOEC (weight loss)		Not reported		
14 day EC_{50} (weight loss):		>1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight		
Reference chemical (chloracetamide)				
% mortality and LC50 and 95% confidence limits		Not reported for sublethal effects		

1. No treatment group mean was significantly different for any concentration when compared to the control group (Dunnett's 2-tailed test, $p>0.05$).

C. REPORTED STATISTICS:

Parameters analysed were mortality, sublethal effects including weight change and burrowing time.

Because no mortality occurred in any treatment group, an LC50 value was not statistically defined and was judged to be greater than the highest concentration tested.

The NOEC was determined by visually inspecting the mortality and clinical observation data. Body weights, and change in body weights, were statistically compared with Dunnett's test ($\alpha = 0.05$) using SAS Version 8. Prior to conducting Dunnett's test, the data were tested for homogeneity of variance and normal distribution. If warranted, the LC50 was determined using an appropriate statistical method.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Because of the lack of treatment-related effects (absence of dose related mortality and dose related sub-lethal effects - weight loss is considered below), the statistical evaluation of the biological data for mortality and sublethal effects was not attempted.

Mortality and sublethal effects (weight change is discussed below) were less than 50% at the concentration tested. Consequently, statistically determination of LC50 and EC50 was not possible. The 14-day LC50 and EC50 values were all empirically determined to be greater than the maximum nominal concentration tested i.e. >1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight basis.

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 5-OH metabolite of pyroxsulam exposed mean earthworm weights at day 0;
- Comparison of control earthworm mean weights at day 0 compared to day 14;
- Comparison of control and 5-OH metabolite of pyroxsulam exposed mean earthworm weights at day 14; and
- Comparison of the total changes in mean earthworm weights after 14 days.

The ToxCalc analyses determined that normal distributions and equal variances occurred in all these comparisons.

Comparison of control and 5-OH metabolite of pyroxsulam exposed mean earthworm weights at day 0;

To confirm the mean weights of the control and 5-OH metabolite of pyroxsulam-exposed worms were not significantly different at the start of the exposure period, the mean control and exposed earthworms weights at day 0 were compared.

The mean earthworm weights (based on 10 earthworms/replicate at day 0) are shown in Table 7.

Table 7. Mean weights of control and 5-OH metabolite of pyroxsulam exposed earthworms at day 0.

	Mean earthworm weights (mg) based on 10 earthworms/replicate at day 0			
Replicate number:	**1**	**2**	**3**	**4**
Control (0*)	0.4500	0.4100	0.4200	0.4100
62.5*	0.4400	0.4200	0.3800	0.4600
125	0.4000	0.4100	0.4700	0.4600
250	0.4500	0.4500	0.4300	0.3800
500	0.4500	0.4000	0.3600	0.3900
1000	0.4500	0.4000	0.4000	0.3700

*** Concentrations of 5-OH metabolite of pyroxsulam/kg soil, dry weight.**

The ToxCalc analysis of these data gave the following results with one and two tailed tests conducted:

One tailed test

			Transform: Untransformed					1-Tailed		
Conc-ppm	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
D-Control	0.4225	1.0000	0.4225	0.4100	0.4500	4.480	4			
62.5	0.4250	1.0059	0.4250	0.3800	0.4600	8.037	4	-0.109	2.410	0.0554
125	0.4350	1.0296	0.4350	0.4000	0.4700	8.073	4	-0.543	2.410	0.0554
250	0.4275	1.0118	0.4275	0.3800	0.4500	7.729	4	-0.217	2.410	0.0554
500	0.4000	0.9467	0.4000	0.3600	0.4500	9.354	4	0.978	2.410	0.0554
1000	0.4050	0.9586	0.4050	0.3700	0.4500	8.189	4	0.761	2.410	0.0554

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95756	0.884	-0.009	-0.9591
Bartlett's Test indicates equal variances (p = 0.94)	1.283	15.0863		

Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs D-Control	1000	>1000			0.05544	0.13122	0.00075	0.00106	0.62676	5, 18

Two tailed test

			Transform: Untransformed					2-Tailed		
Conc-ppm	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
D-Control	0.4225	1.0000	0.4225	0.4100	0.4500	4.480	4			
62.5	0.4250	1.0059	0.4250	0.3800	0.4600	8.037	4	0.109	2.840	0.0653
125	0.4350	1.0296	0.4350	0.4000	0.4700	8.073	4	0.543	2.840	0.0653
250	0.4275	1.0118	0.4275	0.3800	0.4500	7.729	4	0.217	2.840	0.0653
500	0.4000	0.9467	0.4000	0.3600	0.4500	9.354	4	0.978	2.840	0.0653
1000	0.4050	0.9586	0.4050	0.3700	0.4500	8.189	4	0.761	2.840	0.0653

