Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (XDE-742) to rainbow trout (*Oncorhynchus mykiss*)

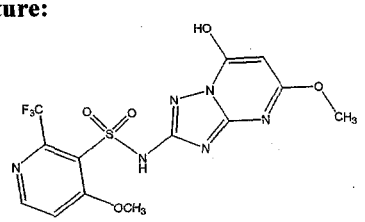
PMRA Submission Number 2006-4727, ID 128xxxx EPA MRID Number 469084-~~xx~~ 24 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.5.2.1
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.2.1.3
	EPA MRID:	469084-xx
	EPA Guideline:	72-1 (OPPTS 850.1075)

Test material: **7-Hydroxy metabolite of pyroxsulam** **Purity (%):** 99%

Common name:	7-OH metabolite of XDE-742 (i.e. 7-OH metabolite of pyroxsulam)
Chemical name:	3-pyridinesulfonamide, N-(7-hydroxy-5-methoxy [1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
IUPAC:	(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:	N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.:	Not available
Synonym:	7-desmethyl XDE-742 metabolite
ID Number.:	TSN 105384

Chemical Structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/02/08 **Date:** 16 January 2007
Australian Government Department of The Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewer(s): Jack Holland 22/2/08 **Date:** 30 January 2007
Australian Government Department of The Environment, Water, Heritage and the Arts

Thomas Steeger, Ph.D., Senior Biologist 4/3/08 **Date:** 19 February 2006
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Anne Gosselin Guillaume for Anne Gosselin 05/03/08 **Date:** 27 April 2007
Pest Management Regulatory Agency, Health Canada

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

CITATION: Sayers, L. E. 2006. 7-OH Metabolite of XDE-742 –Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions. Springborn Smithers Laboratories, Wareham, Massachusetts 02571-1037, Springborn Smithers Study No. 12550.6411 and Sponsor Protocol/Project No. 050165. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences, Indianapolis, Indiana, 46268. 5 April 2006.

Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (XDE-742) to rainbow trout (*Oncorhynchus mykiss*)

PMRA Submission Number 2006-4727, ID 128xxxx EPA MRID Number 469084-24 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.5.2.1
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.2.1.3
	EPA MRID:	469084-24
	EPA Guideline:	72-1 (OPPTS 850.1075)

Test material: **7-Hydroxy metabolite of pyroxsulam** **Purity (%):** 99%

Common name: 7-OH metabolite of XDE-742 (i.e. 7-OH metabolite of pyroxsulam)

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

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

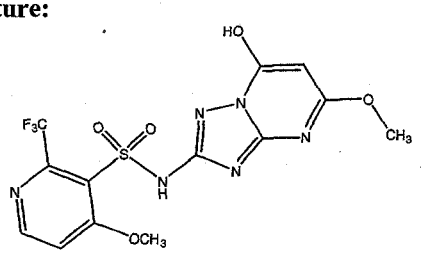
CAS name: N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

CAS No.: Not available

Synonym: 7-desmethyl XDE-742 metabolite

ID Number.: TSN 105384

Chemical Structure:



Primary Reviewer: Daryl Murphy **Date:** 16 January 2007
Australian Government Department of The Environment and Water Resources (DEW)

Secondary Reviewer(s): Jack Holland **Date:** 30 January 2007
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Thomas Steeger, Ph.D., Senior Biologist **Date:** 19 February 2006
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Company Code: DWE
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CITATION: Sayers, L. E. 2006. 7-OH Metabolite of XDE-742 –Acute Toxicity to Rainbow Trout (*Oncorhynchus*

mykiss) Under Static Conditions. Springborn Smithers Laboratories, Wareham, Massachusetts 02571-1037, Springborn Smithers Study No. 12550.6411 and Sponsor Protocol/Project No. 050165. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences, Indianapolis, Indiana, 46268. 5 April 2006. Unpublished report.

EXECUTIVE SUMMARY:

In a 96 h acute toxicity study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to the 7-hydroxy metabolite of pyroxsulam (7-OH metabolite of pyroxsulam) at nominal concentrations of 0 (control), 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L or, as mean measured concentrations over 96 hours, 6.8, 14, 29, 57 and 120 mg 7-OH metabolite of pyroxsulam/L, under static conditions.

