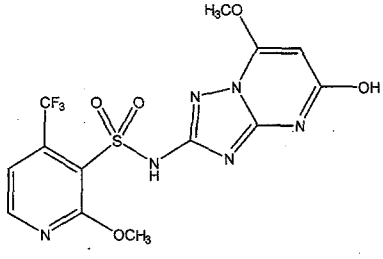
Data Evaluation Report on the acute toxicity of 5-OH metabolite of pyroxsulam (5-OH metabolite of XDE-742) to aquatic vascular plants duckweed, *Lemna gibba*

PMRA Submission Number 2006-4727; 1283258 EPA MRID Number 469084-36 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE: 9.8.5 (TGAI) EPA DP Barcode: D332116 OECD Data Point: IIA 8.6 EPA Guideline: 123-2 (OPPTS 850.4400 (Draft April 1996))
Test material:	5-hydroxy-pyroxsulam or 5-hydroxy-XDE-742
Purity (%):	100% (ID No.: TSN 105231).
Common name:	5-OH Metabolite of XDE-742
Chemical name:	3-pyridinesulfonamide, N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
IUPAC:	N-(5-hydroxy-7-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS name:	3-pyridinesulfonamide, N-(1,35-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
CAS No.:	Not available.
Synonyms:	5-desmethyl XDE-742 metabolite, 5-OH-XDE-742

Chemical Structure:



22/02/07

Primary Reviewer: Daryl Murphy **Date:** 18 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

22/2/08

Secondary Reviewer: Jack Holland **Date:** 19 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

PMRA Reviewer: Émilie Larivière **Date:** 26 July 2007
Environmental Assessment Directorate, PMRA Émilie Larivière 05/03/08

US EPA Reviewer Brian Kiernan **Date:** 22 August 2007
US Environmental Protection Agency

4/10/08

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

CITATION: Hoberg, J. R. 2006. 5-OH Metabolite of XDE-742 - Toxicity to Duckweed, *Lemna gibba*. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6406, Sponsor Protocol/Project No. 050120. The Dow Chemical Company, 1803 Building, Midland, Michigan 48674 for Dow AgroSciences Indianapolis, Indiana 46268. 5 April 2006.Unpublished report.

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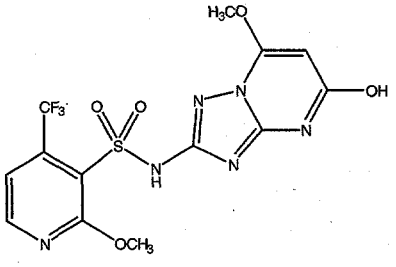

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EXECUTIVE SUMMARY:

In a 7 day acute toxicity study, freshwater floating aquatic vascular plants (duckweed, *Lemna gibba*) were exposed to 5-OH metabolite of pyroxsulam at nominal concentrations of 0 (medium control), 0.94, 1.9, 3.8, 7.55 and 15 mg/L. Mean measured concentrations were 0, 0.80, 1.7, 3.4, 7.1 and 14 mg 5-OH metabolite of pyroxsulam/L. The study was conducted under static renewal conditions at days 3 and 5 in accordance with a protocol reported to meet the general requirements of OECD 221 "Lemna sp. Growth Inhibition Test. Revised Protocol for a New Guideline #221. Draft, April 2004" and US EPA OPPTS draft guideline 850.4400 Aquatic Plant Toxicity Test using *Lemna* sp., Tiers I and II. Draft April 1996.

Effect criteria were reduction in 7-day frond density, biomass (dry weight) and growth rate relative to the control data. With the frond density, response relative to the control ranged from -12 to 91% inhibition of mean frond density. Response relative to the control mean ranged from -4 to 78% inhibition of mean specific growth rate. For biomass based on the day 7 frond dry weights, response relative to the control mean ranged from 4 to 66% inhibition of frond dry weight.

The 7 day EC50 reported for frond density (frond numbers) was 5.7 mg 5-OH metabolite of pyroxsulam/L (mean measured concentration) with 95% confidence limits of 5.6-5.8 mg 5-OH metabolite of pyroxsulam/L. The 0-7 day ErC50 (mean specific growth rate) was 7.4 mg 5-OH metabolite of pyroxsulam/L with 95% confidence limits of 6.9-7.9 mg 5-OH metabolite of pyroxsulam/L. The 7 day EbC50 (biomass, frond dry weight) was 6.6 mg 5-OH metabolite of pyroxsulam/L (mean measured concentration) with 95% confidence limits of 5.8-7.0 mg 5-OH metabolite of pyroxsulam/L. The 7 day NOECs based on frond number, and the 0-7 day specific growth rate were both 3.4 mg 5-OH metabolite of pyroxsulam/L while the biomass (dry weight at 7 days) NOEC was set at 1.7 mg 5-OH metabolite of pyroxsulam/L (mean measured concentration). These EC50 values are considered to classify 5-OH metabolite of pyroxsulam as moderately toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment, Water, Heritage and the Arts **($1 < EC50 \leq 10$ mg/L).**

At test termination, fronds exposed to the 3.4, 7.1 and 14 mg/L treatment levels were curled, while fronds exposed to the 7.1 and 14 mg/L treatment levels were also observed to be slightly chlorotic.

The static exposure (with renewal on days 3 and 5) of duckweed to mean measured concentrations of 0.80 to 14 mg 5-OH metabolite of pyroxsulam/L for seven days is considered to have been satisfactorily conducted according to the requirements of the OECD 221 and US EPA OPPTS 850.4400 guidelines and to have generated acceptable results with respect to effects of 5-OH metabolite of pyroxsulam on the growth of duckweed. As a result, the study is 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:	Static Renewal
Frond count	
7 day EC05:	3.6 mg 5-OH metabolite of pyroxsulam/L
	95% C.I. (confidence limits) 3.4-3.6 5-OH metabolite of pyroxsulam/L
7 day EC50:	5.7 mg 5-OH metabolite of pyroxsulam/L
	95% C.I.: 5.6-5.8 mg 5-OH metabolite of pyroxsulam/L
7 day NOEC:	3.4 mg 5-OH metabolite of pyroxsulam/L
Probit Slope:	Not reported

Mean specific growth rate (day^{-1})
7 day ErC05: 3.8 mg 5-OH metabolite of pyroxsulam/L
95% C.I. (confidence limits) 3.7-3.8 5-OH metabolite of pyroxsulam/L
7 day ErC50: 7.4 mg 5-OH metabolite of pyroxsulam/L
95% C.I.: 6.9 to 7.9 µg 5-OH metabolite of pyroxsulam/L
7 day NOEC: 3.4 mg 5-OH metabolite of pyroxsulam/L
Probit Slope: Not reported

Biomass (frond dry weight)
7 day EbC05: 1.7 mg 5-OH metabolite of pyroxsulam/L
95% C.I. (confidence limits) 0.89-3.6 5-OH metabolite of pyroxsulam/L
7 day EbC50: 6.6 mg 5-OH metabolite of pyroxsulam/L
95% C.I.: 5.8-7.0 mg 5-OH metabolite of pyroxsulam/L
7 day NOEC: 1.7 mg 5-OH metabolite of pyroxsulam/L
Probit Slope: Not reported

Endpoint(s) Effected: Reduction in 7-day frond density, biomass (dry weight) and growth rate relative to the control data.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
The toxicity test was performed according to the Springborn Smithers Laboratories protocol entitled "7-Day Growth Inhibition Test with Duckweed (*Lemna gibba*)," Springborn Smithers Laboratories Protocol No.: 081205/7-Day Lemna/Dow. The protocol was reported as meeting the general requirements of the following OECD and U.S. EPA OPPTS Guidelines:

- OECD Guideline for Testing of Chemicals. *Lemna* sp., Growth Inhibition Test. Revised Protocol for a New Guideline #221. Draft, April 2004, and
- 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.

This DER has assessed the study report primarily against the OECD 221 edition adopted on 23 March 2006 and current US EPA OPPTS 850.4400 requirements.

The protocol stated that the light intensity was to range from 6500 to 10,000 lux and the photosynthetically-active radiation (PAR) from 85 to 120 $\mu E/m^2/s$. During the definitive test, the light intensity ranged from 6500 to 9100 lux and the PAR ranged from 119 to 136 $\mu E/m^2/s$. Since the light intensity was within the appropriate range, the PAR was not adjusted. This deviation was stated by the study report to have had no impact on the results of the study. This matter is considered further in Table 1 on page 18 of this DER.

COMPLIANCE: The data and report presented for the study were reported as produced and compiled in accordance with all pertinent OECD and US EPA Good Laboratory Practice regulations, namely:

- 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. 1998, and
- 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. 1989

with the following exception: routine water contaminant screening analyses were conducted using standard US EPA procedures by GeoLabs, Inc., Braintree, Massachusetts and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. Because the analyses were conducted following standard validated methods, this exception was considered not to have had an impact on the study results.

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

A. MATERIALS:

1. Test Material	5-OH metabolite of XDE-742 (i.e. 5-OH metabolite of pyroxsulam)
Description:	Solid
Lot No./Batch No.:	E2008-48
Purity:	100%
Stability of Compound Under Test Conditions:	The results of the analysis of the exposure solutions for 5-OH metabolite of pyroxsulam concentration closely approximated the desired nominal concentrations and provided the expected concentration gradient. Mean measured concentrations (page 16 of this DER refers) ranged from 85 to 94% of the nominal concentrations (with these results based on the analyses of the 0 hour and 5 day new test solutions and the day 3 and day 7 aged test solutions). Such results indicate the 5-OH metabolite of pyroxsulam was stable under the test conditions.
Storage conditions of test chemicals:	Upon receipt at Springborn Smithers, the test substance (also identified as SSL No. 112-86) was stored at room temperature in the original container in a dark ventilated cabinet.
Physicochemical properties of 5-OH pyroxsulam.	The physicochemical properties of the 5-OH metabolite pyroxsulam were not reported in the study. The study report stated that determination of characterization of the test substance was the responsibility of the Study Sponsor..

