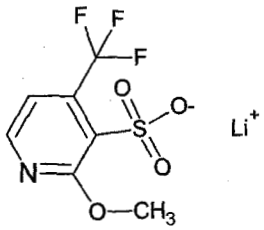
Data Evaluation Report on the acute toxicity of Sulfinic acid metabolite of XDE-742 (Pyroxsulam) to aquatic vascular plants *Lemna gibba*

PMRA Submission Number 2006-4727; ID 1283273 **EPA MRID Number** {.....} 469084-38

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: **Purity (%):** 98%
Common name: X11351479; XDE-742 sulfinic acid metabolite
Chemical name: 3-pyridinesulfinic acid, 2-methoxy-4-(trifluoromethyl)-,lithium salt
ID No: TSN 105138
Lot No. E1960-77
IUPAC name: 2-methoxy-4-(trifluoromethyl)pyridine-3-sulfinic acid
CAS No.: 422556-08-9
Synonyms: None
Chemical structure



22/02/08

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 22/2/08
Australian Government Department of the Environment, Water, Heritage and the Arts
Brian Kiernan Date: 22 August 2007 4/10/08
US Environmental Protection Agency
PMRA Reviewer: Émilie Larivière **Date:** 30 July 2007
Environmental Assessment Directorate, PMRA 05/03/08

Reference/Submission No.: APVMA ATS 40362 NCRIS 61286

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

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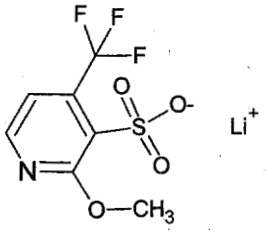

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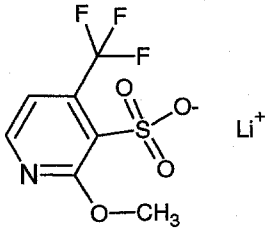

Primary Reviewer: Chris Lee-Steere **Date:** 5 July 2007
Australian Government Department of the Environment and Water Resources

Secondary Reviewers: Jack Holland **Date:** 23 July 2007
Australian Government Department of the Environment and Water Resources
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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater floating aquatic vascular plants (duckweed, *Lemna gibba*) were exposed to XDE-742 sulfinic acid metabolite at nominal concentrations of 0, 6.3, 13, 25, 50 and 100 mg ac/L. Mean measured concentrations were 0, 6.8, 15, 29, 57 and 110 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. An absence of inhibition (0%) or growth stimulation (as high as 26%) conmpared to controls, was observed for all endpoints, at all treatment levels. The 7-d NOECs based on all endpoints was 110 mg ac/L, the highest rate tested, and the 7-d $LOEC_S$ for all endpoints was >110 mg ac/L (mean measured concentration). The 7-d EC50 based on all endpoints was >110 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)	110	110	110
EC05 (mg ac/L) (95% C.I.)	>110	>110	>110
LOEC (mg ac/L)	>110	>110	>110
IC50 or EC50 (mg ac/L) (95% C.I.)	>110	>110	>110

No 95% confidence intervals associated with EC05 or EC50 values as these were determined empirically.

Endpoint(s) Effected: No biological endpoint tested showed adverse effects to exposure.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

OECD, 2000. OECD Guideline for Testing of Chemicals. *Lemna* sp. Growth Inhibition Test. Proposed Guideline #221. Revised Draft, October 2000.

U.S. EPA, 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.4400. Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II. "Public Draft" EPA 712-C-96-156 April 1996. U.S. Environmental Protection Agency. Washington, D.C.

The study report states that no deviations occurred during the study.

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) 17; 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.

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

A. MATERIALS:

1. Test Material Sulfinic acid Metabolite of XDE-742

Description: Solid

Lot No./Batch No.: E1960-77

Purity: 98% (Certificate of analysis provided)

Stability of Compound Under Test Conditions:
At the beginning and end of two renewal periods (i.e., days 0 and 3 and days 5 and 7), one sample was removed from each test solution and the control and analyzed for XDE-742 sulfinic

acid metabolite. Samples analyzed from newly prepared solutions were removed from the volumetric flasks prior to division into the replicate vessels. Test solution samples analyzed at the end of the renewal periods (days 3 and 7) were removed from composited solutions of each treatment level and the control. The following results were found:

Table 1: Measured concentrations of Sulfinic acid Metabolite of XDE-742

Nominal Concentration (mg ac/L)	Measured Concentration (mg ac/L)					% of Nominal
	0 hour (new)	Day 3 (aged)	Day 5 (new)	Day 7 (aged)	Mean (SD)	
Control	<0.30	<0.29	<0.37	<0.19	Not applicable	Not applicable
6.3	6.1	7.4	6.9	6.8	6.8	110
13	14	15	16	14	15	110
25	33	30	28	27	29	120
50	58	62	52	56	57	110
100	110	110	110	110	110	110

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

Storage conditions of test chemicals: Stored frozen (<-4°C) in the original container.