The 96 h LC_{50} was >120 mg 7-OH metabolite of pyroxsulam/L based on mean, measured concentrations. The 96 EC_{50} and NOEC values, based on mortality/sub-lethal effects, were >120 and 120 mg 7-OH metabolite of pyroxsulam/L, respectively, based on mean measured concentrations. No sub-lethal effects were observed. Based on the results of this study, the 7-hydroxy metabolite of pyroxsulam would be classified as practically non-toxic to rainbow trout in accordance with the classification system of the Australian Government Department of The Environment and Water Resources (LC50 or EC50 > 100 mg/L).

This toxicity study is classified as acceptable and is consistent with the guideline requirement for a 96 hour fish acute toxicity study on the rainbow trout.

Results Synopsis

Test Organism Size/Age	
	Mean wet weight: 0.68 g (range 0.22 to 0.98 g)
	Mean total length: 40 mm (range 35 to 43 mm)
	Age: Identified in the study protocol as "Juvenile".
Test Type:	Static 96 hours

The following endpoints are based on mean, measured concentrations.

96 h LC_{50}:	>120 mg 7-OH metabolite of pyroxsulam/L
95% C.I.:	Not applicable
96 h NOEC/NOAEC:	120 mg 7-OH metabolite of pyroxsulam/L
Probit Slope:	Not applicable
96 h EC_{50}:	>120 mg 7-OH metabolite of pyroxsulam/L
95% C.I.:	Not applicable
Endpoint(s) Effected:	There were no compound related effects (survival or sub-lethal) noted during this study.

Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (XDE-742) to rainbow trout (*Oncorhynchus mykiss*)

PMRA Submission Number 2006-4727, ID 128xxxx EPA MRID Number 469084-24 APVMA ATS 40362

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study was reported as conducted to the following test guidelines

OECD Guideline Number 203, Fish, Acute Toxicity Test, 1992.

EC Guideline L383A, Method C.1 Acute Toxicity for Fish, 1992.

U.S. Environmental Protection Agency's Pesticide Assessment Guidelines (Subdivision E, Series 72-1; 1982.

Guidelines appear to have been generally complied with apart from some minor deviations (see relevant text entries below and also the Study Deficiencies/Deviations table on page 15 of this draft DER).

COMPLIANCE:

The study reports stated that the data and report presented were produced and compiled in accordance with all pertinent OECD (OECD, 1998) and US EPA Good Laboratory Practice regulations (40 CFR, Part 160) Good Laboratory Practice regulations with the following exceptions: routine food and water screening analyses were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard US EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. As the analyses were conducted following standard validated methods, these exceptions were considered not to have had an impact on the study results.

The signed and dated GLP Compliance Statement for the study was provided.

The signed and dated Quality Assurance Statement for the study was provided.

The signed and dated Statement of No Data Confidentiality for the study was provided.

A. MATERIALS:

1. Test Material: The 7-hydroxy metabolite of pyroxsulam (XDE-742), generally referred to as the 7-OH metabolite of pyroxsulam in this DER report.

Description: Solid (as described in the product's Certificate of Analysis)

Lot No./Batch No. : 35172-56

Purity: 99%

Stability of Compound Under Test Conditions: Stability stated to be the responsibility of the study sponsor. The company's study profile template (referred to as Sayers, 2006a to distinguish it from the study report (Sayers, 2006)) states that, based on measurements of the test substance concentrations at 0 and 96 hours, the test substance was stable under the test conditions.

Over 96 hours, the measured concentrations of the 7-OH metabolite of pyroxsulam ranged from 91 to 98% of nominal

(page 10 of this draft DER refers), confirming the metabolite's stability under the test conditions.

Storage conditions of test chemicals: The test substance was stored at room temperature in the original container in a dark, ventilated cabinet.

Physicochemical properties of 7-OH metabolite of pyroxsulam.

Parameter	Values	Comments
Water solubility at 20°C	Not available	The company's study profile template (Sayers, 2006a) reported that the physicochemical properties were not available at the time of the study profile template's publication.
Vapour pressure	Not available	
UV absorption	Not available	
pKa	Not available	
Kow	Not available	

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)

Age at test initiation: Not stated, the company's study profile template (Sayers, 2006a) refers to the fish as "juvenile". The study protocol identifies the fish to be used as "juvenile" and actively feeding.

