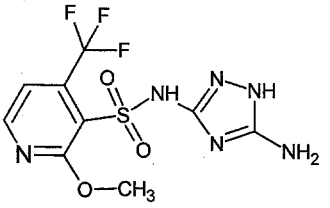
Data Evaluation Report on the acute toxicity of ATSA metabolite of XDE-742 (Pyroxsulam) to aquatic vascular plants *Lemna gibba*

PMRA Submission Number2006-4727; ID 1283262 **EPA MRID Number{.....}** 468084-52

Data Requirement: PMRA DATA CODE: 9.8.5
EPA DP Barcode:
OECD Test Guideline: IIA 8.6
EPA Guideline: 123-2 (OPPTS 850.4400 (Draft April 1996))

Test material: ATSA Metabolite of pyroxulam **Purity (%):** 100%
Common name: ATSA Metabolite of XDE-742
Chemical name: 3-pyridinesulfonamide, N-(5-amino-1H-1,2,4-triazole-3-yl)-2-methoxy-4-(trifluoromethyl)
ID No: TSN 105493
Lot No. 035298-95
CAS No.: Not available
Synonyms: X11265218
Chemical structure



Primary Reviewer: Chris Lee-Steere **Date:** 5 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Secondary Reviewers: Jack Holland **Date:** 23 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts
Brian Kiernan Date: 22 August 2007
US Environmental Protection Agency
PMRA Reviewer: Émilie Larivière **Date:** 30 July 2007
Environmental Assessment Directorate, PMRA

Reference/Submission No.: APVMA ATS 40362 NCRIS 61286

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

CITATION: G. A. Hancock, M.S., B. H. Arnold, B.S., J. R. Najar, B.S., J. M. Sushynski (24 February 2006). ATSA METABOLITE OF XDE-742: Growth Inhibition Test with the Aquatic Plant Duckweed, *Lemna gibba*. Toxicology & Environmental Research and Consulting, The

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Dow Chemical Company, Midland, Michigan. Study ID: 061006, (24 February 2006). Unpublished.

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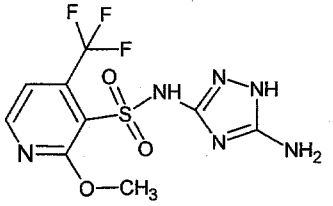

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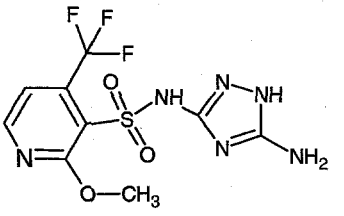

Primary Reviewer: Chris Lee-Steere **Date:** 5 July 2007
Australian Government Department of the Environment and Water Resources

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Plant Duckweed, *Lemna gibba*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Study ID: 061006, (24 February 2006). Unpublished.

EXECUTIVE SUMMARY:

In a 7 day acute toxicity study, freshwater floating aquatic vascular plants (duckweed, *Lemna gibba*) were exposed to pyroxsulam ATSA metabolite at nominal concentrations of 0, 0.112, 0.358, 1.14, 3.66, 11.7, 37.5, and 120 mg ac/L. Mean measured concentrations were 0, 5 0.105, 0.338, 1.09, 3.52, 11.4, 37.1, and 120 mg ac/L. The study was conducted under static renewal conditions at days 3 and 5 in accordance with the guidelines, OECD 221 "Lemna sp. Growth Inhibition Test" (draft, 2002) and US EPA guidelines including U.S. Environmental Protection Agency (1996). Ecological Effects Test Guidelines. OPPTS 850.4400 Aquatic Plant Toxicity Test using Lemna sp., Tiers I and II. Draft April 1996.

The % inhibition was determined for frond number, mean specific growth rate and biomass (frond dry weight). No biological endpoint showed statistically significant inhibition at any concentration tested. A dose-response relationship was not observed for any endpoint. The 7-day NOECs based for all endpoints was 120 mg ac/L, the highest rate tested, and the 7-d LOECs for all endpoints was >120 mg ac/L (mean measured concentration). The 7-d EC50 based on all endpoints was >120 mg ac/L.