Physicochemical properties of Sulfinic acid metabolite of XDE-742: None available at the time of testing.

2. Test organism:

Name: Duckweed (*Lemna gibba*)
Strain, if provided: Not reported.
Source: Obtained from the University of Toronto, Toronto, Canada and maintained in stock culture at Springborn Smithers Laboratories, Wareham, MA.
Method of cultivation: Stock cultures were grown in 270-mL covered crystallizing dishes containing 100 mL of medium. The cultures were maintained in an environmental chamber within the following conditions: a temperature of 24 ± 2°C and continuous illumination of approximately 600 to 930 footcandles (6500 to 10,000 lux). Lighting was supplied by Premira VitaLux® fluorescent bulbs.
Age of inoculum: The fronds used to initiate the test were taken from a stock culture that had been transferred to fresh medium two days prior to testing.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: No range-finding study was performed.

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 two days prior to testing.	It is unclear how long stock cultures were maintained under test conditions. *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 270-mL covered crystallizing dishes containing 100 mL of 20X Algal Assay Procedure (AAP) medium. The cultures were maintained under test conditions.	*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.*
Health: (any toxicity observed)	Not reported. However, control plants demonstrated satisfactory growth indicating healthy plants.	
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	Environmental chamber within the following conditions: a temperature of 24 ± 2°C and continuous illumination of approximately 600 to 930	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>footcandles (6,500 to 10,000 lux). Lighting was supplied by Premira VitaLux® fluorescent bulbs.</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>

Glass crystallizing dishes.
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 Procedure (AAP) medium 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>240</td></tr><tr><td>$CaCl_2 \cdot 2H_2O$</td><td>90</td></tr><tr><td>$MgSO_4 \cdot 7H_2O$</td><td>290</td></tr><tr><td>$K_2HPO4 \cdot 3H_2O$</td><td>30</td></tr><tr><td>$NaHCO_3$</td><td>300</td></tr><tr><td>H_3BO_3</td><td>3.7</td></tr><tr><td>Na_2SeO_4[a]</td><td>0.0376</td></tr><tr><td>$MnCl_2 \cdot 4H_2O$</td><td>8.3</td></tr><tr><td>$ZnCl_2$</td><td>0.066</td></tr><tr><td>$CoCl_2 \cdot 6H_2O$</td><td>0.029</td></tr><tr><td>$CuCl_2 \cdot 2H_2O$</td><td>0.00024</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 growth medium used was based on that recommended in the OECD guideline. The only variation from this guideline was the addition of 0.0376 mg/L Na_2SeO_4.

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*
	a) additional nutrient required	
pH at test initiation: pH at test termination: Chelator used: Carbon source:	7.7 (control medium) 9.0 (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)	Standard AAP medium was used with the addition of 0.0376 mg/L Na_2SeO_4. Full details were provided.	Requirement considered met.
<u>Dilution water</u>		
Source/type:	The 20X AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium using sterile, deionized water and equilibrated to test temperature.	Requirements considered met. OECD 221 and US EPA OPPTS 850.4400 do not address these parameters specifically (other than pH).
pH:	Reported above for control medium.	
Total Organic Carbon:	A representative sample of 20X AAP medium was analyzed monthly for TOC. The TOC concentration for October 2005 (month of testing) was 2.6 mg/L.	
Particulate matter: Metals: Pesticides: Chlorine: Water pretreatment (if any): Intervals of water quality measurement	Not reported. Not reported. Not reported. Not reported. Sterile, deionized water used. Measurements (pH) were made on days 0, 3 and 5 (new solutions) and days 3, 5 and 7 (old solutions).	*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)	A 100 mg ac/L primary stock solution was prepared on the day of test initiation by placing	

Parameter	Details	Remarks *Criteria*
	0.2041 g of test substance (0.20 g as active ingredient) in a 2000-mL volumetric flask and bringing it to volume with 20X AAP medium. The resulting stock solution was observed to be clear and colorless with no visible undissolved test substance. Nominal test solutions were prepared from the primary stock solution.	
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:	 3 Not applicable. 3	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	5	Requirements considered met. *OECD: each test vessel should 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	3	Requirements considered met.