Weight at study initiation: Mean wet weight = 0.68 g (range 0.22 to 0.98 g) with date of weighing not specified.

Length at study initiation: Mean total length = 40 mm (range 35 to 43 mm) with date of measurement not specified.

Note: Mean and range values obtained from 30 fish from the test population – the time of measurement was not identified in the study report.

Source: Troutlodge, Inc., Sumner, Washington.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study:
The following information was provided on the preliminary test conducted:

"Prior to initiating the definitive study, a preliminary test was conducted at Springborn Smithers during which rainbow trout were exposed under static conditions to nominal concentrations of 0.10, 1.0, 10 and 100 mg 7-OH metabolite of pyroxsulam/L and a control. One test vessel containing five fish was established for each treatment level and the control. At test termination, no mortality or adverse effects were observed among fish exposed to any treatment level tested or the control.

During the 96-hour preliminary exposure, a sample of an intermediate nominal treatment level tested (i.e., 1.0 mg/L) was analysed for 7-OH metabolite of XDE-742 concentration at 0, 48 and 96 hours of exposure to ensure that a static test design was appropriate. Three quality control samples were also prepared at each sampling interval.

The sample of the 1.0 mg 7-OH metabolite of pyroxsulam/L (nominal) treatment level analysed at 0, 48 and 96 hours during the preliminary exposure resulted in analytical recoveries of 92, 97 and 85%, respectively, indicating that the test substance was stable under the test conditions maintained. QC samples ranged from 92.3 to 103% of nominal concentrations (0.500, 10.0 and 100 mg 7-OH metabolite of pyroxsulam/L at 0-hour; 0.0500, 0.400 and 2.00 mg 7-OH metabolite of pyroxsulam/L at 48- and 96-hour) during this period.

Based on these results and consultation with the Study Sponsor, the nominal concentrations selected for the definitive exposure were 7.5, 15, 30, 60 and 120 mg a.i./L."

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation: Period: Conditions: (same as test or not)	A minimum of 12 days before testing. Prior to testing, the fish were held in a 500-Lss tank under a photoperiod of 16 hours light and 8 hours darkness. The culture water was described as "soft" water and was drawn from a 100-meter deep bedrock well into an epoxy-coated concrete reservoir where it was aerated and supplemented with well water supplied by the Town of Wareham, Massachusetts. The water which flowed into this holding tank was characterized as having total hardness and total alkalinity ranges as calcium carbonate ($CaCO_3$) of 44 to 58 mg/L and 30 to 34 mg/L, respectively, and a specific conductance range of 150 to 190 micromhos per centimeter (µmhos/cm). Other parameters monitored in the holding tank were pH with a range of 7.3 to 7.5 and dissolved oxygen percent saturation with a range of 87 to 95%. Fish used during the definitive exposure were maintained under these conditions for a minimum of 12 days prior to testing. The temperature in the holding tank was 11 to 13°C during the 12 days prior to test initiation. The dilution water source used during this study was from the same source as the water which flowed into the fish holding tank and was characterized as having a pH of 7.6, total hardness and alkalinity ranges as above, and a specific conductivity range of 170 to 190 µmhos/cm.	See deficiency table (page 15 of this draft DER). *(EPA requires minimum 14 days; no feeding during test; OECD requires minimum of 12 days)*

Parameter	Details	Remarks Criteria
Feeding:	The fish were fed trout chow and brine shrimp, *ad libitum*, generally twice daily. Fish were not fed during the 48-hour period prior to test initiation or during the exposure period.	
Health: (any mortality observed)	No mortality was seen in the test fish population during the 7 day period before testing.	
Duration of the test	96 hours	Requirement met. *(EPA/OECD require 96 hour)*
Test condition: Static/flow through	Static	Requirement met. *(EPA requires: must provide reproducible supply of toxicant)* *(EPA requires: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Type of dilution system- for flow through method Flow rate Renewal rate for static renewal	Not applicable Not applicable Not renewed over 96 hours	
Aeration, if any	Not referred to. The study protocol states that aeration will only be commenced as a last resort, and after sponsor notification, to raise and maintain the dissolved oxygen content at or above 60% of saturation.	Requirement considered met. *(EPA requires: no aeration; OECD permits aeration)*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Each aquarium was made up of glass and silicone adhesive. 39 X 20 X 25 cm (L X W X H) 15 L	Requirement considered met. *(EPA requires: size 19 L (5 gal) or 30 x 60 x 30 cm Fill volume: 15-30 L of solution)*
Source of dilution water	As previously noted, the culture water is described as "soft" water and was drawn from a 100-meter deep bedrock well into an epoxy-coated concrete reservoir where it was aerated and supplemented with well water supplied by the Town of Wareham, Massachusetts.	Requirement considered met. *(EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water); OECD permits dechlorinated tap water)*

