Data Evaluation Report on the acute toxicity effects of the 7-OH metabolite of pyroxsulam (XDE-742) on earthworms

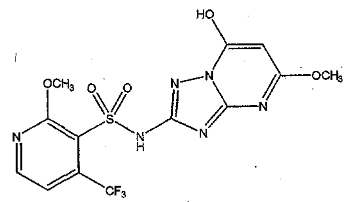
PMRA Submission Number 2006-4727; ID 1283182 EPA MRID Number 4690~~84-xx~~ 85-07 APVMA ATS 40362

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: 7-hydroxy-pyroxsulam or 7-hydroxy-XDE-742 **Purity (%):** 99%

Common name:	7-OH Metabolite of XDE-742
Chemical name:	3-pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
IUPAC:	N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:	N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.:	Not available
Synonyms:	X11250641, 7-desmethyl-XDE-742

Chemical Structure:



Primary Reviewer: Daryl Murphy 22/02/08 **Date:** 30 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland 22/2/08 **Date:** 30 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Ann Lee (#1639) Émilie Larivière ~~for A.~~ 05/03/08 **Date:** 4 May 2007
Environmental Assessment Directorate, PMRA

Émilie Larivière **Date:** 3 December 2007
Environmental Assessment Directorate, PMRA

Christopher Salice 4/10/08 **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. 7-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 379-159 and Dow AgroSciences Study Number 050127. The Dow Chemical Company, Midland MI 48674, USA for Dow AgroSciences, LLC, Indianapolis IN, 46268 USA. February 13, 2006. Unpublished report.

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Data Evaluation Report on the acute toxicity effects of the 7-OH metabolite of pyroxsulam (XDE-742) on earthworms

PMRA Submission Number 2006-4727; ID 1283182 EPA MRID Number 469085-07 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: 7-hydroxy-pyroxsulam or 7-hydroxy-XDE-742 **Purity (%):** 99%

Common name: 7-OH Metabolite of XDE-742

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

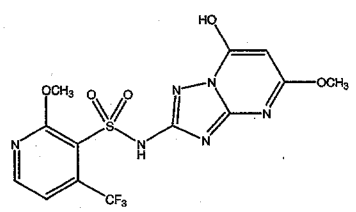
IUPAC: N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

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

CAS No.: Not available

Synonyms: X11250641, 7-desmethyl-XDE-742

Chemical Structure:



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

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

Ann Lee (#1639) **Date:** 4 May 2007
Environmental Assessment Directorate, PMRA

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

In a 14 day acute toxicity study, earthworms (*Eisenia foetida*) were exposed to the 7-OH metabolite of pyroxsulam at 0, 62.5, 125, 250, 500 and 1000 mg/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 carried out in accordance with OECD 207 "Earthworm, Acute Toxicity Test" (1984). Test concentrations in soil were not verified. The control group and the 125 mg 7-OH-XDE-742/kg soil treatment group had 5% and 2.5% mortality, respectively. The 62.5, 250, 500 and 1000 mg 7-OH-XDE-742/kg soil treatment groups had 0% mortality. Since a dose-response relationship was not observed, the mortality in the control and the 125 mg 7-OH-XDE-742/kg soil treatment groups were not considered to be treatment related. All surviving earthworms in the control group and treatment groups were normal in appearance and behaviour throughout the exposure period. Earthworm in both the control and treatment groups exhibited no aversion to the soil when observed on days 0 and 7 for burrowing behaviour.

Mean initial and final body weights in the treatment groups were not identified in the study report as statistically significant ($p > 0.05$) at any concentration when compared to the control group. However, the mean change in body weight (initial - final) was reported by the study, and confirmed by the review, as significant ($p < 0.05$) relative to the control for all of the treatment groups except the 500 mg 7-OH metabolite/kg group. The mean % weight loss after 14 days was 5.8, 14.3, 15.9, 14.3, 11.7 and 17.7% in the control, 62.5, 125, 250, 500 and 1000 mg/kg dw soil treatments, respectively. The study report considered the differences in weight change were incidental to treatment due to the lack of a dose response, lack of an apparent difference in initial or final mean weights, and lack of accompanying signs of toxicity. The statistical significance may have been attributed, according to the study report, to the low control variance caused by an unusually small loss of body weight in the control group.

In contrast, the reviewers of the study have concluded that an alternative interpretation is possible based on the observations that worms in all of the treatment levels showed a larger change in weight compared to the controls and, from a percentage perspective, controls showed a 5.8% reduction in body weight and for treated worms, the change was from 11.7-17.7%. An effect of this magnitude might be considered biologically relevant. The review also considered that, while a dose-response was not observed is cited in the study report as a reason for disregarding the apparent affects, the concentrations of the chemical were not measured and if it degraded quickly – as indicated by reported laboratory half-lives of 1.8 and 14.7 days (Yoder *et al.*, 2006), a dose-response relationship may not be as apparent. In addition, the review of the study considers the multiple statistical tests conducted on this endpoint with similar outcomes further suggests the change in weight loss effect may be real and the NOEC/NOAEC for weight loss should be set to <62.5 mg 7-OH metabolite of pyroxsulam/kg soil (dw) and the corresponding LOEC be set at ≤62.5 mg 7-OH metabolite of pyroxsulam/kg soil (dw).

The 14-day LC_{50} was estimated by the study report as >1000 mg 7-OH metabolite of pyroxsulam/kg dry weight (dw) of soil/substrate. The study's 14-day NOEC, based on mortality and sublethal effects (including weight loss) was 1000 mg 7-OH metabolite of pyroxsulam/kg dw of soil substrate. The study's 14-day LOEC, based on based on mortality and sublethal effects (including weight change), was >1000 mg 7-OH metabolite of pyroxsulam/kg dw of soil/substrate. These study report's LC50 result indicates the 7-OH metabolite of pyroxsulam is very slightly toxic to earthworms above a concentration of 1000 mg active constituent/kg dw of soil/substrate.

This study is classified as supplemental by DEW and satisfies the guideline requirements for an acute toxicity study for 7-OH metabolite of pyroxsulam with earthworms.

The US EPA secondary reviewer has 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.

The PMRA does not share the same study classification scheme as the US EPA and the DEW. This study is acceptable to the PMRA.