2. Test organism:

Name:	Freshwater duckweed, *Lemna gibba*. L.
Strain, if provided:	310
Source:	University of Toronto, Toronto, Canada
Age of inoculum:	Fronds came from a 2 day-old subculture (at test initiation).
Method of cultivation:	The duckweed was maintained in stock culture at Springborn Smithers.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study:
A 7-day preliminary range-finding exposure was conducted at Springborn Smithers at nominal 5-OH metabolite of pyroxsulam concentrations of 0.010, 0.10, 1.0, 10 and 100 mg/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/L treatment levels averaged 393, 327, 359, 43 and 19 fronds/replicate, respectively. Frond density in the control averaged 338 fronds/replicate. Fronds exposed to the 10 mg/L treatment level were observed to be slightly chlorotic and curled, while fronds exposed to the 100 mg/L treatment level were observed to be chlorotic and had less root formation than the control fronds. Fronds exposed to the 0.010, 0.10 and 1.0 mg/L treatment levels and the control were normal. Based on these data, nominal concentrations of 0.94, 1.9, 3.8, 7.5 and 15 mg/L were selected for the definitive exposure.

[b) Definitive Study

The definitive test was conducted from 3 to 13 March 2006 (including dry weight determination) with the exposure phase carried out under static-renewal conditions for seven days (renewals on days 3 and 5).

Note that in the following two tables; Criteria columns (and elsewhere as relevant), entries in italics are those given in the PMRA's Draft Evaluation Report template for acute toxicity to algae. In its examination of the initial drafts of the aquatic invertebrate DERs, the PMRA advised (email of 3/07/2007) that the criteria in the templates were understood to have come from old US guidelines and that failure to comply with these template requirements would not be a deficiency. Provided relevant US EPA or OECD guidelines are complied with, this approach is agreed with.

Table 1. Experimental Parameters

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td><u>Acclimation</u>

Period:</td><td>The fronds used to initiate the toxicity test with 5-OH metabolite of pyroxsulam were taken from a stock culture that had been transferred to fresh medium two days prior to testing.</td><td>See deviations/deficiency table on page 35 of this report.

OECD 221 states that 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.

US EPA OPPTS 850.4400 states axenic stock cultures should be grown in the aquariums for 2 weeks (with necessary transfers) prior to being used in a test. Plants used in a test should be randomly selected from the culturing tank. Inocula should be taken from cultures which are less than 2 weeks old.</td></tr>
<tr><td>Culturing media and conditions: (same as test or not)</td><td>The study protocol states that cultures will be maintained under specified conditions (as shown below) prior to testing for at least the period of time from the last transfer with cultures transferred weekly into fresh medium using aseptic technique.

Typical culturing conditions were described as:
<table>
<tr><th>Conditions:</th><th>Culture:</th></tr>
<tr><td>Temperature (°C):</td><td>24 ± 2°C</td></tr>
<tr><td>Light:</td><td>6500 to 10,000 lux</td></tr>
<tr><td>Photoperiod:</td><td>Continuous</td></tr>
<tr><td>Medium:</td><td>20X Algal Assay Procedure (AAP) medium.</td></tr>
<tr><td>pH:</td><td>AAP medium has a pH adjusted to 7.5 ± 0.1</td></tr>
<tr><td>Culture Vessel:</td><td>270-mL covered crystallizing dishes</td></tr>
</table></td><td>Requirement considered met with the culturing media and conditions the same as those used in the test.

Typical test conditions were described as:
<table>
<tr><th>Conditions:</th><th>Test:</th></tr>
<tr><td>Temperature (°C):</td><td>22 to 25°C</td></tr>
<tr><td>Light:</td><td>6500 to 9100 lux</td></tr>
<tr><td>Photoperiod:</td><td>Continuous</td></tr>
<tr><td>Medium:</td><td>20X Algal Assay Procedure (AAP) medium. The 20X AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium.</td></tr>
<tr><td>pH:</td><td>Adjusted to 7.5 prior to addition of test material.</td></tr>
<tr><td>Culture Vessel:</td><td>Sterile 270-mL crystallizing dishes were used as test vessels with each covered with an</td></tr>
</table></td></tr>
</table>

Parameter	Details	Remarks *Criteria*
	<table><tr><td></td><td></td></tr><tr><td>Inoculation:</td><td>Weekly transfer</td></tr><tr><td>Culture Chamber:</td><td>Environmental chamber</td></tr></table> Comparison of these culture conditions with the test parameters shown in the adjacent "Remarks" column indicates that test conditions can be considered the same as the culture conditions.	<table><tr><td></td><td>inverted, sterile, glass Petri dish.</td></tr><tr><td>Inoculation:</td><td>Single</td></tr><tr><td>Culture Chamber:</td><td>Environmental growth chamber</td></tr></table>
Health: (any toxicity observed)	No specific comment found in the test report but the stock cultures used were maintained by weekly transfer into fresh medium by the testing laboratory. Control growth and absence of toxicity effects in the controls indicate that the duckweed used were healthy.	Requirement considered met. OECD 221 refers to use of monocultures, that are visibly free from contamination by other organisms such as algae and protozoa, should be used. US EPA OPPTS 850.4400 states that inocula should be taken from cultures which are less than 2 weeks old taken from axenic stock cultures that should have been grown in the aquariums for 2 weeks (with necessary transfers) prior to being used in a test.
<u>Test system</u> Static/static renewal	Static-renewal system used.	Requirements considered met. Semi-static (renewal) tests are recognised by OECD 221 while US EPA OPPTS 850.4400 recognises static renewal tests. In both cases, the test refers to a procedure in which the test solution is periodically replaced at specific intervals during the test. These are considered equivalent.
Renewal rate for static renewal:	Renewal of the test media took place on days 3 and 5.	Requirements considered met. OECD 221 refers as follows to the renewal rate, "If a preliminary stability test shows that the test substance concentration cannot be maintained (i.e. the measured concentration falls below 80% of the measured initial concentration) over the test duration (7 days), a semi-static test

Parameter	Details	Remarks *Criteria*
		regime is recommended. In this case, 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; a higher frequency may be needed to maintain near-constant concentrations of highly unstable or volatile substances." US EPA OPPTS 850.4400 states that the colonies should transferred to test solutions on days 3 and 5 and that nutrient medium and test solutions may need to be replaced on day 3 or 5, or as needed to prevent nutrient limitation or depletion of the test chemical. *EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Temperature-controlled environmental chamber with overhead fluorescent lights designed to maintain the test conditions specified in the protocol: a temperature of 24 ± 2°C, continuous lighting with an intensity of 6,500 to 10,000 lux and a photosynthetically active radiation (PAR) of 85 to 120 $\mu E/m^2/S$.	Requirement considered met. OECD 221 states that temperature in the test vessels should be 24 ± 2°C and refers to use of a growth chamber incubator. US EPA OPPTS 850.4400 states that the 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. Recorded temperatures ranged from 22 to 25°C.
Duration of the test	7 days	Requirement considered met. OECD 221 and US EPA OPPTS 850.4400 specify a 7 day exposure period. *EPA requires a duration of 14 days. Seven*

Parameter	Details	Remarks *Criteria*
		day studies will be accepted for review by the Agency. This template requirement is not considered further because of the specification of the 7 day exposure period by the current OECD and US EPA OPPTS guidelines.
Test vessel Material: (glass/polystyrene)	Sterile 270 mL crystallizing dishes were used as test vessels with each covered with an inverted, sterile, glass Petri dish.	Requirement considered met. OECD 221 states 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 and that crystallizing dishes are appropriate test vessels. US EPA OPPTS 850.4400 refers to test containers being glass beakers or Erlenmeyer flasks.
Size:	270 mL	A minimum depth of 20 mm and minimum volume of 100 mL in each test vessel is advised by OECD 221. US EPA OPPTS 850.4400 refers to containers 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
Fill volume:	100 mL	OECD 221 advises there be a minimum fill volume of 100 mL while US EPA OPPTS 850.4400, as stated above, refers to vessels 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.
Details of growth medium Name:	Modified 20X Algal Assay Procedure (AAP) medium The compositions of the 20X AAP medium and the OECD 221 20X AAP medium are provided as Attachment 1 on page 39 of this DER. The	See deviations/deficiency table on page 35 of this report. OECD 221 provides the composition of the 20X AAP medium. US EPA OPPTS 850.4400 refers to use of 20X-AAP medium but does not provide

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td>details provided in the study report were considered to show the two media were equivalent with the following exception:

The test medium contained sodium selenate at 0.0376 mg/L. The study report noted this was an additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.</td><td>the constituents or their percentages. This guideline states that chelating agents such as EDTA are present in 20X AAP medium and that, if it is suspected that the chelating agent will interact with the test material, M-Hoagland's medium, which has no EDTA, should be used.

EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelators are not recommended. The 20X AAP medium (modified by addition of sodium selenate) allows for the presence of the chelating agent, disodium EDTA. Consequently, the template's reference to chelating agents not being recommended is not considered further.</td></tr>
<tr><td>pH (in the fresh exposure solutions) at days 0, 3 and 5:</td><td>The pH of the exposure solutions was measured at test initiation (new solutions), in each aged and new solution at each renewal period, and at test termination (aged solutions).

The portion of the test solution remaining in the volumetric flasks after filling the test vessels was used for initial pH measurements.

In the fresh media, the pH values reported for days 0, 3 and 5 were:

<table>
<tr><th>Nominal concent-rations</th><th>Day 0
New</th><th>Day 3
New</th><th>Day 5
New</th></tr>
<tr><td>Control</td><td>7.7</td><td>7.7</td><td>7.8</td></tr>
<tr><td>0.94</td><td>7.8</td><td>7.8</td><td>7.8</td></tr>
<tr><td>1.9</td><td>7.7</td><td>7.8</td><td>7.8</td></tr>
<tr><td>3.8</td><td>7.8</td><td>7.8</td><td>7.8</td></tr>
<tr><td>7.5</td><td>7.8</td><td>7.9</td><td>7.8</td></tr>
<tr><td>15</td><td>7.8</td><td>7.7</td><td>7.7</td></tr>
</table>
* Nominal concentrations as mg 5-OH metabolite of pyroxsulam/L.</td><td>Requirement considered met.

OECD 221 states that the pH of the 20X AAP growth medium is adjusted to 7.5 ± 0.1 and that the pH of the control medium should not increase by more than 1.5 units during the test.

US EPA OPPTS 850.5400 states that if 20X-AAP medium is used, the pH should be adjusted to 7.5 ± 0.1.

On days 0, 3, and 5, an initial pH was taken from a sample of each bulk test solution.</td></tr>
<tr><td>pH (in spent solution with duckweed) at days 3, 5 and 7:</td><td>As noted above, the pH of the exposure solutions was measured at test initiation (new solutions), in each aged and new solution at each renewal period, and at test termination (aged solutions).

