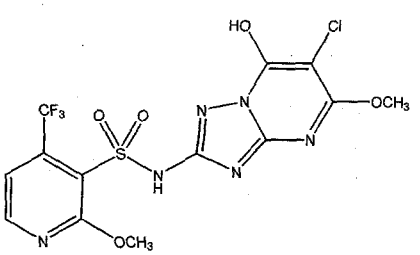
Data Evaluation Report on the effects of 6-Cl-7-OH metabolite of pyroxsulam (XDE-742) on earthworm reproduction in an artificial soil substrate

PMRA Submission Number 2006-4727; ID 1283275 EPA MRID Number 469085-11 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.9
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.9.2
	EPA Guideline:	Non-guideline study
Test material:	6-chloro-7-hydroxy-pyroxsulam metabolite or 6-chloro-7-hydroxy-XDE-742 metabolite	**Purity (%):** 99%
Common name:	6-Cl-7-OH Metabolite of XDE-742	
Chemical name:	3-pyridinesulfonamide, N-(6-chloro-7-hydroxy-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)	
IUPAC:	N-(6-chloro-7-hydroxy-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
CAS name:	N-(6-chloro-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:	X11301338	

Chemical Structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/2/08 **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) Emilie Rainville **Date:** May 8, 2007
Environmental Assessment Directorate, PMRA 05/03/08 for Ann Lee
Christopher Salice **Date:** 5 July 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. 6-Cl-7-OH Metabolite of XDE-742: A Reproduction Study with the Earthworm in an Artificial Soil Substrate. Wildlife International, Ltd, 8598 Commerce Drive, Easton, MD 21601. Wildlife International, Ltd. Project Number 379-162 and Dow AgroSciences Study Number 050134. The Dow Chemical Company, Midland MI 48674, USA for Dow AgroSciences, LLC, Indianapolis IN, 46268 USA. February 16, 2006. Unpublished report.

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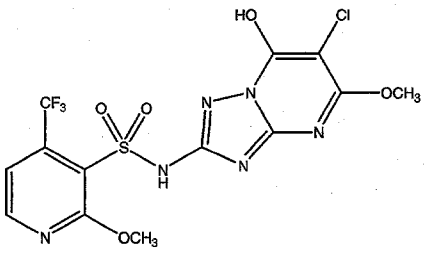
Chemical name:	3-pyridinesulfonamide, N-(6-chloro-7-hydroxy-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)	
IUPAC:	N-(6-chloro-7-hydroxy-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
CAS name:	N-(6-chloro-7-hydroxy-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
CAS No.:	Not available	
Synonyms:	XII301338	

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:** May 8, 2007
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Data Evaluation Report on the effects of 6-Cl-7-OH metabolite of pyroxsulam (XDE-742) on earthworm reproduction in an artificial soil substrate

PMRA Submission Number 2006-4727; ID 1283275 EPA MRID Number 469085-11 APVMA ATS 40362

EXECUTIVE SUMMARY:

In a 56 day reproduction study, earthworms (*Eisenia foetida*) were exposed to the 6-Cl-7-OH metabolite of pyroxsulam at 0, 8.18, 16.3, 32.5, 65.0 and 130 µg/kg dry weight of artificial soil substrate. The adult earthworms were fed cow manure weekly during the first 28 days of the test, removed from the test chambers on day 28, and observed for mortality and signs of toxicity. Test soil and cocoons were returned to test chambers for an additional 28 days in order to determine the numbers of juveniles produced in each replicate by the end of the test on day 56. Manure was also added to the soil once at the beginning of the cocoon exposure period to serve as a source of food for juvenile worms emerging from cocoons. The number of juveniles present in each replicate at test termination was used to determine the NOEC for reproduction. The EC50 value for reproduction, a possible test end-point, is defined as the concentration of test substance that caused a 50% reduction in the mean number of juveniles relative to the control group. In reproduction studies, the NOEC is also a primary end-point, identifying the highest concentration tested which had no observed effects on the species being test compared to the controls.

The experimental protocol was based upon procedures outlined in OECD 222 "Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)" and ISO/FDIS 11268-2. Soil Quality – Effect of Pollutants on Earthworms (Eisenia fetida) – Part 2: Determination of Effects on Reproduction.

There were several mortalities in the control group and most treatment groups during the 28-day adult exposure period, but there was not a dose-response pattern and the mortalities were considered to be incidental and not treatment related. All surviving earthworms in the control and the treatment groups were normal in appearance and behaviour, except for one control group worm that was thin and showed reduced reaction to mechanical stimulus on day 28. Since there was no treatment related mortality of adult earthworms in this study, an LC50 value was not calculated. For the same reasons, 28 day NOEC and LOEC values for mortality was not reported.

Observations of clinical signs (appearance and behaviour) in the adult earthworms were also conducted on day 28 while observations of burrowing behaviour were conducted at test initiation. When compared with the control group, there were no apparent effects upon appearance or behaviour of the surviving earthworms. Earthworms were reported as having showed no aversion to 6-Cl-7-OH metabolite of pyroxsulam treated soils at test initiation. Because of the absence of a dose effect on sub-lethal toxicity endpoints, 28 day EC50, LOEC and NOEC values were not reported.

Weight changes in the control and 6-Cl-7-OH metabolite of pyrasulfatole exposed earthworms were not identified as statistically significantly different at any exposure concentration (Dunnett's 2-tailed test, $p>0.05$) and no EC50, LOEC or NOEC values were reported for this parameter for adult earthworms.

On day 56, no abnormalities were observed among juveniles collected from the control and treatment groups. The mean number of juveniles present in the control group on day 56 was 69.5, while in the 6-Cl-7-OH metabolite of pyroxsulam treatment groups, the mean numbers of juveniles were 71.0, 76.8, 80.0, 72.0 and 49.5 in the 8.18, 16.3, 32.5, 65.0 and 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil treatment groups, respectively.

Because the mean number of juveniles produced was not significantly reduced (Dunnett's 2-tailed t-test, $p=0.05$) relative to the control mean at any test concentration, and no dose response relationship was identified, the EC50 value for reproduction was greater than 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil, the highest concentration tested. The NOEC was set by the study authors as 130 µg of the metabolite/kg dry soil.

The reviewers, in contrast, have set the 56 day NOEC as 65 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil, based on the numbers of juveniles produced. This was on the basis of the 49.5 value appearing a rather large difference compared to control: on the order of 30% reduction in juveniles. Although not statistically significant, such a reduction is likely biologically significant. The 56 day LOEC for reproduction based on the numbers of

juveniles is set by the reviewers as 130 . µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil,.

A reference toxicity test with the toxicant carbendazim was conducted non-concurrently with mortality and sublethal effects on the mature earthworms and the effects on the numbers of juvenile earthworms produced indicated the laboratory test conditions were adequate.

While the data presented in the study report did not enable the nominal concentrations to be calculated and because the stability of the 6-Cl-7-OH metabolite of pyroxsulam in the artificial soil is unknown (laboratory soil tests show that degradation of the 6-Cl-7-OH metabolite of pyroxsulam can occur), OECD 222 does not require that chemical analysis be conducted. The guideline does, however, state that it makes no allowance for possible degradation of the test substance over the period of the test and, consequently, it cannot be assumed that exposure concentrations will be maintained at initial values throughout the test. In such a situation, chemical analysis of the test substance at the start and the end of the test is recommended. Additionally, the OECD guideline states that information on fate and behaviour in the environment should be available where possible. Because of the absence of such information to verify the stability of the 6-Cl-7-OH metabolite of pyroxsulam in the test medium and because the initial and final concentrations of the 6-Cl-7-OH metabolite of pyroxsulam in the soil could not be confirmed, this study is classified as supplemental by the US EPA and DEW.

PMRA Executive Summary:

The effect of 6-Cl-7-OH-XDE-742, a transformation product of pyroxsulam, to the reproduction of the earthworm (*Eisenia foetida*) was studied in an artificial soil substrate for 56 days. The adult earthworms were exposed to the subject chemical for the first 28 days of the test and were removed from the test chambers on day 28 to be examined for signs of toxicity and mortality. Test soil and cocoons were returned to test chambers for an additional 28 days to determine the numbers of juveniles produced in each replicate by the end of the test on day 56. Earthworms were exposed to nominal concentrations of 0, 8.18, 16.3, 32.5, 65.0, and 130 µg 6-Cl-7-OH-XDE-742 /kg dry soil. Test concentrations in soil were not verified. The experiment was conducted in accordance with OECD 222 "Earthworm, Reproduction Test" and ISO/FDIS 11268-2.

After 28 days of exposure to 6-Cl-7-OH-XDE-742, the percent mortalities of adult earthworms in the control, 8.18, 16.3, 32.5, 65.0, and 130 µg 6-Cl-7-OH-XDE-742 /kg treatment groups were 2.5, 5.0, 10, 0, 2.5, and 5.0%, respectively. The mortalities in the treatment groups were not statistically different from those in the control group. All adult earthworms in the control group and treatment groups were normal in appearance and behavior throughout the exposure period except for one worm in the control group appeared to be thin and showed reduced reaction to mechanical stimulus on Day 28. Earthworm in both the control and treatment groups exhibited no aversion to the soil at test initiation. After 28-day exposure period, the mean body weights change of the survived adult worms in the control and 8.18, 16.3, 32.5, 65.0, and 130 µg 6-Cl-7-OH-XDE-742 /kg dry soil treatment groups were 0.13, 0.07, 0.12, 0.12, 0.17, 0.16 g, respectively. Body weight change among earthworms in the treatment groups was not statistically significant ($p>0.05$) at any concentration tested when compared to the control group. The mean number of juveniles in the control and 8.18, 16.3, 32.5, 65.0, and 130 µg 6-Cl-7-OH-XDE-742 /kg dry soil treatment groups were 69.5, 71.0, 76.8, 80.0, 72.0, and 49.5, respectively. The mean number of juveniles produced was not significantly different when compared to the control mean at any test concentration ($p>0.05$).

