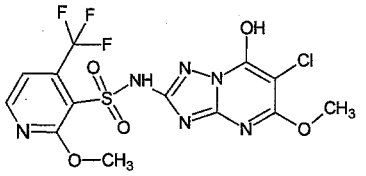
Data Evaluation Report on the acute toxicity of 6-Cl-7-OH metabolite of XDE-742 (Pyroxsulam) to aquatic vascular plants *Lemna gibba*

PMRA Submission Number 2006-4727; ID 1283259 **EPA MRID Number{.....}** 46508437

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 (%):** 99%
Common name: 6-Cl-7-OH Metabolite of pyroxsulam
Chemical name: 3-pyridinesulfonamide, N-(6-chloro-7-hydroxy-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
ID No: TSN 105423
Lot No. E1950-42
IUPAC name: N-(6-chloro-7-hydroxy-5-methoxy[1,2,4]triazolo [1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS No.: Not available
Synonyms: None
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:

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CITATION: Hoberg, James R. (2006): 6-Cl-7-OH Metabolite of XDE-742: Toxicity to Duckweed, *Lemna gibba*. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts, Study No. 12550.6403. Dow AgroSciences, Report No. 050124, 30 March 2006. Unpublished.

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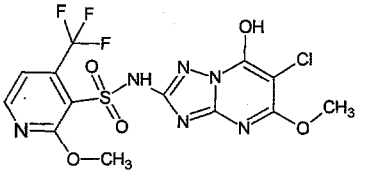

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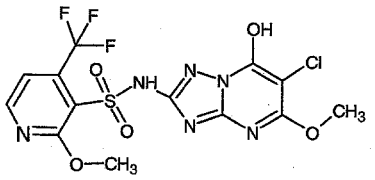
Data Evaluation Report on the acute toxicity of 6-Cl-7-OH metabolite of XDE-742 (Pyroxsulam) to aquatic vascular plants *Lemna gibba*

PMRA Submission Number 2006-4727; ID 1283259 **EPA MRID 469084-37**

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 (%):** 99%
Common name: 6-Cl-7-OH Metabolite of pyroxsulam
Chemical name: 3-pyridinesulfonamide, N-(6-chloro-7-hydroxy-5-methoxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
ID No: TSN 105423
Lot No. E1950-42
IUPAC name: N-(6-chloro-7-hydroxy-5-methoxy[1,2,4]triazolo [1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS No.: Not available
Synonyms: None
Chemical structure



Primary Reviewer: Chris Lee-Steere **Date:** 5 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 6-Cl-7-OH-pyroxsulam, a transformation product of pyroxsulam, at nominal concentrations of 0, 1.0, 2.6, 6.0, 16, 40 and 100 mg ac/L. Mean measured concentrations were 0, 0.93, 2.4, 6.0, 16, 37 and 100 mg ac/L. The study was conducted under static renewal conditions at days 3 and 5, and 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 % growth inhibition was determined for frond number, mean specific growth rate and biomass (frond dry weight). All endpoints showed statistically significant inhibition at the two highest test rates. The 7-day NOECs based for all endpoints was 16 mg ac/L and the 7-day LOECS for all endpoints was 37 mg ac/L (mean measured concentration).

The most sensitive 7-day EC05 was found for frond dry weight (EC05 = 5.2 mg ac/L) and the most sensitive 7-day EC50 was found for frond number (EC50 = 29 mg ac/L). However, sensitivity for all endpoints was of a similar magnitude.

Curled fronds on plants exposed to 37 and 100 mg ac/L were observed at day 7. In addition, some fronds exposed to the highest test concentration of 100 mg ac/L were observed to be slightly chloritic.

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)	16	16	16
EC05 (mg ac/L) (95% C.I.)	16 (2.3-17)	18 (16-18)	5.2 (1.5-18)
LOEC (mg ac/L)	37	37	37
IC50 or EC50 (mg ac/L) (95% C.I.)	29 (27-30)	46 (41-51)	35 (32-42)

Endpoint(s) Effected: 7 day frond number, growth rate and frond dry weight (biomass).