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95756	0.884	-0.009	-0.9591
Bartlett's Test indicates equal variances (p = 0.94)	1.283	15.0863		

Hypothesis Test (2-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs D-Control	1000	>1000			0.06533	0.15463	0.00075	0.00106	0.62676	5, 18

No treatment group mean was significantly different for any concentration when compared to the control group

(Dunnett's 1 or 2-tailed test, p>0.05).

As the t scores are less than the critical one and two tailed t values, no statistically significant differences in the mean earthworm weights at day 0 for the control and 5-OH metabolite of pyroxsulam exposed earthworms are indicated.

Comparison of control earthworm mean weights at day 0 compared to day 14;
To see if the mean weights of the control earthworms varied significantly between day 0 and day 14, the mean control weights at those times were compared. The mean control weights/replicate at days 0 and 14 are shown in Table 8.

Table 8. Mean weights of the control earthworms at days 0 and 14.

	Mean control earthworm weights at days 0 and 14			
Replicate:	**1**	**2**	**3**	**4**
Day 0	0.4500	0.4100	0.4200	0.4100
Day 14	0.3600	0.3800	0.3700	0.3900

The ToxCalc analysis of these data (two tailed test only) gave the following results:

			Transform: Untransformed						2-Tailed			
Conc-ppm	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**		
Control day 0	0.4225	1.0000	0.4225	0.4100	0.4500	4.480	4					
Control day 14	0.3750	0.8876	0.3750	0.3600	0.3900	3.443	4	4.146	2.450	0.0281		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.89822		0.749		0.95238	-0.0016
F-Test indicates equal variances (p = 0.55)							2.15		47.4672			

As the t score is greater than the critical two tailed t value, a statistically significant difference in the mean earthworm weights of the controls at day 0 and day 14 is indicated. As noted in the study report, this effect is not unexpected as the worms were not fed over the 14 day period.

Comparison of control and 5-OH metabolite of pyroxsulam exposed mean earthworm weights at day 14
The day 14 mean weights of the control earthworms were compared to the day 14 mean weights of the 5-OH metabolite of pyroxsulam exposed earthworms with the data used shown in Table 9.

Table 9. Mean weights of control and 5-OH metabolite of pyroxsulam exposed earthworms at day 14.

	Mean earthworm weights (mg) based on 10 earthworms/replicate at day 14			
Replicate number:	**1**	**2**	**3**	**4**
Control (0*)	0.3600	0.3800	0.3700	0.3900
62.5*	0.4000	0.3600	0.3600	0.4200
125	0.3800	0.3700	0.4100	0.3900
250	0.4200	0.4100	0.3900	0.3600
500	0.4000	0.3800	0.3200	0.3600
1000	0.3900	0.3600	0.3500	0.3500

*** Concentrations of 5-OH metabolite of pyroxsulam/kg soil, dry weight.**

The ToxCalc analysis of these data (one and two tailed tests) gave the following results:

One tailed test

ToxCalc gave the following results -

			Transform: Untransformed					1-Tailed		
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.3750	1.0000	0.3750	0.3600	0.3900	3.443	4			
62.5	0.3850	1.0267	0.3850	0.3600	0.4200	7.792	4	-0.579	2.410	0.0416
125	0.3875	1.0333	0.3875	0.3700	0.4100	4.407	4	-0.723	2.410	0.0416
250	0.3950	1.0533	0.3950	0.3600	0.4200	6.698	4	-1.157	2.410	0.0416
500	0.3650	0.9733	0.3650	0.3200	0.4000	9.358	4	0.579	2.410	0.0416
1000	0.3625	0.9667	0.3625	0.3500	0.3900	5.222	4	0.723	2.410	0.0416

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97196	0.884	-0.1479	-0.5908
Bartlett's Test indicates equal variances (p = 0.64)	3.36026	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	>1000			0.04165	0.11106	0.00068	0.0006	0.37876	5, 18