Results Synopsis

Test Organism:	Earthworm, *Eisenia foetida*
Size:	Control earthworms had an initial mean weight of 0.43 g/earthworm. Earthworms exposed to the 7-OH metabolite of pyroxsulam had initial mean weights of 0.43 or 0.44 g/earthworm.
Age:	Adults with clitella
Test Type:	Acute toxicity
14 day LC50:	Not determined but estimated by the study report as >1000 mg 7-OH metabolite of pyroxsulam/kg dry soil.
95% C.I.:	Not determined
14 day NOEC/NOAEC (mortality and sublethal effects, as determined by the study authors):	1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight
14 day NOEC/NOAEC (weight change, reviewer determined):	<62.5 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight
14 day LOEC (for mortality and sublethal effects excluding weight change, reviewer calculated):	>1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight
14 day LOEC (weight change, reviewer calculated):	≤62.5 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight
Probit Slope:	Not determined
95% C.I.:	Not determined
Endpoint(s) Effected:	There were no compound related effects on mortality and sublethal effects reported by the study. The reviewers' assessment identified compound related effects had occurred on weight loss difference.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
The study was conducted based upon the procedures outlined in the Wildlife International, Ltd. protocol, "XDE-742 (7-OH Metabolite): An Acute Toxicity Study With The Earthworm In An Artificial Soil Substrate." The protocol was based upon procedures in the Organization for Economic Cooperation and Development (OECD) Guideline No. 207, *Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests* (1984).

While the acute toxicity template used for the preparation of this DER makes reference on occasion to EPA and/or OECD requirements of unknown provenance, compliance with OECD 207 has been given precedence with the template's requirements noted for information only.

There were a number of deviations from the Guideline and other deficiencies identified (see page 30 of this draft DER).

COMPLIANCE:
The study was conducted in compliance with the 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 Good Laboratory Practice Compliance Statement stated that verification of test concentrations, stability and homogeneity of the test substance in the soil were not determined. Although periodic analyses of soil and water for potential contamination were not conducted according to Good Laboratory Practice Standards, they were reported as being 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 7-OH metabolite of XDE-742 (referred to as the 7-OH metabolite of pyroxsulam in this draft DER).

Description: Solid

Lot No./Batch No. : Lot 35172-56

TSN No.: 105384

Purity: 99% 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, Summary of deficiencies/deviations from guidelines, page 30 of this draft DER.

With respect to the stability of the 7-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 7-OH metabolite of pyroxsulam formed from pyroxsulam degradation was 2.2 days (DT90 7.2 days) in one soil, 14.7 days (DT90 49.0 days) in another soil and 1.8 days (DT90 6.0 days) in a third soil with conversion to the 6-Cl-7-OH metabolite of pyroxsulam or, in the last case to that metabolite and "other". The DT50 in the fourth soil was not reported for the 7-OH metabolite. The terrestrial field dissipation study report for pyroxsulam (and cloquintocet safener) in Canadian soils (Roberts *et al.*, 2006) reported that the 7-OH degradate dissipated with a mean first order field half-life of 32 days (with individual DT50 values of 97, 3, 6 and 21 days reported). Such results indicate that the 7-OH metabolite of pyroxsulam could be expected to have most probably undergone significant degradation in the 14 days of the earthworm exposure study.

Storage conditions of test chemicals: Stored under ambient conditions.

Physicochemical properties of the 7-OH metabolite of pyroxsulam.

Parameter	Values	Comments
Water solubility at 20°C	Not available	Stated in the Study Profile Template

Parameter	Values	Comments
Vapour pressure	Not available	(Sindermann *et al.*, 2006a) as not available at the time of publication of the Study Profile Template.
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: The following table shows the average weights of the earthworms in the controls and test concentrations at test initiation -

Average weights of earthworms at test initiation (as average earthworm body weights in grams based on there being 10 earthworms/replicate, 4 replicates/control and test concentration).						
	Controls	mg 7-OH metabolite of pyroxsulam/kg soil dry weight (nominal)				
		62.5	125	250	500	1000
Mean:	0.43	0.44	0.43	0.43	0.43	0.44
Range:	0.41 to 0.44	0.42 to 0.46	0.41 to 0.45	0.40 to 0.46	0.38 to 0.48	0.41 to 0.46

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.

Reference toxicity tests with the toxicant 2-chloracetamide (referred to as chloracetamide in the study report but as 2-chloracetamide in the study profile template (Sindermann *et al.*, 2006a)) were reported as performed periodically at Wildlife International, Ltd. Tests were said to be conducted under conditions similar to those used in the test now being assessed, and with earthworms from the same source, to monitor the techniques used and sensitivity of the test population. The earthworms in the soil were exposed to nominal concentrations of 13, 25, 50 mg 2-chloroacetamide/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 verified the adequacy and consistency of the methods used in this study.

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 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 the test was conducted from 5 to 19 January 2006. An artificial soil was pre-mixed with the 7-OH metabolite of pyroxsulam to give nominal concentrations of 0, 62.5, 125, 250, 500 and 1,000 mg 7-OH metabolite of pyroxsulam/kg dry soil. Earthworms (*Eisenia foetida*) were exposed to these concentrations of 7-OH metabolite of pyroxsulam in the soil. The objective of this study was to evaluate the acute effects of 7-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 the soil mass). The soil's moisture content was then brought to 34% by addition of 1000 mL 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 34% 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 and 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 7-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 7-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 99% purity of the 7-OH metabolite of pyroxsulam, 62.5, 125, 250, 500 and 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight, i.e. as given in the test report.

Chemical analyses of the treated soil to confirm that dosing had been correct and that the mixing procedure had evenly distributed the 7-OH metabolite of pyroxsulam throughout the treated soils were not conducted (See Table 11, Summary of deficiencies/deviations from guidelines, page 30 of this draft 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): ~70% quartz sand ~20% kaolin clay ~10% sphagnum peat ~1% calcium carbonate	Requirement considered met. *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.* The artificial soil contained ~1% calcium carbonate which is less than the 2% mentioned in the template (above). OECD 207 does not specify a percentage of calcium carbonate to be used.
pH (__:__ soil:water) The study profile template (Sindermann *et al.*, 2006) 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 7.1 to 7.4 in the control and test soils at test initiation and from 7.6 to 7.8 in the controls and test soils at test termination.	See Table 11, Summary of deficiencies/deviations from guidelines, page 30 of this draft 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 ranged from 34 to 35% at test initiation and from	Requirement considered met. OECD 207 refers to the soil having an overall content of about 35%

	31 to 32% at test termination. Not specifically stated to be on a dry weight of soil basis.	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 (31 to 35%) are on a dry soil 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 ~34% 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.