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 following protocol deviations are noted in the study report:

> The protocol states that the light intensity will range from 6500 to 10,000 lux and the photosynthetically-active radiation (PAR) will range from 85 to 120 $\mu E/m^2/s$. During the definitive test, the light intensity ranged from 6500 to 9700 lux and the PAR ranged from 102 to 147 $\mu E/m^2/s$. Since the light intensity was within the appropriate range, the PAR was not adjusted.
>
> The protocol states that at test termination the fronds will be dried at 60 to 70°C for a minimum of two days. Subsequent to test termination, all fronds were dried for four days at 57 to 59°C before dry weights were recorded. Although the drying temperature was inadvertently set slightly lower than required, all fronds were dried at the same temperature and time period, and should have been dry after four days.

These deviations are not expected to influence the study results.

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

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.

A. MATERIALS:

1. Test Material 6-Cl-7-OH Metabolite of pyroxsulam

Description: Solid

Lot No./Batch No.: E1950-42

Purity: 99% (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 6-Cl-7-OH metabolite of pyroxsulam. 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 6-Cl-7-OH Metabolite of pyroxsulam

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	<0.037	<0.034	<0.032	0.037	Not applicable	Not applicable
1.0	0.92	0.94	0.97	0.92	0.93 (0.024)	93
2.6	2.3	2.4	2.6	2.5	2.4 (0.14)	94
6.4	5.2	6.0	6.3	6.6	6.0 (0.59)	94
16	15	17	16	16	16 (0.89)	98
40	31	39	39	40	37 (4.1)	93
100	95	110	100	110	100 (6.1)	100

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

Storage conditions of test chemicals: Stored at in the original container in a dark ventilated cabinet.

Physicochemical properties of 6-Cl-7-OH metabolite of pyroxsulam:
None available at the time of testing.

2. Test organism:

Name: Duckweed (*Lemna gibba*)
Strain, if provided: 310
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: A 7-day preliminary range-finding exposure was conducted at Springborn Smithers at nominal 6-Cl-7-OH metabolite of pyroxsulam concentrations of 0.010, 0.10, 1.0, 10 and 100 mg ac/L and a control. Two exposure vessels were established for each concentration and the control. Test solutions were renewed on days 2 and 4. All test solutions were clear and colorless with no visible undissolved test substance following solution preparation. Following 7 days of exposure, frond densities in the 0.010, 0.10, 1.0, 10 and 100 mg ac/L treatment levels averaged 405, 338, 355, 363 and 27 fronds/replicate, respectively. Frond density in the control averaged 408 fronds/replicate. Fronds exposed to the 100 mg ac/L treatment level were observed to be slightly chlorotic and curled. Fronds exposed to the 0.010, 0.10, 1.0 and 10 mg ac/L treatment levels and the control were normal. Based on these data, nominal concentrations of 1.0, 2.6, 6.4, 16, 40 and 100 mg ac/L were selected for the definitive exposure.

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 footcandles (6,500 to 10,000 lux). Lighting was supplied by	Requirement considered met. *OECD: temperature in the test vessels should be 24 ± 2°C and refers to use of a growth chamber incubator.*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td>Premira VitaLux® fluorescent bulbs.</td><td>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>
a) additional nutrient required</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*
pH at test initiation: pH at test termination: Chelator used: Carbon source:	7.6 (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: pH: Total Organic Carbon: Particulate matter: Metals: Pesticides: Chlorine: Water pretreatment (if any): Intervals of water quality measurement	 The 20X AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium. Several liters of 20X AAP medium were prepared using sterile, deionized water and equilibrated to test temperature. Reported above for control medium. Typically 3 mg/L. 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).	 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)	A 100 mg ac/L primary stock solution was prepared on the day of test initiation by placing 0.2020 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 solution was sonicated for 20 minutes, stirred for 30	

