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

PMRA Submission Number 2006-4727 ID xxxx EPA MRID Number 469084-25 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: **ATSA, metabolite of pyroxsulam (XDE-742)** **Purity (%):** 100%

Common name: ATSA metabolite of pyroxsulam

Chemical name: 3-pyridinesulfonamide, N-(5-amino-1H-1,2,4-triazol-3-yl)-2-methoxy-4-(trifluoromethyl)

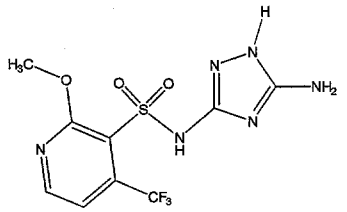
IUPAC: Not given

CAS name: Not given

CAS No.: Not stated

Synonyms: X11265218

Chemical Structure:



Test substance No.: TSN105493

Primary Reviewer: Daryl Murphy D. Murphy 22/02/08 **Date:** 18 January 2007
Australian Government Department of the Environment , Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: 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 T Steeger 4/13/08 **Date** 17 February 2006
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Anne Gosselin Emilie Lavoie for Anne Gosselin 05/03/08 **Date:** 26 April 2007
Pest Management Regulatory Agency, Health Canada

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

CITATION: Marino, T. A. Arnold, B. H. Sushynski, J. M. and Yaroch, A. M. 2006. ATSA Metabolite of XDE-742: An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID 061010. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 15 March 2006. Unpublished report.

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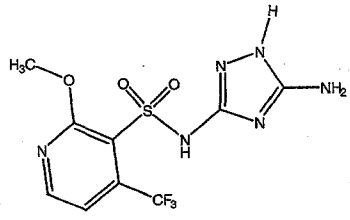

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Reference/Submission No.: APVMA ATS 40362 NCRIS 61286

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

CITATION: Marino, T. A. Arnold, B. H. Sushynski, J. M. and Yaroch, A. M. 2006. ATSA Metabolite of XDE-742: An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID 061010. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 15 March 2006. Unpublished report.

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EXECUTIVE SUMMARY:

In a 96 h acute toxicity limit study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to ATSA, a pyroxsulam metabolite, at 120 mg ATSA/L (nominal) and 119 mg ATSA/L (mean, measured) under static conditions. The 96 h EC_{50} and NOEC values, based on mortality/sub-lethal effects, were >120 mg ATSA/L (nominal) or >119 mg ATSA/L (mean, measured) and 120 mg ATSA/L (nominal) or 119 mg ATSA/L (mean measured), respectively. No sub-lethal effects were observed in the groups exposed to 120 mg ATSA/L (nominal). Based on the results of this study, ATSA would be classified as practically non-toxic to rainbow trout in accordance with the classification system of the US EPA and the Australian Government Department of the Environment and Water Resources (EC50 > 100 mg/L).

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

Results Synopsis
Test Organism Size/Age (mean wet weight or length):
Mean wet weight: 0.607 g (range 0.442-0.755 g)
Mean total length: 4.4 cm (range 4.0-4.6 cm)
Age: Juvenile
Test Type: Static over 96 hours

The following ecotoxicity endpoints are based on mean, measured concentrations.

96 h LC_{50}:	>119 mg ATSA/L;
95% C.I.:	Not applicable
96 h NOEC/NOAEC:	119 mg ATSA/L;
(mortality and sub-lethal effects as appropriate)	
Probit Slope:	Not applicable
96 h EC_{50}:	>119 mg ATSA/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 ATSA, a pyroxsulam (XDE-742) metabolite to rainbow trout (*Oncorhynchus mykiss*)
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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was stated to have generally conformed to current procedures described by

- the Organisation for Economic Cooperation and Development (OECD) guideline for testing of chemicals No. 203 "Fish Acute Toxicity Test",
- the Official Journal of European Communities Annex to Commission Directive 92/69/EEC, C.1 "Acute Toxicity Test for Fish", and
- the U.S. Environmental Protection Agency (U.S. EPA) Pesticide Assessment Guideline 72-1 and Standard Evaluation Procedure, "Acute Toxicity Test for Freshwater Fish".

