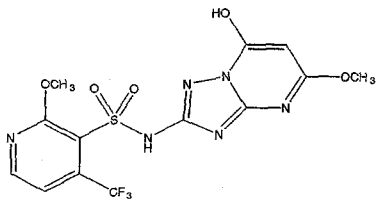
Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (7-OH metabolite of XDE-742) to aquatic vascular plants duckweed, *Lemna gibba*

PMRA Submission Number 2006-4727; 1283260 EPA MRID Number 469084-33 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:	7-OH metabolite of pyroxsulam or 7-OH metabolite of XDE-742)	
Purity (%):	96% (ID No. TSN 105232, used to prepare test solutions and quality control samples), and 99% (ID No. TSN 105384, used to prepare test solutions during preliminary testing and calibration standards during definitive testing).	
Common name:	7-OH metabolite of pyroxsulam (7-OH metabolite of XDE-742)	
Chemical name:	3-pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-	
IUPAC:	N-(7-hydroxy-5-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
CAS name:	3-Pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-	
CAS No.:	Not available	
Synonyms:	7-desmethyl XDE-742 metabolite XR-742, X666742	

Chemical structure:



D. Murphy 22/02/08

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

22/2/08

Secondary Reviewers: 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
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. 7-OH Metabolite of XDE-742 - Toxicity to Duckweed, *Lemna gibba*. . Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts, 02571-1037. Springborn Smithers Study No. 12550.6409, Sponsor Protocol/Project No. 050119. The Dow Chemical Company 1803 Building, Midland, Michigan, 48674 for Dow AgroSciences, Indianapolis, Indiana 46268. 27 April 2006. Unpublished report.

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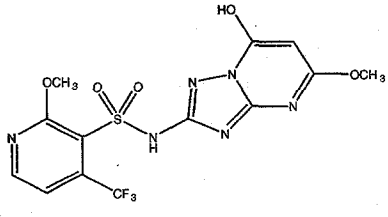
IUPAC:		N-(7-hydroxy-5-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:		3-Pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
CAS No.:		Not available
Synonyms:		7-desmethyl XDE-742 metabolite XR-742, X666742

Chemical structure:



Primary Reviewer:	Daryl Murphy	**Date:** 18 July 2007
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EXECUTIVE SUMMARY:

Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (7-OH metabolite of XDE-742) to aquatic vascular plants duckweed, *Lemna gibba*

PMRA Submission Number 2006-4727; 1283260 EPA MRID Number 469084-33 APVMA ATS 40362

In a 7 day acute toxicity study, freshwater floating aquatic vascular plants (duckweed, *Lemna gibba*) were exposed to 7-OH metabolite of pyroxsulam at nominal concentrations of 0 (medium control), 0.24, 0.81, 2.7, 9.0, 30 and 100 mg 7-OH metabolite of pyroxsulam/L, Mean measured concentrations were 0, 0.21, 0.74, 2.6, 8.6. 31 and 100 mg 7-OH metabolite of pyroxsulam/L. The study was conducted under static conditions with renewal at days 3 and 5, in accordance with a protocol which met the general requirements of guidelines, OECD 221 "Lemna sp. Growth Inhibition Test" (draft, 2004) and US EPA guidelines including U.S. Environmental Protection Agency (1996). Ecological Effects Test Guidelines. OPPTS 850.4400 Aquatic Plant Toxicity Test using *Lemna* sp., Tiers I and II. Draft April 1996.

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 day 7 control mean frond count ranged from 8.9 to 96% inhibition of mean frond density. Response relative to the control mean ranged from 2 to 96% inhibition of mean specific growth rate. For biomass based on the day 7 frond dry weights, response relative to the control mean ranged from 16 to 83% inhibition of frond dry weight.

The 7 day EC50 for frond density (frond numbers) was 1.8 mg 7-OH metabolite of pyroxsulam/L (mean measured concentration) with 95% confidence limits of 1.6-2.0 mg 7-OH metabolite of pyroxsulam/L. The 0-7 day ErC50 (mean specific growth rate) was 4.0 mg 7-OH metabolite of pyroxsulam/L with 95% confidence limits of 3.2-4.5 mg 7-OH metabolite of pyroxsulam/L. The 7 day EbC50 (biomass, frond dry weight) was 2.1 mg 7-OH metabolite of pyroxsulam/L (mean measured concentration) with 95% confidence limits of 1.4-2.5 mg 7-OH metabolite of pyroxsulam/L. The 7 day NOECs based on frond number, 0-7 day specific growth rates and biomass (dry weight at 7 days) were all set at 0.74 mg 7-OH metabolite of pyroxsulam/L (mean measured concentration). These EC50 values are considered to classify 7-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 days 5 and 7 frond appearances in the 2.6 mg 7-OH metabolite of pyroxsulam/L and greater test concentrations were reported as being, variously, curled, slightly chlorotic and having less root formation than the control fronds.

The static exposure (with renewal on days 3 and 5) of duckweed to mean measured concentrations of 0.21 to 100 mg 7-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 7-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: <0.21 mg 7-OH metabolite of pyroxsulam/L
7 day EC50: 1.8 mg 7-OH metabolite of pyroxsulam/L
95% C.I.: 1.6 to 2.0 mg 7-OH metabolite of pyroxsulam/L
7 day NOEC: 0.74 mg 7-OH metabolite of pyroxsulam/L
Probit Slope: Not reported

Mean specific growth rate (day^{-1})
7 day ErC05: 0.82 mg 7-OH metabolite of pyroxsulam/L
7 day ErC50: 4.0 mg 7-OH metabolite of pyroxsulam/L
95% C.I.: 3.2 to 4.5 mg 7-OH metabolite of pyroxsulam/L
7 day NOEC: 0.74 mg 7-OH metabolite of pyroxsulam/L
Probit Slope: Not reported

Biomass (frond dry weight)
7 day EbC05: <0.21 mg 7-OH metabolite of pyroxsulam/L
7 day EbC50: 2.1 mg 7-OH metabolite of pyroxsulam/L
95% C.I.: 1.4 to 2.5 mg 7-OH metabolite of pyroxsulam/L
7 day NOEC: 0.74 mg 7-OH metabolite of pyroxsulam/L
Probit Slope: Not reported

Endpoint(s) Effected: frond count, mean specific growth rate and biomass (dry frond weight)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The toxicity test was reported as 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. This protocol was stated to meet the general requirements of the relevant OECD and U.S. EPA OPPTS guidelines, namely.

- 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 US EPA OPPTS 850.4400 requirements.

The study report identified one protocol deviation. This was that the protocol states that the light intensity will range from 6500 to 10,000 lux and the photosynthetically-active radiation (PAR) will range from 85 to 120 $\mu E/m^2/s$. During the definitive test, the light intensity ranged from 6700 to 8900 lux and the PAR ranged from 120 to 139 $\mu E/m^2/s$. Since the light intensity was within the appropriate range, the PAR was not adjusted. This deviation was reported as having had no impact on the results of the study. This matter is considered on pages 19 and 37 (Table 12) of this DER.

COMPLIANCE: The data and report presented for "7-OH Metabolite of XDE-742 - Toxicity to Duckweed, *Lemna gibba*" were reported as produced and compiled in accordance with all pertinent OECD and US EPA Good Laboratory Practice regulations, namely:

- 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., 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.

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, these exceptions were considered to have had no impact on the study results.

