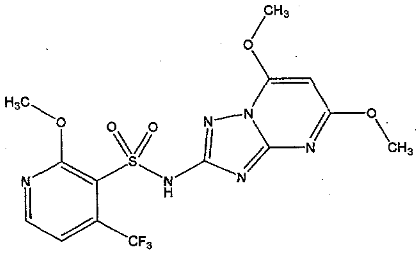
Data Evaluation Report on the acute toxicity of pyroxsulam (XDE-742) to aquatic vascular plants duckweed, *Lemna gibba* (Seven day exposure)

PMRA Submission Number 2006-4727; 1283274 EPA MRID Number 469084-~~xx~~ 42 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.8.5 (TGAI)
	EPA DP Barcode:	D332116
	OECD Data Point:	221
	EPA Guideline:	123-2 (OPPTS 850.4400 (Draft April 1996))

Test material:	**Pyroxsulam (XDE-742)** **Purity (%):** 98%
Common name:	XDE-742
Chemical name:	3-pyridinesulfonamide, N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
IUPAC:	N-(5, 7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.:	422556-08-9
Synonyms:	XR-742, X666742
Test Substance Number:	TSN103826

Chemical structure:



D. Murphy 22/02/08

Primary Reviewer: Daryl Murphy **Date:** 12 June 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

22/2/08

Secondary Reviewers: Jack Holland **Date:** 12 June 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

PMRA Reviewer: Émilie Larivière **Date:** 22 June 2007
Environmental Assessment Directorate, PMRA
Émilie Larivière 05/03/08

US EPA Reviewer: Christopher Salice **Date:** 25 June 2007
Environmental Fate and Effects Division, US Environmental Protection Agency
4/10/08

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

CITATION: Hancock, G. A. McClymont, E. L. and Najar, J. R. 2005. XDE-742: Growth Inhibition Test With The Aquatic Plant Duckweed, *Lemna gibba*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID: 041124. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 28 April 2005. Unpublished report.

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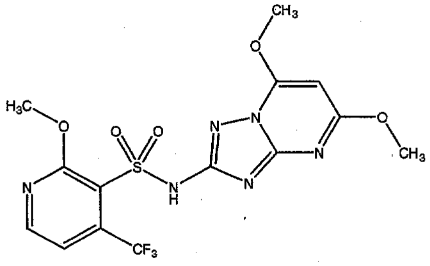
Synonyms: XR-742, X666742
Test Substance Number: TSN103826

Chemical structure:



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EXECUTIVE SUMMARY:

In a 7 day acute toxicity study, freshwater floating aquatic vascular plants (duckweed, *Lemna gibba*) were exposed to pyroxsulam at nominal concentrations of 0 (medium and solvent controls), 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 µg pyroxsulam/L (corresponding mean measured concentrations were 0, 0.335, 0.681, 1.34, 2.81, 5.23 and 10.3 µg pyroxsulam/L) under static renewal conditions at days 3 and 5 in accordance with the guidelines, OECD 221 "Lemna sp. Growth Inhibition Test" (draft, 2002) and US EPA guidelines including U.S. Environmental Protection Agency (1996). Ecological Effects Test Guidelines. OPPTS 850.4400 Aquatic Plant Toxicity Test using Lemna sp., Tiers I and II. Draft April 1996.

The 7 day NOECs based on frond number, specific growth rates and biomass (dry weight at 7 days) were all set at 0.68 µg pyroxsulam/L (mean measured concentration).

The EC50 for frond numbers was 2.57 µg pyroxsulam/L (mean measured concentration) with 95% confidence limits of 1.16-5.70 µg pyroxsulam/L. The ErC50 (mean specific growth rate) was 3.88 µg pyroxsulam/L with 95% confidence limits of 1.68-8.97 µg pyroxsulam/L. The EbC50 (biomass, frond dry weight) was 3.82 µg pyroxsulam/L (mean measured concentration) with 95% confidence limits of 2.23-6.56.

The % growth inhibition was determined for frond number, mean specific growth rate and biomass (frond dry weight). With the frond count, response relative to the pooled controls ranged from 9% stimulation to 89% inhibition of mean frond density. Response relative to the pooled controls ranged from 3% stimulation to 79% inhibition of mean specific growth rate. For biomass based on the day 7 frond dry weights, response relative to the pooled controls ranged from 8% stimulation to 69% inhibition of frond dry weight.

No reference was made in the study report to abnormalities such as any change in frond development or appearance, unusual frond/leaf/plant shape or size, colour differences, aggregation of fronds. Stimulation of growth was identified as having occurred at mean measured concentrations of 0.335 and 0.681 µg pyroxsulam/L. There were dose related effects observed in the three growth parameters determined with growth being adversely affected as the concentration of pyroxsulam increased.

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

Results Synopsis

Test Organism:	Duckweed (*Lemna gibba*)
Test Type:	Static Renewal

Frond count

7 day EC05:	Not reported	
7 day EC50:	2.57 µg pyroxsulam/L	95% C.I.: 1.16 to 5.70 µg pyroxsulam/L
7 day NOEC:	0.68 µg pyroxsulam/L	Probit Slope: Not reported

Mean specific growth rate (day^{-1})

7 day ErC05:	Not reported	
7 day ErC50:	3.88 µg pyroxsulam/L	95% C.I.: 1.68 to 8.97 µg pyroxsulam/L
7 day NOEC:	0.68 µg pyroxsulam/L	Probit Slope: Not reported

Biomass (frond dry weight)

7 day EbC05:	Not reported	
7 day EbC50:	3.82 µg pyroxsulam/L	95% C.I.: 2.23 to 6.56 µg pyroxsulam/L
7 day NOEC:	0.68 µg 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 study generally conformed to procedures described by the Organisation for Economic Cooperation and Development (OECD) draft guideline (at April 2005 and finalised in March 2006):

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

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

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

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

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

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

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

A. MATERIALS:

1. Test Material	XDE-742 (i.e. pyroxsulam)
Description:	Solid

Lot No./Batch No.:	E0952-52-01
Purity:	98%

Stability of Compound Under Test Conditions:

The 26-day stability of pyroxsulam in acetonitrile was determined in a related study (McClymont, 2004) by analysing a stock solution (nominal concentration 515 µg pyroxsulam/mL acetonitrile) that had been stored for 26 days at ~8 °C. The data provided an analysed concentration that was 104% of the expected concentration.

During the study's 7 day exposure phase, the mean measured concentrations of pyroxsulam in the bulk dose solutions (0.335 to 10.3 µg pyroxsulam/L) ranged from 103 to 112% of target (nominal) concentrations, indicative of the pyroxsulam's being stable during the exposure.

In the spent exposure solutions analysed on days 3, 5 and 7, the measured concentrations respectively ranged from 101 to 117, 103 to 115 and 13.7 to 109% of nominal. These latter results indicate that up to day 5, nominal concentrations were exceeded while on day 7, evidence occurred of actual concentrations of pyroxsulam falling, in some cases, well below nominal values.

Similar results were obtained from spent blank solutions analysed on days 3, 5 and 7 with the measured concentrations of pyroxsulam at those days being, respectively, 103-108, 103-115 and 25-109% of nominal. As for the spent exposure solutions, these results indicate stability in the test medium through to day 5 with some pyroxsulam concentrations observed at day 7 falling well below the respective nominal concentrations.

Actual concentrations are shown on page 17 of this DER.