Parameter	Value	Remarks *Criteria*
Test Container Material: Size: Amount of soil or substrate:	 Glass beakers covered with plastic wrap that was perforated for air exchange. Test beakers were held in an environmental chamber during testing. 1 Litre Seven hundred fifty grams of prepared, hydrated soil/test chamber.	Requirement considered met.
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 in the 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 7-OH metabolite of pyroxsulam with the dry artificial soil followed by adjustment to ~34% 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 7-OH metabolite of pyroxsulam/kg soil, dry weight.	Requirement considered met. The weights of 7-OH-pyroxsulam added to known soil weights were provided in the study report and calculated by the reviewer to be equivalent to the nominal

Parameter	Value	Remarks *Criteria*
	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 7-OH metabolite of pyroxsulam/kg).	concentrations reported used.
Measured:	Verification of test concentrations of the 7-OH metabolite of pyroxsulam in the soil did not occur (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 7-OH metabolite of pyroxsulam in the soil did not occur (GLP compliance statement). Information on the stability of the 7-OH metabolite of pyroxsulam (Yoder *et al.*, 2006) shows there is a potential for significant degradation to have occurred during the 14 day test (DT50s ranged between 1.8 and 14.7 days).	See Table 11, Summary of deficiencies/deviations from guidelines, page 30 of this draft 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 and remained at 20°C throughout the test. Air temperature was measured at least once daily in the environmental chamber, except for two days on which the temperature	Requirements considered met. *EPA requirements:* *<u>Temperature</u>: 22 + 2°C*

Parameter	Value	Remarks *Criteria*
	was not recorded. 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 20.2-21.4°C in the test and control groups at test initiation and 20.5 to 21.0°C at test end. Temperature in the environmental chamber was inadvertently not recorded on 7 and 8 January 2006. Because the temperature in the environmental chamber was monitored with a device that retained maximum/minimum readings it was verified that the temperature had not gone out of range on those two days. The information provided in the study report indicates that the temperature range did not exceed 20 ± 2°C on the days the temperature was not measured.	
Lighting conditions:	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 14 of the test. An average intensity of 592 ± 47.5 lux, with a reported range over the surface of the test chambers of 521 to 643 lux.	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.
Moisture:	Soil moisture ranged from 34.1 (control) to 34.9% (250 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight) at day 0. At test termination, the moisture content ranged from 31.3 (125 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight) to 32.2% (the 62.5	See Table 11, Summary of deficiencies/deviations from guidelines, page 30 of this draft DER with respect to relative humidity. *Relative humidity: above 85%*

Parameter	Value	Remarks *Criteria*
	and 250 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight soils). Relative humidity was not reported.	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-chloroacetamid/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 were 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 approximately 30 minutes after test initiation and after observations for signs of toxicity on day 7. Weight determinations were 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. Although a 14 day burrowing time is referred to in the study report (under "Experimental Design", this is considered an error. *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. The study protocol refers to a mechanical stimulus being applied to the earthworms and the reactions recorded. 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 in archives located on the Wildlife International, Ltd. site. Burrowing times were not presented.	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 concentration. Observations of mortality were conducted on days 7 and 14. Treatment related mortality was not observed in the test. There were two dead earthworms in the control group and one in the 125 mg 7-OH metabolite of pyroxsulam/kg treatment group that were considered incidental mortalities. 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 16 of this draft DER.

The following endpoints were reported by the study report:
14 day LC50: > 1000 mg 7-OH metabolite of pyroxsulam/kg dry soil
No Observed Effect Concentration: 1000 mg 7-OH metabolite of pyroxsulam/kg dry soil
(The no-observed-effect-concentration was reported by the study report as determined by visual examination of the mortality and clinical observation data).
)

Table 4. Effect of 7-OH metabolite of pyroxsulam on mortality of the earthworm (*Eisenia foetida*).

Treatment - mg 7-OH metabolite of pyroxsulam/kg soil, dry weight (nominal)	Replicate (10 earthworms/replicate)	Observation period			
		Day 7		Day 14	
		No. dead	% mortality	No. dead	% mortality
Negative control (0)	A	0/10	0	0/10	0
	B	2/10	20	2/10	20
	C	0/10	0	0/10	0
	D	0/10	0	0/10	0
Total number dead and total % mortality in the controls:		**2/40**	**5**	**2/40**	**5**
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	1/10	10	1/10	10
	Total:	**1/40**	**2.5**	**1/40**	**2.5**
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		**1/200**	**0.5%**	**1/200**	**0.5%**
14 day NOEC (mortality)		1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight			

LOEC		Not reported
14 day LC_{50}		>1000 mg 7-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 once at approximately 30 minutes after test initiation and after observations for signs of toxicity on day 7.

The EC50 and no-observed-effect-concentration were reported as 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 normality.

All surviving earthworms in the control group and treatment groups were reported as normal in appearance and behaviour throughout the test period. 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 not provided in the study report).

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

The sublethal effects (apart from changes in weight) results are summarised in Table 5, page 18 of this draft DER.

The study report considered the data supported the following endpoints:

14-Day EC50:	> 1000 mg 7-OH metabolite of pyroxsulam/kg dry soil
No Observed Effect Concentration: (mortality and sublethal effects, based on clinical observation data)	1000 mg 7-OH metabolite of pyroxsulam/kg dry soil

Note that the NOEC reported by the study report is based on the statistically significant mean changes in body weight relative to the control for all treatment groups except the 500 mg/kg soil, dry weight group being interpreted as incidental to the treatment as a result of the lack of a dose response. This is considered further under part "D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER" of this DER (page 21 refers).

Table 5. Sublethal effects (not weight change) of 7-OH metabolite of pyroxsulam on the earthworm (*Eisenia foetida*).