At the end of the study, fronds at all exposure concentrations were observed to be normal.

This toxicity study is classified as acceptable and satisfies the guideline requirement for an acute toxicity study with the aquatic vascular plants *Lemna gibba* (duckweed).

Results Synopsis

Test Organism: Duckweed (*Lemna gibba*)

Test Type (Flow through, Static, Static Renewal): Static renewal

Endpoint	Frond No.	Growth rate	Frond dry weight
NOEC (mg ac/L)	120	120	120
IC50 or EC50 (mg ac/L) (95% C.I.)	>120	>120	>120

No 95% confidence intervals associated with EC_{50} values as these were determined empirically.

Endpoint(s) Effected: No biological endpoint tested in this study was adversely affected.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study generally conformed to procedures described by the Organisation for Economic Cooperation and Development (OECD) draft guideline (at April 2005 and finalised in March 2006):

- Organisation for Economic Co-Operation and Development (2002). OECD Guidelines for the Testing of Chemicals. *Lemna* sp. Growth Inhibition Test. Proposed Guideline 221. Revised Draft July 2002.

and the following U.S. Environmental Protection Agency guidelines:

- U.S. Environmental Protection Agency (1996). *Ecological Effects Test Guidelines*. OPPTS 850.4400 Aquatic Plant Toxicity Test using *Lemna* sp., Tiers I and II. Draft April 1996.
- U.S. Environmental Protection Agency (1982). *Pesticide Assessment Guidelines,* Subdivision J Hazard Evaluation: Non-target Plants, Guideline 123-2, EPA 540/9-82-020, Washington, D.C.
- U.S. Environmental Protection Agency (1986). Hazard Evaluation Division: Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants Tiers 1 and 2. EPA 540/9-86-134, Washington, D.C.

This DER has assessed the study report against the OECD 221 and US EPA OPPTS 850.4400 requirements.

No deviations from the protocol are noted in the study report:

COMPLIANCE: All phases of the study were reported as conducted in compliance with the following Good Laboratory Practice Standards:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENVIMCICHEM (98) 1 7;
- European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004); and
- U.S. Environmental Protection Agency - FIFRA GLPs, Title 40 CFR, Part 160- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Good Laboratory Practice Standards, Final Rule.

Signed and dated Compliance with Good Laboratory Practice Standards, Quality Assurance and No Data Confidentiality Claims statements were provided.

A. MATERIALS:

1. Test Material ATSA Metabolite of pyroxsulam

Description: Solid

Lot No./Batch No.: 035298-95

Purity: 100% (Certificate of analysis provided)

Stability of Compound Under Test Conditions:

The 7-day stability of ATSA Metabolite of pyroxsulam in 75% Milli-Q® water/25% acetonitrile + 0.1% phosphoric acid (diluent) was determined during a related study. The solutions were analyzed after being stored for 7 days at ~ -80°C. The analyzed concentrations of the stability test solutions were within 2% of the initial measured concentrations. During this study, the bulk dose solutions were sampled for analytical confirmation on days 0, 3, and 5 of immediately following preparation. On days 3, 5, and 7 the test solutions containing duckweed at each dose level were pooled to provide one composite duckweed containing sample per dose level for analytical confirmation, while the test solutions at each dose level containing no duckweed were sampled separately. The following results were found using bulk dose solutions and spent exposure solutions containing duckweed:

Table 1: Measured concentrations of ATSA Metabolite of pyroxsulam

Nominal Concentration (mg ac/L)	Measured Concentration (mg ac/L)					% of Nominal
	0 hour (new)	Day 3 (new/aged)	Day 5 (new/aged)	Day 7 (aged)	Mean (SD)	
Control					Not applicable	Not applicable
0.112	0.100	0.103/0.0969	0.127/0.0968	0.104	0.105	93.7
0.358	0.336	0.340/0.319	0.354/0.334	0.343	0.338	94.3
1.14	1.09	0.11/1.05	1.13/1.07	1.11	1.09	95.9
3.66	3.52	3.59/3.37	3.64/3.47	3.54	3.52	96.2
11.7	11.3	11.5/11.0	11.6/11.3	11.4	11.4	97.0
37.5	37.6	37.0/36.4	37.2/37.2	37.4	37.1	99.0
120	117	119/118	120/120	123	120	99.6

There are no stability data for the test substance under light.