At each renewal period and at test termination,</td><td>A final pH of spent solutions was also taken on days 3, 5, and 7 from a pooled sample of the three replicates with fronds

The changes in pH of the control solutions at days 3, 5 and 7 were, respectively, 0.7,</td></tr>
</table>

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td>after frond counts were completed, samples removed from the three replicate vessels of each treatment level and the control were respectively composited, and the pH was measured in each composite solution.

pH values of the aged solutions with duckweed present and measured on days 3, 5 and 7 were:

<table>
<tr><th>Nominal concentrations</th><th>Day 3
Aged</th><th>Day 5
Aged</th><th>Day 7
Aged</th></tr>
<tr><td>Control</td><td>8.4</td><td>8.6</td><td>8.9</td></tr>
<tr><td>0.94</td><td>8.4</td><td>8.7</td><td>9.0</td></tr>
<tr><td>1.9</td><td>8.5</td><td>8.8</td><td>8.9</td></tr>
<tr><td>3.8</td><td>8.5</td><td>8.6</td><td>9.0</td></tr>
<tr><td>7.5</td><td>8.4</td><td>8.3</td><td>8.5</td></tr>
<tr><td>15</td><td>8.4</td><td>8.4</td><td>8.4</td></tr>
</table>
* Nominal concentrations as mg 5-OH metabolite of pyroxsulam/L.</td><td>0.9 and 1.1 pH units. These changes meet the OECD recommendation that the pH of the control medium should not increase by more than 1.5 units during the test.</td></tr>
<tr><td>Chelator used:</td><td>The 20X AAP recipe contained sodium EDTA (which is permitted in the OECD 221 20X AAP recipe).</td><td>Requirement considered met.

OECD 221 identifies the presence of the chelating agent Na_2EDTA in the 20X-AAP medium.

US EPA OPPTS 850.4400 observes that chelating agents, such as EDTA, are present in the 20X-AAP medium to ensure that trace nutrients will be available to the Lemna fronds and that M-Hoagland's medium (which contains no EDTA) should be used for test solution preparation if it suspected that the chelator will interact with the test chemical.

Chelators are not recommended (US EPA).
This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements which allow use of chelating agents (the AAP medium contains sodium EDTA as a chelating agent).</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
Carbon source:	Not identified. Stated to be ambient carbon dioxide by Hancock (2005)	Requirement considered met on the basis of satisfactory growth in the controls. OECD 221 and US EPA OPPTS 850.4400 do not refer to a "carbon source".
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Although the 20X AAP medium is indicated as being a standard medium, the presence of the sodium selenate means that it is in fact a modified 20X AAP medium (see Attachment 1, page 39 of this DER for details on the composition of the 20X AAP medium).	Requirement considered met as full details of the modified 20X AAP medium were provided in the study report.
<u>Dilution water</u> Source/type:	Not identified but the 20X AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium. 20X AAP medium were prepared using sterile, deionised water and equilibrated to test temperature.	Requirement considered met. OECD 221 does not address the quality of the dilution water in specific terms. As the duckweed cultures used had been maintained in stock culture by Springborn Smithers and because the subculture used for the test had satisfactory growth in the controls, the water used is considered to have been acceptable. OECD 221 refers to the use of deionised water or sterile distilled water for stock media preparation. US EPA OPPTS 850.4400 states that stock solutions or growth media should be prepared just prior to use and diluted with water of high quality such as glass-distilled, deionised water, or ASTM Type I to obtain the test solutions.
pH:	The pH of the test medium was adjusted to 7.5 ± 0.1.	Requirement considered met. OECD 221 and US EPA OPPTS 850.4400 state that if 20X-AAP medium is used, the pH should be adjusted to 7.5 ± 0.1. *EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*

Parameter	Details	Remarks *Criteria*
Total Organic Carbon: Particulate matter: Metals and pesticides:	A representative sample of 20X AAP medium was analyzed monthly for total organic carbon (TOC) concentration. The TOC concentration for 20X AAP medium measured in March 2006 was 2.5 mg/L. Not reported Representative samples of the source of the deionised water used in preparing the 20X AAP medium were analysed periodically for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with ASTM (2002) standard practices.	TOC, particulate matter, etc requirements considered met. OECD 221 and US EPA OPPTS 850.4400 do not address these parameters specifically. As the duckweed cultures used had been maintained in stock culture by Springborn Smithers and because the subculture used for the test had satisfactory growth in the controls, the water used is considered to have been acceptable.
Chlorine: Water pretreatment (if any): Intervals of water quality measurement	Not reported. Deionisation Periodically – see above under Metals and pesticides.	
Indicate how the test material is added to the medium (added directly or used stock solution)	A 15 mg/L primary stock solution was prepared on the day of test initiation by placing 0.0305 g of 5-OH metabolite of pyroxsulam in a 2000-mL volumetric flask and bringing it to volume with 20X AAP medium. The resultant stock solution was observed to be clear and colorless with a large amount of visible undissolved test substance. The solution was stirred for approximately two hours and sonicated for five minutes. Following stirring and sonication, the resultant stock solution was observed to be clear and colorless with no visible undissolved test substance. Nominal test solutions at test initiation were prepared from the primary stock solution by serial dilutions. All test solutions were observed to be clear and colorless with no visible undissolved test substance. The nominal test solutions were	Requirements considered met. The primary stock solution was made up taking into account the 100% purity of the 5-OH metabolite of pyroxsulam.

Parameter	Details	Remarks *Criteria*
	prepared from the primary stock solution prepared at each solution renewal (days 3 and 5) following procedures similar to those used at test initiation.	
Aeration or agitation	Agitation and aeration not indicated as having been used.	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) Origin: Textural classification (% sand, silt and clay): Organic carbon (%): Geographic location:	Not applicable as sediment was not used in the duckweed exposure test.	Requirements considered met.
Number of replicates Control: Solvent control: Treatments:	Three replicate vessels were used for the control. A solvent control was not used. Three per treatment level.	Requirement considered met. OECD 221 states the number of replicate control vessels (and solvent vessels, if applicable) should be at least equal to, and ideally twice, the number of vessels used for each test concentration. US EPA OPPTS 850.4400 states that for each concentration and control at least three replicate containers should be used. Requirement not relevant. Requirement considered met.
Number of plants/replicate	Approximately one hour after the test solutions were prepared and added to the test vessels, an inoculum of fifteen fronds was aseptically introduced into each test vessel by placing plants with two to four fronds each into each vessel until each vessel contained 15 fronds.	Requirement considered met. OECD states that 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.

Parameter	Details	Remarks *Criteria*
		Although the number of fronds used was 15, this is not considered a deviation or deficiency of significance. US EPA OPPTS 850.4400 states that for each concentration and control at least three replicate containers should be used, each containing … three to five plants consisting of three to four fronds each … . *EPA requires 5 plants.* This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS regarding the need for a total of 9 to 12 fronds or 9 to 20 fronds respectively.
Number of fronds/plant	2 to 4 fronds/plant (equal to 15 fronds per replicate)	OECD 221 states that 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. US EPA OPPTS 850.4400 refers to use of three to five plants consisting of three to four fronds each. *EPA requires 3 fronds per plant.* This template requirement is not considered further because of the specification of the 2 to 4 fronds by the current OECD guideline and the total number of fronds (15) used satisfying the US OPPTS guideline requirement of 9 to 20 fronds being exposed.
Test concentrations Nominal:	0 (control, 20X AAP medium), 0.94, 1.9, 3.8, 7.5 and 15 mg 5-OH metabolite of pyroxsulam/L. These concentrations are in ratios of approximately 1:2.	Requirement considered met. OECD 221 states that in the definitive toxicity 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, but a larger value may be

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td></td><td>used where the concentration-response curve is flat.

US EPA OPPTS 850.4400 refers to use of 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).

The measured ratios of approximately 1:2 are considered to meet the guideline requirements.

EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</td></tr>
<tr><td>Measured:</td><td>The measured concentrations at 0, 3, 5 and 7 days are shown in the following table together with the overall mean concentrations:

<table>
<tr><th rowspan="2">Nominal concentration, mg/L</th><th colspan="5">Measure concentrations, mg 5-OH metabolite of pyroxsulam/L</th><th rowspan="2">Percent of nominal</th></tr>
<tr><th>0-Hour (new)</th><th>Day 3 (aged)</th><th>Day 5 (new)</th><th>Day 7 (aged)</th><th>Mean[a]</th></tr>
<tr><td>Control</td><td><0.030</td><td><0.034</td><td><0.034</td><td><0.033</td><td>NA[a]</td><td>NA</td></tr>
<tr><td>0.94</td><td>0.75</td><td>0.82</td><td>0.83</td><td>0.80</td><td>0.80</td><td>85</td></tr>
<tr><td>1.9</td><td>1.6</td><td>1.7</td><td>1.7</td><td>1.7</td><td>1.7</td><td>89</td></tr>
<tr><td>3.8</td><td>3.3</td><td>3.5</td><td>3.5</td><td>3.4</td><td>3.4</td><td>90</td></tr>
<tr><td>7.5</td><td>7.2</td><td>7.4</td><td>6.9</td><td>6.7</td><td>7.1</td><td>94</td></tr>
<tr><td>15</td><td>15</td><td>14</td><td>14</td><td>14</td><td>14</td><td>94</td></tr>
</table>
a. Not applicable. Note that the study report's results shown here were calculated using actual analytical data and not the rounded (2 significant figures) data presented in this table.

Reviewer calculated mean values were equivalent to those given in the study report.</td><td>Requirement considered met.

OECD 221 states that test concentrations (nominal and measured) must be included in the test report. The guideline also states that during the test, the concentrations of the test substance are determined at appropriate intervals. In static tests, the minimum requirement is to determine the concentrations at the beginning and at the end of the test.