The 28-day NOEC based on adult worm's mortality and sublethal effects was 130 µg 6-Cl-7-OH-XDE-742 /kg dry soil. The 28-day LC_{50} and EC_{50} based adult mortality and sublethal effect on adult worms was > 130 µg 6-Cl-7-OH-XDE-742 /kg dry soil. The NOEC and EC_{50} based on the numbers of juveniles produced were <130 µg 6-Cl-7-OH-XDE-742 /kg dry soil and > 130 µg 6-Cl-7-OH-XDE-742 /kg dry soil, respectively.

Reproduction studies on earthworms are not currently part of the data requirements of the PMRA. However, this

study is considered acceptable and could provide useful information for risk assessment purposes.

Results Synopsis

Test Organism:	Earthworm, *Eisenia foetida*
Size:	At study initiation, mean weights of 0.69 mg/worm for controls and 0.67 to 0.74 mg/worm for the earthworms exposed to 6-Cl-7-OH metabolite of pyroxsulam were reported.
Age:	Adults with clitella.
Test Type:	Survival and Reproduction
Mature earthworm endpoints	
28 day LC_{50} and EC_{50}:	>130 µg 6-Cl-7-OH-XDE-742 /kg dry soil 95% C.I.: Not determined
28 day NOEC:	130 µg 6-Cl-7-OH-XDE-742 /kg dry soil. Not determined because of the absence of a dose response for mortality and sublethal effects)
28 day LOEC:	>130 µg 6-Cl-7-OH-XDE-742 /kg dry soil
Probit Slope:	Not determined
Endpoint(s) Effected:	There were no compound related effects on mortality and sublethal effects (including weight change).
Juvenile earthworm endpoints	
56 day EC50:	>130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight (numbers of juvenile earthworms produced).
56 day NOEC:	65 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight (numbers of juvenile earthworms produced).
56 day LOEC	130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight (numbers of juvenile earthworms produced).

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
The study was conducted according to the procedures outlined in the protocol, "XDE-742 (6-Cl-7-OH Metabolite): A Reproduction Study with the Earthworm in an Artificial Soil Substrate." The protocol was the Organization for Economic Cooperation and Development (OECD) Guideline No. 222: "*Earthworm Reproduction Test (Eisenia fetida/andrei)*" and ISO/FDIS 11268-2.

Because the study was based on OECD 222 (earthworm reproduction), it has not been evaluated against US EPA OPPTS 850.6200 Earthworm Subchronic Toxicity Test which considers the 28 day toxicity of the test substance to earthworms with observations occurring every seven days. The US EPA guideline has mortality as its primary endpoint.

There were a number of deviations from the Guideline and other deficiencies identified (see page 39 of this DER for further details).

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, stability and homogeneity of the test substance in the soil were not determined in accordance with Good Laboratory Practice Standards. Periodic analyses of soil and well water for potential contaminants were performed using a certified laboratory and standard US EPA analytical methods. Feed was not analysed for potential contaminants.

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

6-Chloro-7-hydroxy-XDE-742 metabolite (referred to as the 6-Cl-7-OH metabolite of pyroxsulam in this DER). This metabolite was identified as formed from the 7-hydroxy metabolite of pyroxsulam in the aerobic degradation of pyroxsulam (Yoder *et al.*, 2006).

Description:	Solid
Lot No./Batch No. :	EI950-42
TSN No.:	TSN105423
Purity:	99% of active ingredient.

Stability of Compound Under Test Conditions:

Not determined (as stated in the GLP compliance statement). See also "Stability and homogeneity of test material in the medium?" in Table 14, Summary of deficiencies/deviations from guidelines on page 39 of this DER.

With respect to the stability of the 6-Cl-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 6-Cl-7-OH metabolite of pyroxsulam formed from 7-OH metabolite of pyroxsulam degradation was 7.4 days (DT90 24.4 days) in one soil, 48.9 days (DT90 162.3 days) in another soil and 9.2 days (DT90 30.7 days) in a third soil with conversion to "other" degradates which include carbon dioxide, bound residues and minor metabolites. DT50 and DT90 for the fourth soil were not reported. Such results indicate that the 6-Cl-7-OH metabolite of pyroxsulam could be expected to have undergone extensive degradation in the 56 days of the earthworm exposure study.

Storage conditions of test chemicals:

Stored under ambient conditions.

Physicochemical properties of 6-Cl-7-OH metabolite of pyroxsulam

Parameter	Values	Comments
Water solubility at 20°C	Not available	Stated in the Study Profile Template (Sindermann *et al.*, 2006) as not available at the time of publication of the Study Profile Template. OECD 222 states that water solubility, Kow etc. should be available to help the design of appropriate test procedures and that the test report must include such information
Vapour pressure	Not available	
UV absorption	Not available	
pKa	Not available	
Kow	Not available	

(See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER).

2. Test organism:

Species:

Earthworms (*Eisenia foetida*)

Age at test initiation:

Adults (with clitella).

Earthworms were from synchronous cultures (individuals not differing in age by more than four weeks) maintained in moist peat moss and fed saturated alfalfa.

OECD 222 states that the earthworms should be between two months and one year old with clitella at the start of the test (See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER).

Weight at study initiation:

Average weights at day 0 of earthworms at test initiation, as average earthworm body weights in grams (10 earthworms/replicate), 4 replicates/control and test concentration.

	Controls	µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil dry weight				
		8.18	**16.3**	**32.5**	**65.0**	**130**
Mean replicate weight:	0.69	0.71	0.67	0.74	0.71	0.71
Range of mean replicate weights:	0.60-0.76	0.66-0.76	0.60-0.73	0.71-0.77	0.68-0.78	0.65-0.76

Note: OECD 222 refers to the wet mass of individual earthworms being between 250 and 600 mg.

Earthworm mean weights are seen to have exceeded the OECD 222 range of 250 to 600 mg on most occasions. This was noted as a Deviation from the protocol (See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER). Additionally, OECD 222 requires that the earthworms be individually weighed at test commencement.

Source: Earthworms for the test were from a Wildlife International Ltd. culture 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.

b. Definitive Study

The in-life portion of the test was conducted from 17 November 2005 to 13 January 2006. Adult earthworms were exposed to a geometric series of five test concentrations of 6-Cl-7-OH metabolite of pyroxsulam in artificial soil. A negative control group was exposed to soil prepared without the addition of the test substance and was maintained concurrently.

The objective of the study was to determine the effects of the 6-Cl-7-OH metabolite of pyroxsulam on the earthworm, *Eisenia fetida*, during an 8-week exposure period in an artificial soil substrate. Adults were exposed for 28 days and then removed to evaluate mortality and sublethal effects including growth (weight change). The cocoons and any juveniles present and the soil were returned to test chambers for another 28 days to evaluate effects upon reproductive output (number of juveniles at test termination).

The adult earthworms were fed cow manure weekly during the first 28 days of the test. Manure was also added to the soil once at the beginning of the cocoon exposure period to serve as a source of food for juvenile worms emerging from cocoons.

1. Soil

The artificial soil was prepared in bulk by blending approximately 70% sand, 20% kaolin clay and 10% sphagnum peat. The pH of the bulk soil prior to hydration was adjusted to 5.6 using calcium carbonate. 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 dissolving the test substance into a portion of the water needed to hydrate the dry artificial soil. Sufficient deionised water was added to the dry artificial soil to achieve a moisture content of approximately 35% by weight.

Test soil components were mixed for a total of 20 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. Seven hundred fifty grams of prepared soil were added to each test chamber. Test soil components were mixed for a total of 20 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. The test concentrations were adjusted for the purity of the test substance, therefore, test concentrations are reported as micrograms of the 6-Cl-7-OH metabolite of pyroxsulam per kilogram of soil on a dry weight basis.

Soil moisture content was determined by measuring the initial weight of the soil sample, and then weighing the soil sample after drying for 5 or more days at approximately 105°C.

The study report referred to the amounts of stock solution of 6-Cl-7-OH metabolite of pyroxsulam added to the weights of soil. No reference was found to the actual concentration of the stock solution and verification of the calculated nominal concentrations of the 6-Cl-7-OH metabolite of pyroxsulam was not possible (See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER under "Rates of application Measured"). OECD 222 does not require that chemical analysis be conducted. The guideline does, however, state that it makes no allowance for possible degradation of the test substance over the period of the test and, consequently, it cannot be assumed that exposure concentrations will be maintained at initial values throughout the test. In such a situation, chemical analysis of the test substance at the start and the end of the test is recommended. Additionally, the OECD guideline states that information on fate and behaviour in the environment should be available where possible. The absence of such information to verify the stability of the 6-Cl-7-OH metabolite of pyroxsulam in the test medium and because the initial concentrations of the 6-Cl-7-OH metabolite of pyroxsulam in the soil could not be confirmed are considered significant deficiencies. Determination of concentrations of the 6-Cl-7-OH metabolite of pyroxsulam in the soil over the exposure period should have been determined in the light of the findings of Yoder *et al.* (2006). These authors reported soil DT50s of this metabolite of 7.4, 48.9 and 9.2 days which show that degradation would have been expected to have occurred over the 56 days of the exposure period.

The nominal soil concentrations of the 6-Cl-7-OH metabolite of pyroxsulam were reported as: 0 (Negative control), 8.18, 16.3, 32.5, 65.0 and 130 µg/kg of soil, dry weight. Selection of the nominal test concentrations was reported as based upon known toxicity data and the physical/chemical properties of the test substance (which although not reported in the study report, were presumably known at the time).

The study report's Deviation from Study Protocol notes that the 8.13 value was actually 8.18 (See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER). The 8.18 value is referred to in this DER.

Analyses of the treated soil to confirm that dosing had been correct and that the mixing procedure had evenly distributed the 6-Cl-7-OH metabolite of pyroxsulam throughout the treated soils were not conducted. (See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER).