Storage conditions of test chemicals: Test solutions were prepared fresh on days 0, 3 and 5. Standards were prepared prior to testing, frozen at ~-80°C and thawed prior to analysis on days 0, 3 and 5.

Physicochemical properties of ATSA metabolite of pyroxsulam:

None available at the time of testing.

2. Test organism:

Name: Duckweed (*Lemna gibba*)
Strain, if provided: G3
Source: Axenic samples of this species were received in May of 1999 from USDA/ARS Beltsville Agricultural Research Center, Beltsville, Maryland. Stock cultures of this organism were maintained axenically by periodic transfer into fresh medium.
Method of cultivation: Typical culturing conditions are described in the report. *Lemna gibba* is cultured in 500 mL Erlenmeyer flasks maintained in an environmental chamber with temperature 25±2°C. A continuous photoperiod is maintained with light intensity of 5400±1100 lux. The growth medium is modified (20X) AAM (described further below) with a pH range of 7.5-8.5. Inoculation is every seven days.
Age of inoculum: The fronds used to initiate the test were taken from a stock culture that had been transferred to fresh medium twelve days prior to testing.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The exposure levels selected for evaluating the effects of ATSA Metabolite of pyroxsulam on the growth of duckweed were based on the results of a probe study. The exposure phase of the probe was conducted as a seven-day static-renewal exposure (renewals on days 3 and 5) from 13 January to 20 January 2006 using five nominal concentrations of 0.00120, 0.0120, 0.120, 1.20, and 12.0 mg ATSA Metabolite of pyroxsulam/L plus a medium control. Percent inhibition of frond growth compared to controls on Day 7 was 11, 5, 8, 18, and 18% for the 0.00120, 0.0120, 0.120, 1.20, and 12.0 mg ATSA Metabolite of pyroxsulam/L test levels, respectively. The percent inhibition compared to the medium control based on the growth rate parameter on Day 7 was 4, 2, 3, 6, and 7% for the 0.00120, 0.0120, 0.120, 1.20, and 12.0 mg/L test concentrations, respectively. Based on this, the empirically derived EC50 values for frond density and specific growth rate were >12.0 mg/L. As a result, the definitive study was conducted under static renewal exposure conditions (renewals on days 3 and 5) at target ATSA Metabolite of pyroxsulam concentrations of 0 (control, 20X AAM medium), 0.112, 0.358, 1.14, 3.66, 11.7, 37.5, and 120 mg ATSA Metabolite of pyroxsulam/L 20X AAM.

b) Definitive Study

Table 2: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period:	The fronds used to initiate the test were taken from a stock culture that had been transferred to fresh medium twelve days prior to testing.	Transfer time was slightly longer than the maximum 10 days stated in the OECD guideline, but within the 2 week time frame provided in the US EPA guideline. *OECD: at least seven days before testing, sufficient colonies are transferred aseptically into fresh sterile medium and cultured for 7-10 days under the conditions of the test.*
Culturing media and conditions: (same as test or not)	Stock cultures were grown in 500-mL in modified (20X) AAM The cultures were maintained under conditions similar to those used in the test	
Health: (any toxicity observed)	No specific comment found in the test report but the stock cultures used were maintained axenically by weekly transfer into fresh medium.	*EPA: axenic stock cultures should be grown in the aquariums for 2 weeks (with necessary transfers) prior to being used in a test. Inocula should be taken from cultures which are less than 2 weeks old.*
Test system Static/static renewal/ Renewal rate for static renewal:	Static renewal Renewals on days 3 and 5.	Requirement considered met. *EPA: Renewals (transfer of colonies to test solution) should occur on days 3 and 5.* *OECD: When using a semi-static test regime the colonies should be exposed to freshly prepared test and control solutions on at least two occasions during the test (e.g. days 3 and 5). The frequency of exposure to fresh medium will depend on the stability of the test substance.*
Incubation facility	The incubator was thermostated at 25 ± 2°C with continuous light at approximately 6600 ± 990 lux.	Requirement considered met. *OECD: temperature in the test vessels should be 24 ± 2°C and refers to use of a growth chamber*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td></td><td>incubator.