US EPA OPPTS 850.4400 refers to use of standard analytical methods, if available, to establish concentrations of the test solutions and that concentrations of the test chemical in the test solutions prior to use and discarding on day 3, 5, and 7 should be reported.</td></tr>
<tr><td></td><td>Results from the quality control samples were:

<table>
<tr><td>QC#1</td><td>0.464</td><td>0.499</td><td>0.465</td><td>0.461</td></tr>
<tr><td>0.500</td><td>(92.9)[a]</td><td>(99.8)</td><td>(93.1)</td><td>(92.3)</td></tr>
<tr><td>QC#2</td><td>4.70</td><td>4.71</td><td>4.69</td><td>4.61</td></tr>
<tr><td>5.00</td><td>(94.1)</td><td>(94.2)</td><td>(93.7)</td><td>(92.2)</td></tr>
<tr><td>QC#3</td><td>18.6</td><td>18.0[b]</td><td>19.0</td><td>18.7</td></tr>
<tr><td>20.0</td><td>(93.1)</td><td>(89.8)</td><td>(95.2)</td><td>(93.4)</td></tr>
</table>
a Percent of nominal is presented in parentheses.
b Area of this sample was above the curve, therefore, the concentration is extrapolated.</td><td>The study report also noted that the analyses of the quality control samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 89.8 to 99.8% (N=12) of the nominal fortified levels (0.5, 5.0 and 20.0 mg/L). Based on the results of these analyses, it was established that the appropriate precision</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
		and quality control was maintained during the analyses of the exposure solutions.
Solvent (type, percentage, if used)	A solvent was not used.	Requirement not applicable as no solvent was used.
Method and interval of analytical verification:	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 analysed for 5-OH metabolite of pyroxsulam. Samples analysed from newly prepared solutions (days 0 and 5) were removed from the volumetric flasks prior to division into the replicate vessels. Test solution samples analysed at the end of the renewal periods (days 3 and 7) were removed from composited solutions of each treatment level and the control. Three quality control (QC) samples were prepared at each sampling interval and remained with the appropriate set of exposure solution samples throughout the analytical process. All exposure solution and QC samples were analysed for 5-OH metabolite of pyroxsulam by high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV) based on methodology validated at Springborn Smithers. The method validation study established an average recovery of 103 ± 5.80% from 20X AAP medium. The acceptable range for evaluating QC sample recovery was set at 80% to 120%.	Requirement considered met. Analysis of the quality control samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 89.8 to 99.8% (N=12) of the nominal fortified levels (0.5, 5.0 and 20.0 mg/L). Based on the results of these analyses, it was established that the appropriate precision and quality control was maintained during the analyses of the exposure solutions. Typical chromatograms of a calibration standard, a 100 mg/L recovery sample fortified with 5-OH metabolite of pyroxsulam during the method validation study and a control sample were presented. The QC samples were prepared in dilution water at nominal concentrations which approximated the test concentration range. The results of the analyses of the QC samples were used to judge the precision and the quality control maintained during the analytical process. Conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation study.
Limit of Quantitation:	The limit of quantitation (LOQ) was reported as 0.0115 mg 5-OH metabolite of pyroxsulam/L.	
Limit of Detection:	Not reported.	

Parameter	Details	Remarks *Criteria*
Test conditions Temperature:	Temperatures during the exposure period ranged from 22 to 25°C.	Requirement considered met. OECD 221 states that the temperature in the test vessels should be 24 ± 2°C. US EPA OPPTS 850.4400 states that the environmental conditions should be maintained at 25 ± 2°C. While a temperature of 22°C was recorded and is just below the US EPA OPPTS 850.4400 range of 23 to 27°C, this occurred only once. This was on day 5. At all other times the minimum temperature was 23°C, complying with the US EPA OPPTS guideline. Consequently, this single excursion outside the recommended limits is not considered to have been of significance. *EPA temperature: 25°C* The template requirement does not provide an acceptable range and has been disregarded in favour of the current guidelines.
Photoperiod:	Continuous light conditions	Requirement considered met. OECD 221 refers to use of continuous warm or cool white fluorescent light. US EPA OPPTS 850.4400 states that continuous warm-white fluorescent lighting should be used. *EPA photoperiod: continuous*
Light intensity and quality:	6,500 to 9,100 lux The photosynthetically active radiation (PAR) measured at test initiation ranged from 119 to 136 $\mu E/m^2/s$.	See deviations/deficiency table on page 35 of this report. OECD 221 refers use of light of an intensity equivalent to 6,500-10,000 lux and to 85-135 $\mu E/m^2/s$ when measured in a photosynthetically active radiation (400-700 nm). The measured PAR is considered to comply with the range of 85-135 $\mu E/m^2/s$

Parameter	Details	Remarks *Criteria*
		specified by the OECD guideline. US EPA OPPTS 850.4400 states that a light intensity in the range of 4,200 and 6,700 lux should be used. *EPA light: 5,000 lux (15%)*
Reference chemical (if used) Name: Concentrations:	No reference chemical mentioned.	Requirement considered met. OECD 221 states that a reference substance(s), such as 3,5-dichlorophenol may be tested as a means of checking the test procedure. The guideline says 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. US EPA OPPTS 850.4400 states that positive controls using zinc chloride as a reference chemical should be run periodically. While it is considered most probable that testing with a reference chemical had been conducted with satisfactory results and it is only an oversight that the relevant results were not provided, provision of the results from the most recent reference chemical study would have added value to the test report.
Other parameters, if any	None identified.	Not applicable.

2. Observations:

Table 2. Observation parameters

Parameters	Details	Remarks *Criteria*
Parameters measured (e.g.: number of fronds, plant dry weight or other toxicity symptoms)	On days 3, 5 and at test termination (day 7), fronds were counted and observations were made. At test termination, frond dry weights were determined for each control and test treatment. Inhibition of cell density, total biomass and average growth rate relative to the control's results were the identified effects criteria. pH, temperature, light intensity and analyte concentrations were determined either continuously or at defined intervals during the study.	Requirement considered met. OECD 221 refers to determination of total frond area and dry and fresh frond weights with frond number the primary measurement variable. The guideline also notes that the test report must include, *inter alia*, temperature during the test, light intensity and homogeneity, pH values of the test and control media and test substance concentrations. The test reported dry frond weights. US EPA OPPTS 850.4400 states 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. As noted above, the test reported dry weight values as one of the other optional endpoint parameters. The US guideline also refers to pH measurement before and after use of the test solutions, measurement of light intensity and a temperature range of 23 to 27°C. Concentration of the test chemical in the test solutions prior to use and discarding on day 3, 5, and 7 should also be reported. Biomass (dry weight) of the plants (fronds and roots) in each replicate was determined by allowing the plants dry at approximately 60°C for at least 48 hours in a drying oven.
Measurement technique for	Counting of fronds.	See deviations/deficiency table on page

frond number and other end points	Frond dry weight (determined at test termination, when the counted fronds were dried at 62 to 69°C for three days).	35 of this report. OECD 221 refers to frond numbers appearing normal or abnormal, need to be determined at the beginning of the test, at least once every 3 days during the exposure period (i.e. on at least 2 occasions during the 7 day period), and at test termination and that total frond area, dry weight (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) and fresh weight may be determined. US EPA OPPTS 850.4400 states that "Any frond which is visible as a bud when viewed under a hand lens or dissecting microscope should be counted." While the study report did not refer to use of such optical aids, it has been assumed that they were used and the omission of this information from the report is not considered a deficiency. While the drying temperature of 62 to 69°C exceeds the current OECD 221 specified temperature of 60°C, the temperature range used to dry the fronds was within the study protocol's specified range of 60 to 70°C.
Observation intervals	On days 3, 5 and at test termination (day 7), fronds were counted and observations were made. The pH of the exposure solutions was measured at test initiation (new solutions), in each aged and new solution at each renewal period, and at test termination (aged solutions). Temperature was measured continuously with a maximum/minimum thermometer located in a flask of water adjacent to the test vessels. Temperature readings	Requirement considered met. OECD 221 refers to frond numbers appearing normal or abnormal, need to be determined at the beginning of the test, at least once every 3 days during the exposure period (i.e. on at least 2 occasions during the 7 day period), and at test termination. OECD 221 also states that if a semi-static test design is used, the pH should be measured in each batch of 'fresh' test solution prior to each renewal and also in the corresponding 'spent'

	were recorded daily. Light intensity was measured at 0 hour and at each subsequent 24-hour interval during the exposure period. Photosynthetically active radiation was measured at the initiation of the exposure phase. 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 5-OH metabolite of pyroxsulam.	solutions and that light intensity measurements should be made at least once during the test. Additionally, the temperature of the medium in a surrogate vessel held under the same conditions in the growth chamber, incubator or room should be recorded at least daily. OECD 221 also states that during the test, the concentrations of the test substance are determined at appropriate intervals.
Other observations, if any	The initial pH of the 20X AAP medium was adjusted, if necessary, to 7.5 ± 0.1 prior to use. The test vessels were assigned new random positions within the environmental chamber after the 3- and 5-day observation intervals.	Requirement considered met. OECD 221 states that the pH of the growth medium is adjusted to pH 7.5 ± 0.1. US EPA OPPTS 850.4400 states that if 20X-AAP medium is used, the pH should be adjusted to 7.5 ± 0.1 with 0.1 N NaOH or HCl. OECD 221 states that the method of light detection and measurement, in particular the type of sensor, will affect the measured value. Spherical sensors (which respond to light from all angles above and below the plane of measurement) and "cosine" sensors (which respond to light from all angles above the plane of measurement) are preferred to unidirectional sensors, and will give higher readings for a multi-point light source of the type described in the 221 guideline. US EPA OPPTS 850.4400 also states that a light intensity in the range of 4,200 and 6,700 lux, as measured adjacent to each test chamber at the surface of the test solution. The light intensity at each position in the

		incubation area should be measured and should not differ by more than 15 percent from the selected light intensity.
Indicate whether there was an exponential growth in the control	The study's protocol required that the doubling time of frond number in the control must be less than 2.5 days, which corresponds to approximately an eight-fold increase in 7 days. The 7-day mean control frond density was 350 fronds, which exceeds the required eight-fold increase of 120 fronds (e.g., 15 fronds/vessel x 8 = 120). Additionally, the 7-day average specific growth rate of the control should exceed 0.275 $days^{-1}$. The 7-day average specific growth rate of the control was 0.46 $days^{-1}$, which exceeds the required rate.	Requirement considered met. OECD 221 states, "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}". No specific requirements were identified in US EPA OPPTS 850.4400.
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?	No. Tabulated results for duckweed growth data (specific growth rate, frond counts, dry weight, percentage inhibition), pH, 5-OH metabolite of pyroxsulam concentrations in the test solutions, light intensity and temperature were provided. All original raw data, the protocol and the original final report produced during this study are archived by the Toxicology and Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan. A copy of the final report is retained at Springborn Smithers Laboratories, Wareham, Massachusetts.	Requirement considered met. With respect to data, OECD 221 states that, *inter alia*, the test report must contain raw data for number of fronds and other measurement variables in each test and control vessel at each observation and occasion of analysis. The guideline also states that the test report must include results relating to any visual signs of phytotoxicity as well as observations of test solutions. While the data presented in the study report is not "raw" data (i.e. in the form of laboratory reports), they were presented as individual replicate values

		which are considered to be sufficient to allow a reliable assessment of the study's results – e.g. individual frond numbers in each replicate at days 0, 3, 5 and 7 were presented as tabulated results as were the dry frond weights for each replicate. The data presented are considered to provide the same information as would have been provided by "raw data". US EPA OPPTS 850.4400 says that the number of fronds per test concentration and control at the end of the test, the percent inhibition and/or stimulation of growth rate, and percent frond mortality for each test concentration compared to controls should be in the data which should be reported. The data presented in the study report is considered to have met the US EPA OPPTS 850.4400 requirements in this respect. US EPA advice was that the tabulated data is considered as "raw" provided it is complete enough to re-run statistical analyses (which in this case it was).