Guidelines appear to have been generally followed with some minor deviations with respect to US EPA guidelines reported on occasion (see relevant text entries below and also the Study Deficiencies entry on page 14 of this draft DER).

COMPLIANCE: All phases of the study were stated to have been conducted in compliance with the following Good Laboratory Practice Standards:

- US Environmental Protection Agency – FIFRA GLPs Title 40 CFR, Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule,
- Organisation for Economic Co-Operation and Development (OECD) – OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles of Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17, and
- European Community (EC) – European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004).

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 ATSA, metabolite of pyroxsulam (XDE-742)

Description: Solid

Lot No./Batch No. : 035298-95

Purity: 100% active constituent

Stability of Compound Under Test Conditions: Analytical determinations over 4 days (see page 9 of this draft DER) in the present study indicated the ATSA metabolite was stable under the test conditions with the day 0 and day 4 mean concentration of

ATSA being 99.2% of nominal.

Storage conditions of test chemicals: Not identified in the study report but reported in the company's study profile template (Marino, 2006) as "Ambient room temperature".

Physicochemical properties of ATSA.

Parameter	Values	Comments
Water solubility at 20°C	Not available	Company's study profile template (Marino, 2006) stated that physicochemical properties of ATSA, a metabolite of pyroxsulam were not available at the time of publication of the company's study profile template document.
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: Juveniles (mean and range of ages not provided)
Weight at study initiation: Mean wet (blotted dry) weight = 0.607 g (range 0.442 to 0.755 g)
Length at study initiation: Mean total length = 4.4 cm (range 4.0 to 4.6 cm)
Note: Mean and range values obtained from 10 fish from the test population measured at day 0.
Source: Thomas Fish Company, Anderson, California.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A probe study was conducted between 09 January and 13 January 2006. One replicate of five fish per test concentration was exposed to nominal concentrations of 0 (water control), 12.0, 60.0, and 120 mg ATSA metabolite of pyroxsulam/L, over a 96 hour static exposure period. Following 96 hours of exposure, no fish mortality or sub-lethal effects were observed in the water control or any of the treatment vessels. Mild aeration was applied to each vessel during the exposure and appeared to have had no adverse impact on test solution concentrations, which remained stable during the study's conduct. Percent of target values for the measured test concentrations ranged from approximately 96-98% on day 0 and 86-87% on day 4.

Based on this information, the definitive study was conducted as a limit test with rainbow trout exposed to a nominal concentration of 120 mg ATSA Metabolite of pyroxsulam/L.

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation: Period: Conditions: (same as test or not)	 Greater than 14 days The study report indicates the waters used for fish testing and culturing were the same. The test lot used for the definitive study was acclimated to 13 ± 1°C for greater than 14 days prior to test initiation and temperature changes did not exceed ± 3°C in 72 hours pre-test. All fish were held on a 16-hour light/8-hour dark transitional photoperiod and observed for greater than 14 days before testing. No specific information was identified with respect to the pH of the water during the acclimatisation period. The company's study profile template (Marino, 2006) stated the conditions were the same as in the test.	See Study deficiencies/deviations table, page 14 of this draft DER. *(EPA requires minimum 14 days; no feeding during test; OECD requires minimum of 12 days)*
Feeding:	During holding, the fish received a standard diet (Aquatic Diet Number 1 Lot #233796, Harlan-Teklad, Madison, Wisconsin) typically once daily when not fasted for testing. Feed was withdrawn 48 hours before testing commenced.	
Health: (any mortality observed)	Mortality did not exceed three percent of the population in the 48 hour period before testing.	
Duration of the test	96 hours	Requirement considered met. *(EPA/OECD require 96 hour)*
Test condition:		Requirement met.