Two tailed test

TheToxCalc results were -

			Transform: Untransformed					2-Tailed		
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.3750	1.0000	0.3750	0.3600	0.3900	3.443	4			
62.5	0.3850	1.0267	0.3850	0.3600	0.4200	7.792	4	0.579	2.840	0.0491
125	0.3875	1.0333	0.3875	0.3700	0.4100	4.407	4	0.723	2.840	0.0491
250	0.3950	1.0533	0.3950	0.3600	0.4200	6.698	4	1.157	2.840	0.0491
500	0.3650	0.9733	0.3650	0.3200	0.4000	9.358	4	0.579	2.840	0.0491
1000	0.3625	0.9667	0.3625	0.3500	0.3900	5.222	4	0.723	2.840	0.0491

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97196	0.884	-0.1479	-0.5908
Bartlett's Test indicates equal variances (p = 0.64)	3.36026	15.0863		

Hypothesis Test (2-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	1000	>1000			0.04908	0.13087	0.00068	0.0006	0.37876	5, 18

Both the one and two tailed results confirm the study report finding that, when comparing the day 14 average earthworm weights, no treatment group mean was significantly different from the control group (Dunnett's 2-tailed test, p>0.05).

Comparison of the total changes in mean earthworm weights after 14 days.

The change in weights of treatments compared to change in weights of controls were statistically analysed. The average total changes in earthworm weights after 14 days of the test are shown in Table 10.

Table 10. Mean total changes in earthworm weights after 14 days.

	Mean change in earthworm weights (mg) at day 14			
Replicate number:	**1**	**2**	**3**	**4**
Control (0*)	-0.0900	-0.0300	-0.0500	-0.0200
62.5*	-0.0400	-0.0600	-0.0200	-0.0400
125	-0.0200	-0.0400	-0.0600	-0.0700
250	-0.0300	-0.0400	-0.0400	-0.0200
500	-0.0500	-0.0200	-0.0400	-0.0300
1000	-0.0600	-0.0400	-0.0500	-0.0200

*** Concentrations of 5-OH metabolite of pyroxsulam/kg soil, dry weight.**

For the analysis of these data, two approaches were used; the first was by the ToxCalc program considering one and two tailed tests and, the second, use of the US EPA's on-line Dunnett's Procedure (at http://www.epa.gov/eerd/stat2.htm).

ToxCalc data analyses

The ToxCalc analysis of these data, following conversion of the data to fractions (i.e. day 14 weight expressed as a fraction of the day 0 weight) gave the following results (note that the ToxCalc results, when referring to untransformed data, refer to the ToxCalc treatment of the already transformed data which were analysed without further transformation):

One tailed test

Conc-ppm	1	2	3	4
S-Control	0.8000	0.9268	0.8810	0.9512
62.5	0.9091	0.8571	0.9474	0.9130
125	0.9500	0.9024	0.8723	0.8478
250	0.9333	0.9111	0.9070	0.9474
500	0.8889	0.9500	0.8889	0.9231
1000	0.8667	0.9000	0.8750	0.9459

			Transform: Untransformed						1-Tailed	
Conc-ppm	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
S-Control	0.8898	1.0000	0.8898	0.8000	0.9512	7.479	4			
62.5	0.9067	1.0190	0.9067	0.8571	0.9474	4.105	4	-0.578	2.410	0.0705
125	0.8932	1.0038	0.8932	0.8478	0.9500	4.925	4	-0.116	2.410	0.0705
250	0.9247	1.0393	0.9247	0.9070	0.9474	2.059	4	-1.195	2.410	0.0705
500	0.9127	1.0258	0.9127	0.8889	0.9500	3.246	4	-0.785	2.410	0.0705
1000	0.8969	1.0080	0.8969	0.8667	0.9459	3.973	4	-0.245	2.410	0.0705

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97112	0.884	-0.2924	0.1765
Bartlett's Test indicates equal variances (p = 0.50)	4.38429	15.0863		

Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs S-Control	1000	>1000			0.07046	0.0792	0.00071	0.00171	0.83357	5, 18

Two tailed test

			Transform: Untransformed					2-Tailed		
Conc-ppm	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
S-Control	0.8898	1.0000	0.8898	0.8000	0.9512	7.479	4			
62.5	0.9067	1.0190	0.9067	0.8571	0.9474	4.105	4	0.578	2.840	0.0830
125	0.8932	1.0038	0.8932	0.8478	0.9500	4.925	4	0.116	2.840	0.0830
250	0.9247	1.0393	0.9247	0.9070	0.9474	2.059	4	1.195	2.840	0.0830
500	0.9127	1.0258	0.9127	0.8889	0.9500	3.246	4	0.785	2.840	0.0830
1000	0.8969	1.0080	0.8969	0.8667	0.9459	3.973	4	0.245	2.840	0.0830

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97112	0.884	-0.2924	0.1765
Bartlett's Test indicates equal variances (p = 0.50)	4.38429	15.0863		

Hypothesis Test (2-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs S-Control	1000	>1000			0.08304	0.09333	0.00071	0.00171	0.83357	5, 18

Note: data for the two-tailed test are as shown for the one-tailed test.