II. RESULTS AND DISCUSSION:

A. INHIBITORY EFFECTS:

The following was information was presented in the study report.

Test concentrations of the 5-OH metabolite of pyroxsulam
The measured concentrations closely approximated the desired nominal concentrations and provided the expected concentration gradient. Mean measured concentrations ranged from 85 to 94% of the nominal concentrations and defined the treatment levels tested as 0.80, 1.7, 3.4, 7.1 and 14 mg/L.

Analysis of the quality control samples was reported to have resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 89.8 to 99.8% (N=12) of the nominal fortified levels (0.5, 5.0 and 20.0 mg/L). Based on the results of these analyses, the study report stated that the appropriate precision and quality control was maintained during the analyses of the exposure solutions.

Frond density
The 7-day frond density in the control averaged 350 fronds per replicate. Frond production in the 0.80, 1.7, 3.4, 7.1 and 14 mg/L treatment levels averaged 355, 391, 383, 76 and 30 fronds per replicate, respectively.

Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set was reported to have passed the requirements for normality and homogeneity of variance, therefore, Williams' Test was used to determine treatment-related effects. Williams' Test determined a significant reduction in frond density in the 7.1 and 14 mg/L treatment levels compared to the control data.

Response relative to the day 7 control mean frond count ranged from -12 to 91% inhibition of mean frond density. The mean frond counts and standard deviations at day 3, 5 and 7 given in the study report were recalculated by the reviewer and found to be identical to those reported. Similarly, the study report's percentage inhibition values for frond count at day 7 were recalculated and verified as correct.

The study report's toxicological results for day 7 frond counts were:

7-Day Results	EC05	EC50	EC90	LOEC	NOEC
EC value (mg/L):	3.6	5.7	13	7.1	3.4
95% confidence limits:	3.4-3.6	5.6-5.8	13-13	Not applicable	Not applicable

The frond counts from days 0 to 7, plus the calculated percentage inhibition based on control counts, as given in the study report, are shown in Table 3. Mean frond counts/control or test solution and associated standard deviations are also shown in the table.

Table 3. Effect of 5-OH metabolite of pyroxsulam on frond number of the freshwater duckweed (*Lemna gibba*) as given in the study report (Hoberg, 2006).

Treatment (nominal and measured concentration), mg 5-OH metabolite of pyroxsulam/L		Replicate No.	Frond number at:				% inhibition from the controls[c]
Nominal	Mean measured		Day 0	Day 3	Day 5	Day 7	
0 (Control)	<LLQ[a]	A	15	62	185	316	
		B	15	73	199	379	
		C	15	71	211	355	
		Mean	15	69	198	350	NA[d]
		SD[b]	0	6	13	32	
0.94	0.80	A	15	62	190	346	
		B	15	63	196	389	
		C	15	61	180	330	
		Mean	15	62	189	355	-1
		SD	0	1	8	31	
1.9	1.7	A	15	72	201	383	
		B	15	62	173	394	
		C	15	65	197	395	
		Mean	15	66	190	391	-12
		SD	0	5	15	7	
3.8	3.4	A	15	56	228	430	
		B	15	50	174	358	
		C	15	64	215	362	
		Mean	15	57[e]	206[e]	383[e]	-10
		SD	0	7	28	40	
7.5	7.1	A	15	30	54	81	
		B	15	27	49	67	
		C	15	30	56	81	
		Mean	15	29[e]	53[ef]	76[efg]	78
		SD	0	2	4	8	
15	14	A	15	23	33	33	
		B	15	22	29	28	
		C	15	23	29	29	
		Mean	15	23[e]	30[ef]	30[efg]	91
		SD	0	1	2	3	

a <LLQ = Less than the limit of quantification. The limit of quantitation was 0.0115 mg/L. b. SD = Standard Deviation. c. Percent inhibition relative to the control. d. NA = Not Applicable. e. Curled fronds were observed. f. Slightly chlorotic fronds were observed. g. Significantly reduced compared to the control, based on Williams' Test.

At test termination, fronds exposed to the 3.4, 7.1 and 14 mg/L treatment levels were curled, while fronds exposed to the 7.1 and 14 mg/L treatment levels were also observed to be slightly chlorotic.

Growth rate

At test termination, growth rate for the control averaged 0.46 days^{-1}. Frond growth rate in the 0.80, 1.7, 3.4, 7.1 and 14 mg/L treatment levels averaged 0.46, 0.48, 0.47, 0.24 and 0.10 days^{-1}, respectively.

Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set passed the requirements for normality and homogeneity of variance, therefore, Williams' Test was used to determine treatment-related effects. Williams' Test determined a significant reduction in growth rate in the 7.1 and 14 mg/L treatment levels compared to the control data. Therefore, the 7 day NOEC and LOEC for growth rate were determined to be 3.4 and 7.1 mg/L, respectively. The 7-day EC50 value was determined to be 7.4 mg/L, with 95% confidence limits of 6.9 to 7.9 mg/L.

The calculated growth rates for days 0-3, 0-5 and 0-7, plus the calculated percentage inhibition based on the mean day 7 control growth rate, as given in the study report, are shown in Table 4.

Table 4. Calculated growth rates of *Lemna gibba* after 7 days of exposure to 5-OH metabolite of pyroxsulam, as reported by Hoberg, 2006.

Mean Measured Concentration (mg/L)	Replicate	Average Growth Rate (days^{-1}) Observation Interval (Days) Day 0-3	Day 0-5	Day 0-7	7-Day Inhibition[a]
Control	A	0.46	0.50	0.45	
	B	0.51	0.51	0.47	
	C	0.50	0.52	0.46	
	Mean (SD[b])	**0.49 (0.03)**	**0.51 (0.01)**	**0.46 (0.01)**	**NA[c]**
0.80	A	0.46	0.50	0.46	
	B	0.46	0.51	0.48	
	C	0.45	0.49	0.45	
	Mean (SD)	**0.46 0.01)**	**0.50 (0.01)**	**0.46 (0.01)**	**0**
1.7	A	0.51	0.51	0.48	
	B	0.46	0.48	0.48	
	C	0.47	0.51	0.48	
	Mean (SD)	**0.48 (0.02)**	**0.50 (0.02)**	**0.48 (0.00)**	**-4**
3.4	A	0.43	0.54	0.49	
	B	0.39	0.48	0.47	
	C	0.47	0.53	0.47	
	Mean (SD)	**0.43 (0.04)**	**0.52 (0.03)**	**0.47 (0.02)**	**-2**
7.1	A	0.22	0.25	0.25	
	B	0.19	0.23	0.22	
	C	0.22	0.26	0.25	
	Mean (SD)	**0.21 (0.02)**	**0.25 (0.01)**	**0.24 (0.02)[d]**	**48**
14	A	0.14	0.16	0.12	
	B	0.12	0.13	0.09	
	C	0.14	0.13	0.10	
	Mean (SD)	**0.13 (0.01)**	**0.14 (0.01)**	**0.10 (0.01)[d]**	**78**

a Percent inhibition relative to the control.
b SD = Standard Deviation.
c NA = Not Applicable.
d Significantly reduced compared to the control, based on Williams' Test.

Frond dry weight

Mean and individual frond dry weight results were presented in the study report. The replicate frond weights and percentage inhibitions based on the control are shown in Table 5.

The 7-day biomass for the control averaged 44.57 mg. Frond biomass in the 0.80, 1.7, 3.4, 7.1 and 14 mg/L treatment levels averaged 41.97, 42.73, 38.20, 19.80 and 15.23 mg, respectively. Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set passed the requirements for normality and homogeneity of variance, therefore, Williams' Test was used to determine treatment-related effects. Williams' Test determined a significant reduction in frond biomass in the 3.4, 7.1 and 14 mg/L treatment levels compared to the control data. Therefore, 7-day NOEC and LOEC values for frond biomass were determined to be 1.7 and 3.4 mg/L, respectively. The 7-day EC50 value was determined to be 6.6 mg/L, with 95% confidence limits of 5.8 to 7.0 mg/L.

Mean and individual frond dry weight results were presented in the study report. The replicate frond weights and percentage inhibitions based on the control are shown in Table 5.

Table 5. Effect of 5-OH metabolite of pyroxsulam on frond dry weight of the freshwater duckweed (*Lemna gibba*) as given in the study report (Hoberg, 2006).

Treatment (mean measured concentration), mg 5-OH metabolite of pyroxsulam/L	Replicate No.	Frond dry weight at day 7, mg	% inhibition[a]
Control	A	39.10	
	B	52.20	
	C	42.40	
	Mean (sd[b])	44.57 (6.8)	NA[c]
0.80	A	38.60	
	B	45.90	
	C	41.40	
	Mean (sd)	41.97 (3.7)	6
1.7	A	40.30	
	B	44.50	
	C	43.40	
	Mean (sd)	42.73 (2.2)	4
3.4	A	41.80	
	B	37.60	
	C	35.20	
	Mean (sd)	38.20 (3.3)[d]	14
7.1	A	19.00	
	B	19.50	
	C	20.90	
	Mean (sd)	19.80 (1.0)[d]	56
14	A	16.40	
	B	15.00	
	C	14.30	
	Mean (sd)	15.23 (1.1)[d]	66

a Percent inhibition relative to the control.
b SD = Standard Deviation.
c NA = Not Applicable.
d Significantly reduced compared to the control, based on Williams' Test.