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

A. MATERIALS:

1. Test Material	7-OH metabolite of XDE-742 (i.e. 7-OH metabolite of pyroxsulam)
Description:	Solid
Lot No./Batch No.:	E2008-46
Purity:	96%
Stability of Compound Under Test Conditions:	The results of the analysis of the exposure solutions for 7-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 88 to 100% 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 7-OH metabolite of pyroxsulam was stable under the test conditions.
Storage conditions of test chemicals:	The test substance (also identified as SSL No. 112-85) was stored at room temperature in the original container in a dark ventilated cabinet. This sample of test substance was used to prepare test solutions and quality control samples during definitive testing.
Physicochemical properties of the 7-OH metabolite of pyroxsulam:	The physicochemical properties of the 7-OH metabolite of pyroxsulam were not reported in the study. The study profile template (Hoberg, 2006a) stated that physicochemical properties were not available at the time of publication of the study profile template.

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 7-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 3 and 5. 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 584, 609, 34, 23 and 20 fronds/replicate, respectively. Frond density in the control averaged 534 fronds/replicate. Fronds exposed to the 1.0 and 10 mg/L treatment level were observed to be slightly chlorotic, while those exposed to the 100 mg/L treatment levels were observed to be chlorotic. Fronds exposed to the 0.010 and 0.10 mg/L treatment levels and the control were normal.

An initial 7-day definitive exposure was conducted at nominal concentrations of 0.10, 0.20, 0.40, 0.80 and 1.6 mg 7-OH metabolite of pyroxsulam/L and a control. Three exposure vessels were established for each concentration and the control. Test solutions were renewed on days 3 and 5. 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 0.10, 0.20, 0.40, 0.80 and 1.6 mg/L treatment levels averaged 442, 432, 436, 425 and 410 fronds/replicate, respectively.

Frond density in the control averaged 451 fronds/replicate. Fronds exposed to the 1.6 mg/L treatment level were curled and smaller than control fronds. Fronds exposed to the remaining treatment levels (0.10, 0.20, 0.40 and 0.80 mg/L) and the control were observed to be normal. Since no concentration resulted in >50% reduction in frond density as compared to the control, EC50 values could not be determined. Based on these data, nominal concentrations of 0.24, 0.81, 2.7, 9.0, 30 and 100 mg 7-OH metabolite of pyroxsulam/L were selected for the definitive exposure in an attempt to determine an EC50 value.

[b) Definitive Study

The definitive test was conducted from 10 to 20 February 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

Parameter	Details	Remarks *Criteria*
Acclimation Period:	The fronds used to initiate the toxicity test with 7-OH metabolite of pyroxsulam were taken from a stock culture that had been transferred to fresh medium two days prior to testing.	See deviations/deficiency table on page 37 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.
Culturing media and conditions: (same as test or not)	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. Culturing conditions were described as: Conditions: Culture: Temperature: 24 ± 2°C Light (lux): 6500-10,000 Photoperiod: Continuous Medium: 20X Algal Assay Procedure (AAP) medium pH: Adjusted to 7.5 ± 0.1 Culture Vessel: 270 mL covered crystallising dishes with 100 mL of medium. Inoculation: Weekly transfer Culture Chamber: Environmental chamber 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.	Requirement considered met with the culturing media and conditions the same as those used in the test. Typical test conditions were described as: Conditions: Test: Temperature: 22 to 23°C Light (lux): 6700 to 8900 lux Photoperiod: Continuous Medium: 20X Algal Assay Procedure (AAP) medium pH: Adjusted to 7.5 prior to addition of test material. Culture Vessel: 270 mL borosilicate crystallizing dish with cover. Inoculation: Single Culture Chamber: Environmental growth chamber

Parameter	Details	Remarks *Criteria*
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.
Test system 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 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

Parameter	Details	Remarks *Criteria*
		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 at a temperature of 24 ± 2°C and continuous lighting with an intensity of 6,500 to 10,000 lux.	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 23°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 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.
<u>Test vessel</u> 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

Parameter	Details	Remarks *Criteria*
		containers being glass beakers or Erlenmeyer flasks.
Size: Fill volume:	270 mL 100 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 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.
<u>Details of growth medium</u> Name:	Modified 20X Algal Assay Procedure (AAP) medium The compositions of the 20X AAP stock medium and the OECD 221 20X AAP medium are provided as Attachment 1 on page 41 of this DER. The 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.	See deviations/deficiency table on page 37 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 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.
pH (in the fresh exposure solutions) at days 0, 3 and 5:	The pH of the exposure solutions was measured at test initiation (new solutions), in each aged and new solution at each renewal	See deviations/deficiency table on page 37 of this report.

Parameter	Details	Remarks *Criteria*
	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. The pH values reported for days 0, 3 and 7 (fresh or new solutions) were: **Conc.*** **Day 0** **Day 3** **Day 5** Control 8.0 7.5 7.5 0.24 7.8 7.8 7.7 0.81 7.9 7.8 7.7 2.7 7.8 7.9 7.7 9.0 7.7 7.8 7.7 30 7.6 7.8 7.7 100 7.4 7.3 7.2 *** Nominal concentrations as mg 7-OH metabolite of pyroxsulam/L.**	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. The reason for the day 0 pH values exceeding 7.5 in the control is unclear. The pH of the AAP was stated to have been adjusted to a pH of 7.5 before addition of any test material or alga and the pH determined on the portion of medium in the volumetric flasks. As a result, a pH of close to 7.5 would have been expected in the control medium at day 0.

<table>
<tr>
<td>pH (in pooled replicates of 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, 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 spent solutions with duckweed present and measured on days 3, 5 and 7 were:

<table>
<tr><th>Conc.*</th><th>Day 3</th><th>Day 5</th><th>Day 7</th></tr>
<tr><td>Control</td><td>8.5</td><td>8.6</td><td>9.0</td></tr>
<tr><td>0.24</td><td>8.5</td><td>8.5</td><td>9.1</td></tr>
<tr><td>0.81</td><td>8.5</td><td>8.6</td><td>9.0</td></tr>
<tr><td>2.7</td><td>8.5</td><td>8.3</td><td>8.4</td></tr>
<tr><td>9.0</td><td>8.5</td><td>8.3</td><td>8.4</td></tr>
<tr><td>30</td><td>8.4</td><td>8.3</td><td>8.3</td></tr>
<tr><td>100</td><td>8.4</td><td>8.3</td><td>8.3</td></tr>
</table>
* Nominal concentrations as µg 7-OH metabolite of pyroxsulam/L.</td>
<td>Requirement considered met.

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, 0.5, 1.1 and 1.5 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 current OECD and US EPA OPPTS requirements which allow use of chelating</td>
</tr>
</table>

		agents (the AAP medium contains sodium EDTA as a chelating agent).
Carbon source:	Not identified.	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 a standard medium, the presence of the sodium selenate means that it is in fact a modified 20X AAP medium (see Attachment 1, page 41 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. Several liters of 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.*
Total Organic Carbon:	A representative sample of 20X AAP medium was analysed monthly for total organic carbon (TOC) concentration. The TOC concentration for 20X AAP medium ranged from 2.2 to 3.1 mg/L for samples analyzed in November, December 2005, and January, March 2006.	TOC, particulate matter, etc. requirements considered met. OECD 221 and US EPA OPPTS 850.4400 do not address these parameters specifically.