EPA: temperature should be maintained at 25 ± 2°C and that a controlled environment growth chamber or an enclosed area capable of maintaining the specified number of test chambers and test parameters is required.</td></tr>
<tr><td>Duration of the test</td><td>7 days.</td><td></td></tr>
<tr><td><u>Test vessel</u>

Material: (glass/polystyrene)

Size:
Fill volume:</td><td>

Borosilicate crystallizing dish with cover
270 mL
100 mL</td><td>Requirement considered met.

OECD: glass beakers, crystallising dishes or glass petri dishes of appropriate dimensions have all proved suitable. This guideline also states the test vessels must be covered. A minimum depth of 20 mm and minimum volume of 100 mL in each test vessel.

EPA: test containers being glass beakers or Erlenmeyer flasks. Containers should be large enough to contain 150 mL of test solution, or enough test solution to result in a volume to-vessel size ratio of 2:5</td></tr>
<tr><td><u>Details of growth medium</u>
Name:</td><td>20 Algal Assay Medium (AAM) as follows:
<table><tr><th>Compound</th><th>Final concentration (mg/L)</th></tr><tr><td>$NaNO_3$</td><td>510</td></tr><tr><td>$MgCl_2 \cdot 6H_2O$</td><td>243</td></tr><tr><td>$CaCl_2 \cdot 2H_2O$</td><td>88.0</td></tr><tr><td>$MgSO_4 \cdot 7H_2O$</td><td>294</td></tr><tr><td>K_2HPO_4</td><td>20.9</td></tr><tr><td>$NaHCO_3$</td><td>300</td></tr><tr><td>H_3BO_3</td><td>3.72</td></tr><tr><td>$MnCl_2 \cdot 4H_2O$</td><td>8.32</td></tr><tr><td>$ZnCl_2$</td><td>0.0654</td></tr><tr><td>$CoCl_2 \cdot 6H_2O$</td><td>0.0286</td></tr><tr><td>$CuCl_2 \cdot 2H_2O$</td><td>0.00022</td></tr><tr><td>$Na_2MoO_4 \cdot 2H_2O$</td><td>0.145</td></tr><tr><td>$FeCl_3 \cdot 6H_2O$</td><td>3.2</td></tr><tr><td>$Na_2EDTA \cdot 2H_2O$</td><td>6.0</td></tr></table></td><td>The composition of 20 AAM used in the study is essentially the same as 20 AAP defined in the OECD Guideline with only very minor differences.
pH of the control medium increased by 1.6 units over the course of the study.
EPA: refers to use of 20X-AAP medium but does not provide the constituents or their percentages.

OECD: For L. gibba, the OECD guideline recommends use of 20 AAP growth medium with the composition well defined in the guideline.</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
pH at test initiation: pH at test termination: Chelator used: Carbon source:	7.6 (control medium) 9.2 (control medium) EDTA. Not reported.	*OECD: The pH of the control medium should initially be 7.5±1 and should not increase by more than 1.5 units through the test.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	The nutrient medium was fully characterized.	Requirement considered met.
<u>Dilution water</u> Source/type: pH: Total Organic Carbon: Particulate matter: Metals: Pesticides: Chlorine: Water pretreatment (if any): Intervals of water quality measurement	 The medium was prepared using deionized water. The source water for the deionized water was Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant. Reported above for control medium. The pH was adjusted to 7.5 prior to adding test substance. Not reported. Not reported. Not reported. Not reported. Not reported. Deionized water used. Measurements (pH) were made on days 0, 3 and 5 (new solutions) and days 3, 5 and 7 (old solutions).	 Requirements considered met. OECD 221 and US EPA OPPTS 850.4400 do not address these parameters specifically (other than pH). *OECD: The pH should be measured in each batch of 'fresh' test solution prior to each renewal and also in the corresponding 'spent' solutions.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Test solutions were prepared on days 0, 3 (renewal), and 5 (renewal). The high-dose test solution was prepared by direct addition of the test material to 20X AAM medium. To prepare the 120 mg/L test solution, a target aliquot of 120 mg (no adjustment for purity, 100%) of ATSA Metabolite of	