STATISTICAL ENDPOINT VALUES REPORTED IN THE STUDY REPORT

The study report's statistical findings are summarized in Table 6.

Table 6. 7 Day statistical endpoint values (NOEC, LOEC and EC50 values for duckweed exposed to various 5-OH metabolite of pyroxsulam concentrations for 7 days in a static renewal test) as reported by Hoberg (2006)

7 day Statistical Endpoint	Frond No.	Mean specific growth rate (per day)	Biomass (frond dry weight)
NOEC (mg 5-OH metabolite of pyroxsulam/L)	3.4	3.4	1.7
LOEC (mg 5-OH metabolite of pyroxsulam/L)	7.1	7.1	3.4
EC50 (mg 5-OH metabolite of pyroxsulam/L), 95% confidence limits in brackets.	5.7 (5.6-5.8)	7.4 (6.9-7.9)	6.6 (5.8-7.0)
Reference chemical **NOEC** **IC50/EC50**	No reference chemical used.		

Validity of test

OECD 221 (2006) requires that, 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/day.

To determine the doubling time *(Td)* of frond number and adherence to this validity criterion by the study (paragraph 12), OECD 221 states that the following formula is used with data obtained from the control vessels:

$T_d = \ln 2/\mu$

where μ is the average specific growth rate

The average specific growth rate for a specific period is calculated as the logarithmic increase in the growth variables -frond numbers and one other measurement variable (total frond area, dry weight or fresh weight) - using the formula below for each replicate of control and treatments:

$$\mu_{i-j} = (\ln(Nj) - \ln(Ni)))/t$$

where:

- μ_{i-j}: average specific growth rate from time i to j
- Ni : measurement variable in the test or control vessel at time i
- Nj : measurement variable in the test or control vessel at time j
- t : time period from i to j For each treatment group and control group

Examination of US EPA OPPTS 850.5400 did not identify validity criteria.

Using the reported mean specific growth rates for the control, the calculated doubling time was as shown in Table 7.

Table 7. Reviewer calculated control doubling time for frond numbers in *Lemna gibba*

Sample	Reported mean specific growth rate, per day	Td (doubling time), days
Control	0.46	1.51

The control Td value all satisfies the OECD 221 requirement that the Td be <2.5 days. The mean specific growth rate of 0.46/day reported in the study report exceeded the OECD 221 requirement that the average specific growth rate be 0.275/day.

Frond number increase over 7 days

OECD 221 also refers to the test being valid if there is an approximately 7-fold increase in frond numbers in seven days. The day 7 mean frond number for the control was 350 fronds. As the initial frond number was 15, the day 7 counts represent a 23-fold increase in frond number, satisfying the OECD 221 criterion.

B. REPORTED STATISTICS:

The study report's results were based on mean measured concentrations (calculated from the initial (0-hour) and day 5 new test solutions and the day 3 and day 7 aged test solutions) of 5-OH metabolite of pyroxsulam and are reported as the 7-day EC05, EC50 and EC90, No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) values for 7-day frond density, growth rate and frond biomass (dry weight). Mean measured concentrations ranged from 85 to 94% of the nominal concentrations and defined the treatment levels tested as 0.80, 1.7, 3.4, 7.1 and 14 mg/L.

The EC05, EC50 and EC90 values were calculated, when possible, for frond densities, average growth rate and biomass at test termination. The EC05, EC50 and EC90 values are defined as the concentration of test substance which caused a 5%, 50% or 90% reduction, respectively, in frond density, average growth rate or biomass, compared to the control data. 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. If no concentration resulted in a 5%, 50% or 90% reduction, the EC values were empirically estimated to be greater than the highest concentration tested.

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 using the equation given under "Validity of test", page 29 of this DER.

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.

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

The statistical re-evaluation of the biological data presented in the study report for frond numbers, mean specific growth rates and biomass (as dry weight) was performed. Toxicity endpoints are expressed as mean measured concentrations. The statistical analyses conducted are shown in Appendix I of this DER.

Verification of frond number (cell density) statistics
Replicate data for frond numbers, specific growth rates and biomass were tested (ToxCalc™ v5.0.23j. Copyright 1994-2005 Tidepool Scientific Software, McKinleyville, CA 95519 USA) for normality and homogeneity, by respectively, the Shapiro-Wilk's and Bartlett's tests and for difference between the mean frond counts, mean specific growth rates and mean biomass results of the 5-OH metabolite of pyroxsulam exposed duckweed and the mean of the controls by William's test. The ToxCalc package was used to determine the EC50 and associated 95% confidence limits by use of linear interpolation methodology and NOEC values.

Frond counts

The ToxCalc analysis used the untransformed day 3, 5 and 7 frond counts with the untransformed data for days 3 and 5 were identified as normally distributed with equal variances. The day 7 frond counts were identified as normally distributed with equality of variances also being confirmed.

The results of these frond analyses are shown in Table 8 (page 32) with the ToxCalc results shown on, respectively, pages 40, 41 and 42 of this DER.

Table 8. Reviewer calculated EC50 and NOEC values for *Lemna gibba* frond counts after 3, 5 and 7 days exposure to 5-OH metabolite of pyroxsulam with the results determined by use of mean measured concentration. EC50, 95% confidence limits and NOEC values are as mg 5-OH metabolite of pyroxsulam/L.

Time	EC50	95% Confidence limits	NOEC	Mean measured concentrations which had statistically significantly lower mean frond counts compared to the mean of the control
Day 3	6.4	5.4-7.2	1.7	≥3.4
Day 5	5.9	5.3-6.1	3.4	≥7.1
Day 7	5.7	5.5-5.8	3.4	≥7.1
Study report's day 7 values	5.7	5.6-5.8	3.4	≥7.1

The study report's 7-day calculated EC50 value (95% confidence interval) for cell density (i.e. frond count) was 5.7 (5.6-5.8) mg 5-OH metabolite of pyroxsulam/L. As shown in Table 8, the reviewer calculated 7 day EC50, 95% confidence limits and NOEC were the same as those given in the study report.

Verification of specific growth rate statistics

The specific growth rates for each replicate and the equivalent mean and standard deviation were recalculated using the day 0 and day 7 frond counts with a time interval of 7 days as per the previously given equation (page 29 of this DER refers).

The recalculated individual replicate values and their associated mean, standard deviations and % inhibition based on the control mean were equivalent to those given in the study report.

The recalculated specific growth rates and associated mean and standard deviations are shown in Table 9 with the calculated % inhibition. Note that negative inhibition indicates greater growth than controls.

Table 9. Reviewer's recalculation of day 0-3, day 0-5 and day 0-7 specific growth rates and % inhibition using the day 7 frond count results and the day 7 % inhibition based on the mean control rates at that time.

Concentration*	Replicate	Average Growth Rate (days^{-1})			Reviewer calculated % inhibition	Study report % inhibition
		day 0-3	day 0-5	day 0-7		
Control	A	0.47	0.50	0.44		
	B	0.53	0.52	0.46		
	C	0.52	0.53	0.45		
	Mean	**0.51**	**0.52**	**0.45**		
	St. dev.	**0.03**	**0.01**	**0.01**	**Not applicable (NA)**	**NA**
0.80	A	0.47	0.51	0.45		
	B	0.48	0.51	0.47		
	C	0.47	0.50	0.44		
	Mean	**0.47**	**0.51**	**0.45**	**0**	**0**
	St. dev.	**0.01**	**0.01**	**0.01**		
1.7	A	0.52	0.52	0.46		
	B	0.47	0.49	0.47		
	C	0.49	0.52	0.47		
	Mean	**0.49**	**0.51**	**0.47**	**-4**	**-4**
	St. dev.	**0.03**	**0.02**	**0.00**		
3.4	A	0.44	0.54	0.48		
	B	0.40	0.49	0.45		
	C	0.48	0.53	0.45		
	Mean	**0.44**	**0.52**	**0.46**	**-2**	**-2**
	St. dev.	**0.04**	**0.03**	**0.01**		
7.1	A	0.23	0.26	0.24		
	B	0.20	0.24	0.21		
	C	0.23	0.26	0.24		
	Mean	**0.22**	**0.25**	**0.23***	**49**	**48**
	St. dev.	**0.02**	**0.01**	**0.02**		
14	A	0.14	0.16	0.11		
	B	0.13	0.13	0.09		
	C	0.14	0.13	0.09		
	Mean	**0.14**	**0.14**	**0.10***	**78**	**78**
	St. dev.	**0.01**	**0.01**	**0.01**		

Notes: The reviewer calculated specific growth rates, standard deviations, and % inhibition were equivalent to those reported in the study report. Percentage inhibition is based on the day 0-7 mean average growth rate (0.50 days^{-1}). Day 0-7 means marked with an asterisk as statistically significantly less than the day 0-7 control mean value of 0.46 days^{-1}.

The % inhibition data in Table 9 indicate a dose response was occurring at the 7.1 and 14 mg 5-OH metabolite of pyroxsulam/L concentrations.

The ToxCalc analysis used the reviewer calculated untransformed day 0-7 specific growth rates. The untransformed data were identified as normally distributed with equality of variances being confirmed. Mean specific growth rates for concentrations ≥7.1 mg 5-OH metabolite of pyroxsulam/L were identified as statistically significantly less than the control mean (Williams' test, 1 tailed).

The ToxCalc output is provided at page 43 of this DER.

The study report's and the reviewer calculated toxicity endpoints based on specific growth rate are considered equivalent as shown in Table 10.

Table 10. Reviewer calculated ErC50 and NOEC values determined from the specific growth rates (as day^{-1}) for *Lemna gibba* frond counts after 7 days exposure to 5-OH metabolite of pyroxsulam. EC50, 95% confidence limits and NOEC values are as mg 5-OH metabolite of pyroxsulam/L. Equivalent study report values are also shown.

	ErC50	95% Confidence limits	NOEC	Mean measured concentrations which had statistically significantly lower mean specific growth rates compared to the mean of the control
Reviewer calculated	7.3	6.4-8.3	3.4	≥7.1
Study report	7.4	6.9-7.9	3.4	≥7.1

Verification of biomass (frond dry weight) statistics

The biomass (day 7 frond dry weight) data reported are shown in Table 5 on page 28 of this DER and were analysed by the TidePool Scientific Software program, ToxCalc (v5.0.23A) as previously described.