	The sample analyzed in February 2006 was artificially high (11 mg/L) because the TOC analyzer was not adequately purged of inorganic carbon prior to analysis. The above results indicate that the TOC in 20X AAP medium is typically approximately 3 mg/L.	
Particulate matter: Metals and pesticides Chlorine: Water pretreatment (if any): Intervals of water quality measurement	Not reported The study report stated that 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. Not reported. Water used to prepare the 20X AAP medium was "sterile, deionised water". Periodically. See above under Metals and pesticides.	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.
Indicate how the test material is added to the medium (added directly or used stock solution)	A 100 mg 7-OH metabolite of pyroxsulam/L primary stock solution was prepared on the day of test initiation by placing 0.1045 g of the 7-OH metabolite of pyroxsulam (0.1003 g as active ingredient) in a 1000-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 sonicated for 15 minutes and stirred for 30 minutes. Following sonication and stirring, the solution was observed to be clear and colorless with no visible undissolved test substance. Nominal test solutions 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 100 mg/L primary stock solution and nominal test solutions were prepared at each solution renewal (days 3 and 5) following similar procedures.	Requirements considered met. The primary stock solution was made up taking into account the 96% purity of the 7-OH metabolite of pyroxsulam.

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. Solvent control 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 two hours after the test solutions were prepared and added to the test vessels, an inoculum of five plants with three fronds each was aseptically introduced into each test vessel.	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. 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.*
Number of fronds/plant	3 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.*
Test concentrations Nominal:	0 (control, 20X AAP medium), 0.24, 0.81, 2.7, 9.0, 30 and 100 mg 7-OH metabolite of pyroxsulam/L. Concentrations were adjusted for the purity of the test substance and are presented as 7-OH metabolite of pyroxsulam. These concentrations are in a ratio of approximately 1:3.3 or 1:3.4.	See deviations/deficiency table on page 37 of this report. 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 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). *EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*

Measured:	Mean measured concentrations in fresh solutions analysed on days 0 and 5 were: **Nominal 7-OH metabolite of pyroxsulam value, mg/L** / **Mean measured 7-OH metabolite of pyroxsulam value, mg/L: 0 hour, new** / **Day 5, new** **Control** <0.014 <0.0091 **0.24** 0.20 0.22 **0.81** 0.78 0.74 **2.7** 2.6 2.7 **9.0** 9.0 9.4 **30** 29 29 **100** 100 100 Mean measured concentrations in aged solutions analysed on days 3 and 7 were: **Nominal 7-OH metabolite of pyroxsulam value, mg/L** / **Mean measured 7-OH metabolite of pyroxsulam value, mg/L#: Day 3, aged** / **Day 7, aged** **Control** <0.016 <0.013 **0.24** 0.21 0.21 **0.81** 0.75 0.68 **2.7** 2.6 2.4 **9.0** 7.0 9.1 **30** 35 29 **100** 100 110	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. None of the analyses of the controls exhibited peaks eluting at the retention times of the analyte exceeding the limit of quantitation (LOQ) of 0.0141 mg 7-OH metabolite of pyroxsulam/L. OECD 221 refers to the situation in which 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

	Measured concentrations of 7-OH metabolite of pyroxsulam in the fresh and spent solutions at days 3, 5 and 7 were reported as: **Nominal 7-OH metabolite of pyroxsulam value, mg/L** / **Mean measured 7-OH metabolite of pyroxsulam value, mg/L** / **Percentage of nominal** **Control**: NA: NA **0.24**: 0.21 (0.21)*: 88 **0.81**: 0.74 (0.74): 91 **2.7**: 2.6 (2.6): 95 **9.0**: 8.6 (8.6): 96 **30**: 31 (30): 100 **100**: 100 (102): 100 **NA = not applicable. * Mean values in brackets are those calculated by the reviewer.** These mean measured concentrations were determined from the day 0 and day 5 fresh/new solutions results and the day 3 and day 7 aged solution results. The means in the study report were recalculated by the reviewer with the reviewer's results considered equivalent to those reported with the small differences found attributed to the reviewer's use of the rounded 2 significant figure results reported by the study. The study's reported values were calculated using actual analytical data and not the rounded (2 significant figures) data presented in the study report.	test regime is recommended. The study complied with this guideline requirement. No specific reference found in US EPA OPPTS 850.4400 other than, "The colonies may have to be transferred more frequently for highly volatile test substances in order to maintain 80 percent of the initial test substance concentration." and "Periodic renewal (static-renewal) will help to maintain constant exposure concentrations of the test chemical over the test period for compounds that are unstable in water." Analysis of the quality control samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 93.4 to 113% (N=12) of the nominal fortified levels (0.100, 5.00 and 100 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.
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 analyzed for 7-OH metabolite of pyroxsulam. Samples analyzed from newly prepared solutions (days 0 and 5) were removed from the volumetric flasks prior to division into the replicate vessels. Test solution samples analyzed at the end of the renewal periods (days 3 and 7) were removed from composited solutions of each treatment level and the control.	Requirement considered met. Methodology was validated (23 January 2006) to quantify the amount of 7-OH metabolite of pyroxsulam present in 20X AAP medium (a freshwater algal medium). This method validation was reported as based on the guidance document SANCO/3029/99 rev.4. Recovery samples were analyzed by automated injection on a high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV). This

	All exposure solution and QC samples were analyzed for 7-OH metabolite of pyroxsulam by high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV) based on methodology validated at Springborn Smithers.	method was validated by fortification of 20X AAP medium with 7-OH metabolite of pyroxsulam at concentrations of 0.05 and 100 mg/L. Recoveries averaged 105 ± 1.95% with a limit of quantitation (LOQ) of 0.0141 mg/L. The quality control sample range for subsequent studies was set at 80 to 120%. 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 stated as similar to those used in the method validation study with the following exception: only one lot of test substance was used in the method validation (Lot No. 35172-56). This lot of test substance was used to fortify both the calibration standards and the recovery samples. During testing, the test substance (Lot No. 35172-56) was used for calibration standards and the other lot of test substance (E2008-46) was used for QC samples. Analysis of the quality control samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 93.4 to 113% (N=12) of the nominal fortified levels (0.100, 5.00 and 100 mg/L). The results of these analyses established that the appropriate precision and quality control was maintained during the analyses of the exposure solutions. Representative chromatograms from the analysis of a calibration standard, recovery sample and a control sample were presented in the study report.
Limit of Quantitation:	The lowest level quantified was set at 0.0141 mg 7-OH metabolite of pyroxsulam/L.	
Limit of Detection:	Not reported.	
<u>Test conditions</u> Temperature:	Temperatures during the exposure period ranged from 22 to 23°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. *EPA temperature: 25°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 7. 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. 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:	The light intensity ranged from 6700 to 8900 lux. The photosynthetically active radiation (PAR) of the test area at test initiation ranged from 120 to 139 $\mu E/m^2/s$.	See deviations/deficiency table on page 37 of this report. 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) 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: 5000 lux (±15%)*
<u>Reference chemical (if used)</u> Name: Concentrations:	No reference chemical used.	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)	Frond numbers were counted on days 0, 3, 5 and 7 in each replicate. 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 frond number and other end points	Counting of fronds with every frond visibly projecting beyond	Requirement considered met.

	the edge of the parent frond counted. At test termination (day 7), after frond density determinations were complete, the fronds were removed from each vessel, blotted dry and transferred to preweighed aluminum pans. Fronds were dried in an oven at 61 to 63°C for three days prior to dry weight determination.	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.
Observation intervals	A count of the total number of fronds was taken of each replicate on days 0, 3, 5 and 7. 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 minimum/maximum thermometer located in a flask of water adjacent to the test vessels. Temperature readings were recorded daily. Light intensity was measured at 0 hour and at each subsequent 24-hour interval during the exposure period.	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' 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

	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 7-OH metabolite of pyroxsulam.	of the test substance are determined at appropriate intervals.
Other observations, if any	pH of the modified 20X AAP medium was adjusted to 7.5 prior to addition of test material. 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 requires 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 484 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 0.50 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, 7-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.	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 7-OH metabolite of pyroxsulam

Results from the chemical analysis of the exposure solutions for 7-OH metabolite of pyroxsulam yielded mean measured concentrations ranging from 88 to 100% of the nominal concentrations which defined the treatment levels tested as 0.21, 0.74, 2.6, 8.6, 31 and 100 mg 7-OH metabolite of pyroxsulam/L and biological results were based on these mean measured values.