Parameter	Details	Remarks *Criteria*
		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, 6.3, 13, 25, 50, 100 0, 6.8, 15, 29, 57, 110	Requirement considered met. 6 test concentrations in geometric series of (nominal) ~2.0. *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: Limit of Detection:	All exposure solutions and QC samples were analyzed for test substance using liquid chromatography/mass spectrometry (LC/MS/MS). Fresh solutions were analysed on days 0, 3 and 5. Old exposure solutions were analysed on days 3, 5 and 7. 0.186 mg ac/L Not reported.	The requirements are considered met. The method validation study was conducted prior to the initiation of definitive testing and established an average recovery of 101± 5.76% from 20X AAP medium.
Test conditions Temperature: Photoperiod:	 24 to 25°C Continuous illumination.	Light intensity within OECD range but largely outside the range provided in the US EPA guideline. *OECD: temperature in the test*

Parameter	Details	Remarks *Criteria*
Light intensity and quality:	6500 to 9700 lux	*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 production; growth rate; frond dry weight.	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	On days 3, 5 and at test termination (day 7), fronds were counted (visual observation). At test termination (day 7), after frond density determinations were complete, the fronds were removed from each vessel, blotted dry and transferred to preweighed aluminum pans. Fronds were dried in an oven at 63-74°C for three days prior to dry weight determination.	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); Days 0-3; 0-5; and 0-7 (growth rate); Day 7 (frond dry weight).	Requirement considered met.
Other observations, if any	Frond appearance was observed at intervals measuring frond numbers. At test termination, fronds exposed to all test concentrations were observed to be normal.	
Indicate whether there was an exponential growth in the control	Yes. Mean frond numbers in the control groups 457 after 7 days, or around a 30 fold increase over the test period. The average growth rate over the 7 day test period in the control was 0.49 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:

Data sets for all end-points were shown statistically to pass requirements for normality and homogeneity of variance and therefore, Williams' Test 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 110 and >110 mg ac/L respectively.

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

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 050122 Sulfinic Acid metabolite of XDE-742 on frond number of Duckweed (*Lemna gibba*).

Mean Measured Concentration (mg ac/L)	Replicate No.	Frond number at:				7 Day Inhibition (%) compared to control.
		Day 0	Day 3	Day 5	Day 7	
Control	A	15	108	248	455	
	B	15	85	227	459	
	C	15	90	220	458	
	Mean	**15**	**94**	**232**	**457**	**Not applicable**
	SD	**0**	**12**	**15**	**2**	
6.8	A	15	96	231	507	
	B	15	79	212	382	
	C	15	89	272	575	
	Mean	**15**	**88**	**238**	**488**	**-7**
	SD	**0**	**9**	**31**	**98**	
15	A	15	94	237	592	
	B	15	98	278	527	
	C	15	87	233	457	
	Mean	**15**	**93**	**249**	**525**	**-15**
	SD	**0**	**6**	**25**	**68**	
29	A	15	107	291	569	
	B	15	80	237	481	
	C	15	87	223	467	
	Mean	**15**	**91**	**250**	**506**	**-11**
	SD	**0**	**14**	**36**	**55**	
57	A	15	107	260	622	
	B	15	84	279	570	
	C	15	103	229	510	
	Mean	**15**	**98**	**256**	**567**	**-24**
	SD	**0**	**12**	**25**	**56**	
110	A	15	102	285	577	
	B	15	101	293	596	
	C	15	105	272	561	
	Mean	**15**	**103**	**283**	**578**	**-26**
	SD	**0**	**2**	**11**	**18**	

Table 5: Effect of 050122 Sulfinic Acid metabolite of XDE-742 on Growth Rate of Duckweed (*Lemna gibba*).