Treatment - mg 7-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	8 appeared normal, 2 not found, presumed dead and decomposed.	8 appeared normal, 2 not found, presumed dead and decomposed.
	C	10 appeared normal	10 appeared normal
	D	10 appeared normal	10 appeared normal
Total number abnormal and total % abnormality in the controls:		2/40, 5%	2/40, 5%
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	9 appeared normal, 1 not found, presumed dead and decomposed.	9 appeared normal, 1 not found, presumed dead and decomposed.
	Total:	1/40, 2.5%	1/40, 2.5%
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		**1/200, 0.5%**	**1/200, 0.5%**
14 day NOEC (sublethal effects apart from weight change)		1000 mg 7-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 7-OH metabolite of pyroxsulam/kg soil, dry weight	
Reference chemical	**% mortality:**	Not reported for sublethal effects	
	14 day LC_{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. The replicate data within each category were normally distributed and the variance among initial body weight and change in body weight were homogeneous.

Although variance in the final body weight data failed to meet the homogeneity criterion (Levene's test, p=0.0345), Dunnett's test was regarded by the study authors as robust with regard to slight departures from homogeneity. Therefore no data transformations were warranted.

Mean initial and final body weights in the treatment groups were not statistically significant ($p > 0.05$) at any concentration tested when compared to the control group. 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. The mean change in body weight (initial - final) was significant ($p < 0.05$) relative to the control for all of the treatment groups except the 500 mg 7-OH metabolite of pyroxsulam/kg group. The differences in weight change were considered by the study authors as incidental to treatment due to the lack of a dose response, lack of an apparent difference in initial or final mean weights, and lack of accompanying signs of toxicity. The statistical significance may have been a result of the low control variance caused by an unusually small loss of body weight in the control group. The US EPA secondary reviewer has identified that as all treatment levels showed more of a weight loss than the control, that only the 500 mg treatment was not statistically significant from the control results and because the highest test concentration showed the largest difference, the NOEC/NOAEC needed reconsideration. This issue is considered further in the "D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER" section below.

The weight change data were considered by the study authors to support the following endpoints:

14-Day EC50: > 1000 mg 7-OH metabolite of pyroxsulam/kg dry soil

No Observed Effect Concentration: 1000 mg 7-OH metabolite of pyroxsulam/kg dry soil
(based on visual examination of the mortality and clinical observation data, and assumed to include weight changes)

Sub-lethal effects on the weights of earthworms exposed to the 7-OH metabolite of pyroxsulam in an artificial soil matrix are shown in Table 6, page 20 of this draft DER.

Table 6. Sub-lethal effects on the weights of earthworms, *Eisenia foetida* exposed to 7-OH metabolite of pyroxsulam in artificial soil matrix.

Treatment (mg 7-OH metabolite of pyroxsulam/kg soil, dry weight, nominal)		Average Earthworm Body Concentration Weights[1] (g)		
		Day 0[2] weight	Day 14[2] weight	Total change in weight
Control (0)	Replicate (10 earthworms/replicate)			
	A	0.42	0.41	-0.01
	B	0.44	0.41	-0.03
	C	0.41	0.39	-0.02
	D	0.43	0.39	-0.04
Mean and standard deviation		0.43 ± 0.013	0.40 ± 0.012	-0.03± 0.013
62.5	A	0.46	0.39	-0.07
	B	0.44	0.39	-0.05
	C	0.43	0.34	-0.09
	D	0.42	0.38	-0.04
Mean and standard deviation		0.44 ± 0.017	0.38± 0.024	-0.06 ± 0.022*[3]
125	A	0.45	0.37	-0.08
	B	0.42	0.35	-0.07
	C	0.42	0.37	-0.05
	D	0.41	0.34	-0.07
Mean and standard deviation		0.43 ± 0.017	0.36 ± 0.013	-0.07 ± 0.012*
250	A	0.43	0.36	-0.07
	B	0.40	0.36	-0.04
	C	0.44	0.38	-0.06
	D	0.46	0.38	-0.08
Mean and standard deviation		0.43 ± 0.025	0.37 ± 0.012	-0.06 ± 0.017*
500	A	0.41	0.34	-0.07
	B	0.38	0.35	-0.03
	C	0.48	0.43	-0.05
	D	0.44	0.39	-0.05
Mean and standard deviation		0.43 ± 0.043	0.38 ± 0.041	-0.05 ± 0.016
1000	A	0.42	0.36	-0.06
	B	0.45	0.35	-0.10
	C	0.41	0.34	-0.07
	D	0.46	0.38	-0.08
Mean and standard deviation		0.44 ± 0.024	0.36 ± 0.017	-0.08 ± 0.017*
14 day NOEC ((based on visual examination of the mortality and clinical observation data, and assumed to include weight changes))		1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight		
LOEC (weight loss)		Not reported		
14 day EC_{50} (weight loss):		>1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight		
Reference	% mortality	Not reported for sublethal effects		

chemical	LC50 and 95% confidence limits	Not reported for sublethal effects

1. Values listed in the study report were from SAS output with the note that manual calculations may be slightly different due to differences in rounding.
2. For the day 14 weight results, no treatment group mean was significantly different for any concentration when compared to the control group (Dunnett's 2-tailed test, p>0.05).
3. For the total change in weight results, treatment group means significantly different from the control group (Dunnett's 2-tailed test, p>0.05) are marked by an asterisk.

C. REPORTED STATISTICS:

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

Because mortality above 50% did not occur 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 ($\alpha = 0.05$). 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 study's results (absence of dose related mortality and dose related sub-lethal effects - weight change is considered below), the statistical evaluation of the biological data for mortality and sublethal effects was not attempted.

Mortality were less than 50% at the concentration tested. Consequently, statistically determination of LC50 values could not be undertaken. The 14 day LC50 values were all empirically determined to be greater than the maximum nominal concentration tested i.e. >1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight basis.

Verification of weight change 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 7-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 7-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 7-OH metabolite of pyroxsulam exposed mean earthworm weights at day 0;

To confirm the mean weights of the control and 7-OH metabolite of pyroxsulam exposed worms were not statistically 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) reported are shown in Table 7.

Table 7. Mean weights of control and 7-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*)	0.4200	0.4400	0.4100	0.4300
62.5*	0.4600	0.4400	0.4300	0.4200
125	0.4500	0.4200	0.4200	0.4100
250	0.4300	0.4000	0.4400	0.4600
500	0.4100	0.3800	0.4800	0.4400
1000	0.4200	0.4500	0.4100	0.4600

* Concentrations of 7-OH metabolite of pyroxsulam/kg soil, dry weight.