Parameter	Details	Remarks Criteria
Static/flow through Type of dilution system- for flow through method Flow rate Renewal rate for static renewal	Static (limit) test Not applicable Not applicable None	*(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)*
Aeration, if any	Mild aeration was applied to each test vessel during the exposure.	See Study deficiencies/deviations table, page 14 of this draft DER. *(EPA requires: no aeration; OECD permits aeration)*
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass cylindrical jars. All vessels were loosely covered with a glass plate lid and uniquely labelled for identification purposes. 17 L (30.5 X 30.5 cm) ~15 L	Requirement considered met. Fill volume considered to meet the lower limit of the US EPA requirement. *(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	The water used for fish testing and culturing (referred to in the study report as laboratory dilution water or LDW) was Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant. The water was obtained from the upper Saginaw Bay of Lake Huron off Whitestone Point.	Requirement considered met. Before use in the laboratory, the water was sand-filtered, pH-adjusted with gaseous CO_2, carbon-filtered, and UV-irradiated. *(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:		See Study deficiencies/deviations table, page 14 of this draft DER.
Hardness	68 mg $CaCO_3$/L in control water at day 0 and 72 mg $CaCO_3$/L in test solutions at day 0.	*Hardness* *EPA : 40 - 48 mg as $CaCO_3$/L* *OECD: 10 -250* mg as $CaCO_3$/L
pH	7.4-7.7 (control solutions, over 96 h) 6.7-7.7 (test solutions, over 96 h)	*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	The dissolved oxygen contents were: 9.3-11.6 mg/L (90-113% oxygen saturation) in the controls over 96 h 9.2-11.7 mg/L (89-114% oxygen saturation) in the test solutions over 96 h (Theoretical dissolved oxygen content at 13°C reported as 10.3 mg/L).	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	The temperature was 13°C in both control and test solutions over the 96 hours.	*Temperature:* *EPA: estuarine/marine: 22 ± 1 □C* *OECD: 21 - 25°C for bluegill and 13 - 17°C for rainbow trout*

Parameter	Details	Remarks Criteria
Total organic carbon Particulate matter (total suspended solids or TSS)	390 µg/L 2000 µg/L	OPTTS 850.1075 refers to testing water having, *inter alia*, a maximum TOC of 2.0 mg/L, a maximum particulate matter concentration of 20.0 mg/L.
Metals Pesticides	A table of inorganic analyses identified most metallic ions as being below their relevant detection levels as were anions such as bromide, chloride, nitrate etc. Where measurable concentrations were found (e.g. Al^{3+}, Ca^{2+}, Cl^- etc.), they had not been identified as of concern. A table of analysis of selected organic species and pesticides in laboratory water indicated all analytes measured were below their relevant limits of detection.	On occasion concentrations of inorganics such as aluminium, arsenic and other analytes were present in the laboratory water at concentrations exceeding the maximum levels set in OPPTS 850.1075. This may be consistent with the source of the water and, based on absence of death or sub-lethal effects in the control fish, not considered to have adversely affected the study.
Chlorine (residual)	Below detection limit of 20 µg/L	OPTTS 850.1075 refers to testing water having a residual chlorine content of 3 µg/L.
{Salinity for marine or estuarine species} Intervals of water quality measurement	Not applicable Dissolved oxygen, pH, and temperature data were measured daily in each test vessel. Water temperature was continuously monitored with a minimum/maximum thermometer placed in a surrogate test vessel and the range recorded daily. Light intensity was measured at each test vessel location on day 0. Water quality parameters such as hardness etc. were measured from a day 0 control and test solution.	*Salinity* *EPA: 30-34 % (parts per thousand) salinity, weekly range < 6 %)* *(EPA water quality: measured at beginning of test and every 48 hours).* The dilution water was typically monitored weekly for pH, alkalinity, conductivity, hardness, and residual chlorine. Periodically, the water was monitored for total organic carbon (TOC), total suspended solids (TSS), and selected inorganic and organic compounds.
Number of replicates/groups: Control (dilution water): Solvent control: Treatments:	Three control replicates (10 fish per replicate) Solvent control not used Three test replicates (10 fish per replicate)	Requirement considered met for a limit test. *(EPA/OECD requires: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geo-metric series)*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td><u>Number of organisms per replicate /groups:</u>

Control (dilution water):

Solvent control:

Treatments:</td><td>

10 fish/replicate
Not applicable

10 fish/replicate with only one test concentration, i.e. a 120 mg/L used (limit test).</td><td>Requirement met.