Parameter	Details	Remarks *Criteria*
	pyroxsulam was weighed out and added to a 1000-mL volumetric flask containing some 20X AAM. The flask was stoppered and shaken vigorously to thoroughly mix. Due to the presence of undissolved test material, these bulk high-dose test solutions were sonicated briefly (approximately three to four minutes) and subsequently appeared clear. The remaining test solutions (37.5, 11.7, 3.66, 1.14, 0.358, and 0.112 mg ATSA Metabolite of pyroxsulam/L) were prepared by serial dilution.	
Aeration or agitation	No aeration or agitation was reported.	Requirements considered met. OECD 221 and US EPA OPPTS 850.4400 do not specifically refer to aeration or agitation. OECD 221 notes that test vessels must be covered to minimise evaporation and accidental contamination, while allowing necessary air exchange.
Sediment used **(for rooted aquatic vascular plants)**	Not applicable.	
Number of replicates Control: Solvent control: Treatments:	 7 Not applicable. 4 (1 serving as a blank).	Requirement considered met. *OECD: at least 3 replicates should be used for each test concentration. The number of replicate control vessels should be at least equal to, and ideally twice, the number of vessels used for each test concentration.* *US EPA: for each concentration and control at least three replicate containers should be used.*
Number of plants/replicate	3	Requirements considered met. *OECD: each test vessel should*

Parameter	Details	Remarks *Criteria*
		contain a total of 9 to 12 fronds. The number of fronds and colonies should be the same in each test vessel. *EPA: 3 to 5 plants per replicate.*
Number of fronds/plant	4	Requirements considered met. *OECD: colonies consisting of 2 to 4 visible fronds are transferred from the inoculum culture and randomly assigned to the test vessels under aseptic conditions. Each test vessel should contain a total of 9 to 12 fronds.* *EPA: 3 to 4 fronds per plant.*
Test concentrations Nominal (mg ac/L): Measured (mg ac/L):	 0, 0.112, 0.358, 1.14, 3.66, 11.7, 37.5, 120. 0, 0.105, 0.338, 1.09, 3.52, 11.4, 37.1, 120.	Seven concentrations were tested in geometric series of (nominal) ~3.2. *OECD: in the definitive test, there should normally be at least five test concentrations arranged in a geometric series. Preferably the separation factor between test concentrations should not exceed 3.2.* *EPA at least five concentrations of chemical, exclusive of controls, should be used in the definitive test and chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, 64 mg/L).*
Solvent (type, percentage, if used)	Not applicable.	
Method and interval of analytical verification: Limit of Quantitation:	Bulk dose solutions on days 0, 3, and 5; Spent exposure and blank solutions on days 3, 5, and 7 using high performance liquid chromatography with an ultraviolet absorbance detector (HPLC/UV) and external standard quantitation. 0.0124 mg/L	The requirements are considered met. The variability associated with the analytical method, as well as solution homogeneity, was assessed by analyzing four replicate samples that were collected from the day 0 bulk dose solutions at nominal concentrations of 0.112 and 120 mg/L. Four repeated measurements (4 samples x 1 injection/sample)

Parameter	Details	Remarks *Criteria*
Limit of Detection:	Not reported.	resulted in percent relative standard deviation (%RSD) values of 2.01% and 1.10%, respectively (data not shown in test report).
Test conditions Temperature: Photoperiod: Light intensity and quality:	 23.5-25.8°C Continuous illumination. 6490 ± 285 lux	Light intensity was satisfactory based on EPA guidance, marginally lower than the recommended range for OECD guidance. *OECD: temperature in the test vessels should be 24 ± 2°C with light intensity equivalent to 6500 to 10000 lux. Photoperiod not specified.* *EPA: environmental conditions should be maintained at 25 ± 2°C. Continuous warm-white fluorescent lighting should be used to provide a light intensity in the range of 4,200 and 6,700 lux*
Reference chemical (if used)	Not applicable.	Requirement considered met. There is no specific requirement to run a reference chemical test in conjunction with the test substance. It is unlikely that provision of the results from the most recent reference chemical study would have added any further value to interpretation of this test report. *OECD: a reference substance such as 3,5-dichlorophenol used in the international ring test may be tested as a means of checking the test procedure. It is advisable to test a reference substance at least twice a year or, where testing is carried out at a lower frequency, in parallel to the determination of the toxicity of a test substance.* *EPA: positive controls using zinc chloride as a reference chemical should be run periodically.*
Other parameters, if any	None.	