The study report considered the results from the spent solutions in detail and reported as follows,

> "The analysis of the spent test solutions containing duckweed, as well as the spent test solutions that contained no duckweed, resulted in measured concentrations that were within ± 20% of the target concentration with the exception of three day 7 samples that were 67.7% (1.25 µg/L spent blank solution), 13.7% (2.50 µg/L spent exposure solution) and 25.0% (2.50 µg/L spent blank solution) of target. The explanation for these low recoveries is unclear; however, these results are inconsistent with all other analyses throughout the study which showed the solutions to be dosed correctly, and to be stable for the period between solution renewals .... Analysis of these original bulk solutions demonstrated that they were prepared correctly and were very close to target concentrations. Since the anomalously low spent solutions are simply aged aliquots of these bulk solutions, and because the test material has demonstrated stability between

solution renewals, the low concentrations measured are clearly an artefact of analytical error. The source of this error may be a mistake in processing of these three samples, a detector matrix effect, or some other factor."

Storage conditions of test chemicals: Not stated in study report. Study profile template (Hancock, 2005), states "Room temperature in the dark".

Physicochemical properties of pyroxsulam.

Parameter	Values	Comments
Water solubility at 20°C		
pH 4	0.0164 g/L	Turner (2004a)
pH 6	0.0626 g/L	Turner (2004a)
pH 7	3.2 g/L	Turner (2004a)
pH 9	13.7 g/L	Turner (2004a)
Vapour pressure	<1E-7	Madsen (2003)
UV absorption: Not available at the time of publication of the company's study profile template.		
pKa	4.670	Cathie (2004)
Kow		
pH 4	12.1 (log Pow = 1.08)	Turner (2004b)
pH 7	0.097 (log Pow = -1.01)	Turner (2004b)
pH 9	0.024 (log Pow = -1.60)	Turner (2004b)

Note: The physicochemical properties of pyroxsulam were not reported in the study. The values recorded in the company's study profile template report (Dow Chemical Company study ID: 041124.SPT (Hancock, 2005) were misordered). The correct values (confirmed by examination of Turner (2004b) in Madsen (2006)) are shown above in the physicochemical properties of pyroxsulam table.

2. Test organism:

Name:	Freshwater duckweed, *Lemna gibba*. L.
Strain, if provided:	G-3
Source:	Axenic samples of this species were received in May of 1999 from USDA/ARS Beltsville Agricultural Research Center, Beltsville, Maryland.
Age of inoculum:	Fronds came from a 16 day-old subculture (at test initiation).
Method of cultivation:	Stock cultures of this organism were maintained axenically by weekly transfer into fresh medium.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study:
The exposure phase of the probe or range-finding study was conducted between 6 and 13 August 2004 (seven-day static exposure) using seven nominal concentrations of 0.0500, 0.100, 0.500, 1.00, 5.00, 10.0 and 500 µg pyroxsulam/L. Percent inhibition of frond growth compared to controls on day 7 was -2, -15, 9, 36, 82 and 86% for the 0.0500, 0.100, 0.500, 1.00, 5.00, 10.0 and 500 µg pyroxsulam/L test levels, respectively (negative inhibition

indicates greater growth than controls). Based on this, the empirically derived EC50 based on frond density was between 1.00 and 5.00 µg/L. A seven-day recovery period was added on to this probe to evaluate the ability of the plants to recover. Growth during the recovery phase was similar to controls for the 0.0500, 0.100 and 0.500 µg/L test levels. A recovery phase was not conducted on the definitive test. The information derived from this probe was used to set the range of concentrations for the definitive test.

The original definitive test was initiated on 5 November 2004 at exposure levels of control (media control), 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 µg pyroxsulam/L. However, due to variable and unacceptable analytical recoveries, the study was considered invalid. Test solutions were renewed on day 3 only. The exposure was carried out for the full seven days and the fronds were enumerated at this time. Percent inhibition of frond growth compared to controls on day 7 was -8, 10, -13, 22, 76, 87 and 91% for the 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 µg pyroxsulam/L test levels, respectively (negative inhibition indicates greater growth than controls). Due to the variability in analytical recoveries it was decided to investigate the use of a solvent in preparation of the test solutions. Preliminary data indicated that test solution preparation using solvent stock solutions was superior to the preparation method without solvent.

The response from the unsuccessful definitive study indicated that the target test concentrations were appropriate. Therefore, the repeat definitive study was conducted under static-renewal exposure conditions.

[b) Definitive Study

The definitive test was conducted from 7 to 14 January 2005 with the exposure phase carried out aseptically 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:	Axenic samples of the *L. gibba* were received in May of 1999 and a sixteen-day-old subculture was used for the test.	See deviations/deficiency table on page 36 of this report. The aquatic vascular plants template does not specify acclimatisation details. 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

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
<i>Criteria</i></th></tr>
<tr><td></td><td></td><td>used in a test should be randomly selected from the culturing tank. Inocula should be taken from cultures which are less than 2 weeks old.</td></tr>
<tr><td>Culturing media and conditions: (same as test or not)</td><td>Stock cultures of the test organism were maintained axenically by weekly transfer into fresh medium.

Typical culturing conditions were described as:

<table>
<tr><td>Conditions:</td><td>Culture:</td></tr>
<tr><td>Temperature (°C):</td><td>25 ± 2°C</td></tr>
<tr><td>Light (lux):</td><td>5400 ± 1100</td></tr>
<tr><td>Photoperiod:</td><td>Continuous</td></tr>
<tr><td>Medium:</td><td>Modified (20X) AAM</td></tr>
<tr><td>pH:</td><td>~7.5 to 8.5</td></tr>
<tr><td>Culture Vessel:</td><td>500 mL Erlenmeyer flask</td></tr>
<tr><td>Inoculation:</td><td>Every seven days</td></tr>
<tr><td>Culture Chamber:</td><td>Environmental chamber</td></tr>
<tr><td>Amount of Transfer:</td><td>Approximately five plants (15 fronds, three fronds/plant)</td></tr>
<tr><td>Transfer:</td><td>Sterile bacteriological loop</td></tr>
</table>

Comparison of these culture conditions with the test parameters shown in the adjacent "Remarks" column indicates that test conditions can be considered the same as the culture conditions.</td><td>Requirement considered met.

Typical test conditions were described as:

<table>
<tr><td>Conditions:</td><td>Test:</td></tr>
<tr><td>Temperature (°C):</td><td>25 ± 2°C</td></tr>
<tr><td>Light (lux):</td><td>6600 ± 990</td></tr>
<tr><td>Photoperiod:</td><td>Continuous</td></tr>
<tr><td>Medium:</td><td>Modified (20X) AAM</td></tr>
<tr><td>pH:</td><td>Adjusted to 7.5 prior to addition of test material.</td></tr>
<tr><td>Culture Vessel:</td><td>270 mL borosilicate crystallizing dish with cover.</td></tr>
<tr><td>Inoculation:</td><td>Single</td></tr>
<tr><td>Culture Chamber:</td><td>Environmental growth chamber</td></tr>
<tr><td>Amount of Transfer:</td><td>Three plants, four fronds per plant.</td></tr>
<tr><td>Transfer:</td><td>Not relevant</td></tr>
</table></td></tr>
<tr><td>Health: (any toxicity observed)</td><td>No specific comment found in the test report but the stock cultures used were maintained axenically by weekly transfer into fresh medium.</td><td>Requirement considered met.

OECD 221 refers to use of monocultures, that are visibly free from contamination by other organisms such as algae and Protozoa.