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

One tailed test

			Transform: Untransformed						1-Tailed	
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.4250	1.0000	0.4250	0.4100	0.4400	3.038	4			
62.5	0.4375	1.0294	0.4375	0.4200	0.4600	3.904	4	-0.705	2.410	0.0427
125	0.4250	1.0000	0.4250	0.4100	0.4500	4.075	4	0.000	2.410	0.0427
250	0.4325	1.0176	0.4325	0.4000	0.4600	5.780	4	-0.423	2.410	0.0427
500	0.4275	1.0059	0.4275	0.3800	0.4800	9.993	4	-0.141	2.410	0.0427
1000	0.4350	1.0235	0.4350	0.4100	0.4600	5.472	4	-0.564	2.410	0.0427

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98462	0.884	0.16767	0.35585
Bartlett's Test indicates equal variances (p = 0.42)	5.00375	15.0863		

Two tailed test

			Transform: Untransformed						2-Tailed	
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.4250	1.0000	0.4250	0.4100	0.4400	3.038	4			
62.5	0.4375	1.0294	0.4375	0.4200	0.4600	3.904	4	0.705	2.840	0.0504
125	0.4250	1.0000	0.4250	0.4100	0.4500	4.075	4	0.000	2.840	0.0504
250	0.4325	1.0176	0.4325	0.4000	0.4600	5.780	4	0.423	2.840	0.0504
500	0.4275	1.0059	0.4275	0.3800	0.4800	9.993	4	0.141	2.840	0.0504
1000	0.4350	1.0235	0.4350	0.4100	0.4600	5.472	4	0.564	2.840	0.0504

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98462	0.884	0.16767	0.35585
Bartlett's Test indicates equal variances (p = 0.42)	5.00375	15.0863		

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

As the t scores are less than the critical one tailed t value and the critical two tailed t value, no statistically significant differences in the mean earthworm weights at day 0 for the control and the 7-OH metabolite of pyroxsulam exposed earthworms are indicated. This conclusion was also reached in the study report with respect to the two tailed analysis.

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 (mg) at days 0 and 14			
Replicate:	**1**	**2**	**3**	**4**
Day 0	0.42	0.44	0.41	0.43
Day 14	0.41	0.41	0.39	0.39

The ToxCalc analysis of these data gave the following results (one-tail analysis only, mean weights expected to decrease):

			Transform: Untransformed						1-Tailed	
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Day 0	0.4250	1.0000	0.4250	0.4100	0.4400	3.038	4			
*Day 14	0.4000	0.9412	0.4000	0.3900	0.4100	2.887	4	2.887	1.943	0.0168

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90446		0.749		5.9E-16	-1.9444
F-Test indicates equal variances (p = 0.86)	1.25		47.4672			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates significant differences Treatments vs Control	0.01683	0.0396	0.00125	0.00015	0.02781	1, 6

As the t score is greater than the critical one tailed t value, a statistically significant decrease 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 7-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 7-OH metabolite of pyroxsulam exposed earthworms with the data used shown in Table 9.

Table 9. Mean weights of control and 7-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*)	0.4100	0.4100	0.3900	0.3900
62.5*	0.3900	0.3900	0.3400	0.3800
125	0.3700	0.3500	0.3700	0.3400
250	0.3600	0.3600	0.3800	0.3800
500	0.3400	0.3500	0.4300	0.3900
1000	0.3600	0.3500	0.3400	0.3800

*** Concentrations of 7-OH metabolite of pyroxsulam/kg soil, dry weight.**

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

One tailed test
For the one tailed test, ToxCalc reported an interrupted dose response. With the option of identifying the first significant treatment as the LOEC, the following results were determined -

(Comparison of control and test mean weights at day 14, one-tailed analysis.)

Conc-ppm	Mean	N-Mean	Transform: Untransformed Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD
D-Control	0.4000	1.0000	0.4000	0.3900	0.4100	2.887	4			
62.5	0.3750	0.9375	0.3750	0.3400	0.3900	6.348	4	1.570	2.410	0.0384
*125	0.3575	0.8938	0.3575	0.3400	0.3700	4.196	4	2.669	2.410	0.0384
250	0.3700	0.9250	0.3700	0.3600	0.3800	3.121	4	1.884	2.410	0.0384
500	0.3775	0.9438	0.3775	0.3400	0.4300	10.895	4	1.413	2.410	0.0384
*1000	0.3575	0.8938	0.3575	0.3400	0.3800	4.777	4	2.669	2.410	0.0384

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.94283		0.884		0.24534	0.99613
Bartlett's Test indicates equal variances (p = 0.20)					7.22295		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	62.5	125	88.3883		0.03837	0.09592	0.00099	0.00051	0.13375	5, 18

With the option of not identifying the first significant treatment as the LOEC, the following results were determined –

Conc-ppm	Mean	N-Mean	Transform: Untransformed Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD
D-Control	0.4000	1.0000	0.4000	0.3900	0.4100	2.887	4			
62.5	0.3750	0.9375	0.3750	0.3400	0.3900	6.348	4	1.570	2.410	0.0384
*125	0.3575	0.8938	0.3575	0.3400	0.3700	4.196	4	2.669	2.410	0.0384
250	0.3700	0.9250	0.3700	0.3600	0.3800	3.121	4	1.884	2.410	0.0384
500	0.3775	0.9438	0.3775	0.3400	0.4300	10.895	4	1.413	2.410	0.0384
*1000	0.3575	0.8938	0.3575	0.3400	0.3800	4.777	4	2.669	2.410	0.0384

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.94283		0.884		0.24534	0.99613
Bartlett's Test indicates equal variances (p = 0.20)					7.22295		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	500	1000	707.107		0.03837	0.09592	0.00099	0.00051	0.13375	5, 18

In this latter situation, the statistically significantly different result at 125 mg 7-OH metabolite of pyroxsulam is disregarded as biologically without significance when the absence of statistical significance in the 250 and 500 mg/kg soil (dw) is noted. Because the 1000 mg 7-OH metabolite of pyroxsulam/kg of soil, dry weight mean results are identified as statistically significantly less than the mean control weights, the NOEC is set at 500 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight.