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): 69.3% quartz sand 19.8% kaolin clay 9.9% sphagnum peat 0.99% calcium carbonate	Requirement considered met. *OECD 222 refers to 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 considered to satisfy the 0.3 to 1.0% range specified by OECD 222.
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 was 7.0 in the control soil and 7.0 or 7.1 in test soils at test initiation and 7.2 in the controls and either 7.3 or 7.4 in the test soils at test termination.	See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER. OECD 222 refers to the pH of the artificial soil being initially 6.0 ± 0.5.
Organic carbon (%)	Not reported.	OECD 222 states that it has been demonstrated that *Eisenia foetida* can comply with the validity criteria on reproduction when tested in field soils with lower organic carbon content (e.g. 2.7%) and there is experience that this can also be achieved in artificial soil with 5% peat. As a result, the guideline indicates it is not necessary before using such a soil

		in a definitive test to demonstrate the suitability of the artificial soil for allowing the test to comply with the validity criteria unless the peat content is lowered more than specified above i.e. an artificial soil with 5% peat. As 10% sphagnum peat was used, it is accepted that the organic carbon content was satisfactory.
Moisture (%)	Soil moisture content was reported as 36.0% in the controls and 35.6 to 35.8% in each test group at test initiation. At test termination, the control moisture was 32.2% while that of the test groups ranged from 32.0 to 35.5%.	See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER. OECD 222 refers to the soil having a final moisture content of 40 to 60% of maximum water holding capacity corresponding to 40-60% moisture dry mass. This guideline also states that the soil's maximum water holding capacity must be included in the test report.

Table 2. Experimental Design

Parameter	Value	Remarks Criteria
Acclimation: Duration:	Ten days pre-test acclimatisation in bedding followed by placing the earthworms into five one-litre glass beakers containing prepared artificial soil substrate adjusted to a moisture content of approximately 35% by weight for 24 hours.	Requirement considered met. OECD 222 states that earthworms be acclimatised for at least one day.
Conditions (state if same as the test conditions):	Only the bedding conditions were not the same as those of the test. The 24 hours pre-test conditions were reported as in an environmental room at 18-22°C with a 16 hours light/8 hours dark photoperiod in the artificial soil substrate adjusted to a moisture content of approximately 35% by weight, for the acclimation period. The earthworms were fed in both the acclimatisation and test periods.	Requirement considered met. Conditions of the 24 hours pre-test acclimatisation period considered equivalent to the test conditions.
Health:	All surviving earthworms in the control group and treatment groups were normal in appearance and behaviour except for one control worm which was thin and showed reduced reaction to mechanical stimulus. Sindermann *et al.* (2006), in the Study Profile Template referred to the earthworms being apparently healthy at the completion of acclimation.	Requirement considered met. OEDC 222 states that worms can be considered to be healthy if they move through the substrate, do not try to leave the substrate and reproduce continuously. The guideline also states that healthy worms normally burrow immediately into substrate.
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	Requirement considered met.

Parameter	Value	Remarks Criteria
	wrap that was perforated for air exchange.	
Size: Amount of soil or substrate:	1 Litre Seven hundred fifty grams of prepared, hydrated soil/test chamber.	
<u>No. of replicates</u> Per treatment group: Per control:	 Four test chambers for each of the treatment groups. Eight test chambers for the control group.	Requirement met. OECD 222 recommends four replicates for each test concentration and eight control replicates if the NOEC is to be determined.
No. of earthworms per treatment	Forty earthworms per treatment level and, in the control, 80 earthworms (Each test and control chamber contained 10 earthworms).	Requirement met. OECD 222 states that groups of 10 earthworms should be used per replicate.
Co-solvents used or not (if yes report the name and concentration)	No solvent used.	Requirement met.
<u>Rates of application</u> Nominal:	 Nominal concentrations of 0 (control), 8.18, 16.3, 32.5, 65.0 and	See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER with respect to analytical confirmation of the nominal concentrations and verification of the measured concentrations.

Parameter	Value	Remarks Criteria
Measured:	130 µg 6-Cl-7-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 micrograms of test substance per kilogram of soil on a dry weight basis (µg 6-Cl-7-OH metabolite of pyroxsulam/kg). Note that the 8.13 nominal weight value was actually 8.18. Verification of test concentrations of the 7-OH metabolite of pyroxsulam in the soil did not occur (GLP compliance statement).	OECD 222 requires exposure to at least five test concentrations, in geometric series, in which the concentrations should be spaced by a factor not exceeding 2.0. The appropriate amount of 6-Cl-7-OH metabolite of pyroxsulam (the amount was not found in the study report) dissolved and brought to volume in a 1 L flask. An appropriate amount of the stock solution was then brought to 1000 mL with deionised water and blended with a known weight of the artificial soil. The remaining amount of deionised water needed to hydrate the soil, was then added to the soil/6-Cl-7-OH metabolite of pyroxsulam mixture and mixed for approximately five minutes. Negative control soil was prepared in a similar manner, but without the addition of test substance.
Stability and homogeneity of test material in the medium?	Verification of the stability and homogeneity of the 6-Cl-7-OH metabolite of pyroxsulam in the soil did not occur.	See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER.
Test conditions: Temperature:	During the test, the worms were maintained in an environmental room set to maintain a temperature of approximately 20 ± 2°C. Air temperature was measured daily in the environmental room.	Requirements considered met.

Parameter	Value	Remarks Criteria
	Soil temperature was measured in one replicate of each treatment and control group at test initiation and termination using a hand-held digital thermometer. Air temperature in the environmental room was within the desired range of approximately 20 ± 2°C on all but two days when mean daily temperature was 23°C. Mean air temperature was 20.2 ± 1.17 °C (mean ± standard deviation) during the test. Soil temperature ranged from 21 to 22°C in each of the groups at test initiation and was 22°C at test termination, and was therefore within the desired range of 20 ± 2°C.	OECD 222 states the test temperature is 20 ± 2°C. The mean daily temperature of 23°C occurred on days 12 and 13 of the first 28 days of the study and, based on the satisfactory performance of the controls, is not considered to have adversely affected the study.
Lighting conditions:	The photoperiod during the test was 16 hours of light and 8 hours of dark per day provided by overhead fluorescent bulbs. The target light intensity during the test was approximately 400 to 800 lux, and was verified after the in-life phase of the test was completed. Average light intensity during the 16-hour light period was 509 ± 36.8 lux.	Requirement met. OECD 222 states the test is carried out under controlled light-dark cycles (preferably 16 hours light and 8 hours dark) with illumination of 400 to 800 lux in the area of the test containers.

Parameter	Value	Remarks Criteria
Moisture:	Soil moisture ranged from 35.6 to 36% at day 0. At test termination, the moisture content ranged from 32.0 to 35.5%.	See Table 14, Summary of deficiencies/deviations from guidelines, page 39 of this DER. OECD 222 refers to the final soil moisture content being 40 to 60% of maximum water holding capacity (corresponding to 50 ± 10% moisture dry mass, i.e. 40 to 60%) and states that the test report must record the maximum water holding capacity of the soil. OECD 222 also states that the water content should not vary by more than 10 % from that at the start of the test.
Duration of the study	Adult earthworms were exposed for 28 day and then removed. Cocoons were kept in the soil in the test chambers for a further 28 days.	Requirement met. OECD 222 refers to mortality and growth effects on the adult worms being determined after 4 weeks of exposure. The adults are then removed from the soil and effects on reproduction assessed after a further 4 weeks by counting the number of offspring present in the soil.
Reference chemical, if used Name:	Carbendazim	Requirement considered met.

Parameter	Value	Remarks Criteria
Concentration:	The study profile template (Sindermann *et al.,* 2006) refers to nominal rates of 0.3, 0.5, 1, 2, 4 and 6 mg carbendazim/kg dry soil; the study was not conducted concurrently with the 6-Cl-7-OH metabolite of pyroxsulam test. This information was not identified in the study report.	OECD 222 states that the NOEC and/or EC10 or EC50 etc. of a reference substance must be determined and that significant effects must be seen between 1 and 5 mg active substance/kg dry mass. Wildlife International, Ltd. conducted a reference toxicity test with carbendazim in 2005. The LC50 value for the mortality of the adult earthworms exposed to carbendazim for 28 days was 5 mg/kg dry soil, with a 95% confidence interval of 4 and 8 mg/kg dry soil. There were effects upon adult earthworm weight at concentrations of 2 mg/kg and higher, and effects upon the numbers of juveniles produced at 2 mg/kg and above. The EC50 value for reproduction was calculated to be 1.85 mg carbendazim/kg dry soil, with a 95% confidence interval of 1.792 and 1.913 mg/kg dry soil. The NOEC was 1 mg carbendazim/kg dry soil, and the LOEC was 2 mg carbendazim/kg dry soil, based on the numbers of juveniles at test termination.

2. Observations:

Table 3. Observations

Parameters	Details	Remarks Criteria
Observation intervals	Observations of mortality and clinical signs were conducted on day 28. Observations of burrowing behaviour were conducted once at test initiation. Body weights of the earthworms were measured on day 28. On day 56, juveniles were removed from the test soil, counted and examined for physical abnormalities. Soil temperature was measured in one replicate of each treatment and control group at test initiation and termination (Day 56) using a hand-held thermometer. Moisture content and pH measurements were made on soil samples collected from each batch of soil prepared for treatment and control groups at the time of test soil mixing. At test termination, samples for moisture content and pH measurements were collected from one replicate of each control and treatment group. Air temperature was recorded daily in the environmental room.	Requirement considered met. On day 56, juveniles were removed from the test soil, counted and examined for physical deficiencies. The Deviations from Study Protocol note that the juveniles from the nominal 65 and 130 µg/kg soil, dry weight were extracted from the soil on day 56 but held overnight and counted on day 57. This deviation is not considered to be of significance with respect to the test conduct or outcomes.
Parameters measured including	Burrowing behaviour, mortality, gross	Requirement considered met.

the sublethal effects/toxicity symptoms	physical and behavioural abnormalities of parental earthworms, body weights of parental worms, numbers of juvenile earthworms and examination of juvenile earthworms for physical abnormalities.	OECD 222 refers to test results for adult mortality, the total mass of adults at the beginning of the test in each test container; changes in body weight of live adults (% of initial weight) in each test container after the first four weeks of the test; the number of juveniles produced in each test container at the end of the test; a description of obvious or pathological symptoms or distinct changes in behaviour; and the results obtained with the reference test substance.
Were raw data included?	Raw data in the form of laboratory notes were not provided. Tabulated soil moisture, pH and temperature data were presented as were mortality, effects and weight change data. Data generated in the study are filed in archives at the Wildlife International, Ltd. site.	Provision of raw data is not specifically required by OECD 222.
Other observations, if any	In addition to the manure added to the soils on a weekly basis during the first 28 days of the study and at the start of the second 28 days, the control and test soils also had manure mixed with them in their preparation. Test chambers were weighed periodically to monitor soil moisture loss. Lost soil moisture was replaced by adding water to the soil surface until weights approximated those at the start of the test.	Requirement considered met. OECD 222 states that after the addition of the test worms, the weight of each test container should be measured to ensure that there is an initial weight that can be used as the basis for monitoring soil moisture content throughout the test. The guideline then goes on to state that the water content of the soil substrate in the test containers is maintained throughout the test by re-weighing the test containers (minus their covers) periodically. Losses are replenished as necessary with deionised water. The water content should not vary by more than 10 % from that at the start of the test.