2. Observations:

Table 3: Observation parameters

Parameters	Details	Remarks *Criteria*
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Frond number, frond dry weight, general health observations; growth rate derived from frond number	Requirement considered met. *OECD: Frond numbers are the primary parameter measured In addition to determinations of frond number during the test, effects of the test substance on one or more of total frond area, dry weight or fresh weight are also assessed.* *EPA: observations of frond numbers and appearance should be made of the colonies on day 0, 3, 5, and 7 and refers to other (optional) growth inhibition endpoints such as chlorophyll values and biomass (dry weight at 60°C) at the end of the test.*
Measurement technique for frond number and other end points	For quantitative purposes and for statistical evaluation, a count of the total number of fronds was taken of each replicate on days 0, 3, 5, and 7. Every frond visibly projecting beyond the edge of the parent frond was counted. At test termination, the fronds for each replicate were placed on a predried, preweighed weigh boat. The fronds were dried for at least 48 hours at approximately 60°C in a drying oven. After drying, the weight of the fronds plus the weigh boat for each replicate was recorded.	Requirement considered met. *OECD: Dry weight measurement - All colonies are collected from each of the test vessels and rinsed with distilled or deionised water. They are blotted to remove excess water and then dried at 60°C to a constant weight.*
Observation intervals	Days 0, 3, 5 and 7 (frond numbers); Day 7 (frond dry weight).	Requirement considered met.
Other observations, if any	Frond appearance was observed at intervals measuring frond numbers. At all observation	

	periods, all fronds were noted as normal.	
Indicate whether there was an exponential growth in the control	Yes. Mean frond numbers in the control groups 263 after 7 days, or around a 22 fold increase over the test period. The average growth rate over the 7 day test period in the control was 0.441 d^{-1}.	Requirement considered met. *OECD: For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}".* *EPA: No specific requirements identified.*
Water quality was acceptable (Yes/No)	Not specifically recorded in the test report but the successful control growth indicates the quality was acceptable.	Requirement considered met.
Were raw data included?	Yes, in transcribed form. Results for test end-points were provided in tabulated form and included results for individual replicates. Test condition parameters (pH, temperature and light intensity) were provided in tabulated form.	Requirement considered met. The transcribed data provided correspond to the OECD description of raw data requirements. While the EPA guideline does not comment on raw data, the reporting requirements outlined in this guideline were met *OECD: raw data: number of fronds and other measurement variables in each test and control vessel at each observation and occasion of analysis.* *EPA: No comment on raw data.*

II. RESULTS AND DISCUSSION:

A. INHIBITORY EFFECTS:

The raw data for frond density, growth rate, and biomass (dry weight) met the assumptions of homogeneity of variance and normality. Dunnets test (• = 0.05) was used to determine treatment-related effects.

No inhibitory effects were found for any biological end-points evaluated in this study. For all biological end-points, the 7-day NOEC and LOEC were determined to be 120 and >120 mg ac/L respectively.

At test termination, fronds exposed to all treatment levels were observed to be normal.

The pH values ranged from 7.3 to 8.0 in bulk exposure solutions, from 8.5 to 9.2 in pooled replicates of spent exposure solutions with duckweed, and from 8.4 to 8.5 in spent solutions (blank replicates) without duckweed.

The frond counts from days 0 to 7 plus the calculated % inhibition compared to control counts, as given in the study report, are shown in Table 4. The growth rates for days 0-3, 0-5 and 0-7 plus the calculated % inhibition compared to control growth rates as given in the study report, are shown in Table 5. The day 7 frond dry weights plus the calculated % inhibition compared to control frond dry weight, as given in the study report, are shown in Table 6.