Two tailed test

For the two-tailed test, ToxCalc did not report an interrupted dose response.

The ToxCalc results are as follow:

(Comparison of control and test mean weights at day 14, two-tailed analysis.)

			Transform: Untransformed						2-Tailed	
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.4000	1.0000	0.4000	0.3900	0.4100	2.887	4			
62.5	0.3750	0.9375	0.3750	0.3400	0.3900	6.348	4	1.570	2.840	0.0452
125	0.3575	0.8938	0.3575	0.3400	0.3700	4.196	4	2.669	2.840	0.0452
250	0.3700	0.9250	0.3700	0.3600	0.3800	3.121	4	1.884	2.840	0.0452
500	0.3775	0.9438	0.3775	0.3400	0.4300	10.895	4	1.413	2.840	0.0452
1000	0.3575	0.8938	0.3575	0.3400	0.3800	4.777	4	2.669	2.840	0.0452

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.94283		0.884		0.24534	0.99613
Bartlett's Test indicates equal variances (p = 0.20)					7.22295		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			0.04522	0.11304	0.00099	0.00051	0.13375	5, 18

The two tailed result confirms the study report finding that, when comparing the day 14 average earthworm weights, 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 study report had stated that although variance in the final body weight data failed to meet the homogeneity criterion (Levene's test, p=0.0345), Dunnett's test was robust with regard to slight departures from homogeneity and, therefore no data transformations were warranted. The ToxCalc analysis of the final weight data reported that Bartlett's Test indicated equality of variances, a finding different to that reported by the study report.

This difference is not considered to have affected the NOEC decided upon.

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

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*)	-0.01	-0.03	-0.02	-0.04
62.5*	-0.07	-0.05	-0.09	-0.04
125	-0.08	-0.07	-0.05	-0.07
250	-0.07	-0.04	-0.06	-0.08
500	-0.07	-0.03	-0.05	-0.05
1000	-0.06	-0.1	-0.07	-0.08

*** Concentrations of 7-OH metabolite of pyroxsulam/kg soil, dry weight.**

For the analysis of these data, two approaches were used:

The first was with 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

For the analyses, the following transformed data were used:

Weight changes over 14 days as a fraction of the initial weights:

Conc-ppm	1	2	3	4
D-Control	0.9762	0.9318	0.9512	0.9070
62.5	0.8478	0.8864	0.7907	0.9048
125	0.8222	0.8333	0.8810	0.8293
250	0.8372	0.9000	0.8636	0.8261
500	0.8293	0.9211	0.8958	0.8864
1000	0.8571	0.7778	0.8293	0.8261

The ToxCalc analysis of these data, 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
For the one tailed test, ToxCalc reported an interrupted dose response. With the option of identifying the first significant treatment as the LOEC, the following results were determined (with transformation of the original data as described above) -

			Transform: Untransformed						1-Tailed			
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
S-Control	0.9416	1.0000	0.9416	0.9070	0.9762	3.117	4					
*62.5	0.8574	0.9106	0.8574	0.7907	0.9048	5.879	4	3.303	2.410	0.0614		
*125	0.8414	0.8937	0.8414	0.8222	0.8810	3.177	4	3.930	2.410	0.0614		
*250	0.8567	0.9099	0.8567	0.8261	0.9000	3.836	4	3.330	2.410	0.0614		
500	0.8831	0.9380	0.8831	0.8293	0.9211	4.391	4	2.293	2.410	0.0614		
*1000	0.8226	0.8736	0.8226	0.7778	0.8571	4.007	4	4.671	2.410	0.0614		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.95962		0.884		-0.3189	-0.6144
Bartlett's Test indicates equal variances (p = 0.92)							1.45835		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			<62.5	62.5			0.06139	0.0652	0.00691	0.0013	0.00354	5, 18

The study report's findings for the total change in weight results, that the treatment group means for the 62.5, 125, 250 and 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight are statistically significantly different from the control mean are confirmed by these ToxCalc results.

With the option of not identifying the first significant treatment as the LOEC, the following results were determined -

			Transform: Untransformed						1-Tailed			
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
S-Control	0.9416	1.0000	0.9416	0.9070	0.9762	3.117	4					
*62.5	0.8574	0.9106	0.8574	0.7907	0.9048	5.879	4	3.303	2.410	0.0614		
*125	0.8414	0.8937	0.8414	0.8222	0.8810	3.177	4	3.930	2.410	0.0614		
*250	0.8567	0.9099	0.8567	0.8261	0.9000	3.836	4	3.330	2.410	0.0614		
500	0.8831	0.9380	0.8831	0.8293	0.9211	4.391	4	2.293	2.410	0.0614		
*1000	0.8226	0.8736	0.8226	0.7778	0.8571	4.007	4	4.671	2.410	0.0614		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.95962		0.884		-0.3189	-0.6144
Bartlett's Test indicates equal variances (p = 0.92)							1.45835		15.0863			
Hypothesis Test (1-tail, 0.05)			**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test			500	1000	707.107		0.06139	0.0652	0.00691	0.0013	0.00354	5, 18
Treatments vs S-Control												

Again, the study report's results with respect to statistically significant total change in weight are consistent with these ToxCalc results.

Two tailed test
For the two tailed test with data transformation, ToxCalc again reported an interrupted dose response. With the option of identifying the first significant treatment as the LOEC, the following results were determined -

Conc-ppm	1	2	3	4
S-Control	0.9762	0.9318	0.9512	0.9070
62.5	0.8478	0.8864	0.7907	0.9048
125	0.8222	0.8333	0.8810	0.8293
250	0.8372	0.9000	0.8636	0.8261
500	0.8293	0.9211	0.8958	0.8864
1000	0.8571	0.7778	0.8293	0.8261

			Transform: Untransformed						2-Tailed			
Conc-ppm	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
S-Control	0.9416	1.0000	0.9416	0.9070	0.9762	3.117	4					
*62.5	0.8574	0.9106	0.8574	0.7907	0.9048	5.879	4	3.303	2.840	0.0723		
*125	0.8414	0.8937	0.8414	0.8222	0.8810	3.177	4	3.930	2.840	0.0723		
*250	0.8567	0.9099	0.8567	0.8261	0.9000	3.836	4	3.330	2.840	0.0723		
500	0.8831	0.9380	0.8831	0.8293	0.9211	4.391	4	2.293	2.840	0.0723		
*1000	0.8226	0.8736	0.8226	0.7778	0.8571	4.007	4	4.671	2.840	0.0723		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.95962		0.884		-0.3189	-0.6144
Bartlett's Test indicates equal variances (p = 0.92)							1.45835		15.0863			
Hypothesis Test (2-tail, 0.05)			**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test			<62.5	62.5			0.07235	0.07684	0.00691	0.0013	0.00354	5, 18
Treatments vs S-Control												

Again, the study report's results with respect to statistically significant total change in weight are consistent with these ToxCalc results.