II. RESULTS AND DISCUSSIONS

A. MORTALITY:

Eighty earthworms (10 per replicate) were exposed to a control and forty earthworms to each pyroxsulam containing test soil and observations of mortality conducted on day 28. There were several mortalities in the control group and most treatment groups during the 28-day adult exposure period, but there was no apparent dose-response pattern and the mortalities were considered to be incidental and not treatment related. All surviving earthworms in the control and the treatment groups were normal in appearance and behaviour except for one control group worm that was thin and showed reduced reaction to mechanical stimulus on day 28. Since there was no treatment related mortality of adult earthworms in this study, an LC50 value was not calculated.

The mortality results are summarised in Table 4, page 20 of this DER.

28-Day LC50:	Not calculated as there were no treatment related mortalities of the adult earthworms
28 day Lowest Observed Effect Concentration:	Not reported (there were no treatment related mortalities of the adult earthworms).
28 day No Observed Effect Concentration (mortality):	Not reported (there were no treatment related mortalities of the adult earthworms).

Table 4. Effect of 6-Cl-7-OH metabolite of pyroxsulam on mortality of the earthworm (*Eisenia foetida*) after a 28 days reproduction study.

Treatment - µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight (nominal)	Replicate (10 earthworms/replicate)	Mortalities after 28 days	
		No. dead	% mortality
Negative control (0)	A	0/10	0
	B	1/10	10
	C	0/10	0
	D	0/10	0
	E	1/10	10
	F	0/10	0
	G	0/10	0
	H	0/10	0
Total number dead and total % mortality in the controls:		**2/80**	**2.5**
8.18	A	0/10	0
	B	1/10	10
	C	0/10	0
	D	1/10	10
	Total:	**2/40**	**5.0**
16.3	A	2/10	20
	B	0/10	0
	C	2/10	20
	D	0/10	10
	Total:	**4/40**	**10**
32.5	A	0/10	0
	B	0/10	0
	C	0/10	0
	D	0/10	0
	Total:	**0/40**	**0**
65.0	A	0/10	0
	B	1/10	10
	C	0/10	0
	D	0/10	0
	Total:	**1/40**	**2.5**
130	A	0/10	0
	B	2/10	20
	C	0/10	0
	D	0/10	0
	Total:	**2/40**	**5.0**
Total number dead and total % mortality in all exposed earthworms		**9/200**	**4.5**

28 day NOEC (mortality)		Not calculated in the study report (lack of dose response observed)
28 day LC50 and LOEC (mortality)		Not calculated in the study report (lack of dose response observed)
Reference chemical carbendazim	**28 day LC_{50}:**	5 mg carbendazim/kg dry soil, with a 95% confidence interval of 4 and 8 mg/kg dry soil. Percentage mortality not reported.
	28 day EC50 (reproduction)	1.85 mg carbendazim/kg dry soil, with a 95% confidence interval of 1.792 and 1.913 mg/kg dry soil.
	28 day NOEC and LOEC (reproduction)	The NOEC was 1 mg carbendazim/kg dry soil, and the LOEC was 2 mg carbendazim/kg dry soil, based on the numbers of juveniles at test termination.

B. SUB-LETHAL TOXICITY ENDPOINTS:

Observations of clinical signs (appearance and behaviour) in the adult earthworms were conducted on day 28. Observations of burrowing behaviour were conducted at test initiation. When compared with the control group, there were no apparent effects upon appearance or behaviour of the surviving earthworms. Earthworms were reported as having showed no aversion to 6-Cl-7-OH metabolite of pyroxsulam treated soils at test initiation (burrowing time data were not provided).

Because of the absence of a dose effect on sub-lethal toxicity endpoints, 28 day EC50, LOEC and NOEC values were not reported.

The sublethal effects results are summarised in Table 5, page 22 and Table 6, page 24 (changes in weight) of this DER.

Table 5. Sublethal effects (not weight loss) of 6-Cl-7-OH metabolite of pyroxsulam on the earthworm (*Eisenia foetida*) following a 28 day exposure period.

Treatment - µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight (nominal)	Replicate (10 earthworms/replicate)	Observation period	
		Observed effects at day 28	Comment
Negative control (0)	A	10 appeared normal*	No sublethal effects reported
	B	9 appeared normal, 1 missing	Missing worm presumed dead.
	C	10 appeared normal*	No sublethal effects reported
	D	9 appeared normal, 1 affected.	The one worm was thin and had a reduced reaction to mechanical stimulus
	E	9 appeared normal, 1 missing	Missing worm presumed dead.
	F	10 appeared normal*	No sublethal effects reported
	G	10 appeared normal*	No sublethal effects reported
	H	10 appeared normal*	No sublethal effects reported
Total number abnormal (including those earthworms missing and assumed dead) and total % abnormality in the controls:		**3/80 (3.8%)**	
8.18	A	10 appeared normal	No sublethal effects reported
	B	9 appeared normal, 1 missing	Missing worm presumed dead.
	C	10 appeared normal	No sublethal effects reported
	D	9 appeared normal, 1 missing	Missing worm presumed dead.
Total (including those earthworms missing and assumed dead) and total % abnormality:		**2/40, 5%**	
16.3	A	8 appeared normal, 2 missing	Missing worms presumed dead
	B	10 appeared normal	No sublethal effects reported
	C	8 appeared normal, 2 missing	Missing worms presumed dead
	D	10 appeared normal	No sublethal effects reported
Total (including those earthworms missing and assumed dead) and total % abnormality:		**4/40, 10%**	
32.5	A	10 appeared normal	No sublethal effects reported
	B	10 appeared normal	No sublethal effects reported
	C	10 appeared normal	No sublethal effects reported
	D	10 appeared normal	No sublethal effects reported
Total (including those earthworms missing and		**0/40, 0%**	

assumed dead) and total % abnormality:		**0/40, 0%**	
65.0	**A**	10 appeared normal	No sublethal effects reported
	B	9 appeared normal, 1 missing	Missing worm presumed dead.
	C	10 appeared normal	No sublethal effects reported
	D	10 appeared normal	No sublethal effects reported
Total (including those earthworms missing and assumed dead) and total % abnormality:		**1/40, 2.5%**	
130	**A**	10 appeared normal	No sublethal effects reported
	B	8 appeared normal, 2 missing	Missing worms presumed dead
	C	10 appeared normal	No sublethal effects reported
	D	10 appeared normal	No sublethal effects reported
Total (including those earthworms missing and assumed dead) and total % abnormality: **Total number and total % of effected earthworms in exposed earthworms, including those missing, presumed dead:** **28 day, EC50, LOEC and NOEC (sublethal effects apart from weight change)**		**2/40, 5%** **9/200, 4.5%** **If the worms presumed dead are excluded, the number of effected worms is 1, and the percentage 0.5%** Not reported because of lack any dose effect.	
Reference chemical carbendazim	**% mortality and 28 day EC50:**	The EC50 value for reproduction was calculated to be 1.85 mg carbendazim/kg dry soil, with a 95% confidence interval of 1.792 and 1.913 mg carbendazim/kg dry soil. The NOEC was 1 mg carbendazim/kg dry soil, and the LOEC was 2 mg carbendazim/kg dry soil, based on the numbers of juveniles at test termination.	

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

C. CHANGES IN BODY WEIGHT OF THE PARENT EARTHWORMS OVER 28 DAYS:

The body weights of the adult worms available for use in the test ranged from 0.471 to 0.751 g with a mean of 0.606 ± 0.0798 (SD) grams based on 20 individually weighed worms that had been acclimatised for the test.

Group weights for all earthworms in each replicate were collected prior to being placed in the test chambers on day 0. On day 28, all surviving earthworms were removed from each replicate test chamber, rinsed with deionised water, blotted on dry paper and group body weights were measured for the earthworms in each test chamber.

Average adult body weights at initiation and termination of the adult exposure period, and the change in body weight from initiation to termination, were calculated from the day 0 and day 28 measurements.

When compared with the control group, there were no apparent effects upon earthworm weight in the treatment groups during the 28-day adult exposure period (weight results given in Table 6, page 24 of this DER).

All replicates (control and test) except one of the 130 µg/kg soil, dry weight replicates, showed small increases in weight had occurred over the 28 days as a result of the earthworms' being able to feed on the added manure. In the

replicate that showed a loss of weight, the day 0 weight was 0.75 g/earthworm and the day 28 result, 0.71 g/earthworm.

Mean body weights, and change in body weights, were statistically compared with Dunnett's 2-tailed 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.

Initial and final body weight means were not statistically significant (p>0.05) for any group when compared to the control group using Dunnett's 2-Tailed Test. Similarly, change in body weight values were not statistically significant (p> 0.05) for any group when compared to the control group using Dunnett's 2-Tailed Test.