Both the one and two tailed results confirm the study report finding that, when comparing the weight losses over 14 days, no treatment group mean was significantly different for any concentration when compared to the control group (Dunnett's 2-tailed test, p>0.05).

US EPA Dunnett's Procedure data analyses
The Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory of the US EPA at Cincinnati, Ohio 45268 provides an online access to a Dunnett Program (Version 1.5).

Using the total change in earthworm weight values after 14 day, the program gave the following results:

Summary Statistics and ANOVA

Transformation = None

Concentration#	n	mean	s.d.	CV%
1 = control	**4**	**-.0475**	**.0310**	**65.2**
2 (62.5)	**4**	**-.0400**	**.0163**	**40.8**
3 (125)	**4**	**-.0475**	**.0222**	**46.7**
4 (250)	**4**	**-.0325**	**.0096**	**29.5**
5 (500)	**4**	**-.0350**	**.0129**	**36.9**
6 (1000)	**4**	**-.0425**	**.0171**	**40.2**

 # mg 5-OH metabolite of pyroxsulam/kg soil, dry weight.

No means were identified as significantly less than the control mean.

Minimum detectable difference for Dunnett's test = -.033122. This difference corresponds to -69.73 percent of control

Between concentrations sum of squares = .000783 with 5 degrees of freedom.

Error mean square = .000378 with 18 degrees of freedom.

Bartlett's test p-value for equality of variances = .485.

These results are considered consistent with the ToxCalc finding that, for the single tailed Dunnett's test, none of the 5-OH metabolite of pyroxsulam mean weight changes were statistically significantly lower than the control mean weight change.

Statistical Method:

14 day LC50 and EC50:	Not determined, estimated as >1000 mg 5-OH metabolite of pyroxsulam/kg dry soil for mortality and sublethal effects.
95% C.I.:	Not determined
14 day NOEC: (mortality and sublethal effects including weight loss)	1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight.
14 day LOEC:	>1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight.
Probit Slope:	Not determined
95% C.I.:	Not determined
Endpoint(s) affected:	There were no compound related effects on mortality or sublethal effects.

E. STUDY DEFICIENCIES:

Table 11 identifies the deviations from guidelines or deficiencies noted. These are considered to be of such a nature as to not to have significantly affected the study's conduct. Reference to US EPA OPPTS 850.6200 is not made in the table because 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 11. Summary of deficiencies/deviations from the OECD 207 guideline.

Parameter	Study report result	OECD Guideline for Testing of Chemicals, "Earthworm, Acute Toxicity Tests", 207, adopted 4 April 1984
Stability and homogeneity of test material in the medium?	**Not verified. Information on the stability of the 5-OH metabolite of pyroxsulam shows there is potential for degradation during the 14 day test.**	**OECD 207 indicates chemical stability in water, soil and light are known, i.e. such parameters are considered "Guidance information".**
Physicochemical properties of soil. pH (__:__ soil:water)	**Soil water ratio not provided. In the control and test soils pH was 7.2-7.3 at test start and 7.4-7.6 at test end.**	**OECD 207 refers to the pH of the artificial soil being 6.0 ± 0.5.**

With respect to OECD 207, the significant deviation with respect to effect on the results is considered to be the lack

of information of the stability of the 5-OH metabolite of pyroxsulam under the test conditions. While the absence of results confirming the test doses of the 5-OH metabolite of pyroxsulam/kg soil, dry weight and that the 5-OH metabolite of 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. However, the short half-life (see below, under "Reviewer's Comments") does mean that a significant degradation of the 5-hydroxy material could have occurred and there has to be some doubt as to the actual exposure concentrations over the 14 days of exposure.

F. REVIEWER'S COMMENTS:

This study was conducted as a 14-day acute toxicity test with a nominal concentration of 0 to 1000 mg 5-OH metabolite of pyroxsulam/kg technical grade material/kg soil, dry weight. Although there were no actual measurements of the metabolite concentrations in the treated soil conducted, the amounts of 5-OH metabolite of pyroxsulam and soil mixed together were provided and were recalculated to show that the initial test concentrations referred to were correct.