Parameter	Details	Remarks Criteria
Water parameters: Hardness	The dilution water had a total hardness, as $CaCO_3$, of 44 to 58 mg/L.	See deficiency table (page 15 of this draft DER). *(Hardness* *EPA : 40 - 48 mg as $CaCO_3$/L* *OECD: 10 -250* mg as $CaCO_3$/L
pH	During the test, the pH in the control vessels ranged from 6.7 (96 hours) to 7.3 (0 hours). In the test concentrations, the pHs at 0 hours ranged from 6.4 (100 mg/L) to 7.2 (15 and 30 mg/L). At 96 hours, the test concentrations had pH values of 6.4 (100 mg/L) to 6.7 (7.5, 15, and 30 mg/L).	See deficiency table (page 15 of this draft DER). *pH* *(EPA: 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8)* *OECD: 6.0 - 8.5*
Dissolved oxygen	87-95% of saturation in the holding tank water. Over 96 hours, the dissolved oxygen content ranged from 7.7 to 9.9 mg/L (75 to 98% saturation) in the controls and from 6.4 to 10.1 mg/L (62 to 100% saturation) in the test solutions. No concentration fell below 60% of the saturation value over the 96 hours.	Dissolved Oxygen *EPA: Static: 60% during 1st 48 hrs and 40% during 2nd 48 hrs, flow-through: 60%)* *OECD: at least 80% saturation value.*
Temperature	14 to 15°C	See deficiency table (page 15 of this draft DER). *Temperature:* *EPA: estuarine/marine: 22 ± 1°C* *OECD: 21 - 25°C for bluegill and 13 - 17°C for rainbow trout.*
Total organic carbon	0.26 mg/L for the dilution water source.	
Particulate matter	Not reported. Stated (Sayers, 2006a) to be "within normal limits".	
Metals	Not reported. Sayers (2006a) states "within normal limits".	

Parameter	Details	Remarks Criteria
Pesticides	Representative samples of the dilution water source were analysed periodically for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analysed, in agreement with ASTM (2002) standard practice.	
Chlorine	Not reported. Sayers (2006a) states "Within normal limits".	
Salinity for marine or estuarine species	Not applicable	*Salinity* *EPA: 30-34 % (parts per thousand) salinity, weekly range < 6 %)*
Intervals of water quality measurement	0, 24, 48, 72 and 96 hour results reported for pH, dissolved oxygen and temperature measurements.	*(EPA water quality: measured at beginning of test and every 48 hours)*
Number of replicates/groups: Control (dilution water): Solvent control: Treatments:	 1 replicate Not applicable 1 replicate per treatment level	Requirement met. *(EPA/OECD requires: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series)*
Number of organisms per replicate /groups: Control (dilution water): Solvent control: Treatments:	 10 fish Not applicable 10 fish	Requirement met. *(EPA: 10/concentration); OECD requires at least 7 fish/concentration)*
Biomass loading rate	0.45 g of biomass/L of test solution	Requirement considered met. *(EPA: static: 0.8 g/L at 17□C, 0.5 g/L at > 17□C; flow-through: 1 g/L/day; OECD requires: maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through)*
Test concentrations: Nominal:	 0 (control), 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L.	Requirement met.