There was satisfactory growth in the controls, indicative of the duckweed being healthy. No phytotoxicity effects noted (Hancock, 2005).</td></tr>
</table>

Parameter	**Details**	**Remarks** ***Criteria***
		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 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

Parameter	**Details**	**Remarks** ***Criteria***
		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	Environmental chamber thermostatically controlled at 25 ± 2°C.	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 24.2 to 24.5°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.*
<u>Test vessel</u>		Requirement considered met.
Material: (glass/polystyrene)	Borosilicate crystallizing dish with cover	OECD 221 states glass beakers, crystallising dishes or glass Petri dishes of appropriate dimensions have all proved suitable. This guideline also states the test vessels must be covered and that crystallizing dishes are appropriate test vessels. US EPA OPPTS 850.4400 refers to test containers being glass beakers or Erlenmeyer flasks.
Size:	270 mL	A minimum depth of 20 mm and minimum volume of 100 mL in each test vessel is advised by OECD 221.

Parameter	**Details**	**Remarks** ***Criteria***
Fill volume:	100 mL	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.
Details of growth medium Name:	Modified 20X AAM. The growth and test medium used (twenty strength algal assay medium or 20X AAM) was stated to be based on that designated for the EPA Algal Assay Bottle Test and recommended by the American Society for Testing and Materials. The compositions of the 20X AAM stock medium and the OECD 221 20X AAP medium are provided as Attachment 1 on page 41 of this DER.	See deviations/deficiency table on page 36 of this report. Hancock (2005) states that the study report refers to the 20X AAP medium as 20X AAM. Comparison of the composition of the OECD 221 recipe for 20X AAP with the study report's modified 20X AAM recipe indicates that concentrations of some of the constituents in the stock solutions are similar but others vary. Comparison of the modified 20X AAM medium's composition with the 20X AAP medium composition described in OECD 221 indicates the same components are present and, in the made-up medium, at concentrations equivalent to those in the made-up OECD 221 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.*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
<i>Criteria</i></th></tr>
<tr>
<td>pH (in the bulk exposure solutions) at days 0, 3 and 5:</td>
<td>In the bulk media control, the pH values reported for days 0, 3 and 7 were:
<table>
<tr><th>Conc.*</th><th>Day 0</th><th>Day 3</th><th>Day 5</th></tr>
<tr><td>Medium control</td><td>7.9</td><td>7.5</td><td>7.7</td></tr>
<tr><td>Solvent (DMF) control</td><td>7.9</td><td>7.5</td><td>7.7</td></tr>
<tr><td>0.313</td><td>7.9</td><td>7.5</td><td>7.7</td></tr>
<tr><td>0.625</td><td>7.9</td><td>7.5</td><td>7.7</td></tr>
<tr><td>1.25</td><td>7.9</td><td>7.5</td><td>7.7</td></tr>
<tr><td>2.50</td><td>7.9</td><td>7.5</td><td>7.7</td></tr>
<tr><td>5.00</td><td>7.9</td><td>7.5</td><td>7.7</td></tr>
<tr><td>10.0</td><td>7.8</td><td>7.5</td><td>7.7</td></tr>
</table>
* Nominal concentrations as µg pyroxsulam/L.</td>
<td>See deviations/deficiency table on page 36 of this report.

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 bulk medium control having a pH of 7.9 is unclear. The pH of the AAM was stated to have been adjusted to a pH of 7.5 before addition of any test material or alga and, as a result, a pH of close to 7.5 would have been expected in the control medium at day 0.</td>
</tr>
<tr>
<td>pH (in pooled replicates of spent solution with duckweed) at days 3, 5 and 7:</td>
<td>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>Media control</td><td>7.1</td><td>7.2</td><td>8.0</td></tr>
<tr><td>Solvent (DMF) control</td><td>7.1</td><td>7.2</td><td>8.0</td></tr>
<tr><td>0.313</td><td>7.2</td><td>7.3</td><td>8.1</td></tr>
<tr><td>0.625</td><td>7.2</td><td>7.4</td><td>8.2</td></tr>
<tr><td>1.25</td><td>7.3</td><td>7.4</td><td>8.2</td></tr>
<tr><td>2.50</td><td>7.4</td><td>7.5</td><td>8.2</td></tr>
<tr><td>5.00</td><td>7.4</td><td>7.5</td><td>8.3</td></tr>
<tr><td>10.0</td><td>7.4</td><td>7.5</td><td>8.3</td></tr>
</table>
* Nominal concentrations as µg pyroxsulam/L.</td>
<td>A final pH of spent solutions was also taken on days 3, 5, and 7 from a pooled sample of the three replicates with fronds</td>
</tr>
<tr>
<td>pH (in pooled replicates of spent solution without duckweed) at days 3, 5 and 7:</td>
<td>pH values of the spent solutions which did not have 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>
</table></td>
<td>A final pH of spent solutions was also taken on days 3, 5, and 7 from each replicate without fronds at each test concentration and control group.</td>
</tr>
</table>

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
<i>Criteria</i></th></tr>
<tr><td></td><td>
<table>
<tr><td>Media control</td><td>7.2</td><td>7.3</td><td>7.8</td></tr>
<tr><td>Solvent (DMF) control</td><td>7.2</td><td>7.3</td><td>7.6</td></tr>
<tr><td>0.313</td><td>6.9</td><td>6.8</td><td>7.6</td></tr>
<tr><td>0.625</td><td>6.9</td><td>6.7</td><td>7.6</td></tr>
<tr><td>1.25</td><td>6.9</td><td>6.7</td><td>7.6</td></tr>
<tr><td>2.50</td><td>6.9</td><td>6.7</td><td>7.7</td></tr>
<tr><td>5.00</td><td>6.9</td><td>6.7</td><td>7.7</td></tr>
<tr><td>10.0</td><td>6.9</td><td>6.7</td><td>7.7</td></tr>
</table>
* Nominal concentrations as µg pyroxsulam/L.
</td><td></td></tr>
<tr><td>Chelator used:</td><td>The 20X AAM 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 <i>Lemna</i> 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.

<i>Chelators are not recommended (US EPA).</i></td></tr>
<tr><td>Carbon source:</td><td>Not identified. Stated to be ambient carbon dioxide by Hancock (2005)</td><td>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".</td></tr>
<tr><td>If non-standard nutrient medium was used, detailed composition provided (Yes/No)</td><td>Although the 20X AAM medium is not indicated as identical to the 20X AAP medium, the requirement is still met as the 20X AAM medium's detailed composition was provided and there are only minor differences.

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(see Attachment 1, page 41 of this DER for details on the composition of the 20X AAM medium).</td><td>Requirement considered met.</td></tr>
</table>

Parameter	**Details**	**Remarks** ***Criteria***
<u>Dilution water</u>		
Source/type:	Not identified. Sterile deionised water was used to prepare the 20X AAM medium with the study report identifying the dilution water as the modified (20X) algal assay medium (AAM).	OECD 221 does not address the quality of the dilution water in specific terms. As the duckweed cultures used had been maintained since 1999 and a sixteen-day-old subculture was used for the test with the controls growing satisfactorily, 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. pH value at day 0 in the bulk medium control was 7.9 with the reason for this not known. In the test bulk medium solutions, the pH ranged from 7.8 to 7.9, presumably related to the presence of the pyroxsulam added to these solutions.	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. OECD 221 also states that the pH of the control medium should not increase by more than 1.5 units during the test. *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:	Not reported.	Requirements considered met. OECD 221 and US EPA OPPTS 850.4400 do not address these parameters specifically. As the duckweed cultures used had been maintained since 1999 and a sixteen-day-old subculture was used for the test with the controls growing satisfactorily, the water used is considered to have been acceptable.
Particulate matter:	Not reported	
Metals:	Not reported	
Pesticides:	Not reported	
Chlorine:	Not reported.	
Water pretreatment (if any):	Deionisation	
Intervals of water quality measurement	Not reported.	