The final body weights and change values were analysed for normality and homogeneity of variances (results of these tests were not identified in the study report).

Because of the absence of a dose effect on weight changes in the earthworms, 28 day EC50, LOEC and NOEC values were not reported for this parameter.

Table 6. Sub-lethal effect on the weights of earthworms, *Eisenia foetida* exposed to 6-Cl-7-OH metabolite of pyroxsulam in an artificial soil matrix over 28 day. There were 10 earthworms/replicate.

Treatment (µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight, nominal)		Average Earthworm Body Concentration Weights (g)		
		Day 0[1]	Day 28[1]	Total change[1]
Control (0)	Replicate	weight	weight	difference in initial and final weights
	A	0.70	0.81	+0.11
	B	0.60	0.83	+0.23
	C	0.76	0.79	+0.03
	D	0.72	0.89	+0.17
	E	0.66	0.76	+0.10
	F	0.72	0.75	+0.03
	G	0.65	0.84	+0.19
	H	0.71	0.92	+0.21
Mean and standard deviation		0.69 ± 0.050	0.82 ± 0.060	+0.13 ± 0.079
8.18	A	0.76	0.83	+0.07
	B	0.68	0.76	+0.08
	C	0.66	0.76	+0.1
	D	0.73	0.78	+0.05
Mean and standard deviation		0.71 ± 0.046	0.78 ± 0.034	+0.07 ± 0.021
16.3	A	0.73	0.85	+0.12
	B	0.6	0.74	+0.14
	C	0.66	0.73	+0.07
	D	0.7	0.84	+0.14
Mean and standard deviation		0.67 ± 0.056	0.79 ± 0.065	+0.12 ± 0.035
32.5	A	0.77	0.84	+0.07

	B	0.72	0.80	+0.08
	C	0.75	0.91	+0.16
	D	0.71	0.86	+0.15
Mean and standard deviation		0.74 ± 0.028	0.85 ± 0.046	+0.12 ± 0.047
65.0	A	0.68	0.83	+0.15
	B	0.78	0.97	+0.19
	C	0.69	0.86	+0.17
	D	0.69	0.85	+0.16
Mean and standard deviation		0.71 ± 0.047	0.88 ± 0.061	+0.17 ± 0.016
130	A	0.68	0.92	+0.24
	B	0.76	0.99	+0.23
	C	0.65	0.87	+0.22
	D	0.75	0.71	-0.04
Mean and standard deviation		0.71 ± 0.054	0.87 ± 0.118	+0.16 ± 0.135
28 day NOEC (weight loss)		Not reported (lack of a dose response)		
28 day LOEC (weight loss)		Not reported (lack of a dose response)		
28 day EC_{50} (weight loss):		Not reported (lack of a dose response)		
Reference chemical Carbendazim	**% mortality and LC50 and 95% confidence limits**	There were effects upon adult earthworm weight at concentrations of 2 mg carbendazim/kg and higher.		

1. Initial and final body weight means were not statistically significant (p>0.05) for any group when compared to the control group using Dunnett's 2-Tailed Test

2. Change in body weight values were not statistically significant (p> 0.05) for any group when compared to the control group using Dunnett's 2-Tailed Test.

D. REPRODUCTIVE OUTPUT ENDPOINT:

The EC50 value for reproduction, the main end-point of the test, is defined as the concentration of test substance that caused a 50% reduction in the mean number of juveniles relative to the control group.

Because the mean number of juveniles produced was not significantly reduced (Dunnett's 2-tailed t-test, p=0.05) relative to the control mean at any test concentration, and no dose response relationship was identified, the study's reported EC50 value for reproduction was greater than 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil, the highest concentration tested. The study's reported NOEC was 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil, based on the numbers of juveniles produced.

On day 56, no abnormalities were observed among juveniles collected from the control and treatment groups. The mean number of juveniles present in the control group on day 56 was 69.5, while in the 6-Cl-7-OH metabolite of pyroxsulam treatment groups, the mean numbers of juveniles were 71.0, 76.8, 80.0, 72.0 and 49.5 in the 8.18, 16.3, 32.5, 65.0 and 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil treatment groups, respectively. The mean number of juveniles produced was not significantly reduced (Dunnett's 2-tailed t-test, p=0.05) relative to the control mean at any test concentration.

The study's reported end-points for reproduction were:

56 day EC50 (reproduction): > 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil.

56 day No Observed Effect Concentration: 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil. (reproduction) where "reproduction" refers to the numbers of juvenile earthworms produced.

The numbers of juveniles produced in the controls and replicates is given in Table 7 on page 26 of this DER.

Table 7. Number of Juveniles Produced by Earthworms Exposed to 6-Cl-7-OH metabolite of XDE-742 in an Artificial Soil Substrate.

Nominal Concentration (•g 6-Cl-7-OH metabolite of pyroxsulam /kg dry soil)	Replicate	Number of Juveniles[1] (Day 56)	Mean Number of Juveniles[2] (Mean ± Standard Deviation)
Negative control (0)	A	67	69.5 ± 14.05[3]
	B	58	
	C	73	
	D	84	
	E	67	
	F	47	
	G	68	
	H	92	
8.18	A	109	71.0 ± 29.13
	B	78	
	C	53	
	D	44	
16.3	A	115	76.8 ± 28.92
	B	46	
	C	79	
	D	67	
32.5	A	77	80.0 ± 32.95
	B	98	
	C	35	
	D	110	
65.0	A	40	72.0 ± 44.65
	B	31	
	C	126	
	D	91	
130	A	44	49.5 ± 10.25
	B	38	
	C	60	
	D	56	

1 All juveniles were normal in appearance and behaviour. 2 Number of juveniles was not statistically significantly different ($p>0.05$) for any group when compared to the control using Dunnett's Test. 3 Coefficient of Variation = S.D./mean *100 = 20.2%.

E. **REPORTED STATISTICS:**

As previously noted in this DER, the EC50 value for reproduction is the main end-point of the test and, if warranted, the EC50 was determined using an appropriate statistical method. Differences in mean fecundity, body weight, and change in body weight between the control and each treatment group were evaluated for statistical significance with Dunnett's 2-tailed t test (•=0.05) using SAS Version 8.

The no-observed-effect-concentration was determined by visually inspecting the juvenile output, adult earthworm mortality and body weight data and clinical observation data.

Body weights, and change in body weights, were statistically compared with Dunnett's test (•=0.05) using SAS Version 8. Prior to conducting Dunnett's test, the data were tested for homogeneity of variance and normal distribution.

F. **VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:**

Although the study results showed an absence of dose related mortality and dose related sub-lethal effects (apart from weight loss), the statistical evaluation of the biological data for mortality and sublethal effects was performed. Statistical verification of the changes in weight and reproductive output was also undertaken.

Verification of mortality statistics

The earthworm mortality data reported were analysed by the TidePool Scientific Software program, ToxCalc (v5.0.23A) and, on occasion, also using Dunnett's one-tailed test (earthworm mortalities are expected to increase as a result of exposure to the 6-Cl-7-OH metabolite of pyroxsulam). The numbers of earthworms alive at the start and end of the initial 28 day exposure period are shown in Table 8.

Table 8. Numbers of earthworms alive in the control and 6-Cl-7-OH metabolite of pyroxsulam test soils at the start and end of the 28 day exposure period. There were eight control replicates and four replicates per test concentration.

Test concentration:	Control		8.1		16.3		32.5		65.0		130	
Numbers of worms alive initially and at end of 28 days:	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Replicate A	10	10	10	10	10	8	10	10	10	10	10	10
Replicate B	10	9	10	9	10	10	10	10	10	9	10	8
Replicate C	10	10	10	10	10	8	10	10	10	10	10	10
Replicate D	10	10	10	9	10	10	10	10	10	10	10	10
Replicate E	10	9										
Replicate F	10	10										
Replicate G	10	10										
Replicate H	10	10										

The ToxCalc analysis of this data compared the final numbers of earthworms alive as a fraction with the initial numbers and with an arc sine square root transformation of this data gave the results shown below.

The transformed data were identified as normally distributed but with equality of variances not being able to be confirmed.

No statistically significant differences between the control means and the test means were identified by the Dunnett's test analysis and the 28 day NOEC was set at 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight, the highest concentration tested. The 28 day LC50 would be set as >130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight. This result is considered consistent with the study report's conclusion that, since there was no treatment related mortality of adult earthworms in this study an LC50 value was not calculated.

ToxCalc results from the analysis of the earthworm mortality (as survival) over 28 day's exposure to 6-Cl-7-OH metabolite of pyroxsulam/kg soil dry weight.

Conc-ppb	1	2	3	4	5	6	7	8
S-Control	1.0000	0.9000	1.0000	1.0000	0.9000	1.0000	1.0000	1.0000
8.13	1.0000	0.9000	1.0000	0.9000				
16.3	0.8000	1.0000	0.8000	1.0000				
32.5	1.0000	1.0000	1.0000	1.0000				
65	1.0000	0.9000	1.0000	1.0000				
130	1.0000	0.8000	1.0000	1.0000				

			Transform: Arcsin Square Root						1-Tailed	
Conc-ppb	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
S-Control	0.9750	1.0000	1.3713	1.2490	1.4120	5.501	8			
8.13	0.9500	0.9744	1.3305	1.2490	1.4120	7.072	4	0.625	2.374	0.1546
16.3	0.9000	0.9231	1.2596	1.1071	1.4120	13.974	4	1.715	2.374	0.1546
32.5	1.0000	1.0256	1.4120	1.4120	1.4120	0.000	4	-0.625	2.374	0.1546
65	0.9750	1.0000	1.3713	1.2490	1.4120	5.942	4	0.000	2.374	0.1546
130	0.9500	0.9744	1.3358	1.1071	1.4120	11.411	4	0.545	2.374	0.1546

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90398	0.896	-0.698	-0.1733
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs S-Control	130	>130			0.08098	0.08429	0.01117	0.01132	0.44811	5, 22

Verification of other effects (excluding weight change) statistics

The earthworm effects data (weight change excluded) reported were analysed by the TidePool Scientific Software program, ToxCalc (v5.0.23A) and, on occasion, also by using Dunnett's one-tailed test (effects on the earthworms are expected to increase as a result of exposure to the 6-Cl-7-OH metabolite of pyroxsulam).