Analysis of the quality control samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 93.4 to 113% (N=12) of the nominal fortified levels (0.100, 5.00 and 100 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.

Frond density

Mean frond count results and individual replicate data were presented in the study report. Mean frond counts after seven days of exposure were 484, 441, 424, 114, 29, 18 and 18 fronds for the media control, 0.21, 0.74, 2.6, 8.6, 31 and 100 mg 7-OH metabolite of pyroxsulam/L test levels, respectively. Response relative to the day 7 control mean frond count ranged from 8.9 to 96% 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 stated that, based on the results of Shapiro-Wilks' and Bartlett's Tests, the day 7 frond count data set passed the requirements for normality and homogeneity of variance and Williams' Test was used to determine treatment-related effects. Williams' Test determined a significant difference in frond density in the 2.6, 8.6, 31 and 100 mg/L treatment levels compared to the control data.

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

7-Day Results	EC05	EC50	EC90	LOEC	NOEC
EC value (mg/L):	<0.21	1.8	7.2	2.6	0.74
95% confidence limits:	NA[a]	1.6-2.0	6.7-7.6	NA	NA

[a] **NA = Not Applicable. EC05 value was empirically estimated, therefore, 95% confidence limits could not be calculated.**

The frond counts from days 0, 3, 5 and 7, plus the calculated percentage inhibition based on the mean day 7 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 7-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 7-OH metabolite of pyroxsulam/L	Replicate	Frond number at:			% Inhibition*
		Day 3	Day 5	Day 7	
Control/<LOQ#	A	85	229	483	
	B	90	229	538	
	C	79	208	432	
	Mean	**85**	**222**	**484**	**NA****
	St. dev.	**6**	**12**	**53**	
0.24/0.21	A	80	197	477	
	B	77	211	441	
	C	85	187	405	
	Mean	**81**	**198**	**441**	**8.9**
	St. dev.	**4**	**12**	**36**	
0.81/0.74	A	61	169	412	
	B	77	207	425	
	C	67	193	434	
	Mean	**68**	**190**	**424**	**13**
	St. dev.	**8**	**19**	**11**	
2.7/2.6	A	39	54	94	
	B	44	62	117	
	C	46	75	132	
	Mean	**43**	**64[a]**	**114[a, d]**	**76**
	St. dev.	**4**	**11**	**19**	
9.0/8.6	A	23	24	30	
	B	24	25	27	
	C	24	26	29	
	Mean	**24**	**25[a]**	**29[a, d]**	**94**
	St. dev.	**1**	**1**	**2**	
30/31	A	16	16	15	
	B	17	16	19	
	C	19	18	19	
	Mean	**17**	**17[a]**	**18[a, c, d]**	**96**
	St. dev.	**2**	**1**	**2**	
100/100	A	15	15	16	
	B	20	21	20	
	C	18	18	18	
	Mean	**18**	**18[b]**	**18[a, c d]**	**96**
	St. dev.	**3**	**3**	**2**	

 # LOQ = limit of quantitation = 0.0141 mg 7-OH metabolite of pyroxsulam/L)* % inhibition relative to the day 7 control mean. ** NA = not applicable. a. Curled fronds were observed. b. Slightly chlorotic fronds were seen. c. Fronds were seen to have less root formation than the control fronds. d. Significantly reduced to the control based on Williams' test.

Growth rate

At test termination, growth rate for the control averaged 0.50 days^{-1}. Frond growth rate in the 0.21, 0.74, 2.6, 8.6, 31 and 100 mg/L treatment levels averaged 0.49, 0.49, 0.29, 0.09, 0.02 and 0.03 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 difference in growth rate in the 2.6, 8.6, 31 and 100 mg/L treatment levels compared to the control data. Therefore, the 7 day NOEC and LOEC for growth rate were determined to be 0.74 and 2.6 mg 7-OH metabolite of pyroxsulam/L, respectively. The 7-day EC50 value was determined to be 4.0 mg/L, with 95% confidence limits of 3.2 to 4.5 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 7-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	0.55	0.55	0.50	
	B	0.57	0.55	0.52	
	C	0.53	0.53	0.49	
	Mean (SD[b])	**0.55 (0.02)**	**0.54 (0.01)**	**0.50 (0.02)**	**NA[c]**
0.21	A	0.53	0.52	0.50	
	B	0.52	0.53	0.49	
	C	0.55	0.51	0.48	
	Mean (SD)	**0.54 (0.02)**	**0.52 (0.01)**	**0.49 (0.01)**	**2**
0.74	A	0.45	0.49	0.48	
	B	0.52	0.53	0.49	
	C	0.48	0.51	0.49	
	Mean (SD)	**0.48 (0.04)**	**0.51 (0.02)**	**0.49 (0.00)**	**2**
2.6	A	0.30	0.26	0.27	
	B	0.34	0.29	0.30	
	C	0.36	0.32	0.32	
	Mean (SD)	**0.33 (0.03)**	**0.29 (0.03)**	**0.29 (0.03)[d]**	**42**
8.6	A	0.14	0.09	0.10	
	B	0.15	0.10	0.09	
	C	0.15	0.11	0.10	
	Mean (SD)	**0.15 (0.01)**	**0.10 (0.01)**	**0.09 (0.01)[d]**	**82**
31	A	0.02	0.01	0.00	
	B	0.04	0.01	0.03	
	C	0.08	0.04	0.03	
	Mean (SD)	**0.05 (0.03)**	**0.02 (0.01)**	**0.02 (0.02)[d]**	**96**
100	A	0.00	0.00	0.01	
	B	0.09	0.07	0.04	
	C	0.06	0.04	0.03	
	Mean (SD)	**0.05 (0.05)**	**0.03 (0.03)**	**0.03 (0.02)[d]**	**94**

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 59.30 mg. Frond biomass in the 0.21, 0.74, 2.6, 8.6, 31 and 100 mg 7-OH metabolite of pyroxsulam/L treatment levels averaged 49.73, 47.57, 22.70, 14.37, 10.53 and 10.27 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 difference in frond biomass in the 2.6, 8.6, 31 and 100 mg/L treatment levels compared to the control data. The 7-day calculated EbC50 value (95% confidence interval) for frond dry weight was 2.1 (1.4-2.5) mg/L. Based on the William's test ($\alpha = 0.05$), the 7-day mean frond dry weight was significantly less than the controls at test levels $\geq$ 2.6 mg/L; therefore, the 7-day NOEC value for mean frond dry weight was determined to be 0.74 mg/L. Response relative to the controls ranged from 16% to 83% inhibition of frond dry weight.

At days 5 and 7 frond appearances in the 2.6 mg 7-OH metabolite of pyroxsulam/L and greater test concentrations were reported as being, variously, curled, slightly chlorotic and having less root formation than the control fronds.