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td>Measured:</td><td>Measured concentrations were as shown in the following table (next page):
<table>
<tr><th rowspan="2">Nominal concentrations mg/L[a]</th><th colspan="3">Measured concentrations, mg 7-OH metabolite of pyroxsulam/L</th><th rowspan="2">Percent of nominal</th></tr>
<tr><th>0 hours</th><th>96 hours</th><th>Mean</th></tr>
<tr><td>Control</td><td><0.31</td><td><0.26</td><td>na[c]</td><td>na</td></tr>
<tr><td>7.5</td><td>6.7</td><td>7.0</td><td>6.8</td><td>91</td></tr>
<tr><td>15</td><td>14</td><td>14</td><td>14</td><td>93</td></tr>
<tr><td>30</td><td>28</td><td>29</td><td>29</td><td>96</td></tr>
<tr><td>60</td><td>56</td><td>57</td><td>57</td><td>94</td></tr>
<tr><td>120</td><td>120</td><td>120</td><td>120</td><td>98</td></tr>
<tr><td>3.00[b]</td><td>3.93 (131)</td><td>3.02 (101)</td><td colspan="2" rowspan="3">na</td></tr>
<tr><td>30.0[b]</td><td>29.0 (96.6)</td><td>30.3 (101)</td></tr>
<tr><td>100[b]</td><td>96.2 (96.2)</td><td>103 (103)</td></tr>
</table>
a. As 7-OH metabolite of pyroxsulam.
b. Quality control samples with % recovery in brackets.
c. Not applicable.

Calculated results reported as based on actual, not rounded, analytical results.

Mean percent recoveries of 7-OH metabolite of pyroxsulam ranged from 91 to 98% of the nominal concentrations and defined the mean measured concentrations as 6.8, 14, 29, 57 and 120 mg/L.

The quality control sample for 0 hours, 3.00 mg/L recovery of 131% is outside the acceptable range (80-120%). This is not considered a significant issue given the acceptability of the 96 hour results for the 3.00 mg/L sample and the acceptability of the other quality control samples' recoveries.</td><td>Note: The stock solution used to prepare the nominal concentrations was corrected for the 99% purity of the 7-OH metabolite of pyroxsulam.</td></tr>
<tr><td>Solvent (type, percentage, if</td><td>Solvent not used</td><td>Requirement met.</td></tr>
</table>

Parameter	Details	Remarks
		Criteria
used)		*(EPA requires: not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD requires solvent not exceed 100 mg/L)*
Lighting	16 hours light, 8 hours dark. 840-970 lux at the surface of the aquaria (individual lux results were not presented).	Requirement considered met. *(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod)*
Feeding	Fish not fed during the 48 hour period before exposure or during the exposure period.	Requirement met. *(EPA/OECD requires: no feeding during the study)*
Recovery of chemical: Frequency of determination Level of Detection Level of Quantitation	 At 0 and 96 hours Not reported Limit of quantitation set at 0.0141 mg 7-OH metabolite of pyroxsulam/L from 20X AAP medium for the method validation.	Requirement considered met.
Positive control {if used, indicate the chemical and concentrations}	Positive control not used	
Other parameters, if any	None identified	

2. Observations:

Table 2. Observations

Parameter	Details	Remarks Criteria
Other observations, if any	The 120 mg 7-OH metabolite of pyroxsulam/L stock solution used to prepare the other test concentrations was stated to be clear and colourless and to have no visible undissolved test material after mixing. All test solutions were stated to be clear and colourless with no visible undissolved test substance.	Requirement considered met.
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortalities, biological observations, including adverse effects (e.g., darkened pig-mentation) of the exposed rainbow trout and observations of the physical characteristics of the test solutions (e.g., presence of precipitate, film on the solution's surface). Effects for the study were based on death, defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding).	Requirement considered met.
Observation intervals	0, 2, 3, 6, 24, 48, 72 and 96 hours	Requirement met. (EPA/OECD requires: minimally every 24 hours)
Water quality was acceptable (Yes/No)	Yes	Requirement considered met.
Were raw data included?	Tabulated mortality and behaviour data were presented. All original raw data, the protocol and the original final report produced during this study are archived by the Toxicology and Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan. A copy of the final report is retained at Springborn Smithers Laboratories,	The absence of raw data is not considered a deficiency even though US EPA OPPTS 850.1075 states that "Raw data must be available to support study author's conclusions and should be presented with the study report." (OPPTS 850.1075 (4) Observations (g) Data and reporting (2) Test report (xv)). This deficiency decision is on the basis of advice from the US EPA that tabulated results are considered sufficient as they allow recalculation of dose response if necessary.

	Wareham, Massachusetts.	

II. RESULTS and DISCUSSION:

A. MORTALITY:

There were no deaths in any control or treatment (Table 3).