Parameter	Details	Remarks *Criteria*
	minutes then sonicated for a further 4 minutes. The solution was then stirred for an additional two hours, allowed to settle, and the soluble fraction was siphoned out. The soluble fraction was observed to be clear and colorless with no visible undissolved test substance. Nominal test solutions were prepared from the primary stock solution. At each solution renewal (days 3 and 5), a 100 mg ac/L primary stock solution was prepared by placing 0.1010 g of 6-Cl-7-OH metabolite of pyroxsulam (0.10 g as active ingredient) in a 1000-mL volumetric flask and bringing it to volume with 20X AAP medium. Following sonication and stirring, these primary stock solutions were observed to be clear and colorless with no visible undissolved test substance.	
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*

Parameter	Details	Remarks *Criteria*
		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. *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.*
<u>Test concentrations</u> Nominal (mg ac/L): Measured (mg ac/L):	 0, 1.0, 2.6, 6.4, 16, 40, 100 0, 0.93, 2.4, 6.0, 16, 37, 100	Six concentrations were tested in a geometric series of (nominal) ~2.5. *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:	All exposure solutions and QC samples were analyzed for test substance using high	The requirements are considered met. The method validation study was

Parameter	Details	Remarks *Criteria*
	performance liquid chromatography with ultraviolet detection (HPLC/UV). Fresh solutions were analysed on days 0, 3 and 5. Old exposure solutions were analysed on days 3, 5 and 7.	conducted prior to the initiation of definitive testing and established an average recovery of 101± 4.12% from 20X AAP medium.
Limit of Quantitation:	0.0133 mg ac/L	
Limit of Detection:	Not reported.	
Test conditions		Light intensity within OECD range but largely outside the range provided in the US EPA guideline.
Temperature:	22 to 24 °C	
Photoperiod:	Continuous illumination.	*OECD: temperature in the test vessels should be 24 ± 2°C with light intensity equivalent to 6500 to 10000 lux. Photoperiod not specified.*
Light intensity and quality:	6500 to 9700 lux	*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.*

Parameter	Details	Remarks *Criteria*
		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 57-59°C for four days prior to dry weight determination.	Requirement considered met. Drying temperature slightly lower than recommended in the guideline, but drying was performed over 4 days. *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	Requirement considered met.

	rate); Day 7 (frond dry weight).	
Other observations, if any	Frond appearance was observed at intervals measuring frond numbers. At test termination, fronds exposed to the 37 and 100 mg ac/L treatment levels were curled, while fronds exposed to the 100 mg ac/L treatment level were also observed to be slightly chlorotic.	
Indicate whether there was an exponential growth in the control	Yes. Mean frond numbers in the control groups 458 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:

Inhibitory effects were found for all biological end-points evaluated in this study. 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.

For all biological end-points, the 7-day NOEC and LOEC were determined to be 16 and 37 mg ac/L, respectively. The 7-day EC50 values for frond production, growth rate and frond dry weight were determined to be 29 mg ac/L (95% CI of 27-30 mg ac/L), 46 mg ac/L (95% CI of 41-51 mg ac/L) and 35 mg ac/L (95% CI of 32-42 mg ac/L) respectively.

At test termination, fronds exposed to the 37 and 100 mg ac/L treatment levels were curled, while fronds exposed to the 100 mg ac/L treatment level were also observed to be slightly chlorotic.

The pH of the newly formulated test and control solutions (day 0, 3 and 5) ranged from 7.3 to 8.0. The aged test and control solution pH (day 3, 5 and 7) ranged from 8.4 to 9.2. No other observations were made in the test report.