Parameter	**Details**	**Remarks** ***Criteria***
Indicate how the test material is added to the medium (added directly or used stock solution)	Test solutions were prepared from concentrated stock solutions which were prepared as serial dilutions from a primary stock solution of 100 µg pyroxsulam/mL primary stock solution prepared by dissolving 25.5 mg pyroxsulam (corrected for percent active ingredient) in 250 mL of dimethylformamide (DMF). Exposure solutions were prepared by injecting 100 µL of each corresponding DMF stock solution into 1 L of 20X AAM.	Requirements considered met. The primary stock solution was made up taking into account the 98% purity of the 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:	Four, three with plants, one without. The fourth replicate for each exposure group was not inoculated with *Lemna gibba* and served as a blank. These blanks were used to monitor test material concentration and pH in the absence of the test organism.	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.
Solvent control:	Four, three with plants, one without	Requirement considered met.
Treatments:	Four, three with plants, one without	Requirement considered met.
Number of plants/replicate	3 plants/replicate	Requirement considered met.

Parameter	**Details**	**Remarks** ***Criteria***
		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. 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	4 fronds/plant (equal to 12 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 AAM medium), 0 (DMF solvent control), 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 µg/L 20X AAM These concentrations are in a ratio of 1:2.	Requirement considered met. OECD 221 states that in the definitive toxicity test, there should normally be at least five test concentrations arranged in a geometric series. Preferably the separation factor between test concentrations should not exceed 3.2, but a larger value may be 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.*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
<i>Criteria</i></th></tr>
<tr>
<td>Measured:</td>
<td>Mean measured concentrations (based on the mean of the bulk dose measured concentrations for analysis on days 0, 3 and 5) were:
<table>
<tr><th>Nominal pyroxsulam value,µg/L</th><th>Mean measured pyroxsulam value, µg/L#</th><th>% of nominal</th></tr>
<tr><td>Control</td><td><LLQ*</td><td>N/A**</td></tr>
<tr><td>Solvent (DMF) control</td><td><LLQ*</td><td>N/A**</td></tr>
<tr><td>0.313</td><td>0.335</td><td>107</td></tr>
<tr><td>0.625</td><td>0.681</td><td>109</td></tr>
<tr><td>1.25</td><td>1.34</td><td>107</td></tr>
<tr><td>2.50</td><td>2.81</td><td>112</td></tr>
<tr><td>5.00</td><td>5.23</td><td>105</td></tr>
<tr><td>10.0</td><td>10.3</td><td>103</td></tr>
</table>
Values are from the bulk dose test solutions.
* Less than the lowest level quantified (0.101 µg pyroxsulam/L 20X AAM).
** Not applicable.
</td>
<td>Requirement considered met.

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

US EPA OPPTS 850.4400 refers to use of standard analytical methods, if available, to establish concentrations of the test solutions and that concentrations of the test chemical in the test solutions prior to use and discarding on day 3, 5, and 7 should be reported.

None of the analyses of the water controls exhibited peaks eluting at the retention times of the analyte at concentrations exceeding the LLQ (0.101 µg pyroxsulam/L 20 x AAM).

These analytical results indicate that target concentrations were reached and that the pyroxsulam was stable over the 7 days of exposure.</td>
</tr>
</table>

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td>
<p>Measured concentrations of pyroxsulam in the spent exposure and spent blank solutions at days 3, 5 and 7 were reported as:</p>
<table>
<tr><th>Spent solution tested</th><th>Day 3</th><th>Day 5</th><th>Day 7</th></tr>
<tr><td colspan="4">Control</td></tr>
<tr><td>Exposure</td><td><LLQ</td><td><LLQ</td><td><LLQ</td></tr>
<tr><td>Blank</td><td><LLQ</td><td><LLQ</td><td><LLQ</td></tr>
<tr><td colspan="4">Solvent (DMF) control</td></tr>
<tr><td>Exposure</td><td><LLQ</td><td><LLQ</td><td><LLQ</td></tr>
<tr><td>Blank</td><td><LLQ</td><td><LLQ</td><td><LLQ</td></tr>
<tr><td colspan="4">0.313</td></tr>
<tr><td>Exposure</td><td>0.321
103%</td><td>0.333
106%</td><td>0.311
99.4%</td></tr>
<tr><td>Blank</td><td>0.336
107%</td><td>0.321
103%</td><td>0.309
98.7%</td></tr>
<tr><td colspan="4">0.625</td></tr>
<tr><td>Exposure</td><td>0.672
108%</td><td>0.705
113%</td><td>0.616
98.6%</td></tr>
<tr><td>Blank</td><td>0.674
108%</td><td>0.691
111%</td><td>0.681
109%</td></tr>
<tr><td colspan="4">1.25</td></tr>
<tr><td>Exposure</td><td>1.46
117%</td><td>1.40
112%</td><td>1.26
101%</td></tr>
<tr><td>Blank</td><td>1.35
108%</td><td>1.37
110%</td><td>0.846
67.7%</td></tr>
<tr><td colspan="4">2.50</td></tr>
<tr><td>Exposure</td><td>2.76
110%</td><td>2.82
113%</td><td>0.342
13.7%</td></tr>
<tr><td>Blank</td><td>2.65
106%</td><td>2.87
115%</td><td>0.626
25.0%</td></tr>
<tr><td colspan="4">5.00</td></tr>
<tr><td>Exposure</td><td>5.28
106%</td><td>5.28
106%</td><td>4.86
97.2%</td></tr>
<tr><td>Blank</td><td>5.28
106%</td><td>5.16
103%</td><td>4.71
94.2%</td></tr>
<tr><td colspan="4">10.0</td></tr>
<tr><td>Exposure</td><td>10.1
101%</td><td>10.3
103%</td><td>9.02
90.2%</td></tr>
<tr><td>Blank</td><td>10.3
103%</td><td>10.3
103%</td><td>8.95
89.5%</td></tr>
</table>
</td><td>
<p>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 test regime is recommended. The study complied with this guideline requirement.</p>
<p>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.”</p>
<p>The study report noted that the reason for the low recoveries at day 7 in the 1.25 and 2.50 mg/L solutions was unclear and inconsistent with all other analytical results.</p>
<p>The report also said that analysis of the original bulk solutions had demonstrated they were prepared correctly and close to target concentrations. Because the anomalously low spent blank solutions are actually aged aliquots of these bulk solutions, and because the test material had demonstrated stability between solution renewals, the low concentrations measured were considered an artefact of analytical error.</p>
<p>The study authors’ comments are noted.</p>
</td></tr>
<tr><td></td><td></td><td></td></tr>
</table>