(EPA: □10/concentration); OECD requires at least 7 fish/concentration)</td></tr>
<tr><td>Biomass loading rate</td><td>0.4 g fish/L of test solution (mean fish weight of 0.607 X 10 fish/vessel all divided by 15 L (test solution volume)).</td><td>Requirement considered met.

(EPA: static: not exceeding 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)</td></tr>
<tr><td><u>Test concentrations:</u>

Nominal:

Measured:</td><td>0 (water control) and 120 mg ATSA/L (limit test, so only one test concentration examined)
Concentrations (as mg ATSA/L) recorded over the 96 hours were as shown in the following table:
<table><tr><th><u>Day 0</u></th><th colspan="3"><u>Replicate Number</u></th></tr><tr><td><u>Nominal</u></td><td><u>1</u></td><td><u>2</u></td><td><u>3</u></td></tr><tr><td><u>Control</u></td><td><u><LLQ</u></td><td><u><LLQ</u></td><td><u><LLQ</u></td></tr><tr><td><u>120 mg ATSA/L</u></td><td><u>118</u></td><td><u>118</u></td><td><u>118</u></td></tr><tr><td><u>Day 4</u></td><td colspan="3"></td></tr><tr><td>Control</td><td><LLQ</td><td><LLQ</td><td><LLQ</td></tr><tr><td>120 mg ATSA/L</td><td>119</td><td>118</td><td>119</td></tr></table>Note: <LLQ = less than the lowest level quantified; LLQ = 0.254 mg ATSA/L of laboratory dilution water.

The mean concentration of ATSA at day 0 was 118 mg/L (98.3% of nominal), and at day 4, 119 mg/L (99.2% of nominal) with a combined (day 0 and day 4) mean concentration of 119 mg/L</td><td>Requirement considered met for a limit test.

The test material (ATSA Metabolite of pyroxsulam) was a solid with a purity of 100% and no correction for purity was performed.

There was no evidence of incomplete dissolution of the test material was reported as absent following preparation of the test solutions.</td></tr>
</table>

Parameter	Details	Remarks Criteria
	or 99.2% of nominal.	
Solvent (type, percentage, if used)	Solvent not used	Requirement met. *(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	The photoperiod was set at 16 hours light/8 hours dark per day.	Requirement met. *(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod)*
Feeding	Fish were not fed during the test with feed withdrawn at least 48 hours before the testing commenced.	Requirement met. *(EPA/OECD requires: no feeding during the study)*
Recovery of chemical: Frequency of determination Level of Detection Level of Quantitation	 Measured at day 0 and day 4 Not reported Lowest level quantified was set at 0.254 mg ATSA metabolite of pyroxsulam/L LDW based on the concentration of analyte in the lowest standard analysed multiplied by the lowest dilution factor.	Requirement considered met. OECD 203 requires that there must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80 per cent of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20 per cent, results should be based on the measured concentration. OPPTS 850.1075 requires that there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period.
Positive control {if used, indicate the chemical and concentrations}	Positive control not used.	Requirement met.
Other parameters, if any	Light intensity (individual data not reported) ranged from 721-988 lux.	OPPTS 850.1075 refers to 30-100 Lumen.

2. Observations:

Table 2. Observations

Parameter	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects. Any behavioural effects *e.g.*, swimming at the surface, complete, or partial loss of body equilibrium, lethargy, hyperactivity, and erratic movement. were noted and reported as were any gross pathological conditions *e.g.*, exophthalmia (bulging eyes), ascites (fluid accumulation in the abdomen), hemorrhage (discharge of blood), excess mucus, sloughing of epidermis, and melanosis (increased amount of dark pigmentation).	Requirement considered met.
Observation intervals	At 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 on basis of water parameters reported and absence of mortality or sub-lethal effects in controls.
Were raw data included?	Tabulated mortality and behaviour results presented. The data, protocol, protocol changes/revisions, and final report are archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.	The absence of raw data is not considered a deficiency even though US EPA OPPTS 850.1075 requires 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 decision on the absence of a deficiency 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.
Other observations, if any	Evidence of incomplete dissolution of the test material was absent following preparation of the test solutions.	