The ToxCalc analysis used the untransformed day 7 frond dry weight values given in the study report and identified the dry weight data as normally distributed with equality of variances confirmed. The ToxCalc output is provided on page 44 of this DER.

The study report's and the reviewer calculated toxicity endpoints based on biomass (as day 7 frond dry weight) are considered equivalent as shown in Table 11.

Table 11. Reviewer calculated EbC50 and NOEC values determined from the measured dry frond weight (i.e. biomass as mg) for *Lemna gibba* frond counts after 7 days exposure to 5-OH metabolite of pyroxsulam. EC50, 95% confidence limits and NOEC values are as mg 5-OH metabolite of pyroxsulam/L. Equivalent study report values are also shown.

	EbC50	95% Confidence limits	NOEC	Mean measured concentrations which had statistically significantly lower mean biomass (as frond dry weight) compared to the mean of the controls
Reviewer calculated	6.6	5.0-7.4	1.7	≥3.4
Study report	6.6	5.8-7.0	1.7	≥3.4

D. STUDY DEFICIENCIES:

Table 12 summarises deficiencies and deviations from the OECD 221 and US EPA OPPTS 850.4400 Guidelines.

Table 12. Deviation from Guidelines and other deficiencies

Parameter	Study reported results	OECD 221	US EPA OPPTS 850.4400
Acclimation Period:	The fronds used to initiate the toxicity test with 5-OH metabolite of pyroxsulam were taken from a stock culture that had been transferred to fresh medium two days prior to testing.	OECD 221 states that 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.	US EPA OPPTS 850.4400 states axenic stock cultures should be grown in the aquariums for 2 weeks (with necessary transfers) prior to being used in a test. Plants used in a test should be randomly selected from the culturing tank. Inocula should be taken from cultures which are less than 2 weeks old.
Details of growth medium Name:	Modified 20X AAP. Contains sodium selenate as an additional nutrient identified as required via a personal communication. Dr. R.R.L. Guillard, June 1991.	OECD 221 provides the composition of the 20X AAP medium with sodium selenate not identified as a constituent.	US EPA OPPTS 850.4400 refers to use of 20X-AAP medium but does not provide the constituents or their percentages. This guideline states that chelating agents such as EDTA are present in 20X AAP medium and that, if it is suspected that the chelating agent will interact with the test material, M-Hoagland's medium, which has no EDTA, should be used.
Light intensity and quality:	6500 to 9100 lux The photosynthetically active radiation (PAR) measured at test initiation ranged from 119 to 136 $\mu E/m^2/s$.	OECD 221 refers use of light of an intensity equivalent to 6500-10000 lux and to 85-135 $\mu E/m^2/s$ when measured in a photosynthetically active radiation (400-700 nm). The measured PAR is considered to comply with the range of 85-135 $\mu E/m^2/s$ specified by the OECD guideline.	US EPA OPPTS 850.4400 states that a light intensity in the range of 4,200 and 6,700 lux should be used.
Measurement technique for frond number and other end points	Fronds were dried at 62 to 69°C for three days.	OECD 221 refers to frond numbers appearing normal or abnormal, need to be determined at the beginning of the test, at least once every 3 days during the exposure period (i.e. on at least 2 occasions during the 7 day period), and at	US EPA OPPTS 850.4400 states that "Any frond which is visible as a bud when viewed under a hand lens or dissecting microscope should be counted." While the study report did not refer to use of such optical aids, it has been assumed that they were used and the omission of this information from the report is not considered a

		test termination and that total frond area, dry weight (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) and fresh weight may be determined.	deficiency.

The use of a 2 day old subculture for the test fails the 7 to 10 days acclimatisation referred to by OECD 221 but would be considered to meet the US EPA OPPTS 850.4400 requirement that inocula should be taken from cultures which are less than 2 weeks old. As there was acceptable growth of the duckweed in the controls, this deviation from the OECD 221 requirements is not considered to have adversely affected the study's conduct or outcomes. However, the reason for using the 2 day old subculture could have been profitably included in the study report.

The medium used, 20X AAP was identified as having the same constituents as the OECD (2006) 20X AAP medium recipe with the exception that the study medium also contained sodium selenate. While the presence of this chemical is not expected to have adversely affected the study's conduct or outcomes, the study report should have identified the presence of the sodium selenate in the body of the report to make it clear that a modified 20X AAP medium had been used.

The 6500 to 10,000 lux range exceeds the 4,200 to 6,700 lux which US EPA OPPTS 850.4400 states should be used. The higher light intensity was not identified as detrimental to the study's conduct or results.

While the drying temperature range of 62 to 69°C used to determine frond dry weights exceeds the current OECD 221 specified temperature of 60°C, the temperature range used to dry the fronds was within the study protocol's specified range of 60 to 70°C. Because controls and 5-OH metabolite of pyroxsulam exposed fronds were subjected to the same drying range, the frond dry weights are considered valid even though the drying temperature exceeds the 60°C recommended by the current OECD 221 guideline.

E. REVIEWER'S COMMENTS:

The study is considered to have been satisfactorily conducted following the requirements of OECD 221 and US EPA OPPTS 850.4400 and to have yielded reliable results. The OECD 221 validity requirement with respect to doubling time of frond numbers in the controls being less than 2.5 days is considered met. The deficiencies/deviations found are not considered to have adversely affected either the study's conduct or its results.

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

F. CONCLUSIONS:

The static 3 and 5 day renewal exposure of duckweed to mean measured concentrations of 0.80 to 14 mg 5-OH metabolite of pyroxsulam/L for seven days is considered to have been satisfactorily conducted according to the requirements of the OECD 221 and US EPA OPPTS 850.4400 guidelines and to have generated acceptable results with respect to effects of the 5-OH metetabolite of pyroxsulam on the growth of duckweed. As a result, the study is

acceptable.

Three duckweed growth parameters were determined, frond number over seven days, mean specific growth rates (day^{-1}) and biomass (as day 7 dried frond weight) with a medium control.

Analytical concentrations of 5-OH metabolite of pyroxsulam in the test solutions, pH, temperature and lighting intensity were satisfactorily determined during the study's exposure phase.

The toxicity EC50 endpoints from the study report were as follows:

7 day duckweed growth endpoints, as mg of 5-OH metabolite of pyroxsulam/L with 95% confidence limits shown in brackets:		
	Study report	
Frond number EC50	5.7 (5.6-5.8)	
Mean specific growth rate (day^{-1}) ErC50	7.4 (6.9-7.9)	
Biomass (frond dry weight) EbC50	6.6 (5.8-7.0)	

The EC50 values are considered to classify the 5-OH metabolite of pyroxsulam as moderately toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment, Water, Heritage and the Arts ($1 < EC50 \leq 10$ mg/L).

The study report values are acceptable and will be used in the risk assessment.

III. REFERENCES:

American Society for Testing and Materials (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. 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.

OECD, 2004. OECD Guideline for Testing of Chemicals. *Lemna* sp., Growth Inhibition Test. Revised Protocol for a New Guideline #221. Draft, April 2004.

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.

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.

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.

Attachment 1
Comparison of the 20X AAP medium used in the duckweed study of Hoberg (2006) and the 20X AAP medium described by OECD 221 (2006).

Compound	Final Concentration (mg/L)	
	As described in the study report (Hoberg, 2006)	As described in OECD 221, 23 March 2006
$NaNO_3$	510	510
$MgCl_2 \cdot 6H_2O$	240	240
$CaCl_2 \cdot 2H_2O$	90	90
$MgSO_4 \cdot 7H_2O$	290	290
$K_2HPO_4 \cdot 3H_2O$	30	30
$NaHCO_3$	300	300
H_3BO_3	3.7	3.7
Na2SeO4[a]	0.0376	Not present
$MnCl_2 \cdot 4H_2O$	8.3	8.3
$ZnCl_2$	0.066	0.066
$CoCl_2 \cdot 6H_2O$	0.029	0.029
$CuCl_2 \cdot 2H_2O$	0.00024	0.00024
$Na_2MoO_4 \cdot 2H_2O$	0.145	0.145
$FeCl_3 \cdot 6H_2O$	3.2	3.2
$Na_2EDTA \cdot 2H_2O$	6.0	6.0

a Additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.

Notes: The study report stated, for the 20X AAP medium, the following:

- Ingredients added to sterile, deionised water.
- If required, pH was adjusted to 7.5 ± 0.1 with 0.1 N NaOH, 0.1 N HCl or 5 N HCl
- Source: OECD, 2004. OECD Guideline for Testing of Chemicals. *Lemna* sp., Growth Inhibition Test. Revised Protocol for a New Guideline #221. Draft, April 2004.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Frond number at 72 hours (3 days)

The ToxCalc calculations were as follows with frond count numbers at 72 hours shown:

Conc-mg/L	1	2	3
D-Control	62.000	73.000	71.000
0.8	62.000	63.000	61.000
1.7	72.000	62.000	65.000
3.4	56.000	50.000	64.000
7.1	30.000	27.000	30.000
14	23.000	22.000	23.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	68.667	1.0000	68.667	62.000	73.000	8.533	3				68.667	1.0000
0.8	62.000	0.9029	62.000	61.000	63.000	1.613	3	1.263	1.780	6.344	64.167	0.9345
1.7	66.333	0.9660	66.333	62.000	72.000	7.736	3	1.263	1.870	6.665	64.167	0.9345
*3.4	56.667	0.8252	56.667	50.000	64.000	12.395	3	3.367	1.900	6.772	56.667	0.8252
*7.1	29.000	0.4223	29.000	27.000	30.000	5.973	3	11.129	1.920	6.843	29.000	0.4223
*14	22.667	0.3301	22.667	22.000	23.000	2.547	3	12.906	1.930	6.879	22.667	0.3301