Table 5. Effect of 7-OH metabolite of pyroxsulam on frond dry weight of the freshwater duckweed (*Lemna gibba*) as given in the study report (Hoberg, 2006).

Treatment (nominal and measured concentration), mg 7-OH metabolite of pyroxsulam/L	Replicate No.	Frond dry weight at day 7, mg	% inhibition relative to the control mean[a]
Control/<LOQ[b]	A	51.30	
	B	77.00	
	C	49.60	
	Mean	59.30	NA[c]
	Standard deviation	15.4	
0.24/0.21	A	56.60	
	B	48.90	
	C	43.70	
	Mean	49.73	16
	Standard deviation	6.5	
0.81/0.74	A	43.30	
	B	44.10	
	C	55.30	
	Mean	47.57	20
	Standard deviation	6.7	
2.7/2.6	A	20.00	
	B	22.50	
	C	25.60	
	Mean	22.70[d]	62
	Standard deviation	2.8	
9.0/8.6	A	14.60	
	B	15.40	
	C	13.10	
	Mean	14.37[d]	76
	Standard deviation	1.2	
30/31	A	10.90	
	B	10.30	
	C	10.40	
	Mean	10.53[d]	82
	Standard deviation	0.3	
100/100	A	8.90	
	B	11.00	
	C	10.90	
	Mean	10.27[d]	83
	Standard deviation	1.2	

a Percent inhibition relative to the control.
b. <LLQ = Less than Lowest Level Quantified = <0.0141 mg 7-OH metabolite of pyroxsulam/L.
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 7-OH metabolite of pyroxsulam concentrations for 7 days in a static renewal test) as reported by Hoberg, 2006.

Statistical Endpoint	Day 7 Frond No.	Day 7 mean specific growth rate (per day)	Day 7 biomass (frond dry weight)
NOEC **EC_{05}** **(mg 7-OH metabolite of pyroxsulam /L) (95% C.I.)**	0.74 <0.21 (NA*)	0.74 0.82 (0.53-0.94)	0.74 <0.21 (NA)
LOEC (mg 7-OH metabolite of pyroxsulam/L)	2.6	2.6	2.6
EC_{50} (mg 7-OH metabolite of pyroxsulam /L) (95% C.I.)	1.8 (1.6-2.0)	4.0 (3.2-4.5)	2.1 (1.4-2.5)
EC90 (95% C.I.)	7.2 (6.7-7.6)	23 (21-25)	>100 (NA)
Reference chemical **NOEC** **IC_{50}/EC_{50}**	No reference chemical used.		

*** NA = Not applicable. EC values were empirically estimated and, as a result, 95% confidence limits could not be calculated.**

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	0.50	1.38

The control Td value satisfies the OECD 221 requirement that the Td be <2.5 days.

The mean specific growth rates for the control replicates over 0-7 days were 0.50, 0.52 and 0.49 day^{-1}, all of which exceed the OECD 221 requirement that the average specific growth rate be 0.275 day^{-1}.

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 484 fronds. As the initial frond number was 15, the day 7 counts represent an approximate 32 fold increase in frond number, satisfying the OECD 221 criterion.

B. REPORTED STATISTICS:

The frond numbers, mean specific growth rate and biomass data from the study were evaluated based on mean measured 7-OH metabolite of pyroxsulam concentrations measured on days 0 (new), 3 (aged), 5 (new) and 7 (aged). The measured concentrations of the 7-OH metabolite closely approximated the desired nominal concentrations and provided the expected concentration gradient. Mean measured concentrations ranged from 88 to 100% of the nominal concentrations and defined the treatment levels tested as 0.21, 0.74, 2.6, 8.6, 31 and 100 mg 7-OH metabolite of pyroxsulam/L.

The statistical endpoints determined were the EC50 value for frond number, the ErC50 value for mean specific growth rate, and the EbC50 value for dry weight (biomass). In addition, the no-observed-effect-concentration (NOEC) values for each of the three endpoints were determined.

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 in frond density, average growth rate or biomass, compared to the control data. 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. 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.

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 on page 31 of this DER, under "Validity of test".

Based on the results of statistical analysis performed for 7-day frond density, growth rate and biomass (as dry weight), 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 reported as 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 number, 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 7-OH metabolite of pyroxsulam exposed duckweed and the mean of the controls by Williams' test. The ToxCalc package was used to determine the EC50 and associated 95% confidence limits (by use of liner interpolation methodology) and NOEC values.

Frond counts

The ToxCalc analysis used the untransformed day 3, 5 and 7 frond counts. The untransformed data for days 3 and 5 were identified as normally distributed with equal variances. The log transformed 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 with the ToxCalc results shown on, respectively, pages 42, 43 and 44 of this DER.

Table 8. Reviewer calculated EC50 and NOEC values for *Lemna gibba* frond counts after 3, 5 and 7 days exposure to 7-OH metabolite of pyroxsulam with the results determined by use of mean measured concentrations. EC50, 95% confidence limits and NOEC values are as mg 7-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 controls
Day 3	2.8	0.44-1.9	0.21	≥0.74
Day 5	1.9	0.08-1.5	<0.21	≥0.21
Day 7	1.8	1.5-2.1	0.74	≥2.6
Study report's day 7 EC50 etc values:	1.8	1.6-2.0	0.74	≥2.6

The study report's 7-day calculated EC50 value (95% confidence interval) for cell density (i.e. frond count) was 1.8 (1.6-2.0) mg 7-OH metabolite of pyroxsulam/L. The study report's NOEC was 0.74 mg 7-OH metabolite of pyroxsulam/L. As shown in Table 8, the reviewer calculated 7 day EC50, 95% confidence limits and NOEC were

1.8, 1.5 to 2.1 and 0.74 mg 7-OH metabolite of pyroxsulam/L with these results considered equivalent to 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 deviations were recalculated using the day 0, day 5 and day 7 frond counts with a time interval of 3, 5 and 7 days as per the study report formula given on page 31 of this DER.

The recalculated individual replicate values and their associated mean, standard deviations and % inhibition based on the control mean were the same as those given in the study report. Specific growth rates for days 0-3, 0-5 and 0-7 days were recalculated and found to be equivalent to the study report's values for specific growth rates on those days.

The recalculated specific growth rates and associated mean and standard deviations are shown in Table 9 with the calculated % inhibition.

Table 9. Reviewer's recalculation of day 0-3, day 0-5 and day 0-7 specific growth rates determined from the reported frond counts 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	0.58	0.55	0.50		
	B	0.60	0.55	0.51		
	C	0.55	0.53	0.48		
	Mean	0.58	0.54	0.50		
	St. dev.	0.02	0.01	0.02	Not applicable	Not applicable
0.21	A	0.52	0.52	0.49		
	B	0.55	0.53	0.48		
	C	0.58	0.50	0.47		
	Mean	0.55	0.52	0.48	3	2
	St. dev.	0.03	0.01	0.01		
0.74	A	0.47	0.48	0.47		
	B	0.55	0.52	0.48		
	C	0.50	0.51	0.48		
	Mean	0.50	0.51	0.48	5	2
	St. dev.	0.04	0.02	0.00		
2.6	A	0.32	0.26	0.26		
	B	0.36	0.28	0.29		
	C	0.37	0.32	0.31		
	Mean	0.35	0.29	0.29*	42	42
	St. dev.	0.03	0.03	0.02		
8.6	A	0.14	0.09	0.10		
	B	0.16	0.10	0.08		
	C	0.16	0.11	0.09		
	Mean	0.15	0.10	0.09*	82	82
	St. dev.	0.01	0.01	0.01		
31	A	0.02	0.01	0.00		
	B	0.04	0.01	0.03		
	C	0.08	0.04	0.03		
	Mean	0.05	0.02	0.02*	95	96
	St. dev.	0.03	0.01	0.02		
100	A	0	0	0.01		
	B	0.10	0.07	0.04		
	C	0.06	0.04	0.03		
	Mean	0.05	0.03	0.03*	95	94
	St. dev.	0.05	0.03	0.02		

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 0.50 days^{-1}.