The numbers of earthworms identified as normal in appearance and behaviour at the start and end of the initial 28 day exposure period are identical to the mortality data except for one control replicate containing an earthworm reported as thin and showing a reduced reaction to mechanical stimulus. The other earthworms identified as being affected were all earthworms identified as missing and presumed dead with this being classed as an effect rather than mortality in the study report.

The ToxCalc statistics (summarised below) indicate no statistically significant differences between any of the test concentrations and the control means. The 28 day NOEC for effects other than mortality and weight change is 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight and the 28 day EC50 would be set as >130 µg 6-Cl-7-

OH metabolite of pyroxsulam/kg soil, dry weight. These results are consistent with those reported by the study.

			Transform: Arcsin Square Root					1-Tailed		
Conc-ppb	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
S-Control	0.9625	1.0000	1.3509	1.2490	1.4120	6.244	8			
8.13	0.9500	0.9870	1.3305	1.2490	1.4120	7.072	4	0.307	2.374	0.1577
16.3	0.9000	0.9351	1.2596	1.1071	1.4120	13.974	4	1.375	2.374	0.1577
32.5	1.0000	1.0390	1.4120	1.4120	1.4120	0.000	4	-0.920	2.374	0.1577
65	0.9750	1.0130	1.3713	1.2490	1.4120	5.942	4	-0.307	2.374	0.1577
130	0.9500	0.9870	1.3358	1.1071	1.4120	11.411	4	0.227	2.374	0.1577

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91616	0.896	-0.6064	-0.4494
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	130	>130			0.08834	0.09276	0.01027	0.01177	0.51524	5, 22
Treatments vs S-Control										

The data set for these analyses is as shown in the ToxCalc mortality analyses except that control replicate 4 has a value of 0.9 rather than 1.0.

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 6-Cl-7-OH metabolite of pyroxsulam exposed mean earthworm weights at day 0;
- Comparison of control earthworm mean weights at day 0 compared to day 28;
- Comparison of control and 6-Cl- 7-OH metabolite of pyroxsulam exposed mean earthworm weights at day 28; and
- Comparison of the total changes in mean earthworm weights after 28 days.

The ToxCalc analyses determined normal distributions and equal variances occurred in all these comparisons except the last (total changes in earthworm weights after 28 days). Here, some ToxCalc transformations indicated that Bartlett's Test indicated unequal variances. In contrast, the transformation of log x + 1 reported equal variances for the transformed data according to Bartlett's Test.

Comparison of control and 6-Cl-7-OH metabolite of pyroxsulam exposed mean earthworm weights at day 0;

To confirm the mean weights of the control and 6-Cl-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 9.

Table 9. 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**	**5**	**6**	**7**	**8**
Control (0*)	0.7000	0.6000	0.7600	0.7200	0.6600	0.7200	0.6500	0.7100
62.5*	0.7600	0.6800	0.6600	0.7300	**			
125	0.7300	0.6000	0.6600	0.7000				
250	0.7700	0.7200	0.7500	0.7100				
500	0.6800	0.7800	0.6900	0.6900				
1000	0.6800	0.7600	0.6500	0.7500				

*** Concentrations of 6-Cl- 7-OH metabolite of pyroxsulam as µg/kg soil, dry weight. ** 8 control replicates and 4 test substance replicates/test concentrations were used.**

The ToxCalc analysis of these data gave the following results:

One tailed test

Conc-ppb	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
Control	0.6900	1.0000	0.6900	0.6000	0.7600	7.308	8					
8.13	0.7075	1.0254	0.7075	0.6600	0.7600	6.464	4	-0.593	2.374	0.0700		
16.3	0.6725	0.9746	0.6725	0.6000	0.7300	8.357	4	0.593	2.374	0.0700		
32.5	0.7375	1.0688	0.7375	0.7100	0.7700	3.734	4	-1.611	2.374	0.0700		
65	0.7100	1.0290	0.7100	0.6800	0.7800	6.606	4	-0.678	2.374	0.0700		
130	0.7100	1.0290	0.7100	0.6500	0.7600	7.541	4	-0.678	2.374	0.0700		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.96038		0.896		-0.1466	-0.8128
Bartlett's Test indicates equal variances (p = 0.92)							1.446		15.0863			
Hypothesis Test (1-tail, 0.05)			**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs Control			130	>130			0.07	0.10145	0.00206	0.00232	0.50571	5, 22

Two tailed test

			Transform: Untransformed					2-Tailed				
Conc-ppb	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
Control	0.6900	1.0000	0.6900	0.6000	0.7600	7.308	8					
8.13	0.7075	1.0254	0.7075	0.6600	0.7600	6.464	4	0.593	2.783	0.0821		
16.3	0.6725	0.9746	0.6725	0.6000	0.7300	8.357	4	0.593	2.783	0.0821		
32.5	0.7375	1.0688	0.7375	0.7100	0.7700	3.734	4	1.611	2.783	0.0821		
65	0.7100	1.0290	0.7100	0.6800	0.7800	6.606	4	0.678	2.783	0.0821		
130	0.7100	1.0290	0.7100	0.6500	0.7600	7.541	4	0.678	2.783	0.0821		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.96038		0.896		-0.1466	-0.8128
Bartlett's Test indicates equal variances (p = 0.92)							1.446		15.0863			
Hypothesis Test (2-tail, 0.05)			**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs Control			130	>130			0.08207	0.11894	0.00206	0.00232	0.50571	5, 22

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 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 pyroxsulam exposed earthworms are indicated.

Comparison of control earthworm mean weights at day 0 compared to day 28

To see if the mean weights of the control earthworms varied significantly between day 0 and day 28, the mean control weights at those times were compared. The mean control weights/replicate at days 0 and 28 are shown in Table 10.

Table 10. 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**	**5**	**6**	**7**	**8**
Day 0	0.7000*	0.6000	0.7600	0.7200	0.6600	0.7200	0.6500	0.7100
Day 28	0.8100	0.8300	0.7900	0.8900	0.7600	0.7500	0.8400	0.9200

*** Concentrations of 6-Cl- 7-OH metabolite of pyroxsulam as µg/kg soil, dry weight.**

The ToxCalc analysis of these data gave the following results:

			Transform: Untransformed					2-Tailed				
Conc-ppb	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**		
Day 0	0.6900	1.0000	0.6900	0.6000	0.7600	7.308	8					
*Day 28	0.8238	1.1938	0.8238	0.7500	0.9200	7.224	8	4.850	2.140	0.0590		
Auxiliary Tests							**Statistic**		**Critical**		**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							0.97632		0.844		0.03612	-0.6512
F-Test indicates equal variances (p = 0.67)							1.39256		8.88539			

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 28 is indicated. As noted in the study report, this effect is not unexpected as the earthworms were fed over the 28 day period.

Comparison of control and 6-Cl-7-OH metabolite of pyroxsulam exposed mean earthworm weights at day 28

The day 28 mean weights of the control earthworms were compared to the day 28 mean weights of the 6-Cl-7-OH metabolite of pyroxsulam exposed earthworms with the data used shown in Table 11.

Table 11. 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**	**5**	**6**	**7**	**8**
Control (0*)	0.8100	0.8300	0.7900	0.8900	0.7600	0.7500	0.8400	0.9200
62.5*	0.8300	0.7600	0.7600	0.7800	**			
125	0.8500	0.7400	0.7300	0.8400				
250	0.8400	0.8000	0.9100	0.8600				
500	0.8300	0.9700	0.8600	0.8500				
1000	0.9200	0.9900	0.8700	0.7100				

*** Concentrations of 6-Cl- 7-OH metabolite of pyroxsulam as µg/kg soil, dry weight. . ** 8 control replicates and 4 test substance replicates/test concentrations were used.**

The ToxCalc analysis of these data gave the following results:

One tailed test

ToxCalc gave the following results -

			Transform: Untransformed						1-Tailed	
Conc-ppb	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
Control	0.8238	1.0000	0.8238	0.7500	0.9200	7.224	8			
8.13	0.7825	0.9499	0.7825	0.7600	0.8300	4.222	4	0.995	2.374	0.0984
16.3	0.7900	0.9590	0.7900	0.7300	0.8500	8.072	4	0.814	2.374	0.0984
32.5	0.8525	1.0349	0.8525	0.8000	0.9100	5.365	4	-0.693	2.374	0.0984
65	0.8775	1.0653	0.8775	0.8300	0.9700	7.170	4	-1.296	2.374	0.0984
130	0.8725	1.0592	0.8725	0.7100	0.9900	13.638	4	-1.176	2.374	0.0984

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96962	0.896	-0.2252	0.56934
Bartlett's Test indicates equal variances (p = 0.38)	5.27051	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	130	>130			0.09843	0.11949	0.00678	0.00459	0.23669	5, 22

Two tailed test

The ToxCalc results were -

			Transform: Untransformed						2-Tailed	
Conc-ppb	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
Control	0.8238	1.0000	0.8238	0.7500	0.9200	7.224	8			
8.13	0.7825	0.9499	0.7825	0.7600	0.8300	4.222	4	0.995	2.783	0.1154
16.3	0.7900	0.9590	0.7900	0.7300	0.8500	8.072	4	0.814	2.783	0.1154
32.5	0.8525	1.0349	0.8525	0.8000	0.9100	5.365	4	0.693	2.783	0.1154
65	0.8775	1.0653	0.8775	0.8300	0.9700	7.170	4	1.296	2.783	0.1154
130	0.8725	1.0592	0.8725	0.7100	0.9900	13.638	4	1.176	2.783	0.1154

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96962	0.896	-0.2252	0.56934
Bartlett's Test indicates equal variances (p = 0.38)	5.27051	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	130	>130			0.1154	0.14009	0.00678	0.00459	0.23669	5, 22

Both the one and two tailed result confirms the study report finding that, when comparing the day 28 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).