Table 3. Effect of 7-OH metabolite of pyroxsulam on mortality of rainbow trout. Mean measured concentrations tested, corresponding cumulative percent and number of mortalities, and observations made during the 96-hour static acute exposure of rainbow trout (*Oncorhynchus mykiss*) to 7-OH metabolite of pyroxsulam.

Mean Measured Concentration, mg 7-OH metabolite of pyroxsulam/L	Number of fish at start of study	Cumulative percent mortality (number of dead fish)						
		2 hours	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Control (dilution water only)	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.8	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
14	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
29	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
57	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
120	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NOEC, mg 7-OH metabolite of pyroxsulam/L	120	120	120	120	120	120	120	120
LC_{50}, mg 7-OH metabolite of pyroxsulam/L	>120	>120	>120	>120	>120	>120	>120	>120
Positive control, if used, mortality LC_{50}:	Positive control not used							

Note: 95% confidence intervals for the LC50 could not be calculated because the corresponding LC50 values were empirically estimated.

Effects for the study were based on death, defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding).

Following 96 hours exposure no mortality was seen in any of the fish exposed to any treatment level or the control.

The study report concluded that, "Additional testing to further define the LC50 value was not performed since the highest nominal concentration tested exceeds the maximum test concentration required by the U.S. EPA and OECD guidelines (100 mg/L)."

B. NON-LETHAL TOXICITY ENDPOINTS:

There were no sub-lethal effects observed in any control or treatment (Table 4).

Table 4. Sub-lethal effect of 7-OH metabolite of pyroxsulam on rainbow trout. Mean measured concentrations tested, corresponding cumulative percent and number of sub-lethal effects observed during the 96-hour static acute exposure of rainbow trout (*Oncorhynchus mykiss*) to 7-OH metabolite of pyroxsulam.

Mean Measured Concentration, mg 7-OH metabolite of pyroxsulam/L	Number of fish at start of study	Cumulative percent of observed sub-lethal effects (number of affected fish)						
		2 hours	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Control (dilution water only)	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6.8	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
14	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
29	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
57	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
120	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NOEC/NOAEC, mg 7-OH metabolite of pyroxsulam/L		120	120	120	120	120	120	120
LOEC/LOAEC, mg 7-OH metabolite of pyroxsulam/L		>120	>120	>120	>120	>120	>120	>120
EC_{50}, mg 7-OH metabolite of pyroxsulam/L		>120	>120	>120	>120	>120	>120	>120
Positive control, if used, mortality LC_{50}:		Positive control not used						

Note: 95% confidence intervals for the EC50 could not be calculated because the corresponding EC50 values were empirically estimated.

C. REPORTED STATISTICS:

Mean-measured analyte concentrations over 96 hours were determined.

Due to the study results (absence of mortality and sub-lethal effects), the statistical evaluation of the biological data was not attempted. The 24-, 48-, 72-, and 96-hour LC50, EC50 and LOEC values were all empirically determined to be greater than the maximum mean measured concentration tested over 96 hours. The 96-hour NOEC was determined as the maximum mean measured concentration tested that exhibited no mortality or sub-lethal effects.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

The mortality and sub-lethal results reported support the study author's results and the non-use of statistical analyses.

Statistical Method: None used.

Statistical analysis was not considered necessary based on the absence of death and sub-lethal effects in the controls and test concentrations. The LC50, EC50, LOEC and NOEC values were estimated from visual inspection of the mortality and sub-lethal effects data.

E. STUDY DEFICIENCIES:

The following deficiencies or deviations from guidelines were noted but not considered to have significantly affected the study's outcome:

Table 5. Study deficiencies/deviations from the guidelines

Parameter	Study report result	Template reference to US/OECD Guideline	US EPA OPPTS 850.1075 Fish Acute Toxicity Test, Freshwater and Marine, April 1996	OECD Guideline 203 for Testing Chemicals, Fish Acute Toxicity Test, 203, adopted 17/07/92
Acclimation period	A minimum of 12 days before testing. During acclimatisation, the temperature of the holding tank was 11 to 13°C.	*EPA requires minimum 14 days; OECD requires minimum of 12 days*	A minimum 12–day acclimation period is required with 14 days recommended. A minimum of 7 days of the acclimation period must be performed in test dilution water. US EPA OPPTS 850.1075 states that fish should be acclimatized at the test temperature, which for rainbow trout is 12±2°C.	All fish must be obtained and held in the laboratory for at least 12 days before they are used for testing. OECD 203 prefers holding water to have temperature range of 13-17°C for rainbow trout. The temperature range of 11-13°C is not expected to have adversely affected the fish or the study outcome.
Hardness	Total hardness, as $CaCO_3$, of 44 to 58 mg/L	*(Hardness EPA : 40 - 48 mg as $CaCO_3$/L OECD: 10 -250* mg as $CaCO_3$/L	Hardness should range between 40 and 180 mg/L as $CaCO_3$ for freshwater species.	Waters with total hardness of between 10 and 250 mg $CaCO_3$ per liter.
pH	7.6 (in the test phase, the control pH ranged from 7.3 (0 hours) to 6.7 (96 hours). In the test solutions at 0 hours, the pH ranged from 6.4-7.3 and, at 96 hours, 6.4-6.7.	*EPA: 7.2 - 7.6 OECD: 6.0 - 8.5*	The pH must be monitored in low, medium, and high test concentrations and must remain > 6.0 and < 8.0 for freshwater testing.	Waters with a pH 6.0 to 8.5 are preferable.
Temperature	14 to 15°C	*Temperature: EPA: estuarine/marine: 22 ± 1°C OECD: 21 - 25°C for bluegill and 13 - 17°C for rainbow trout*	12 ± 2.0°C	13–17°C

This table shows that all but one of the deviations/deficiencies relate to either the template's references to EPA/OECD requirements (which the PMRA has advised separately are based on old guidelines and now not used with preference given to the OECD and more recent OPPTS guidelines).. In the case of temperature, the study was conducted at 14-15°C compared to the 10-14°C recommended by US EPA OPPTS 850.1075.

F. REVIEWER'S COMMENTS:

This study was conducted to determine the toxicity of the 7-OH metabolite of pyroxsulam to the rainbow trout. Based on mean measured concentrations over 96 hours and mortality and sub-lethal effects observed, the 96 hour LC50 and 96 hour EC50 are both determined as >120 mg 7-OH metabolite of pyroxsulam/L.

The in-life portion of the definitive toxicity test was conducted between March 10 and March 14, 2006.

Details of the analytical methodology used in the study to determine the concentrations of the analyte of concern were contained as an appendix to the report. Samples were withdrawn from the test solutions and after appropriate work-up, analysed by HPLC with UV detection. Conditions and procedures used in the analyses of the exposure solutions and quality control samples of the study were the same as those used in the method validation study.

Typical chromatograms of a calibration standard, a recovery sample and a control sample were presented. The absence of the 7-OH pyroxsulam in the control sample was verified while the presence of the 7-OH metabolite in the standard and sample was clearly demonstrated.

The validity criteria for OECD 203 (adopted 17.07.92) and US EPA OPPTS 850.1075 were considered to have been met by the study.

G. CONCLUSIONS:

This study is acceptable. The 96-h acute static toxicity study resulted in a 96 hour LC50 of the 7-hydroxy metabolite of pyroxsulam in rainbow trout of >120 mg 7-OH metabolite of pyroxsulam/L based on the mean analytically determined concentrations.

The 96 h EC50 for sub-lethal effects in rainbow trout was determined to have the same values as reported for the 96 h LC50, i.e. >120 mg 7-OH metabolite of pyroxsulam/L based on the mean analytically determined concentrations.

Consequently, the 7-OH metabolite of pyroxsulam is considered as practically non-toxic to the rainbow trout (96 hour LD50 and EC50 both >100 mg/L) based on mean measured concentrations of the 7-OH metabolite of pyroxsulam and according to the classification system of the Australian Government Department of the Environment and Water Resources.

The 96 hour NOEC and NOAEC (for mortality and sub-lethal effects as appropriate) were both set at 120 mg 7-OH metabolite of pyroxsulam/L based on mean analytically determined concentrations.

III. REFERENCES:

Note: for the purpose of this parallel process work, references to standard guidelines or methodologies have been included at this time in the list of references.

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(Note that Sayers (2006) refers to the cited study report (page 1 of this draft DER refers) which is not included in these references).

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Approved 04/01/01 C.K.