Parameter	**Details**	**Remarks** ***Criteria***
Solvent (type, percentage, if used)	Dimethyl formamide (DMF). Exposure solutions were prepared by injecting 100 µL of each corresponding DMF stock solution into 1 L of 20X AAM, for a consistent DMF concentration in solvent control and exposure solutions of 0.100 mL/L (100 µL/L).	Requirement considered met. OECD 221 states that commonly used solvents which do not cause phytotoxicity at concentrations up to 100 µL/L include dimethyl-formamide. US EPA OPPTS 850.4400 states that the upper limit of carrier volume is 0.5 mL/L and the same amount of carrier should be added to each test concentration.
Method and interval of analytical verification:	The bulk dose solutions were sampled for analytical confirmation on days 0, 3, and 5 of the study. On days 3, 5, and 7, the spent test solutions containing duckweed at each dose level (three replicates per dose level) were pooled to provide one composite duckweed containing sample per dose level for analytical confirmation while the test solutions at each dose level not containing duckweed were sampled separately. Pyroxsulam extracted from the solutions was determined by liquid chromatography/positive electrospray ionization mass spectrometry (LC/PESI-MS).	Requirement considered met. To assess analytical method precision and solution homogeneity, three additional samples were collected on day 0 from the 0.313 and 10.0 µg/L bulk dose solutions. Assessment of extraction efficiency yielded average recovery values of 103%, 107%, 100% and 109% for days 0, 3, 5 and 7, respectively, which were used to adjust the analysed concentrations of the extracted test solutions for method recovery on each analysis day. The LC/PESI-MS instrumentation exhibited a linear response for pyroxsulam over a concentration range extending from approximately 2.02 to 114 µg/L diluent. This range encompassed the expected range of concentrations in the test solutions following appropriate sample preparation. None of the analyses of the 20X AAM control or DMF solvent control samples exhibited a peak eluting at the retention time and mass of pyroxsulam at a concentration exceeding the lowest level quantified of 0.101 µg/L 20X AAM, which was the concentration of the lowest standard quantified times the lowest dilution factor. Typical chromatograms of a control, a
Limit of Quantitation:	The lowest level quantified was set at 0.101 µg pyroxsulam/L 20 X AAM.	
Limit of Detection:	Not reported.	

Parameter	**Details**	**Remarks** ***Criteria***
		standard, and a renewal bulk dose solution sample were presented.
<u>Test conditions</u> Temperature:	Temperatures during the exposure period ranged from 24.2–24.5°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*
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 mean (± standard deviation) light intensity was 6565 ± 43 lux with a range of 6440–6690 lux.	Requirement considered met. OECD 221 refers use of light of an intensity equivalent to 6500-10000 lux and to 85-135 μE/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: 5.0 Klux (15%)*
<u>Reference chemical (if used)</u> Name: Concentrations:	No reference chemical mentioned.	See deviations/deficiency table on page 36 of this report. 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

Parameter	**Details**	**Remarks** ***Criteria***
		substance. US EPA OPPTS 850.4400 states that positive controls using zinc chloride as a reference chemical should be run periodically. 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. 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 (but not other endpoint parameters such as chlorophyll values). 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. OECD 221 refers to frond numbers

	the edge of the parent frond counted. Dry weight (at least 48 hours at 60°C).	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. On days 0, 3 and 5, an initial pH was taken from a sample of each bulk test solution. 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 and from each replicate without fronds at each test concentration and control group. Light intensity was measured at test initiation. Pyroxsulam determinations in bulk dose solutions were made on days 0, 3 and 5 and in spent exposure and spent blank solutions on days 3, 5 and 7. Temperature was monitored continuously during the test.	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 of the test substance are determined at appropriate intervals.

Other observations, if any	pH of the modified (20X) AAM medium was adjusted to 7.5 prior to addition of test material. The light intensity was measured at test initiation at each position where inoculated replicates were placed during the in-life phase (*i.e.*, only designated positions were used during the test). The light intensity at each position was then applied to each replicate that occupied that position during the exposure period. This allowed a mean light intensity for each replicate and an overall mean light intensity to be calculated for the exposure period.	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	After 7 days, the mean frond counts in the control and solvent controls were, respectively, 203 and 187. These values represent, respectively, a 16.9 and a 15.6 increase over 7 days of the initial frond number (12) in the control and solvent control replicates. The mean specific growth rates for the control and solvent	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.

	control were reported as, respectively, ~0.404 and 0.392 day^{-1}. These criteria meet the OECD 221 requirements for growth and show that exponential growth occurred in the control.	
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, pyroxsulam concentrations in the test solutions, light intensity and temperature were provided. The data, protocol, protocol changes/revisions, and final report are archived by the Toxicology & 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. The study report stated that the raw data for the cell density and growth rate and endpoints met the assumptions of homogeneity and normality. 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).

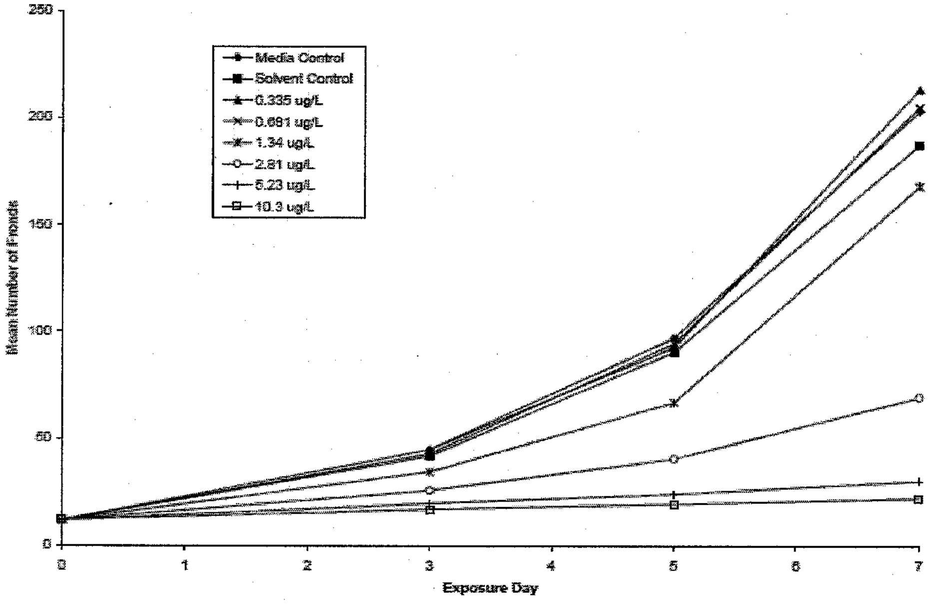
II. RESULTS AND DISCUSSION:

A. INHIBITORY EFFECTS:

Results from the chemical analysis of the bulk exposure solutions for pyroxsulam yielded percent of target values ranging from 100 to 115%. Three recoveries in spent exposure and blank solutions were less than 80% of nominal on Day 7. These recoveries appeared to be spurious and, as a result, biological results were based on mean measured bulk pyroxsulam concentrations. The mean measured bulk concentrations were 0.335, 0.681, 1.34, 2.81, 5.23 and 10.3 µg/L for the 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 µg/L nominal test concentrations, respectively.

Mean specific growth rates after seven days of exposure were 0.404, 0.393, 0.398, 0.411, 0.405, 0 376, 0.249, 0.131 and 0.0844 day^{-1} for the media control, solvent control, pooled control, 0.335, 0.681, 1.34, 2.81, 5.23 and 10.3 µg/L test levels, respectively. Response relative to the pooled controls ranged from 3% stimulation to 79% inhibition of mean specific growth rate. The 7-day calculated ErC50 value (95% confidence interval) for mean specific growth rate was 3.88 (1.68-8.97) µg/L. Based on Dunnett's test ($\alpha = 0.05$), the 7-day mean specific growth rate was significantly less than the controls at test levels ≥ 1.34 µg/L; therefore, the 7-day NOEC value for mean specific growth rate was determined to be 0.681 µg/L.

Mean frond count results and individual replicate data were presented in the study report. A graphical representation of these data (*i.e.*, growth curves) presented in the study report is reproduced below:



Graphical representation of duckweed growth for each exposure level and the control group of the 7 day exposure period (as presented in the study report).