II. RESULTS and DISCUSSION:

A. MORTALITY:

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

Table 3. Effect of ATSA, a metabolite of pyroxsulam, on mortality of rainbow trout (*Oncorhynchus mykiss*).

Treatment (mg ATSA/L) [record measured and nominal conc. used]	No. of fish at start of study	Observation period							
		24 h		48 h		72 h		96 h	
		Number Dead	% mortality	Number Dead	% mortality	Number Dead	% mortality	Number Dead	% mortality
Control (dilution water only), if used	30	0	0	0	0	0	0	0	0
Solvent control, if used	Not used								
Test concentration 120 mg ATSA/L (nominal) or 119 mg ATSA/L (mean, measured over 96 h)	30	0	0	0	0	0	0	0	0
NOEC		120 mg/L (nominal) or 119 mg/L (measured)		120 mg/L (nominal) or 119 mg/L (measured)		120 mg/L (nominal) or 119 mg/L (measured)		120 mg/L (nominal) or 119 mg/L (measured)	
LC_{50}		>120 mg/L (nominal) or >119 mg/L (measured)		>120 mg/L (nominal) or >119 mg/L (measured)		>120 mg/L (nominal) or >119 mg/L (measured)		>120 mg/L (nominal) or >119 mg/L (measured)	
Positive control, if used, mortality LC_{50}	Not used								

Mortality was defined as no response to touching of the caudal peduncle and no opercula movement. No mortality was recorded in any of the control or test solutions.

B. NON-LETHAL TOXICITY ENDPOINTS:

Sub-lethal effects were not recorded in any of the control or test solutions (Table 4).

Table 4. Sub-lethal effect of ATSA, a metabolite of pyroxsulam, on rainbow trout.

Treatment (mg ATSA/L) [record measured and nominal concentrations used]	Observation period			
	endpoints[a] at Day 1	endpoints at Day 2	endpoints at Day 3	endpoints at Day 4
	% affected	% affected	% affected	% affected
Control (dilution water only), if used	0	0	0	0
Solvent control, if used	Not used			
Test concentration 120 mg ATSA/L (nominal) or 119 mg ATSA/L (mean, measured over 96 h)	0	0	0	0
NOEC/NOAEC	120 mg/L (nominal) or 119 mg/L (measured)	120 mg/L (nominal) or 119 mg/L (measured)	120 mg/L (nominal) or 119 mg/L (measured)	120 mg/L (nominal) or 119 mg/L (measured)
LOEC/LOAEC	>120 mg/L (nominal) or >119 mg/L (measured)	>120 mg/L (nominal) or >119 mg/L (measured)	>120 mg/L (nominal) or >119 mg/L (measured)	>120 mg/L (nominal) or >119 mg/L (measured)
EC_{50}	>120 mg/L (nominal) or >119 mg/L (measured)	>120 mg/L (nominal) or >119 mg/L (measured)	>120 mg/L (nominal) or >119 mg/L (measured)	>120 mg/L (nominal) or >119 mg/L (measured)
Positive control, if used % sub-lethal effect: EC_{50}:	Not used			

a. Behavioural effects reported as including swimming at the surface, complete, or partial loss of body equilibrium, lethargy, hyperactivity, and erratic movement. Gross pathological conditions referred to were exophthalmia (bulging eyes), ascites (fluid accumulation in the abdomen), hemorrhage (discharge of blood), excess mucus, sloughing of epidermis, and melanosis (increased amount of dark pigmentation).

C. REPORTED STATISTICS:

Due to the study design (i.e., limit test) and 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 mean measured limit concentration tested. The 96 hour NOEC was determined based on the mean measured limit concentration tested exhibiting no mortality or sub-lethal effects.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Statistical Method: The lack of mortality and sub-lethal effects precluded the use of statistical analyses. All toxicity values were therefore determined visually based on the mean-measured concentration.