With the option of not identifying the first significant treatment as the LOEC, the following results were determined -

			Transform: Untransformed						2-Tailed			
Conc-ppm	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**		
S-Control	0.9416	1.0000	0.9416	0.9070	0.9762	3.117	4					
*62.5	0.8574	0.9106	0.8574	0.7907	0.9048	5.879	4	3.303	2.840	0.0723		
*125	0.8414	0.8937	0.8414	0.8222	0.8810	3.177	4	3.930	2.840	0.0723		
*250	0.8567	0.9099	0.8567	0.8261	0.9000	3.836	4	3.330	2.840	0.0723		
500	0.8831	0.9380	0.8831	0.8293	0.9211	4.391	4	2.293	2.840	0.0723		
*1000	0.8226	0.8736	0.8226	0.7778	0.8571	4.007	4	4.671	2.840	0.0723		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.95962		0.884		-0.3189	-0.6144
Bartlett's Test indicates equal variances (p = 0.92)							1.45835		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			500	1000	707.107		0.07235	0.07684	0.00691	0.0013	0.00354	5, 18

Again, the study report's results with respect to statistically significant total change in weight are consistent with these ToxCalc results.

The ToxCalc NOEC for weight change of 500 mg differs from the study report NOEC taken as 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight where the study report reasoned that lack of dose response and lack of apparent differences in initial and final weights led to the conclusion of setting the NOEC at the 1000 mg/kg soil, dry weight concentration. However, the more significant NOEC for weight change is the 62.5 mg/kg value indicated in the above data set analysis (this is considered below).

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	-.0250	.0129	51.6
2* (62.5)	4	-.0625	.0222	35.5
3* (125)	4	-.0675	.0126	18.6
4* (250)	4	-.0625	.0171	27.3
5 (500)	4	-.0500	.0163	32.7
6* (1000)	4	-.0775	.0171	22.0

 # mg 7-OH metabolite of pyroxsulam/kg soil, dry weight.

For concentrations marked with an asterisk (*), the mean for these concentrations are significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test.

Minimum detectable difference for Dunnett's test = -.028402
This difference corresponds to -113.61 percent of control

Between concentrations sum of squares = .006650 with 5 degrees of freedom.

Error mean square = .000278 with 18 degrees of freedom.

Bartlett's test p-value for equality of variances = .944

These results are considered consistent with the ToxCalc and study report findings.

Conclusion with respect to total weight change over 14 days

The statistical assessment of the total weight change over 14 days using ToxCalc and the US EPA Dunnett's test program give results equivalent to those found in the study report, namely, that the treatment group means for the 62.5, 125, 250 and 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight are statistically significantly different from that of the mean weight loss of the controls.

Examination of the actual mean weight losses, i.e.

Concentration#	Mean weight loss over 14 days	Mean % weight loss over 14 days
Control	-0.0250	5.8
62.5	-0.0625	14.3
125	-0.0675	15.9
250	-0.0625	14.3
500	-0.0500	11.7
1000	-0.0775	17.7

 # mg 7-OH metabolite of pyroxsulam/kg soil, dry weight.

is not considered to show any apparent dose response. Consequently, those statistical results indicating a NOEC of <62.5 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight could be considered most probably as biologically irrelevant (as was argued by the study authors). Similarly, the absence of a dose response could be taken to indicate that the NOEC can be set at 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight rather than the ToxCalc NOEC of 500 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight.

Consequently, the study report reasoned that the lack of a dose response, lack of apparent weight and other sublethal effects allowed setting the NOEC at the 1000 mg/kg soil, dry weight concentration.

In contrast, the US EPA secondary reviewer identified that worms in all of the treatment levels showed a larger change in weight compared to the controls. From a percentage perspective, controls showed a 5.8% reduction in body weight and for treated worms, the change was from 11.7-17.7%, with an effect of this magnitude might be considered biologically relevant

The secondary reviewer also noted that the fact that a dose-response was not observed was cited as a reason for disregarding the apparent affects. However, the concentrations of the chemical were not measured and if the chemical degraded quickly, a dose-response relationship may not be as apparent. In addition, the multiple statistical tests conducted on this endpoint with similar outcomes further suggests the effect may be real.

Consequently, the DEW reviewer agrees that the NOEC (NOAEC) for weight loss is set to <62.5 mg 7-OH metabolite of pyroxsulam/kg soil (dry weight).

Statistical Method (as determined by either the study report or the review of the study report):

14 day LC50:	Not determined, estimated by the study authors as >1000 mg 7-OH metabolite of pyroxsulam/kg dry soil for mortality and sublethal effects (including weight loss).
95% C.I.:	Not determined
14 day NOEC (mortality and sublethal effects, as determined by the study authors))	1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight.
14 day NOEC/NOAEC (weight changes, reviewer determined)	<62.5 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight
14 day LOEC (for mortality and sublethal effects excluding weight change, reviewer calculated):	>1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight
14 day LOEC (weight change, reviewer calculated):	≤62.5 7-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 and sublethal effects reported by the study. Th reviewers' assessment identified compound related effects had occurred on weight loss difference.

E. STUDY DEFICIENCIES:

The deviations from guidelines or deficiencies shown in Table 11 were noted but are considered to be of such a nature as to not to have significantly affected the study's conduct. 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 7-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 7.1-7.6 at test start and 7.6-7.8 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 7-OH metabolite of pyroxsulam under the test conditions and the failure to have analytically determined concentrations of the 7-OH metabolite over the exposure period.. While the absence of results confirming the test doses of the 7-OH metabolite of pyroxsulam/kg soil, dry weight and that the 7-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 7-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 7-OH metabolite of pyroxsulam/kg technical grade material/kg soil, dry weight. Although there were no actual measurements of the 7-OH metabolite of pyroxsulam concentrations in the treated soil conducted, the amounts of 7-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 (apart from loss of weight) result in 7-OH metabolite of pyroxsulam being considered as very slightly toxic to the earthworm, *Eisenia foetida* (14 day LC50 >1000 mg/kg soil, dry weight) based on a concentrations of 7-OH metabolite of pyroxsulam corrected for its active constituent content of 99%.