The % inhibition data in Table 9 indicate a dose response was occurring.

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 ≥2.6 mg 7-OH metabolite of pyroxsulam/L were identified as statistically significantly less than the control mean (Williams' test, 1 tailed).

The ToxCalc calculations for the specific growth rate results are shown in Table 10 along with the study report's equivalent results. The ToxCalc output is provided at page 45 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 7-OH metabolite of pyroxsulam. EC50, 95% confidence limits and NOEC values are as mg 7-OH metabolite of pyroxsulam/L. Equivalent study report values are also shown.

	0-7 days ErC50	95% Confidence limits	0-7 days NOEC	Mean measured concentrations which had statistically significantly lower mean specific growth rates compared to the mean of the control
Reviewer calculated	3.8	2.6-4.9	0.74	≥2.6
Study report	4.0	3.2-4.5	0.74	≥2.6

Verification of biomass (frond dry weight) statistics

The biomass (day 7 frond dry weight) data reported are shown in Table 5 on page 30 of this DER and were analysed by the TidePool Scientific Software program, ToxCalc (v5.0.23A) as previously described.

The ToxCalc analysis used the log transformed day 7 frond dry weight values with the log transformed data identified as normally distributed and equality of variances. Untransformed data were indicated as having a normal distribution but unequal variances. The ToxCalc output is provided on page 46 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 day 7 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 7-OH metabolite of pyroxsulam. EC50, 95% confidence limits and NOEC values are as mg 7-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	2.1	0.63-3.1	0.74	≥2.6
Study report	2.1	1.4-2.5	0.74	≥2.6

D. STUDY DEFICIENCIES:

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

Table 12. Deviations 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 7-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.
(Initial) pH at days 0, 3 and 5:	The pH values reported for days 0, 3 and 7 (fresh or new solutions) were, respectively, 8.0, 7.5 and 7.5.	OECD 221 states that the pH of the 20X AAP growth medium is adjusted to 7.5 ± 0.1.	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.
Test concentrations Nominal:	The nominal test concentrations are in a ratio of approximately 1:3.3 or 1:3.4.	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 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).
Light intensity and quality:	The light intensity ranged from 6700 to 8900 lux. The photosynthetically	OECD 221 refers use of light of an intensity equivalent to 6500-10000 lux and to 85-135 $\mu E/m^2/s$ when	US EPA OPPTS 850.4400 states that a light intensity in the range of 4,200 and 6,700 lux should be used.

	active radiation (PAR) of the test area at test initiation ranged from 120 to 139 $\mu E/m^2/s$.	measured in a photosynthetically active radiation (400-700 nm)	

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 reason for the day 0 pH value exceeding 7.5 in the control is unclear. The pH of the AAP was stated to have been adjusted to a pH of 7.5 before addition of any test material or alga and the pH determined on the portion of medium in the volumetric flasks. As a result, a pH of close to 7.5 would have been expected in the control medium at day 0, rather than the reported pH of 8.0. This matter is not considered to have had any significant adverse effect on the study or its outcomes.

The nominal test concentrations are in a ratio of ~1:3.3 or 1:3.4. This is considered to only just exceed the maximum OECD 221 recommended ratio of 1:3.2 but to exceed the US EPA OPPTS 850.4400 ratio of 1:1.5 to 2.0. The OECD and US EPA OPPTS requirements are not considered mandatory and the satisfactory control growth is taken to indicate that the deviation from the US EPA OPPTS range did not result in any significant adverse effect on the study or its results.

The protocol states that the light intensity will range from 6500 to 10,000 lux and the photosynthetically-active radiation (PAR) will range from 85 to 120 $\cdot E/m^2/s$. During the definitive test, the light intensity ranged from 6700 to 8900 lux and the PAR ranged from 120 to 139 $\cdot E/m^2/s$. Since the light intensity was within the appropriate range, the decision was taken no to adjust the PAR. This decision is not expected to have had any significantly adverse effect, especially as there was satisfactory control growth. 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.

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 exposure (with renewal on days 3 and 5) of duckweed to mean measured concentrations of 0.21 to 100 mg 7-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 7-OH metabolite 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 7-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 7-OH metabolite of pyroxsulam/L with 95% confidence limits shown in brackets:		
	Study report	
Frond number EC50	1.8 (1.6-2.0)	
Mean specific growth rate (day^{-1}) 0-7 days ErC50	4.0 (3.2-4.5)	
Biomass (frond dry weight) EbC50	2.1 (1.4-2.5)	

These EC50 values are considered to classify 7-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:

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.

Gulley, D.D., Boetler, A.M. and Bergman, H.L. 1996 TOXSTAT® Release 3.5. University of Wyoming, Laramie, Wyoming.

Hoberg, J. R. (2006a). 7-OH Metabolite of XDE-742 - Toxicity to Duckweed, *Lemna gibba.* Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6409.SPT, Dow Study No. 050119.SPT. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences Indianapolis, Indiana 46268. 27 April 2006. Unpublished report.

(Note that the above reference is to the study profile template prepared in support of the study under evaluation. It has been designated 2006a to distinguish it from the study report itself, which would be references as Hoberg (2006)).

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

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

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

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.

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.

Pesticide Assessment Guidelines, Subdivision J. Hazard Evaluation: Nontarget Plants. Report No. EPA 540/9-82-020, PB83-153940. U.S. Environmental Protection Agency, Washington, D.C. 1982.

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.

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.

Attachment 1

Composition of 20X Algal Assay Procedure (AAP) medium used in the study with 7-OH metabolite of pyroxsulam compared to the 20X AAP growth medium described in OECD 221.

20X AAP used in the study with 7-OH metabolite of pyroxsulam		20X AAP growth medium as described in OECD 221		
Ingredient	**Medium concentrations**	**Ingredient**	**Stock concentrations**	**Medium concentrations**
$NaNO_3$	510 mg/L	$NaNO_3$	26 g/L	510 mg/L
$MgCl_2 \cdot 6H_2O$	240 mg/L	$MgCl_2 \cdot 6H_2O$	12 g/L	240 mg/L
$CaCl_2 \cdot 2H_2O$	90 mg/L	$CaCl_2 \cdot 2H_2O$	4.4 g/L	90 mg/L
$MgSO_4 \cdot 7H_2O$	290 mg/L	$MgSO_4 \cdot 7H_2O$	15 g/L	290 mg/L
$NaHCO_3$	300 mg/L	$NaHCO_3$	15 g/L	300 mg/L
$K_2HPO_4.3H_2O$	30 mg/L	K_2HPO_4	1.4 g/L	30 mg/L
H_3BO_3	3.7 mg/L	H_3BO_3	0.19 g/L	3.7 mg/L
$MnCl_2 \cdot 4H_2O$	8.3 mg/L	$MnCl_2 \cdot 4H_2O$	0.42 g/L	8.3 mg/L
$ZnCl_2$	0.066 mg/L	$ZnCl_2$	3.3 mg/L	0.066 mg/L
$Na_2MoO_4 \cdot 2H_2O$	0.145 mg/L	$Na_2MoO_4 \cdot 2H_2O$	7.3 mg/L	0.145 mg/L
$CoCl_2 \cdot 6H_2O$	0.029 mg/L	$CoCl_2 \cdot 6H_2O$	1.4 mg/L	0.029 mg/L
$CuCl_2 \cdot 2H_2O$	0.00024 mg/L	$CuCl_2 \cdot 2H_2O$	0.012 mg/L	0.00024 mg/L
$FeCl_3 \cdot 6H_2O$	3.2 mg/L	$FeCl_3 \cdot 6H_2O$	0.16 g/L	3.2 mg/L
$Na_2EDTA.2H_20$	6.0 mg/L	$Na_2EDTA.2H_20$	0.30 g/L	6.0 mg/L
Na_2SeO_4*	0.0376 mg/L	Na_2SeO_4	Not present	Not present

*** 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:

EC50 values etc. are reported as mg 7-OH metabolite of pyroxsulam/L.