Mean frond counts after seven days of exposure were 203, 187, 195, 213, 205, 168, 69, 30, and 22 fronds for the media control, solvent control, pooled control, 0.335, 0.681, 1.34, 2.81, 5.23 and 10.3 µg/L test levels, respectively. Response relative to the pooled controls ranged from 9% stimulation to 89% inhibition of mean frond density. The 7-day calculated EC50 value (95% confidence interval) for cell density was 2.57 (1.16-5.70) µg/L. Based on Dunnett's test (α = 0.05), the 7-day mean cell density was significantly less than the pooled controls at test levels ≥ 1.34 µg/L; therefore, the 7-day NOEC value for mean cell density was determined to be 0.681 µg/L.

The frond counts from days 0 to 7, plus the calculated percentage inhibition based on pooled 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 pyroxsulam on frond number of the freshwater duckweed (*Lemna gibba*) as given in the study report (Hancock *et al.*, 2005).

Treatment (nominal and measured concentration), µg pyroxsulam/L	Replicate No.	Frond number at:				% inhibition from the pooled controls
		Day 0	Day 3	Day 5	Day 7	
Negative control/<LLQ[1]	1	12	40	91	186	
	2	12	47	104	218	
	3	12	47	96	206	
	Mean	**12**	**45**	**97**	**203**	----[2]
	SD[3]	**0**	**4**	**7**	**16**	
Solvent control (if used)/<LLQ	5	12	43	88	190	
	6	12	43	97	187	
	7	12	39	86	185	
	Mean	**12**	**42**	**90**	**187**	----
	SD	**0**	**2**	**6**	**3**	
Pooled control	**Mean**	**12**	**43**	**94**	**195**	----
	SD	**0**	**3**	**7**	**14**	
0.313/0.335	9	12	51	96	222	
	10	12	44	97	220	
	11	12	39	85	198	
	Mean	**12**	**45**	**93**	**213**	**-9**
	SD	**0**	**6**	**7**	**13**	
0.625/0.681	13	12	49	100	216	
	14	12	41	93	205	
	15	12	38	90	194	
	Mean	**12**	**43**	**94**	**205**	**-5**
	SD	**0**	**6**	**5**	**11**	
1.25/1.34	17	12	36	69	167	
	18	12	33	65	151	
	19	12	34	66	186	
	Mean	**12**	**34**	**67**	**168**	**14**
	SD	**0**	**2**	**2**	**18**	
2.50/2.81	21	12	26	42	73	
	22	12	27	40	65	
	23	12	24	39	68	
	Mean	**12**	**26**	**40**	**69**	**65**
	SD	**0**	**2**	**2**	**4**	
5.00/5.23	25	12	15	23	30	
	26	12	21	24	30	
	27	12	23	25	30	
	Mean	**12**	**20**	**24**	**30**	**85**
	SD	**0**	**4**	**1**	**0**	
10.0/10.3	29	12	16	20	21	
	30	12	19	20	22	
	31	12	16	18	22	
	Mean	**12**	**17**	**19**	**22**	**89**
	SD	**0**	**2**	**1**	**1**	

1 <LLQ = Less than Lowest Level Quantified; 0.101 µg analyte/L 20X AAM. 2 "----" = Not Applicable. 3 SD = Standard Deviation.

Mean and individual frond dry weight results were presented in the study report. The replicate frond weights and percentage inhibitions based on the pooled control are shown in Table 4.

Table 4. Effect of pyroxsulam on frond dry weight of the freshwater duckweed (*Lemna gibba*) as given in the study report (Hancock *et al.*, 2005).

Treatment (nominal and measured concentration), µg pyroxsulam/L	Replicate No.	Frond dry weight at day 7, mg	% inhibition from the pooled controls
Negative control/<LLQ[1]	1	21.64	
	2	26.25	
	3	22.35	
	Mean	**23.41**	----[2]
	SD[3]	**2.48**	
Solvent control (if used)/<LLQ	5	22.01	
	6	21.96	
	7	21.15	
	Mean	**21.71**	----
	SD	**0.48**	
Pooled control	**Mean**	**22.56**	----
	SD	**1.85**	
0.313/0.335	9	25.37	
	10	25.00	
	11	22.52	
	Mean	**24.30**	-8
	SD	**1.55**	
0.625/0.681	13	22.14	
	14	23.28	
	15	21.93	
	Mean	**22.45**	0
	SD	**0.73**	
1.25/1.34	17	16.95	
	18	16.86	
	19	17.85	
	Mean	**17.22**	24
	SD	**0.55**	
2.50/2.81	21	11.36	
	22	12.01	
	23	12.40	
	Mean	**11.92**	47
	SD	**0.53**	
5.00/5.23	25	7.52	
	26	8.24	
	27	9.00	
	Mean	**8.25**	63
	SD	**0.74**	
10.0/10.3	29	6.96	
	30	7.10	
	31	7.21	
	Mean	**7.09**	69
	SD	**0.13**	

1 <LLQ = Less than Lowest Level Quantified; 0.101 µg analyte/L 20X AAM. 2 "----" = Not Applicable. 3 SD = Standard Deviation

Mean frond dry weights after seven days of exposure were 23.41, 21.71, 22.56, 24.30, 22.45, 17.22, 11.92, 8.25 and 7.09 mg for the media control, solvent control, pooled control, 0.335, 0.681, 1.34, 2.81, 5.23 and 10.3 µg/L test levels, respectively. Response relative to the controls ranged from 8% stimulation to 69% inhibition of frond dry weight. The 7-day calculated EbC50 value (95% confidence interval) for frond dry weight was 3.82 (2.23-6.56) µg/L. Based on the Dunnett's test ($\alpha = 0.05$), the 7-day mean frond dry weight was significantly less than the controls at test levels ≥ 1.34 µg/L; therefore, the 7-day NOEC value for mean frond dry weight was determined to be 0.681 µg/L.

No changes in frond development or appearance (e.g. increase or decrease in size, necrosis, chlorosis, sedimentation of test solutions, sinking of fronds, other abnormalities) were reported.

STATISTICAL ENDPOINT VALUES REPORTED IN THE STUDY REPORT

The study report's statistical findings are summarized in Table 7.

Table 5. 7 Day statistical endpoint values (NOEC, LOEC and EC50 values for duckweed exposed to various pyroxsulam concentrations for 7 days in a static renewal test) as reported by Hancock *et al.*, 2005.

7 day Statistical Endpoint	Frond No.	Mean specific growth rate (per day)	Biomass (frond dry weight)
NOEC (µg pyroxsulam/L)	0.681	0.681	0.681
LOEC (µg pyroxsulam/L)	Not reported	Not reported	Not reported
EC50 (µg pyroxsulam/L) (95% C.I.)	2.57 (1.16-5.70)	3.88 (1.68-8.97)	3.82 (2.23-6.56)
Reference chemical **NOEC** **IC50/EC50**	No reference chemical used.		

Validity of test

OECD 221 (2006) requires that, for the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275/day.

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

$T_d = \ln 2/\mu$

where µ is the average specific growth rate

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

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

where:

- $\mu_{i\text{-}j}$: 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, solvent control and pool controls, the calculated doubling times were as shown in Table 6.

Table 6. Reviewer calculated control doubling time for frond numbers in *Lemna gibba*

Sample	Reported mean specific growth rate, per day	Td (doubling time), days
Control	0.404	1.72
Solvent control	0.393	1.76
Pooled control	0.398	1.74

These control Td values all satisfy the OECD 221 requirement that the Td be <2.5 days. The mean specific growth rates reported in the study report all exceed the OECD 221 requirement that the average specific growth rate be 0.275/day.