The demonstration of no mortality and absence of adverse effects in the clinical observation data, resulted in the study authors' considering that there was no dose response for weight loss after 14 days in the 7-OH metabolite of pyroxsulam exposed earthworms and that a NOEC of 1000 mg 7-OH hydroxy metabolite of pyroxsulam/kg soil, dry weight could be established. Although statistically significant weight losses were seen at the 62.5, 125, 250 and 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight, the study authors observed that no equivalent observations were seen with regard to the actual mean weights of the exposed earthworms at either the start or end of the exposure when compared to the mean control weights. Coupled with the absence of a dose response, the 14 day NOEC for weight loss was considered by the study authors to be 1000 mg 7-OH metabolite of 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. 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 as being required by OECD 207, the provision of data on the amounts of 7-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 7-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 7-OH metabolite of pyroxsulam formed from pyroxsulam degradation was 2.2 days (DT90 7.2 days) in one soil, 14.7 days (DT90 49.0 days) in another soil and 1.8 days (DT90 6.0 days) in a third soil with conversion to the 6-Cl-7-OH metabolite of pyroxsulam or, in the last case to that metabolite and "other". No 7-OH metabolite of pyroxsulam DT50 for the fourth soil was provided. In contrast, the terrestrial field dissipation study report for pyroxsulam (and cloquintocet-mexyl safener) in Canadian soils (Roberts *et al.*, 2006) reported that the 7-OH degradate dissipated with a mean first order field half-life of 32 days (with individual DT50 values of 97, 3, 6 and 21 days reported). Such results indicate that the 7-OH metabolite of pyroxsulam could be expected to have undergone significant degradation in the 14 days of the earthworm exposure study.

Because verification of the 7-OH metabolite's stability over the exposure period was not provided, the review of the study has resulted in an alternative interpretation of the weight change data. Thus the short half-life reported elsewhere for the 7-OH metabolite, the consequent expectation of possibly significant degradation of this metabolite in the soil over the 14 days exposure period and the observation that worms in all of the treatment levels showed a larger change in weight compared to the controls and, from a percentage perspective, controls showed a 5.8% reduction in body weight and for treated worms, the change was from 11.7 to 17.7% has lead to an alternative conclusion with respect to the NOEC for weight change. Because an effect of this magnitude (weight change) might be considered biologically relevant and because a dose-response relationship may not be as apparent if the chemical, as expected, degraded quickly, the NOEC/NOAEC for weight change should be set at 62.5 mg 7-OH metabolite of pyroxsulam. This is supported by the multiple statistical tests conducted on this endpoint with similar outcomes further suggests the effect may be real. Consequently, the NOEC/NOAEC for weight change should be set to <62.5 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight.

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 2 earthworms dead out of forty in the controls, i.e. 5%.

F. CONCLUSIONS:

The study is supplementary because of the possibility that significant degradation of the 7-OH metabolite occurred over the 14 days of the earthworm exposure and analytical determinations were not conducted to confirm this.. The 7-OH metabolite of pyroxsulam is very slightly toxic to the earthworm, *Eisenia foetida* (LC50 >1000 mg active constituent/kg soil, dry weight).

In earthworms exposed to 62.5 to 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight for 14 days, there was one mortality in the exposed worms at 125 mg 7-OH metabolite of pyroxsulam/kg soil (dw), i.e. 2.5% of the earthworms exposed to that concentration or a 0.5% mortality based on the total number of exposed earthworms. In the controls over the same period exhibited, there were 2 mortalities in a total of 40 worms, i.e. 5%. Sublethal effects also showed no dose related responses (99.5% of the exposed earthworms and 95% of the controls were identified as showing no sublethal effects, weight loss excluded).

Control and exposed earthworms all lost weight over the 14 days of the exposure period but with no clear dose related effect identified. While changes in mean earthworm weights after 14 days exposure to the 7-OH metabolite of pyroxsulam were statistically significantly different from the mean weight loss seen in the controls, the study authors' concluded that the absence of a dose response and the demonstration that no treatment group mean weight (in contrast to the change in weight) was significantly different for any concentration when compared to the control group (Dunnett's 2-tailed test, $p>0.05$) the weight change differences were not biologically significant.

In contrast, the reviewers of the study have concluded that an alternative interpretation is possible. Thus, worms in all of the treatment levels showed a larger change in weight compared to the controls and, from a percentage perspective, controls showed a 5.8% reduction in body weight and for treated worms, the change was from 11.7-17.7%. An effect of this magnitude might be considered biologically relevant. The fact that a dose-response was not observed is cited as a reason for disregarding these apparent affects although the concentrations of the chemical were not measured; if the chemical degraded quickly (as has been suggested by other data), a dose-response relationship may not be as apparent. In addition, the multiple statistical tests conducted on this endpoint with similar outcomes further suggests the effect may be real and the NOEC/NOAEC for weight loss should be set to <62.5 mg 7-OH metabolite of pyroxsulam/kg soil (dw).

The 14 day LC50 was set at >1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight in the study report. The 14 day NOECs for mortality and sublethal effects were both set at 1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight in the study report. Similarly, the study report did not establish 14 day LOECs for mortality and sublethal effects including weight change.

Based on the review's assessment of the data, the 14 day LC_{50} , EC50 and LOECs, could all be set at >1000 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight for mortality and sublethal effects other than weight change. The LOEC based on weight loss is set by study reviewers as ≤62.5 mg 7-OH metabolite of pyroxsulam/kg soil (dry weight).

The EPA reviewer concluded 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. Furthermore, no NOAEC was determined; based on a body weight changes, the NOAEC is <62.5 mg 7-OH metabolite of pyroxsulam/kg soil, dry weight.

The PMRA does not share the same study classification scheme as the US EPA and the DEW. This study is acceptable to the PMRA.

III. REFERENCES:

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