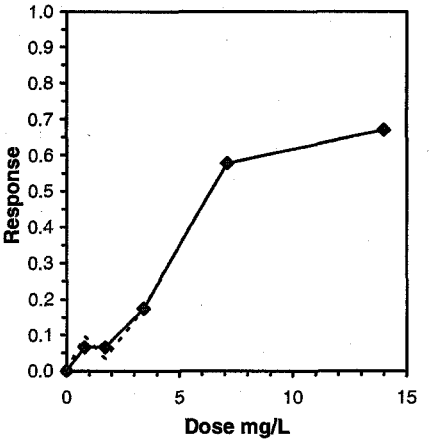
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95113	0.858	-0.0167	0.38677
Bartlett's Test indicates equal variances (p = 0.04)	11.6851	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	1.7	3.4	2.40416		6.87896	0.10018	1192.22	19.0556	3.6E-08	5, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	0.6104	0.8644	0.0000	5.3784	0.9287
IC10	2.2364	0.9138	0.0000	5.2013	-0.2322
IC15	3.0147	0.6128	0.7919	5.1462	0.0734
IC20	3.6318	0.5162	1.2643	5.2841	-0.2822
IC25	4.0910	0.4645	1.7471	5.5737	-0.3761
IC40	5.4684	0.3198	3.9086	6.4572	-0.4498
IC50	6.3867	0.2340	5.3507	7.1568	-0.3659

* indicates IC estimate less than the lowest concentration



Frond number at 120 hours (5 days)

The ToxCalc calculations were as follows with frond count numbers at 120 hours also shown:

Conc-mg/L	1	2	3
D-Control	185.00	199.00	211.00
0.8	190.00	196.00	180.00
1.7	201.00	173.00	197.00
3.4	228.00	174.00	215.00
7.1	54.00	49.00	56.00
14	33.00	29.00	29.00

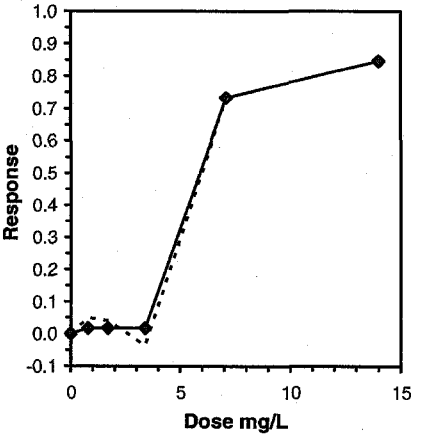
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	198.33	1.0000	198.33	185.00	211.00	6.561	3				198.33	1.0000
0.8	188.67	0.9513	188.67	180.00	196.00	4.284	3	0.289	1.780	21.20	194.89	0.9826
1.7	190.33	0.9597	190.33	173.00	201.00	7.956	3	0.289	1.870	22.27	194.89	0.9826
3.4	205.67	1.0370	205.67	174.00	228.00	13.704	3	0.289	1.900	22.63	194.89	0.9826
*7.1	53.00	0.2672	53.00	49.00	56.00	6.803	3	12.202	1.920	22.87	53.00	0.2672
*14	30.33	0.1529	30.33	29.00	33.00	7.613	3	14.106	1.930	22.99	30.33	0.1529

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94583	0.858	-0.8669	1.71368
Bartlett's Test indicates equal variances (p = 0.05)	10.8812	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	3.4	7.1	4.91325		22.9866	0.1159	19258.2	212.778	4.2E-09	5, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.5688	1.2162	0.0000	3.7565	-1.3038
IC10	3.8274	0.4955	0.0000	4.0142	-4.4987
IC15	4.0860	0.1839	2.9546	4.2719	-2.3559
IC20	4.3446	0.1590	3.3048	4.5296	-1.5544
IC25	4.6032	0.1470	3.6551	4.7892	-1.5333
IC40	5.3789	0.1126	4.6829	5.5657	-1.4151
IC50	5.8961	0.0920	5.3121	6.0878	-1.2490



Frond number at 168 hours (7 days)

The ToxCalc calculations were as follows with frond counts at 7 days also shown:

Conc-mg/L	1	2	3
D-Control	316.00	379.00	355.00
0.8	346.00	389.00	330.00
1.7	383.00	394.00	395.00
3.4	430.00	358.00	362.00
7.1	81.00	67.00	81.00
14	33.00	28.00	29.00

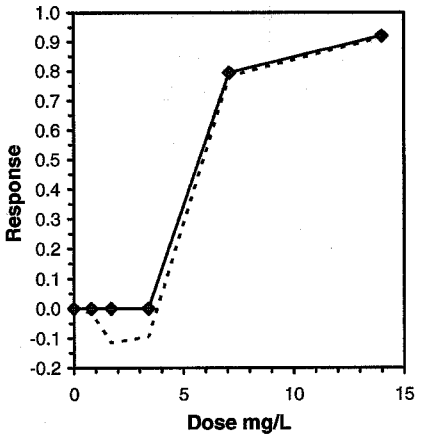
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	350.00	1.0000	350.00	316.00	379.00	9.085	3				369.75	1.0000
0.8	355.00	1.0143	355.00	330.00	389.00	8.595	3	-1.299	1.780	36.07	369.75	1.0000
1.7	390.67	1.1162	390.67	383.00	395.00	1.704	3	-1.299	1.870	37.90	369.75	1.0000
3.4	383.33	1.0952	383.33	358.00	430.00	10.556	3	-1.299	1.900	38.50	369.75	1.0000
*7.1	76.33	0.2181	76.33	67.00	81.00	10.589	3	13.504	1.920	38.91	76.33	0.2064
*14	30.00	0.0857	30.00	28.00	33.00	8.819	3	15.791	1.930	39.11	30.00	0.0811

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93264	0.858	0.60503	0.445
Bartlett's Test indicates equal variances (p = 0.03)	12.5021	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	3.4	7.1	4.91325		39.1113	0.11175	81561.8	616	4.6E-10	5, 12

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.6331	0.0682	3.2294	3.6431	-3.2525
IC10	3.8663	0.0538	3.4799	3.8862	-2.1987
IC15	4.0994	0.0508	3.7304	4.1293	-2.1375
IC20	4.3325	0.0482	3.9810	4.3724	-2.0314
IC25	4.5656	0.0460	4.2317	4.6155	-1.8758
IC40	5.2650	0.0425	4.9845	5.3448	-1.1888
IC50	5.7313	0.0430	5.4834	5.8309	-0.7608



Specific growth rate at 168 hours (7 days)

The ToxCalc calculations were as follows with the individual replicate results for specific growth rate (as re-calculated by the reviewer) also shown. Units for specific growth are day^{-1}:

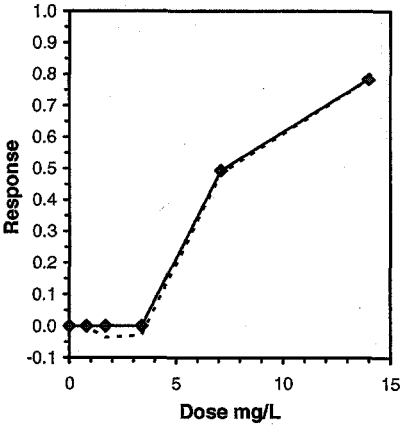
Conc-mg/L	1	2	3
D-Control	0.4354	0.4614	0.4520
0.8	0.4483	0.4651	0.4416
1.7	0.4629	0.4669	0.4673
3.4	0.4794	0.4532	0.4548
7.1	0.2409	0.2138	0.2409
14	0.1126	0.0892	0.0942

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.4496	1.0000	0.4496	0.4354	0.4614	2.926	3				0.4573	1.0000
0.8	0.4517	1.0046	0.4517	0.4416	0.4651	2.678	3	-1.014	1.780	0.0182	0.4573	1.0000
1.7	0.4657	1.0358	0.4657	0.4629	0.4673	0.525	3	-1.014	1.870	0.0191	0.4573	1.0000
3.4	0.4625	1.0287	0.4625	0.4532	0.4794	3.174	3	-1.014	1.900	0.0194	0.4573	1.0000
*7.1	0.2319	0.5158	0.2319	0.2138	0.2409	6.750	3	21.319	1.920	0.0196	0.2319	0.5070
*14	0.0987	0.2194	0.0987	0.0892	0.1126	12.529	3	34.365	1.930	0.0197	0.0987	0.2157

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95615	0.858	0.0614	-1.1215
Bartlett's Test indicates equal variances (p = 0.53)	4.15873	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	3.4	7.1	4.91325		0.01971	0.04384	0.07368	0.00016	2.5E-13	5, 12

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.7753	0.0299	3.5665	3.8126	-1.7459
IC10	4.1505	0.0365	3.9268	4.2252	-0.9295
IC15	4.5258	0.0464	4.2891	4.6378	-0.5783
IC20	4.9010	0.0579	4.6187	5.0511	-0.4598
IC25	5.2763	0.0702	4.9459	5.4640	-0.4180
IC40	6.4021	0.1092	5.9097	6.7066	-0.3878
IC50	7.2660	0.2868	6.4225	8.3002	0.2502



Biomass (Frond dry weight) values at 168 hours (7 days)

The ToxCalc calculations were as follows with the individual replicate results for biomass, as frond dry weight in milligrams, also shown:

Conc-mg/L	1	2	3
D-Control	39.100	52.200	42.400
0.8	38.600	45.900	41.400
1.7	40.300	44.500	43.400
3.4	41.800	37.600	35.200
7.1	19.000	19.500	20.900
14	16.400	15.000	14.300

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	44.567	1.0000	44.567	39.100	52.200	15.288	3				44.567	1.0000
0.8	41.967	0.9417	41.967	38.600	45.900	8.776	3	0.753	1.780	5.240	42.350	0.9503
1.7	42.733	0.9589	42.733	40.300	44.500	5.097	3	0.753	1.870	5.505	42.350	0.9503
*3.4	38.200	0.8571	38.200	35.200	41.800	8.745	3	2.163	1.900	5.594	38.200	0.8571
*7.1	19.800	0.4443	19.800	19.000	20.900	4.974	3	8.413	1.920	5.652	19.800	0.4443
*14	15.233	0.3418	15.233	14.300	16.400	7.019	3	9.964	1.930	5.682	15.233	0.3418

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.95866		0.858		0.72877	1.34114
Bartlett's Test indicates equal variances (p = 0.15)					8.15813		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Williams' Test	1.7	3.4	2.40416		5.68189	0.12749	493.492	13.0006	6.0E-07	5, 12
Treatments vs D-Control										

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	1.7048	1.0842	0.0000	5.4459	0.2812
IC10	2.6176	1.1729	0.0000	5.2100	-0.3995
IC15	3.4640	1.0158	0.0000	5.0784	-1.1044
IC20	3.9121	0.7215	0.5490	5.4545	-1.6114
IC25	4.3602	0.5209	1.8729	5.7891	-0.8649
IC40	5.7045	0.3742	3.9706	6.7698	-0.6022
IC50	6.6006	0.3113	5.0122	7.4393	-0.5271