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 6-Cl-7-OH metabolite of pyroxsulam 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	69	179	452	Not applicable
	B	15	82	199	486	
	C	15	65	184	436	
	Mean	**15**	**72**	**187**	**458**	
	SD	**0**	**9**	**10**	**26**	
0.93	A	15	73	215	548	-5
	B	15	70	181	420	
	C	15	80	178	474	
	Mean	**15**	**74**	**191**	**481**	
	SD	**0**	**5**	**21**	**64**	
2.4	A	15	72	215	534	-7.2
	B	15	77	211	507	
	C	15	72	189	432	
	Mean	**15**	**74**	**205**	**491**	
	SD	**0**	**3**	**14**	**53**	
6.0	A	15	78	217	498	-0.87
	B	15	78	219	495	
	C	15	61	156	394	
	Mean	**15**	**72**	**197**	**462**	
	SD	**0**	**10**	**36**	**59**	
16	A	15	69	184	482	0.87
	B	15	75	200	465	
	C	15	63	167	415	
	Mean	**15**	**69**	**184**	**454**	
	SD	**0**	**6**	**17**	**35**	
37	A	15	36	48	101	78
	B	15	33	42	90	
	C	15	41	55	114	
	Mean	**15**	**37**	**48[a]**	**102[a c]**	
	SD	**0**	**4**	**7**	**12**	
100	A	15	21	21	22	94
	B	15	21	20	32	
	C	15	24	23	33	
	Mean	**15**	**22**	**21[a]**	**29[a b c]**	
	SD	**0**	**2**	**2**	**6**	

a) Curled fronds were observed; b) slightly chloritic fronds were observed c) Significantly reduced compared to the control, based on Williams' Test.

Table 5: Effect of 6-Cl-7-OH metabolite of pyroxsulam 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	0.53	0.50	0.49	
	B	0.59	0.52	0.50	
	C	0.51	0.50	0.48	
	Mean	**0.55**	**0.51**	**0.49**	**Not applicable**
	SD	**0.04**	**0.01**	**0.01**	
0.93	A	0.55	0.53	0.52	
	B	0.54	0.50	0.48	
	C	0.58	0.50	0.50	
	Mean	**0.56**	**0.51**	**0.50**	**-2**
	SD	**0.02**	**0.02**	**0.02**	
2.4	A	0.55	0.53	0.51	
	B	0.57	0.53	0.51	
	C	0.55	0.51	0.48	
	Mean	**0.56**	**0.52**	**0.50**	**-2**
	SD	**0.01**	**0.01**	**0.02**	
6.0	A	0.58	0.54	0.50	
	B	0.58	0.54	0.50	
	C	0.49	0.47	0.47	
	Mean	**0.55**	**0.51**	**0.49**	**0**
	SD	**0.05**	**0.04**	**0.02**	
16	A	0.53	0.50	0.50	
	B	0.56	0.52	0.49	
	C	0.50	0.48	0.48	
	Mean	**0.53**	**0.50**	**0.49**	**0**
	SD	**0.03**	**0.02**	**0.01**	
37	A	0.31	0.23	0.27	
	B	0.28	0.21	0.26	
	C	0.35	0.26	0.29	
	Mean	**0.31**	**0.23**	**0.27[a]**	**45**
	SD	**0.04**	**0.03**	**0.27**	
100	A	0.12	0.07	0.06	
	B	0.12	0.06	0.11	
	C	0.16	0.09	0.11	
	Mean	**0.13**	**0.07**	**0.09[a]**	**82**
	SD	**0.03**	**0.01**	**0.03**	

a) Significantly reduced compared to the control, based on Williams' Test.

Table 6: Effect of 6-Cl-7-OH metabolite of pyroxsulam 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	42.60	
	B	43.80	
	C	42.00	
	Mean	**42.80**	**Not applicable**
	SD	**0.9**	
0.93	A	61.70	
	B	40.60	
	C	51.00	
	Mean	**51.10**	**-19**
	SD	**10.6**	
2.4	A	53.00	
	B	46.20	
	C	42.20	
	Mean	**47.13**	**-10**
	SD	**5.5**	
6.0	A	48.20	
	B	47.90	
	C	35.80	
	Mean	**43.97**	**-3**
	SD	**7.1**	
16	A	46.60	
	B	44.40	
	C	34.60	
	Mean	**41.87**	**2**
	SD	**6.4**	
37	A	21.10	
	B	20.70	
	C	24.40	
	Mean	**22.07[a]**	**48**
	SD	**2.0**	
100	A	11.80	
	B	14.00	
	C	16.30	
	Mean	**14.03[a]**	**67**
	SD	**2.3**	

a) Significantly reduced compared to the control, based on Williams' Test.