Mean Measured Concentration (mg ac/L)	Replicate No.	Average Growth Rate ($days^{-1}$) Observation interval (days) Day 0-3	Day 0-5	Day 0-7	7 Day Inhibition (%) compared to control.
Control	A	0.62	0.57	0.49	
	B	0.54	0.55	0.49	
	C	0.56	0.54	0.49	
	Mean	**0.57**	**0.55**	**0.49**	**Not applicable**
	SD	**0.04**	**0.01**	**0.00**	
6.8	A	0.58	0.55	0.50	
	B	0.52	0.54	0.46	
	C	0.56	0.59	0.52	
	Mean	**0.55**	**0.56**	**0.49**	**0**
	SD	**0.03**	**0.03**	**0.03**	
15	A	0.57	0.56	0.52	
	B	0.59	0.59	0.51	
	C	0.55	0.55	0.49	
	Mean	**0.57**	**0.57**	**0.51**	**-4**
	SD	**0.02**	**0.02**	**0.02**	
29	A	0.61	0.60	0.52	
	B	0.52	0.56	0.49	
	C	0.55	0.55	0.49	
	Mean	**0.56**	**0.57**	**0.50**	**-2**
	SD	**0.05**	**0.03**	**0.02**	
57	A	0.61	0.58	0.53	
	B	0.54	0.59	0.52	
	C	0.60	0.55	0.50	
	Mean	**0.58**	**0.57**	**0.52**	**-6**
	SD	**0.04**	**0.02**	**0.01**	
110	A	0.60	0.60	0.52	
	B	0.60	0.60	0.52	
	C	0.61	0.59	0.52	
	Mean	**0.60**	**0.59**	**0.52**	**-6**
	SD	**0.01**	**0.01**	**0.00**	

Table 6: Effect of 050122 Sulfinic Acid metabolite of XDE-742 on Frond Dry Weight Duckweed (*Lemna gibba*).

Mean Measured Concentration (mg ac/L)	Frond dry weight (g)		
	Replicate No.	Day 7	7 Day Inhibition (%) compared to control.
Control	A	0.0458	
	B	0.0520	
	C	0.0525	
	Mean	**0.0501**	**Not applicable**
	SD	**0.0037**	
6.8	A	0.0579	
	B	0.0388	
	C	0.0639	
	Mean	**0.0535**	**-7**
	SD	**0.0131**	
15	A	0.0696	
	B	0.0564	
	C	0.0499	
	Mean	**0.0586**	**-17**
	SD	**0.010**	
29	A	0.0587	
	B	0.0537	
	C	0.0537	
	Mean	**0.0554**	**-11**
	SD	**0.0029**	
57	A	0.0614	
	B	0.0590	
	C	0.0562	
	Mean	**0.0589**	**-17**
	SD	**0.0026**	
110	A	0.0640	
	B	0.0773	
	C	0.0405	
	Mean	**0.0606**	**-21**
	SD	**0.0186**	

Table 7: Statistical endpoint values.

Statistical Endpoint	Frond No.	Growth rate	Frond dry weight
NOEC (mg ac/L)	110	110	110
EC05 (mg ac/L) (95% C.I.)	>110	>110	>110
LOEC (mg ac/L)	>110	>110	>110
IC50 or EC50 (mg ac/L) (95% C.I.)	>110	>110	>110

EC05 and EC50 values empirically estimated. 95% confidence intervals could not be calculated.

B. REPORTED STATISTICS:

Means and standard deviations of frond densities and growth rate 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 for the period from test initiation to each observation time.

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 ($p \leq 0.05$) 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). If the data sets passed the tests for homogeneity and normality, then Williams' Test (Williams, 1971, 1972) was used to determine the NOEC and LOEC. If the data did not pass the tests for homogeneity and normality, then Kruskal-Wallis' Test was used to determine the NOEC. All statistical determinations were made at the 95% level of certainty, except in the case of Shapiro-Wilks' and Bartlett's Tests, where the 99% level of certainty was applied.

The EC05, EC50 and EC90 values were calculated, when possible, for frond densities, average growth rate and biomass at test termination. TOXSTAT® version 3.5 (Gulley et al., 1996) was used to perform both the statistical (LOEC and NOEC determinations) and EC05, EC50 and EC90 calculations. However, during this study, no concentration resulted in a 5% or 50% reduction, therefore, the EC values were empirically estimated to be greater than the highest concentration tested.

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 growth medium used was essentially the AAP medium recommended in OECD TG 221. However, in addition, 0.0376 mg/L selenate (Na_2SeO_4) was added as an additional nutrient, noted in the test report as based on personal communication. The need for this additional nutrient was not given. However, given the satisfactory growth of control plants this is not considered to have resulted in an impact on the study outcomes.
Test conditions.	Light intensity within OECD range but largely outside the range provided in the US EPA guideline.
Acclimation period:	It is unclear how long stock cultures were maintained at the test facility prior to transferring fronds from the stock culture to fresh medium for use in the test. Given the strong growth of control plants, 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 sulfinic acid metabolite of pyroxsulam is considered practically non-toxic to duckweed, *Lemna gibba*.

EC50/IC50: >110 mg ac/L (all endpoints); NOEC: 110 mg ac/L (all endpoints)

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Approved 04/01/01 C.K.