The mortality and sublethal effects (including weight change) result in 5-OH metabolite of pyroxsulam being considered as very slightly toxic to the earthworm, *Eisenia foetida* (14 day LC50 and EC50 both >1000 mg/kg soil, dry weight) based on a concentrations of 5-OH metabolite of pyroxsulam corrected for its active constituent content of 97%.

The demonstration of a no dose response for weight loss after 14 days in the 5-OH metabolite of pyroxsulam exposed earthworms resulted in a NOEC of 1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight being established.

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. There were no analytical determinations of the treated soil made to confirm that the nominal test doses were achieved and that the active constituent was evenly distributed in the treated soils were used. While such information is not stipulated by OECD 207, the provision of data on the amounts of 5-OH metabolite of pyroxsulam mixed with known amounts of soil and the description of the mixing procedure supports the consideration that the nominal concentrations were actually achieved.

With respect to the stability of the 5-OH metabolite of pyroxsulam in soil, the aerobic soil degradation study of radiolabelled pyroxsulam in four European soils (Yoder *et al.*, 2006) reported that the DT50 for the 5-OH metabolite of pyroxsulam formed from pyroxsulam degradation was 3.4 days (DT90 11.2 days) in one soil and 2.7 days (DT90 9.1 days) in a second oil with conversion to "other" degradates with DT50 values for the other two soils not reported. Such results indicate that the 5-OH metabolite of pyroxsulam could be expected to have undergone significant degradation in the 14 days of the earthworm exposure 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 with there being 0 earthworms dead out of forty in the controls, i.e. 0%.

F. CONCLUSIONS:

The study is supplemental. The 5-OH metabolite of pyroxsulam is very slightly toxic to the earthworm, *Eisenia foetida* (LC50 >1000 mg active constituent/kg soil, dry weight). The study satisfies the guideline requirements for an acute toxicity study for 5-OH metabolite of pyroxsulam with earthworms.

In earthworms exposed to 62.5 to 1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight for 14 days, there were no mortalities in any treatment groups, i.e. 0% mortality. In the controls over the same period exhibited, there were also no mortalities in a total of 40 worms, i.e. 0%. No sublethal effects were observed (including weight loss).

Control and exposed earthworms all lost weight over the 14 days of the exposure period but with no dose related effect identified. No treatment group mean was significantly different for any concentration when compared to the control group (Dunnett's 2-tailed test, $p>0.05$).

The 14-day LC50 was set at >1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight in the study report. While an EC50 could not be determined for sublethal effects including weight change, this parameter could also considered >1000 mg 5-OH metabolite of pyroxsulam.

The 14-day NOECs for mortality and sublethal effects including weight change were both 1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight in the study report.

The study did not establish 14-day LOECs for mortality and sublethal effects including weight change. Based on the data assessed, the LOECs for these parameters are >1000 mg 5-OH metabolite of pyroxsulam/kg soil, dry weight.

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

The US EPA secondary reviewer stated that the study is scientifically sound but classified as supplemental since there are no current EPA guideline requirements for an earthworm toxicity test. In addition, the lack of measured concentrations indicates some uncertainty regarding the toxicity estimates.

III. REFERENCES:

OECD Guideline 207. 1984. *Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests*. Organization for Economic Cooperation and Development.

SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8. Cary, NC, SAS Institute, Inc.

Sindermann, A.B. Porch, J.R. and Krueger, H.O. 2006a. Study Profile Template (SPT) for: 5-OH metabolite of XDE-742: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate. Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601. Wildlife International, Ltd. Project No.: 379-160.SPT and Dow AgroSciences Study Number: 050128.SPT. The Dow Chemical Company Midland, MI 48674 USA for Dow AgroSciences, LLC Indianapolis, IN 46268 USA. 16 February 2006. Unpublished report.

Stephan, C.E. 1977. Methods for Calculating an LC50. *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Pub. No. STP 634:65-84.

Wildlife International, Ltd. 2004. *Chloroacetamide: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate*. Project No. 100R-110.

Yoder, R. N. Smith, K. P. Balcer, J. L. and Linder, S. J. 2006. Aerobic Soil Degradation of 14C-XDE-742 in Four European Soils. Regulatory Laboratories—Indianapolis Lab, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054. 12-Jan-06. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054. Unpublished report.

Approved 04/01/01 C.K.