Frond number increase over 7 days

OECD 221 also refers to the test being valid if there is an approximately 7-fold increase in frond numbers in seven days. The day 7 mean frond numbers for the control, solvent control and pool controls were, respectively, 203, 187 and 195 fronds. As the initial frond number was 12, the day 7 counts represent 16 to 17 fold increases 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 bulk pyroxsulam concentrations of freshly prepared media on days 0, 3 and 5 (100-115% of nominal, i.e. within ± 20% of nominal concentrations). The bulk data were used as three recoveries in spent exposure and blank solutions on day 7 were less than 80% of nominal. These recoveries appeared, the study report stated, to be spurious artefacts of analytical error when compared to the remainder of the data set which showed pyroxsulam to be stable under test conditions (all other values 89.5-117% of nominal).

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 EC50 value for frond number (the concentration estimated to limit frond growth to 50% of that observed in the control population) was determined by a least squares linear regression of cell density at 7 days against the log of the concentration for test concentrations.

The ErC50 value (the concentration estimated to inhibit the mean specific growth rate to 50% of that observed in the control population) was calculated by regressing the percent reduction in mean specific growth rate for each exposure group compared to the control group against the natural logarithm of the concentrations for the 0 to 7 day exposure period.

The following formula was used to calculate mean specific growth rate:

$$\mu_{i-j} = \frac{\ln N_j - \ln N_i}{t_j - t_i}$$

Where: μ = mean specific growth rate from moment i to j (days-1)
ln = natural logarithm
N_i = initial frond number at time i
N_j = frond number at time j
t_i = the moment time for the start of the period
t_j = the moment time for the end of the period

The EbC50 value (the concentration that inhibited the frond dry weight of this species to 50% of the test population compared to the control population) was calculated by regressing the percent inhibition of biomass, compared to the control, against the natural logarithm of the concentration.

The data were tested for normality using Shapiro-Wilk's Test and for homogeneity of variance using Bartlett's Test. The raw data for the cell density and growth rate endpoints met the assumptions of homogeneity and normality. The log-transformed data for the biomass (dry weight) endpoint also met the assumptions of homogeneity and normality. Based on this, these data were analysed using analysis of variance and Dunnett's test ($\alpha = 0.05$) to determine NOEC values.

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 pyroxsulam exposed algae and the mean of the controls by Dunnett's test. The ToxCalc package was used to determine the EC50 and associated 95% confidence limits by use of maximum likelihood-probit methodology and NOEC values.

Frond counts

The ToxCalc analysis used the untransformed day 3, 5 and 7 frond counts with the means of the dilution and solvent controls frond counts not identified as significantly different ($p = 0.17$) and therefore pooled.

The untransformed data for days 3 and 5 were identified as normally distributed with equal variances. The day 7 frond counts were identified as normally distributed but with equality of variances not being able to be confirmed.

The results of these frond analyses are shown in Table 7 with the ToxCalc results shown on, respectively, pages 43, 43 and 44 of this DER.

Table 7. Reviewer calculated EC50 and NOEC values for *Lemna gibba* frond counts after 3, 5 and 7 days exposure to pyroxsulam with the results based on a pooling of the control and solvent control results. EC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L.

Time	EC50	95% Confidence limits	NOEC	Mean measured concentrations which had statistically significantly lower mean frond counts compare to the mean of the pooled controls
Day 3	5.1	2.5-28	0.68	≥1.34
Day 5	2.7	1.5-4.9	0.68	≥1.34
Day 7	2.4	1.7-3.3	0.68	≥1.34

The only frond number statistics presented in the study report were for the 7 day endpoint.

The study report's 7-day calculated EC50 value (95% confidence interval) for cell density (i.e. frond count) was 2.57 (1.16-5.70) µg pyroxsulam/L, determined by a least squares linear regression of cell density at 7 days against the log of the concentration for test concentrations, i.e. an approach differing from the ToxCalc determination. As shown in Table 7, the reviewer calculated 7 day EC50, 95% confidence limits and NOEC were 2.4, 1.7 to 3.3 and 0.68 µg 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 deviation were recalculated using the day 0 and day 7 frond counts with a time interval of 7 days as per the study report formula:

$$\mu_{i-j} = \frac{\ln N_j - \ln N_i}{t_j - t_i}$$

The recalculated individual replicate values and their associated mean, standard deviations and % inhibition based on the pooled controls were the same as those given in the study report. Specific growth rates for days 3 and 5 were not recalculated and the study report's values for specific growth rates on those days are unverified.

The recalculated specific growth rates and associated mean and standard deviations are shown in Table 8 with the calculated % inhibition. Note that negative inhibition indicates greater growth than controls.

Table 8. Reviewer's recalculation of day 7 specific growth rates and % inhibition using the day 7 frond count results.

Mean measured concentration µg pyroxsulam/L	Day 7 replicate frond count	Specific growth rate (day^{-1})	Mean growth rate (day^{-1})	Standard deviation	% Inhibition from pooled control (one significant figure)
<LLQ1, media (dilution or media control)	186	0.39155	0.40397	0.01149	na (not applicable)
	218	0.41423			
	206	0.40614			
<LLQ, solvent	190	0.39459	0.39256	0.00192	na
	187	0.39231			
	185	0.39078			
Pooled control	na	na	0.39827	0.00966	na
0.335	222	0.41682	0.41095	0.00909	-3%
	220	0.41553			
	198	0.40048			
0.681	216	0.41291	0.40531	0.00767	-2%
	205	0.40544			
	194	0.39756			
1.34	167	0.37616	0.37649	0.01489	+5
	151	0.36177			
	186	0.39155			
2.81	73	0.25794	0.24903	0.00836	+37
	65	0.24135			
	68	0.24780			
5.23	30	0.13090	0.13090	0.00000	+67
	30	0.13090			
	30	0.13090			
10.3	21	0.07995	0.08438	0.00384	+79
	22	0.08659			
	22	0.08659			

Note: The reviewer calculated specific growth rates, standard deviations, and % inhibition were the same as those reported in the study report.

The % inhibition data in Table 8 indicate a dose response was occurring.

The ToxCalc analysis used the log transformed day 7 specific growth rates with the means of the dilution and solvent controls frond counts not identified as significantly different ($p = 0.17$) and therefore pooled. The transformed data were identified as normally distributed but with equality of variances not being able to be confirmed. The ToxCalc calculations for the specific growth rate results are shown in Table 9 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 9.

Table 9. 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 pyroxsulam with the results based on a pooling of the control and solvent control results. EC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L. Equivalent study report values are also shown.

	ErC50	95% Confidence limits	NOEC	Mean measured concentrations which had statistically significantly lower mean specific growth rates compared to the mean of the pooled controls
Reviewer calculated	3.97	2.44-6.52	0.68	≥1.34
Study report	3.88	1.68-8.97	0.68	≥1.34

The study report stated the ErC50 value (the concentration estimated to inhibit the mean specific growth rate to 50% of that observed in the control population) was calculated by regressing the percent reduction in mean specific growth rate for each exposure group compared to the control group against the natural logarithm of the concentrations for the 0- to 7-day exposure period, i.e. an approach differing from the ToxCalc determination.

Verification of biomass (frond dry weight) statistics

The biomass (day 7 frond dry weight) data reported are shown in Table 4 on page 28 of this DER and were analysed by the TidePool Scientific Software program, ToxCalc (v5.0.23A) as previously described.

The ToxCalc analysis used the log transformed day 7 frond dry weight values with the means of the dilution and solvent controls frond dry weights not identified as significantly different (p = 0.30) and therefore pooled. The transformed data were identified as normally distributed with equality of variances confirmed. Untransformed data were indicated as having a non-normal distribution but equal 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 10.