96 h LC_{50}:	>119 mg ATSA/L (mean, measured);
95% C.I.:	Not applicable
96 h NOEC (mortality and:	119 mg ATSA/L (mean, measured);

sub-lethal effects)
Probit Slope: Not applicable
96 h EC_{50}: >119 mg ATSA/L (mean, measured);
95% C.I.: Not applicable

E. STUDY DEFICIENCIES:
The following deviations from guidelines were noted but not considered to have significantly affected the study's outcome:

Table 5. Study deficiencies/deviations from 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 for Testing Chemicals, Fish, Acute Toxicity Test, 203, adopted 17/07/92
Acclimation:	Acclimatisation temperature 13±1°C.		US EPA OPPTS 850.1075 states that fish should be held for a minimum of 7 days at the test temperature prior to testing. For rainbow trout, this is 12±2.0°C.	OECD 203 prefers holding water to have a temperature range of 13-17°C for rainbow trout. The temperature range of 13±1°C is not expected to have adversely affected the fish or the study outcome.
Water parameters: Hardness	68 mg $CaCO_3$/L in control water at day 0 and 72 mg $CaCO_3$/L in test solutions at day 0	*Hardness* *EPA : 40 - 48 mg as $CaCO_3$/L* *OECD: 10 -250* mg as $CaCO_3$/L NOTE: PMRA advised that the 40-48 mg/L range in the template is outdated as it is from an old guideline. PMRA do not use that range any more; instead referring to OECD and the more recent OPPTS guidelines.	Hardness should range between 40 and 180 mg/L as CaCO3 for freshwater species.	Hardness should range between 10 and 250 mg CaCO3/L).
pH	7.5-7.7 (control solutions, over 96 h) 6.7-7.7 (test solutions, over 96 h)	*pH (EPA: 7.2 - 7.6; OECD: 6.0 - 8.5*	pH must remain > 6.0 and < 8.0 for freshwater testing	A pH of 6.0 to 8.5 is preferable.
Aeration	Mild aeration applied to the test vessels during the exposure.	*EPA requires: no aeration; OECD permits aeration.*	Gentle aeration of test vessels used in static systems during the exposure period	Aeration can be used provided that it does not lead to a significant loss of

			is permitted only in cases where oxygen levels are in danger of dropping below 60 percent saturation due to chemical characteristics of the test material. This was not indicated as happening in the exposure period.	test substance.

F. REVIEWER'S COMMENTS: This study was conducted as a limit test with a nominal concentration of 120 mg ATSA/L with a mean-measured concentration over 96 hours of 119 mg ATSA/L. Consequently, ATSA, a pyroxsulam metabolite is considered as practically non-toxic to the rainbow trout (96 hour LC50 and EC50 both >100 mg/L) based on mean measured concentrations of ATSA.

The in-life portion of the definitive toxicity test was conducted between January 30, 2006 and February 3, 2006.

In the study under assessment, details of the analytical methodology used to determine the concentrations of ASTA, aliquots were withdrawn from the test solutions and after appropriate work-up, analysed by HPLC with UV detection. None of the LDW controls exhibited a peak eluting at the retention time of ATSA metabolite of pyroxsulam at a concentration exceeding the lowest level quantified of 0.254 mg pyroxsulam/L LDW, which was the concentration of analyte in the lowest standard analysed times the lowest dilution factor. Typical chromatograms of a control, a standard and a sample were presented which confirmed the absence of pyroxsulam in the controls and clearly identified its presence in the standard and sample.

The HPLC/UV instrumentation exhibited a linear response over a concentration range of 0.231 to 57.7 mg ATSA metabolite of pyroxsulam/L diluent on both days 0 and 4.

The validity criteria for OECD 203 (adopted 17.07.920 and US EPA OPPTS 850.1075 were considered to have been met by the study and the study deficiencies or deviations from the guidelines identified are not considered to have adversely affected the study or its outcomes.

G. CONCLUSIONS: This study is acceptable. The 96 h acute static toxicity (limit test) study resulted in the 96 hour LC50 and EC50 of ATSA, a metabolite of pyroxsulam, in rainbow trout both being set at >120 mg ATSA/L based on nominal concentration of the test substance and >119 mg/L based on the mean analytically determined concentration.

The 96 hour NOEC and NOAEC (for mortality and sub-lethal effects as appropriate) were both determined as 120 mg ATSA/L based on nominal concentrations and 119 mg ATSA/L based on mean analytically determined concentration of ATSA.

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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Approved 04/01/01 C.K.