D-Control	85.000	90.000	79.000
0.21	80.000	77.000	85.000
0.74	61.000	77.000	67.000
2.6	39.000	44.000	46.000
8.6	23.000	24.000	24.000
31	16.000	17.000	19.000
100	15.000	20.000	18.000

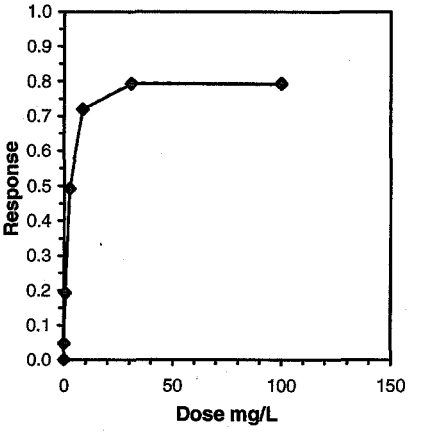
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	84.667	1.0000	84.667	79.000	90.000	6.505	3				84.667	1.0000
0.21	80.667	0.9528	80.667	77.000	85.000	5.010	3	1.120	1.760	6.287	80.667	0.9528
*0.74	68.333	0.8071	68.333	61.000	77.000	11.829	3	4.572	1.850	6.609	68.333	0.8071
*2.6	43.000	0.5079	43.000	39.000	46.000	8.385	3	11.664	1.880	6.716	43.000	0.5079
*8.6	23.667	0.2795	23.667	23.000	24.000	2.440	3	17.075	1.890	6.752	23.667	0.2795
*31	17.333	0.2047	17.333	16.000	19.000	8.813	3	18.802	1.900	6.788	17.500	0.2067
*100	17.667	0.2087	17.667	15.000	20.000	14.245	3	18.802	1.910	6.823	17.500	0.2067

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97765	0.873	0.24285	0.75692
Bartlett's Test indicates equal variances (p = 0.11)	10.2801	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	0.21	0.74	0.39421		6.82325	0.08059	2651.3	19.1429	1.2E-11	6, 14

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.2200	0.1014	0.0000	0.6666	0.6461
IC10	0.4019	0.1442	0.0000	1.1358	0.7199
IC15	0.5839	0.1654	0.0619	1.4539	0.7778
IC20	0.7841	0.2060	0.1708	1.6951	0.5382
IC25	1.0949	0.2414	0.1320	1.9104	-0.0143
IC40	2.0273	0.1719	1.1391	2.6400	-0.4131
IC50	2.8069	0.4364	1.8611	4.9497	0.6347



Frond number at 120 hours (5 days)

The ToxCalc calculations were as follows with frond count numbers at 120 hours also shown:
EC50 values etc. are reported as mg 7-OH metabolite of pyroxsulam/L.

Conc-mg/L	1	2	3
D-Control	229.00	229.00	208.00
0.21	197.00	211.00	187.00
0.74	169.00	207.00	193.00
2.6	54.00	62.00	75.00
8.6	24.00	25.00	26.00
31	16.00	16.00	18.00
100	15.00	21.00	18.00

			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	222.00	1.0000	222.00	208.00	229.00	5.461	3				222.00	1.0000
*0.21	198.33	0.8934	198.33	187.00	211.00	6.078	3	2.737	1.760	15.22	198.33	0.8934
*0.74	189.67	0.8544	189.67	169.00	207.00	10.133	3	3.739	1.850	16.00	189.67	0.8544
*2.6	63.67	0.2868	63.67	54.00	75.00	16.647	3	18.308	1.880	16.26	63.67	0.2868
*8.6	25.00	0.1126	25.00	24.00	26.00	4.000	3	22.779	1.890	16.35	25.00	0.1126
*31	16.67	0.0751	16.67	16.00	18.00	6.928	3	23.665	1.900	16.43	17.33	0.0781
*100	18.00	0.0811	18.00	15.00	21.00	16.667	3	23.665	1.910	16.52	17.33	0.0781

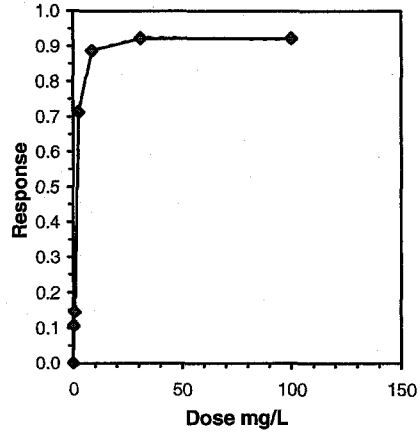
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95423	0.873	-0.3586	0.69315
Bartlett's Test indicates equal variances (p = 0.01)	16.7225	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	<0.21	0.21			16.5183	0.07441	26524.2	112.19	3.0E-13	6, 14

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	0.0985	0.0940	0.0346	0.6854	4.3118
IC10*	0.1970	0.2158	0.0691	1.4748	1.4035
IC15	0.7543	0.2260	0.0000	1.1690	-0.5988
IC20	0.9181	0.1407	0.1554	1.3085	-0.6681
IC25	1.0820	0.1225	0.5380	1.4597	-0.4997
IC40	1.5736	0.0935	1.1376	1.9055	-0.4174
IC50	1.9013	0.0805	1.5373	2.2054	-0.2468

* indicates IC estimate less than the lowest concentration



Frond number at 168 hours (7 days)

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

Note that equality of variance and normality of distribution of counts was achieved by log transformations. EC50 values etc. are reported as mg 7-OH metabolite of pyroxsulam/L.