Table 4: Effect of ATSA metabolite of pyroxsulam on frond number of Duckweed (*Lemna gibba*).

		Frond Number at:				
Mean Measured Concentration (mg ac/L)	Replicate No.	Day 0	Day 3	Day 5	Day 7	7 Day Inhibition (%) compared to control
Control	1	12	53	126	274	
	2	12	51	143	276	
	3	12	52	128	259	
	4	12	48	122	260	
	5	12	46	112	233	
	6	12	53	90	273	
	Mean	**12**	**51**	**120**	**263**	**Not applicable**
	SD	**0**	**3**	**18**	**16**	
0.105	7	12	51	126	253	
	8	12	51	128	252	
	9	12	49	117	246	
	Mean	**12**	**50**	**124**	**250**	**5**
	SD	**0**	**1**	**6**	**4**	
0.338	10	12	52	121	258	
	11	12	55	126	259	
	12	12	46	108	229	
	Mean	**12**	**51**	**118**	**249**	**5**
	SD	**0**	**5**	**9**	**17**	
1.09	13	12	58	127	261	
	14	12	53	120	243	
	15	12	44	114	241	
	Mean	**12**	**52**	**120**	**248**	**5**
	SD	**0**	**7**	**7**	**11**	
3.52	16	12	52	117	248	
	12	12	48	111	234	
	18	12	48	114	255	
	Mean	**12**	**49**	**114**	**246**	**6**
	SD	**0**	**2**	**3**	**11**	
11.4	19	12	49	116	251	
	20	12	51	120	234	
	21	12	49	109	247	
	Mean	**12**	**50**	**115**	**244**	**7**
	SD	**0**	**1**	**6**	**9**	
37.1	22	12	55	130	276	
	23	12	53	135	271	
	24	12	44	112	251	
	Mean	**12**	**51**	**126**	**266**	**-1**
	SD	**0**	**6**	**12**	**13**	
120	25	12	60	139	269	
	26	12	56	128	259	
	27	12	58	142	287	
	Mean	**12**	**58**	**136**	**272**	**-3**
	SD	**0**	**2**	**7**	**14**	

Table 5: Effect of ATSA metabolite of pyroxsulam on Growth Rate of Duckweed (*Lemna gibba*).

Mean Measured Concentration (mg ac/L)	Replicate No.	7 day specific growth rate (d^{-1})	7 Day Inhibition (%) compared to control
Control	1	0.447	
	2	0.448	
	3	0.439	
	4	0.439	
	5	0.424	
	6	0.446	
	Mean	**0.441**	**Not applicable**
	SD	**0.009**	
0.112	7	0.435	
	8	0.435	
	9	0.431	
	Mean	**0.434**	**1**
	SD	**0.002**	
0.358	10	0.438	
	11	0.439	
	12	0.421	
	Mean	**0.433**	**2**
	SD	**0.010**	
1.41	13	0.440	
	14	0.430	
	15	0.429	
	Mean	**0.433**	**2**
	SD	**0.006**	
3.66	16	0.433	
	12	0.424	
	18	0.437	
	Mean	**0.431**	**2**
	SD	**0.006**	
11.7	19	0.434	
	20	0.424	
	21	0.432	
	Mean	**0.430**	**2**
	SD	**0.005**	
37.5	22	0.448	
	23	0.445	
	24	0.434	
	Mean	**0.443**	**0**
	SD	**0.007**	
120	25	0.444	
	26	0.439	
	27	0.454	
	Mean	**0.446**	**-1**
	SD	**0.007**	

Table 6: Effect of ATSA metabolite of pyroxsulam on Frond Dry Weight Duckweed (*Lemna gibba*).