Comparison of the total changes in mean earthworm weights after 28 days.

The average total changes in earthworm weights after 28 days of the test are shown in Table 12.

Table 12. Mean total changes in earthworm weights after 14 days.

	Mean change in earthworm weights (mg) at day 14							
Replicate number:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Control (0*)	0.1100	0.2300	0.0300	0.1700	0.1000	0.0300	0.1900	0.2100
8.18*	0.0700	0.0800	0.1000	0.0500	**			
16.3	0.1200	0.1400	0.0700	0.1400				
32.5	0.0700	0.0800	0.1600	0.1500				
65.0	0.1500	0.1900	0.1700	0.1600				
130	0.2400	0.2300	0.2200	-0.04				

*** Concentrations of 6-Cl- 7-OH metabolite of pyroxsulam as µg/kg soil, dry weight. ** 8 control replicates and 4 test substance replicates/test concentrations were used.**

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

The ToxCalc analysis of these data, following transformation to the form log(x+1) of the data gave the following results:

One tailed test

			Transform: Log (X + 1)					1-Tailed		
Conc-ppb	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
Control	0.1338	1.0000	0.0536	0.0128	0.0899	56.433	8			
8.13	0.0750	0.5607	0.0313	0.0212	0.0414	26.833	4	1.425	2.374	0.0371
16.3	0.1175	0.8785	0.0481	0.0294	0.0569	27.015	4	0.352	2.374	0.0371
32.5	0.1150	0.8598	0.0470	0.0294	0.0645	38.601	4	0.423	2.374	0.0371
65	0.1675	1.2523	0.0672	0.0607	0.0755	9.426	4	-0.872	2.374	0.0371
130	0.1725	1.2897	0.0674	0.0000	0.0934	66.804	4	-0.885	2.374	0.0371

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93483	0.896	-1.0689	1.60705
Bartlett's Test indicates equal variances (p = 0.02)	13.7	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	130	>130			0.09259	0.08184	0.00075	0.00065	0.36251	5, 22

Two tailed test

			Transform: Log (X + 1)						2-Tailed	
Conc-ppb	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.1338	1.0000	0.0536	0.0128	0.0899	56.433	8			
8.13	0.0750	0.5607	0.0313	0.0212	0.0414	26.833	4	1.425	2.783	0.0435
16.3	0.1175	0.8785	0.0481	0.0294	0.0569	27.015	4	0.352	2.783	0.0435
32.5	0.1150	0.8598	0.0470	0.0294	0.0645	38.601	4	0.423	2.783	0.0435
65	0.1675	1.2523	0.0672	0.0607	0.0755	9.426	4	0.872	2.783	0.0435
130	0.1725	1.2897	0.0674	0.0000	0.0934	66.804	4	0.885	2.783	0.0435

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93483	0.896	-1.0689	1.60705
Bartlett's Test indicates equal variances (p = 0.02)	13.7	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	130	>130			0.10776	0.09525	0.00075	0.00065	0.36251	5, 22

Both the one and two tailed result confirms the study report finding that, when comparing the weight losses over 28 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).

The -0.4 value for one of the 130 µg/kg dry soil replicates is treated as 0 by the transformation.

US EPA Dunnett's Procedure data analysis

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 28 day, the US EPA program gave the following results:

Summary Statistics and ANOVA
Transformation = None

Concentration number and corresponding concentration, µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil	Number of replicates	Mean weight	Standard deviation	CV%
1 = control	8	.1338	.0782	58.5
2 (8.18)	4	.0750	.0208	27.8
3 (16.3)	4	.1175	.0330	28.1
4 (32.5)	4	.1150	.0465	40.5
4 (65.0)	4	.1675	.0171	10.2
5 (130)	4	.1625	.1352	83.2

For concentrations marked with an asterisk (*) the mean for this conc. is significantly less than the control mean at alpha = 0.05 (1-sided) by a t – test with Bonferroni adjustment of alpha level. In this case, no significantly different values are indicated.

Minimum detectable difference for t-tests with Bonferroni adjustment = -.088545. This difference corresponds to -66.20 percent of control

Note - the above value for the minimum detectable difference is approximate as the sample sizes are not the same for all of the concentrations.

Between concentrations sum of squares = 0.023573 with 5 degrees of freedom.

Error mean square = .004982 with 22 degrees of freedom.

Bartlett's test p-value for equality of variances = .007

The test program warned that the test for equality of variances is significant (p less than 0.01) and that the results of this analysis should be interpreted with caution.

It is noted that the US EPA program warns of a significant deviation from equality of variances. While this was not found in the ToxCalc log(x + 1) transformation, presumably because of the normalizing effect of the log transformation, other ToxCalc transformations reported that Bartlett's Test indicated unequal variances.

Overall, the weight statistics reported in the study report are considered verified.

Verification of reproductive output statistics

To determine whether the exposure to the 6-Cl-7-OH metabolite of pyroxsulam affected the number of offspring produced, the numbers of juveniles produced in the 6-Cl-7-OH metabolite of pyroxsulam treated soils were compared with the numbers in the control soils. The numbers of juvenile earthworms per replicate after 28 days used for this statistical analysis are shown in Table 13.

Table 13. Number of juvenile earthworms produced by earthworms exposed to 6-Cl-7-OH metabolite of pyroxsulam in an artificial soil substrate for 28 days.

Concentration, µg/kg soil, dry weight	Replicate number (8 control replicates and 4 replicates/test concentration)							
	1	2	3	4	5	6	7	8
Control	67	58	73	84	67	47	68	92
8.18	109	78	53	44				
16.3	115	46	79	67				
32.5	77	98	35	110				
65	40	31	126	91				
130	44	38	60	56				

The ToxCalc one-tailed analysis of these data gave the following results:

One tailed analysis

			Transform: Untransformed						1-Tailed	
Conc-ppb	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	69.500	1.0000	69.500	47.000	92.000	20.217	8			
8.13	71.000	1.0216	71.000	44.000	109.000	41.031	4	-0.091	2.374	39.189
16.3	76.750	1.1043	76.750	46.000	115.000	37.678	4	-0.439	2.374	39.189
32.5	80.000	1.1511	80.000	35.000	110.000	41.193	4	-0.636	2.374	39.189
65	72.000	1.0360	72.000	31.000	126.000	62.020	4	-0.151	2.374	39.189
130	49.500	0.7122	49.500	38.000	60.000	20.701	4	1.211	2.374	39.189

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98554	0.896	0.16953	-0.2127
Bartlett's Test indicates equal variances (p = 0.12)	8.77906	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	130	>130			39.1892	0.56387	456.7	726.898	0.68004	5, 22

The two-tailed analysis gave equivalent results, namely:

Two tailed analysis

			Transform: Untransformed						2-Tailed	
Conc-ppb	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	69.500	1.0000	69.500	47.000	92.000	20.217	8			
8.13	71.000	1.0216	71.000	44.000	109.000	41.031	4	0.091	2.783	45.943
16.3	76.750	1.1043	76.750	46.000	115.000	37.678	4	0.439	2.783	45.943
32.5	80.000	1.1511	80.000	35.000	110.000	41.193	4	0.636	2.783	45.943
65	72.000	1.0360	72.000	31.000	126.000	62.020	4	0.151	2.783	45.943
130	49.500	0.7122	49.500	38.000	60.000	20.701	4	1.211	2.783	45.943

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98554	0.896	0.16953	-0.2127
Bartlett's Test indicates equal variances (p = 0.12)	8.77906	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	130	>130			45.9434	0.66106	456.7	726.898	0.68004	5, 22

These results indicate the number of juveniles was not statistically significantly different ($p>0.05$) for any group when compared to the control using Dunnett's Test, as was reported in the study report.

Additionally, the results confirm the study report's establishing the 28 day NOEC (reproduction) at 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil, the highest concentration tested, based on the numbers of juveniles produced.

US EPA Dunnett's Procedure data analysis of the number of juveniles
This procedure returned the following:

Summary Statistics and ANOVA

Transformation = None

Concentration number and corresponding concentration, µg 6-Cl-7-OH metabolites of pyroxsulam/kg dry soil	Number of replicates	Mean numbers of juveniles	Standard deviation	CV%
1 = control	8	69.5000	14.0509	20.2
2 (8.18)	4	71.0000	29.1319	41.0
3 (16.3)	4	76.7500	28.9180	37.7
44 (32.5)	4	80.0000	32.9545	41.2
4 (65.0)	4	72.0000	44.6542	62.0
5 (130)	4	49.5000	10.2470	20. 7

In this case, no values significantly different from the control value are indicated.

Minimum detectable difference for t-tests with Bonferroni adjustment = -33.820700. This difference corresponds to -48.66 percent of control.

Note - the above value for the minimum detectable difference is approximate as the sample sizes are not the same for all of the concentrations.

Between concentrations sum of squares = 2283.500000 with 5 degrees of freedom.

Error mean square = 726.897727 with 22 degrees of freedom.

Bartlett's test p-value for equality of variances = .115

The US EPA Dunnett's analysis confirms that the number of juveniles was not statistically significantly different ($p>0.05$) for any group when compared to the control using Dunnett's Test and that the 28 day NOEC (reproduction) can be set at 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg dry soil, the highest concentration tested, based on the numbers of juveniles produced.

The Dunnett's test results for the mean numbers of juveniles and the associated standard deviations are equivalent to those reported in the study report.

However, the reviewers considers that the reduction in numbers of juveniles seen at the 130 µg/kg soil (dw) exposure level, while not statistically significantly different from the control results, never-the-less could well be biologically significant. As a result, the review considers the 56 day LOEC for earthworm reproduction, based on the numbers of juveniles produced, can be set at 130 µg/kg soil (dw) with the corresponding NOEC being 65 µg/kg soil (dw).

Statistical Method:
For the adult earthworms
28 day LC50 and EC50: Since there was no treatment related mortality of adult earthworms in this study an LC50 value was not calculated. Similarly, the absence of a dose effect on sub-lethal toxicity endpoints resulted in no EC50 being calculated.