Conc-mg/L	1	2	3
D-Control	483.00	538.00	432.00
0.21	477.00	441.00	405.00
0.74	412.00	425.00	434.00
2.6	94.00	117.00	132.00
8.6	30.00	27.00	29.00
31	15.00	19.00	19.00
100	16.00	20.00	18.00

			Transform: Log					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	484.33	1.0000	2.6834	2.6355	2.7308	1.776	3				484.33	1.0000
0.21	441.00	0.9105	2.6435	2.6075	2.6785	1.345	3	1.034	1.760	0.0680	441.00	0.9105
0.74	423.67	0.8747	2.6269	2.6149	2.6375	0.433	3	1.463	1.850	0.0714	423.67	0.8747
*2.6	114.33	0.2361	2.0540	1.9731	2.1206	3.639	3	16.299	1.880	0.0726	114.33	0.2361
*8.6	28.67	0.0592	1.4570	1.4314	1.4771	1.603	3	31.759	1.890	0.0730	28.67	0.0592
*31	17.67	0.0365	1.2445	1.1761	1.2788	4.763	3	37.144	1.900	0.0734	17.83	0.0368
*100	18.00	0.0372	1.2535	1.2041	1.3010	3.868	3	37.144	1.910	0.0738	17.83	0.0368

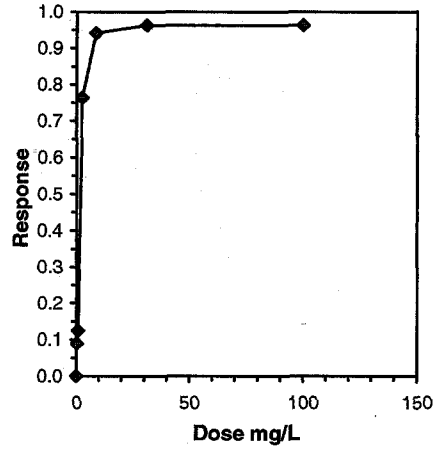
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96354	0.873	-0.4452	-0.4156
Bartlett's Test indicates equal variances (p = 0.46)	5.71687	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	0.74	2.6	1.38708		75.3499	0.1562	1.34988	0.00224	4.5E-16	6, 14

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	0.1174	0.1906	0.0000	1.4435	1.8340
IC10	0.3659	0.2881	0.0000	1.4432	0.4777
IC15	0.8121	0.2787	0.0000	1.2254	-0.8414
IC20	0.9577	0.1940	0.0000	1.3386	-2.0182
IC25	1.1033	0.1173	0.5903	1.4747	-0.1421
IC40	1.5401	0.0949	1.1274	1.8825	-0.1220
IC50	1.8313	0.0819	1.4817	2.1460	-0.1019

* indicates IC estimate less than the lowest concentration



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 rate are day^{-1}:

Conc-mg/L	1	2	3
D-Control	0.4960	0.5114	0.4801
0.21	0.4942	0.4830	0.4708
0.74	0.4733	0.4777	0.4807
2.6	0.2622	0.2934	0.3107
8.6	0.0990	0.0840	0.0942
31	0.0000	0.0338	0.0338
100	0.0092	0.0411	0.0260

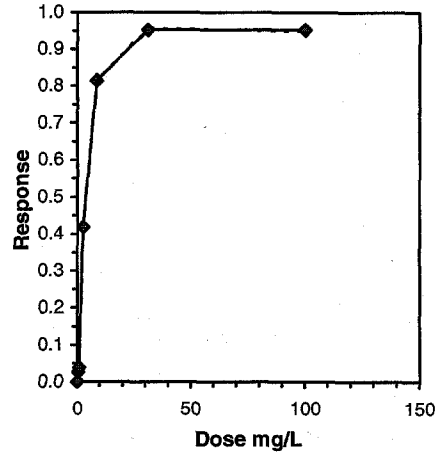
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.4958	1.0000	0.4958	0.4801	0.5114	3.161	3				0.4958	1.0000
0.21	0.4827	0.9735	0.4827	0.4708	0.4942	2.422	3	1.034	1.760	0.0224	0.4827	0.9735
0.74	0.4772	0.9625	0.4772	0.4733	0.4807	0.783	3	1.463	1.850	0.0235	0.4772	0.9625
*2.6	0.2888	0.5824	0.2888	0.2622	0.3107	8.514	3	16.299	1.880	0.0239	0.2888	0.5824
*8.6	0.0924	0.1863	0.0924	0.0840	0.0990	8.316	3	31.759	1.890	0.0240	0.0924	0.1863
*31	0.0225	0.0454	0.0225	0.0000	0.0338	86.603	3	37.144	1.900	0.0241	0.0240	0.0484
*100	0.0255	0.0513	0.0255	0.0092	0.0411	62.650	3	37.144	1.910	0.0243	0.0240	0.0484

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96354	0.873	-0.4452	-0.4156
Bartlett's Test indicates equal variances (p = 0.46)	5.71687	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	0.74	2.6	1.38708		0.02426	0.04893	0.14606	0.00024	4.5E-16	6, 14

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.8013	0.2299	0.0000	1.0301	-1.4852
IC10	1.0460	0.0697	0.7485	1.3087	-0.0274
IC15	1.2906	0.0706	0.9928	1.5766	0.0197
IC20	1.5353	0.0752	1.2349	1.8435	0.0341
IC25	1.7799	0.0829	1.4677	2.1376	0.0266
IC40	2.5139	0.1740	2.1118	3.5149	0.9078
IC50	3.8484	0.3012	2.6124	4.9490	-0.4610



EC50 values etc. are reported as mg 7-OH metabolite of pyroxsulam/L.

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	51.300	77.000	49.600
0.21	56.600	48.900	43.700
0.74	43.300	44.100	55.300
2.6	20.000	22.500	25.600
8.6	14.600	15.400	13.100
31	10.900	10.300	10.400
100	8.900	11.000	10.900

			Transform: Log					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	59.300	1.0000	1.7640	1.6955	1.8865	6.026	3				59.300	1.0000
0.21	49.733	0.8387	1.6942	1.6405	1.7528	3.325	3	1.431	1.760	0.0859	49.733	0.8387
0.74	47.567	0.8021	1.6746	1.6365	1.7427	3.534	3	1.833	1.850	0.0903	47.567	0.8021
*2.6	22.700	0.3828	1.3538	1.3010	1.4082	3.961	3	8.405	1.880	0.0918	22.700	0.3828
*8.6	14.367	0.2423	1.1564	1.1173	1.1875	3.096	3	12.450	1.890	0.0922	14.367	0.2423
*31	10.533	0.1776	1.0224	1.0128	1.0374	1.287	3	15.195	1.900	0.0927	10.533	0.1776
*100	10.267	0.1731	1.0094	0.9494	1.0414	5.153	3	15.462	1.910	0.0932	10.267	0.1731

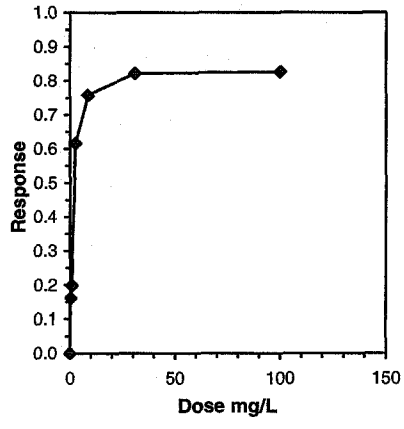
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94883	0.873	0.64694	0.10422
Bartlett's Test indicates equal variances (p = 0.43)	5.94046	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	0.74	2.6	1.38708		11.2194	0.19317	0.32441	0.00357	2.1E-10	6, 14

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	0.0651	0.2468	0.0000	1.8135	1.8286
IC10*	0.1302	0.3239	0.0000	2.0839	1.3127
IC15*	0.1953	0.3910	0.0000	2.3697	0.8981
IC20	0.7495	0.4425	0.0000	2.1221	0.2867
IC25	0.9713	0.4652	0.0000	2.2279	-0.1820
IC40	1.6366	0.3785	0.0000	2.6085	-0.5773
IC50	2.0801	0.3121	0.6325	3.0674	-0.4289

* indicates IC estimate less than the lowest concentration



Note that untransformed data had a normal distribution (Shapiro-Wilk's Test (p >0.01)). However, Bartlett's test indicated unequal variances (p = 8.52E-04). A log transformation resulted in normality of distribution and equal variances being achieved and the data so transformed were used for the ToxCalc analysis rather than the untransformed dry weight data.