Mean Measured Concentration (mg ac/L)	Frond weight (mg)		
	Replicate No.	Day 7	7 Day Inhibition (%) compared to control
Control	1	29.94	
	2	30.38	
	3	26.71	
	4	28.25	
	5	25.77	
	6	29.73	
	Mean	**28.30**	**Not applicable**
	SD	**1.77**	
0.112	7	28.68	
	8	26.68	
	9	26.80	
	Mean	**27.39**	**3**
	SD	**1.12**	
0.358	10	30.43	
	11	27.52	
	12	26.66	
	Mean	**28.20**	**0**
	SD	**1.98**	
1.41	13	28.87	
	14	27.21	
	15	27.37	
	Mean	**27.82**	**2**
	SD	**0.92**	
3.66	16	28.69	
	12	26.82	
	18	25.67	
	Mean	**27.06**	**4**
	SD	**1.52**	
11.7	19	27.57	
	20	24.70	
	21	26.56	
	Mean	**26.82**	**7**
	SD	**1.46**	
37.5	22	30.76	
	23	27.97	
	24	27.07	
	Mean	**28.60**	**-1**
	SD	**1.92**	
120	25	29.19	
	26	27.84	
	27	32.40	
	Mean	**29.81**	**-5**
	SD	**2.34**	

Table 7: Statistical endpoint values.

Statistical Endpoint	Frond No.	Growth rate	Frond dry weight
NOEC (mg ac/L)	120	120	120
IC50 or EC50 (mg ac/L) (95% C.I.)	>120	>120	>120

EC50 calculated empirically so no confidence intervals calculated.

B. REPORTED STATISTICS:

Means and standard deviations of frond numbers were calculated for each treatment level and the control at each observation interval. Means and standard deviations for dry weight were also calculated for each treatment level and the control and were based on the dry plant weight determined after 7 days of exposure.

The growth rate (μ) for each replicate flask was calculated based on day 7 frond numbers.

Based on the results of statistical analysis performed for 7-day frond density, growth rate and biomass, the No-Observed-Effect Concentration (NOEC), the highest test concentration which demonstrated no statistically adverse effect (p • 0.05) when compared to the control data, was determined. Additionally, the Lowest-Observed-Effect Concentration (LOEC), the lowest concentration tested with a statistically significant reduction relative to the control data, was determined. The data were first checked for normality using Shapiro-Wilks' Test (Weber, et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). The data were then analyzed using analysis of variance and Dunnett's test (α = 0.05) to determine NOEC values.

Due to a lack of effects, the EC05 and EC50 were determined empirically.

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Due to a lack of inhibition observed in this study to all biological endpoints, no verification of statistical results is required.

D. STUDY DEFICIENCIES:

Study component	Deficiency
Details of Growth Medium:	The composition of 20 AAM used in the study is essentially the same as 20 AAP defined in the OECD Guideline with only very minor differences in proportions of some of the nutrients. No additional nutrients were added. None of the recommended 20 AAP nutrients were omitted. The pH of the control medium increased by 1.6 units over the course of the study. The OECD guideline states the pH of the control medium should not increase by more than 1.5 units through the study. These deficiencies are not expected to adversely impact the results of the study.
Test concentrations:	Seven test concentrations were used in a geometric series of (nominal) ~3.2. This at the maximum ratio of 3.2 allowed in the OECD guideline and does not comply with EPA guidance where it states the geometric series should have a ratio between 1.5 and 2.0. Given the lack of results at the highest test concentration, this deficiency is not expected to adversely impact the results of the study.
Test conditions.	Light intensity within EPA range but marginally lower than the bottom of the range provided in the OECD guideline. This is not expected to have resulted in an impact on the study outcomes.
Acclimation period:	The fronds used to initiate the test were taken from a stock culture that had been transferred to fresh medium twelve days prior to testing. This transfer time was slightly longer than the maximum 10 days stated in the OECD guideline, but within the 2 week time frame provided in the US EPA guideline. This is not expected to have resulted in an impact on the study outcomes.

E. REVIEWERS COMMENTS: Nothing additional.

The PMRA reviewer agrees with the conclusions of the Australian reviewer. This study is acceptable to the PMRA.

F. CONCLUSIONS: The study is acceptable. Based on the results of this study, the ATSA metabolite of pyroxsulam is considered practically non-toxic to duckweed, *Lemna gibba*.

EC50/IC50: >120 mg ac/L (all endpoints) NOEC: 120 mg ac/L (all endpoints).

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Approved 04/01/01 C.K.