95% C.I.:	Not determined
28 day NOEC:	There was an the absence of dose effects on mortality and (mortality and sublethal effects and weight loss) and the NOEC was set at 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight, the maximum concentration tested.
28 day LOEC:	There was an absence of dose effects on mortality and (mortality and sublethal effects and weight loss) and the LOEC was set at >130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight, the maximum concentration tested.
Probit Slope:	Not determined
95% C.I.:	Not determined
Endpoint(s) affected:	There were no compound related effects on mortality and sublethal effects including weight change.

For the juvenile earthworms (reproduction as measured by number of juveniles produced)

56 day EC50:	>130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight.
56 day LOEC	130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight
56 day NOEC:	65 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight.

G. STUDY DEFICIENCIES:

The following deviations from OECD Guideline 222 or other deficiencies were noted.

Table 14. Summary of deficiencies/deviations from the OECD Guideline 222.

Parameter	Study report result	OECD Guideline for the Testing of Chemicals, Earthworm, Reproduction Test (*Eisenia fetida/Eisenia andrei*), 222, adopted 13 April 2004
Physicochemical properties of 6-Cl-7-OH metabolite of pyroxsulam	Values of water solubility, etc. were not reported.	OECD 222 states water solubility, Kow etc. should be available to help the design of appropriate test procedures and that the test report must include such information.
Age at test initiation	Referred to as adult. The study protocol states the earthworms will be between 2 months and 1 year old.	OECD 222 states the age of the earthworms must be included in the test report and specifies that adult worms between two months and one year old and with a clitellum are required to start the test.
Weight at study initiation	Earthworm weights (600-780 mg) exceeded the OECD 222 range. This was noted in the study report as a Deviation from the Study Protocol. Based on the control performance, the study report considered this had not had an adverse impact on the study. Earthworms not individually weighed at the start of the study.	OECD 222 states that the wet mass of the earthworms should be between 250 and 600 mg. OECD 222 states that groups of 10 earthworms should be weighed individually at the start of the test. On day 28, the guideline directs that the living adult earthworms be weighed but does not specify that individual weights be measured.
Stability and homogeneity of test material in the medium?	Not verified	OECD 222 states that information on fate and behaviour in the environment should be available where possible and that it cannot be assumed that exposure concentrations will be maintained at initial values throughout the test. US EPA OPPTS 850.6200 recommends that test concentrations in artificial soil should be analysed for test chemical concentrations prior to the start of the test and at days 7, 14, 21 and 28 as a minimum.
Analytical verification of nominal concentrations	Not conducted. Soil metabolism studies indicate potential for degradation and analyses should have been done.	OECD 222 notes that it cannot be assumed that exposure concentrations will be maintained at initial values throughout the test and recommends chemical analysis for the test substance at the start and end of the test.
Nominal concentration of 8.13 µg/kg soil, dry weight	The study report's Deviation from Study Protocol notes that the 8.13 value was actually 8.18. This was stated to be a result of the need to round up	

	the amount of active constituent measured for practical reasons. No adverse effect was stated to be expected from this. This amendment could profitably have been noted in the study report at the first occurrence to the nominal 8.13 µg/kg dry soil.	
Physicochemical properties of soil. pH (__:__ soil:water)	The pH of the bulk soil prior to hydration was adjusted to 5.6 using calcium carbonate. Soil water ratio not provided. In the control and test soils pH was 7.4-7.6 at test start and 7.3-7.5 at test end. pH measurements were said to have been made on soil samples with details on how the pH was measured (apart from use of aThermo Orion 525 Aplus pH meter) not recorded.	OECD 222 refers to the pH of the artificial soil being initially 6.0 ± 0.5. It also states that pH is measured in a mixed sample in a 1 M solution of potassium chloride or a 0.01 M solution of calcium chloride.
Rates of application **Measured:**	Verification of test concentrations of the 7-OH metabolite of pyroxsulam in the soil did not occur (GLP compliance statement) and could not be verified by the study report's data on the weights and volumes used to prepare the test soils. Reference was made to a "stock" solution but its concentration was not identified.	No specific reference identified regarding the need to verify the nominal concentrations. The failure to identify the concentration of the stock solution has presented confirmation that the actual doses were equivalent to the nominal doses.
Moisture:	The maximum water holding capacity of the soil was not reported.	OECD 222 states that the test report must contain the maximum water holding capacity of the soil and that this value corresponds to 40 to 60% moisture on a dry mass basis. The recorded soil moisture contents ranged from 35.6 to 36% at day 0 and, at test termination, 32.0 to 35.5%, i.e. from 4 to 8% below the minimum required moisture content.

The failure to measure the actual concentrations of 6-Cl-7-OH metabolite in the soils or to provide sufficient data to

confirm the nominal concentrations prepared were as stated, are considered significant deficiencies as is the indication that the soil moisture content was apparently 4 to 8% below the minimum required moisture content. Consequently, the study is considered supplemental noting that OECD 222 acknowledges that possible degradation of the test substance can occur and recommends chemical analysis of the test substance at the start and end of the test. Other deviations from the guideline or deficiencies were noted but are not considered to have significantly affected the study's conduct or outcomes.

With respect to the stability of the 6-Cl-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 6-Cl-7-OH metabolite of pyroxsulam formed from 7-OH metabolite of pyroxsulam degradation was 7.4 days (DT90 24.4 days) in one soil, 48.9 days (DT90 162.3 days) in another soil and 9.2 days (DT90 30.7 days) in a third soil with conversion to "other" degradates which include carbon dioxide, bound residues and minor metabolites. DT50 and DT90 for the fourth soil were not reported. Such results indicate that the 6-Cl-7-OH metabolite of pyroxsulam could be expected to have undergone degradation in the 56 days of the earthworm exposure study.

H. REVIEWER'S COMMENTS:

This study was conducted as a reproduction test over an initial 28 days to determine parental earthworm mortality, weight change and other adverse effects followed by a further 28 days exposure to determine the number of juveniles produced by the earthworms in the first 28 days exposure and whether any obvious or pathological symptoms or distinct changes in behaviour occurred in these juveniles.

The earthworms were exposed to nominal concentrations of 8.18 to 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight for 28 days and then removed from the test systems. Cocoons and juveniles produced in the first 28 days were then exposed to concentrations of 0 to 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight for a further 28 days.

There were no measurements of the metabolite concentrations in the treated soil conducted and the data provided did not permit calculation of the amounts of 6-Cl-7-OH metabolite of pyroxsulam mixed with the soil to confirm the nominal concentrations.

Stability of the 6-Cl-7-OH metabolite of pyroxsulam over the exposure periods and the homogeneous distribution of the 6-Cl-7-OH metabolite in the soil were not confirmed.

These are considered significant omissions. Because of these issues, the study is considered supplemental especially as OECD 222, in discussing possible degradation of the test substance, recommends chemical analysis of the test substance at the start and end of the test. Information in other parts of the dossier indicates this is possible.

The validity criterion for OECD 222 (adopted 13 April 2004) with respect to the controls of:

- each replicate (containing 10 adults) to have produced ≥ 30 juveniles by the end of the test;
- the coefficient of variation of reproduction to be ≤30 %; and
- adult mortality over the initial 4 weeks of the test to be ≤10 %.

were met, with:

- The numbers of juveniles in the 8 control replicates at day 56 of the study ranged from 47 to 92 with a mean of 69.5 juveniles/replicate (standard deviation ±14.05).

- The coefficient of variation for reproduction of the controls was 20.2%.
- The adult mortality after the first 4 weeks (28 days) was 2.5% (2 dead worms out of 80).

I. CONCLUSIONS:

The study is supplemental to the US EPA and DEW because of the inability to confirm the nominal concentrations used and the lack of measurement to shown the stability of the 6-Cl-7-OH metabolite of pyroxsulam in the artificial soil over the 56 days of the exposure period and its homogeneous distribution in the test soils. Reproduction studies on earthworms are not currently part of the data requirements of the PMRA. The PMRA does not share the same study acceptability classification scheme as the APVMA or the US EPA. For the PMRA, a study is either acceptable or invalid. This study is considered acceptable and could provide useful information for risk assessment purposes.

In earthworms exposed to 8.18 to 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight for 28 days, there were 9 mortalities in 200 exposed worms, i.e. 4.5% mortality. In the controls over the same period exhibited, there were 2 mortalities in a total of 80 worms, i.e. 2.5%.

Sublethal effects (including weight loss and worms identified as "not found") showed no dose related responses (the percentages of the exposed earthworms identified as effected in the 8.18, 16.3, 32.5, 65.0 and 130 µg/kg soil test concentrations were, respectively, 5, 10, 0, 2.5 and 5% and, in the controls, 3.75%).

If earthworms identified as "not found" are excluded from these sublethal effects, then 100% of the 6-Cl-7-OH metabolite of pyroxsulam exposed earthworms showed no effect while 98.7% of the control earthworms showed no effects. In the controls, 1 of 78 worms after 28 days was identified as thin and showing reduced reaction to mechanical stimulus.

Except in one of the four replicates treated at 130 µg 6-Cl-7-OH metabolite of pyroxsulam, control and exposed earthworms all gained weight over the 28 days of the exposure period as a result of their feeding on manure added to the artificial soil. There were no dose related effects identified with respect to adult earthworm mortality or sublethal effects including weight change. Furthermore, 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 28 day LC50, EC50, LOEC and NOEC (for mortality and sublethal effects including weight change) were not determined in the study report but could be set as >130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight for the LC50, EC50 and LOEC and 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight for the NOEC.

The 56 day EC50 for reproduction could be set at >130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight. The NOEC for reproduction (numbers of juvenile earthworms produced) was set by the reviewers as 65 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight and the LOEC at 130 µg 6-Cl-7-OH metabolite of pyroxsulam/kg soil, dry weight. These juvenile earthworm endpoints are based on the consideration that the reduction in the numbers of juveniles seen at the 130 µg/kg soil, dry weight exposure concentration could be biologically significant.

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Approved 04/01/01 C.K.