Table 7: Statistical endpoint values.

Statistical Endpoint	Frond No.	Growth rate	Frond dry weight
NOEC (mg ac/L)	16	16	16
EC_{05} (mg ac/L) (95% C.I.)	16 (2.3-17)	18 (16-18)	5.2 (1.5-18)
LOEC (mg ac/L)	37	37	37
IC_{50} or EC_{50} (mg ac/L) (95% C.I.)	29 (27-30)	46 (41-51)	35 (32-42)

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 • 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.

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

To verify the statistics, the data have been re-analysed using TOXCALC – Toxicity Data Analysis Software v5.0.26. The data set was established using individual replicate values and

EC05 and EC50 values for all biological end-points were verified using non-linear interpolation. The following results were found:

Table 8: Statistical endpoint values – verification results by reviewer.

Statistical Endpoint	Frond No.	Growth rate	Frond dry weight
NOEC (mg ac/L)	16	16	16
EC05 (mg ac/L) (95% C.I.)	16.1 (0.0-18.7)	17.8 (14.6-19.1)	5.2 (0.0-32)
LOEC (mg ac/L)	37	37	37
IC50 or EC50 (mg ac/L) (95% C.I.)	28.9 (25.6-30.2)	46.0 (35.0-56.0)	35.5 (28.8-46.5)

The results are in good agreement with those found in the study.

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 concentrations:	6 test concentrations were used in a geometric series of (nominal) ~2.5. This is within OECD guidance (separation factor between test concentrations should not exceed 3.2), but does not comply with EPA guidance where it states the geometric series should have a ratio between 1.5 and 2.0.
Test conditions.	Light intensity within OECD range but largely outside the range provided in the US EPA guideline. The protocol states that the light intensity will range from 6500 to 10,000 lux and the photosynthetically-active radiation (PAR) will range from 85 to 120 $\mu E/m^2/s$ (with the OECD guideline requirement being 85 to 135 $\mu E/m^2/s$). During the definitive test, the light intensity ranged from 6500 to 9700 lux and the PAR ranged from 102 to 147 $\mu E/m^2/s$. Since the light intensity was within the appropriate range, the PAR was not adjusted. This is not expected to have resulted in an impact on the study outcomes.
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.
Test monitoring – plant growth.	The protocol states that at test termination the fronds will be dried at 60 to 70°C for a minimum of two days. Subsequent to test termination, all fronds were dried for four days at 57 to 59°C before dry weights were recorded. Although the drying temperature was inadvertently set slightly lower than required, all fronds were dried at the same temperature and time period, and should have been dry after four days. 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, 6-Cl-7-OH metabolite of pyroxsulam is considered slightly toxic to duckweed, *Lemna gibba*.

EC50/IC50: 29 mg ac/L (7 day frond number); EC05: 5.2 mg ac/L (7 day frond dry weight).

III. REFERENCES:

ASTM. 2002. Standard Guide for Conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19426.

Gulley, D.D., Boetler, A.M. and Bergman, H.L. 1996 TOXSTAT® Release 3.5. University of Wyoming, Laramie, Wyoming. Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014.

Hoberg J, 2006. Study Profile Template (SPT) for 6-Cl-7-OH Metabolite of XDE-742 – Toxicity to Duckweed, *Lemna gibba*. Study No. 12550.6403.SPT. Springborn Smithers Laboratories, Massachusetts. 30 March 2006.

Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp.

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.

U.S. EPA. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* 27: 103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. *Biometrics* 28: 519-531.

Approved 04/01/01 C.K.