Table 10. Reviewer calculated EbC50 and NOEC values determined from the measured dry frond weight (i.e. biomass as mg) for *Lemna gibba* frond counts after 7 days exposure to pyroxsulam with the results based on a pooling of the control and solvent control results. EC50, 95% confidence limits and NOEC values are as µg 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 pooled controls
Reviewer calculated	3.8	1.9-9.3	0.68	≥1.34
Study report	3.82	2.23-6.56	0.68	≥1.34

The EbC50 value (the concentration that inhibited the frond dry weight of this species to 50% of the test population compared to the control population) was calculated in the study report by regressing the percent inhibition of biomass, compared to the control, against the natural logarithm of the concentration, i.e. an approach differing from that used by ToxCalc. However, the study report's results for biomass are considered equivalent to those determined by the reviewer.

Statistical Method:
The following summarises the results of the statistical verification of the study report's results:

Day 7 frond number

EC50:	2.43 µg pyroxsulam/L	**95% C.I.:**	1.73-3.28 µg pyroxsulam/L
NOEC:	0.68 µg pyroxsulam/L		
Probit Slope:	3.81 (standard error 1.143)	**95% C.I.:**	1.57-6.05

Mean specific growth rate

ErC50:	3.96 µg pyroxsulam/L	**95% C.I.:**	2.44-6.52 µg pyroxsulam/L
NOEC:	0.68 µg pyroxsulam/L		
Probit Slope:	2.64 (standard error 0.879)	**95% C.I.:**	0.92-4.37

Biomass (day 7 frond dry weight)

EbC50:	3.82 µg pyroxsulam/L	**95% C.I.:**	1.93-9.30 µg pyroxsulam/L
NOEC:	0.68 µg pyroxsulam/L		
Probit Slope:	1.80 (standard error 0.646)	**95% C.I.:**	0.53-3.07

These calculated EC50 values classify pyroxsulam as very highly toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment and Water Resources (EC50 <100 µg/L).

D. STUDY DEFICIENCIES:

Table 11 summarises deficiencies and deviations from the OECD 221 and US EPA OPPTS 850.4400 Guidelines

Table 11. Deviation from Guidelines and other deficiencies

Parameter	Study reported results	OECD 221	US EPA OPPTS 850.4400
Acclimation Period:	Axenic samples of the *L. gibba* were received in May of 1999 and a sixteen-day-old subculture was used for the test.	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 AAM.	OECD 221 does not refer to 20X AAM medium.	US EPA OPPTS 850.4400 does not refer to 20X AAM medium.
pH (in the bulk exposure solutions) at days 0, 3 and 5:	On days 0, the initial pH from a sample of bulk medium control was 7.9.	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.
Reference chemical (if used)	No reference chemical mentioned.	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.

The use of a 16 day old subculture for the test exceeded the 7 to 10 days acclimatisation referred to by OECD 221 and the 2 weeks referred to by US EPA OPPTS 850.4400. As there was acceptable growth of the duckweed in the controls, this deviation is not considered to have adversely affected the study's conduct or outcomes.

The medium used, 20X AAM, is not specifically referred to in either OECD 221 or US EPA OPPTS 850.4400 but the reviewer's calculations indicated it is the same as 20X AAP medium described in OECD 221 (see "Recipes" on page 41 of this DER). Consequently, the use of 20X AAM is not considered to have adversely affected the study or its outcomes. Therefore, use of 20X AAM is not considered a deficiency of significance.

The pH of the AAM was stated to have been adjusted to a pH of 7.5 before addition of any test material or alga and, as a result, a pH of close to 7.5 would have been expected in the bulk control medium at day 0. While the reason for the reported pH being 7.9 was not provided in the study report, such occurrence is not considered to have adversely affected the study's conduct or results.

While testing of a reference chemical at the same time as the pyroxsulam exposure study took place is not obligatory, both the OECD and US EPA OPPTS guidelines recommend such testing. Provision of the results from the most recent reference chemical study conducted by the testing laboratory would have added value to the test report. This is assumed to have been an oversight and the absence of results from a reference chemical is taken as a minor deficiency.

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.

F. CONCLUSIONS:

The static renewal exposure of duckweed to mean measured concentrations of 0.335 to 10.3 µg 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 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) using a dilution or medium control and a solvent (dimethylformamide) control. In all three cases, the means of the dilution and solvent controls were not identified as significantly different and were pooled.

The statistical analyses of the data generated indicated that, again for all three growth parameters, the means of concentrations $\geq$1.34 µg pyroxsulam/L were statistically significantly different from the control means and dose effects were apparent. The reviewer's recalculation of statistical endpoints are considered to have been in accord with the values give in the study report with minor differences attributed to the use of different statistical methods.

The NOECs for frond number, specific growth rates and biomass (frond dry weight) were all set at 0.68 mg pyroxsulam by the study report and by the reviewer calculated values.

Analytical concentrations 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 µg pyroxsulam/L with 95% confidence limits shown in brackets:		
	Study report	
Frond number EC50	2.57 (1.16-5.70)	
Mean specific growth rate (day^{-1}) ErC50	3.88 (1.68-8.97)	
Biomass (frond dry weight) EbC50	3.82 (2.23-6.56)	

The EC50 values are considered to classify pyroxsulam as very highly toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment and Water Resources (EC50 <100 µg/L).

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

III. REFERENCES:

American Society for Testing and Materials (1991). Standard Guide for Conducting Static Acute Toxicity Tests with *Lemna gibba*, p3, E 1415-91, ASTM Annual Book of Standards, Vol. 11.04, Philadelphia, Pennsylvania.

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Attachment 1

20X AAM Recipe (Duckweed Medium) and 20X AAP Growth Medium

	Duckweed 20X AAM medium stock and final (medium) solutions as reported by Hancock *et al.* (2005)			**OECD 221 20X AAP growth medium stock and final (medium) solutions**			
Stock solution	**Ingredient**	**Stock concentrations**	**Medium concentrations**	**Stock solution**	**Ingredient**	**Stock concentrations**	**Medium concentrations**
A.	$NaNO_3$ $MgCl_2{\cdot}6H_2O$ $CaCl_2{\cdot}2H_2O$	12.75 g/500 mL 6.08 g/500 mL 2.20 g/500 mL	0.51 g/L 0.24 g/L 0.088 g/L	**A1**	$NaNO_3$ $MgCl_2{\cdot}6H_2O$ $CaCl_2{\cdot}2H_2O$	26 g/L 12 g/L 4.4 g/L	0.52 g/L 0.24 g/L 0.088 g/L
B1.	$MgSO_4{\cdot}7H_2O$	7.35 g/500 mL	0.29 g/L	**A2**	$MgSO_4{\cdot}7H_2O$	15 g/L	0.3 g/L
B2.	$NaHCO_3$	7.5 g/500 mL	0.3 g/L	**C**	$NaHCO_3$	15 g/L	0.3 g/L
B3.	K_2HPO_4	0.522 g/500 mL	0.021g/L	**A3**	K_2HPO_4	1.4 g/L	0.028 g/L
C1.	H_3BO_3 $MnCl_2{\cdot}4H_2O$ $ZnCl_2$ $Na_2MoO_4{\cdot}2H_2O$	1.86 g/L 4.16 g/L 0.0327 g/L 0.0726 g/L	0.0037 g/L 0.0083 g/L 0.065 mg/L 0.145 mg/L	**B** **B** **B** **B**	H_3BO_3 $MnCl_2{\cdot}4H_2O$ $ZnCl_2$ $Na_2MoO_4{\cdot}2H_2O$	0.19 g/L 0.42 g/L 3.3 mg/L 7.3 mg/L	0.0038 g/L 0.0084 g/L 0.066 mg/L 0.146 mg/L
C2.	$CoCl_2{\cdot}6H_2O$ $CuCl_2{\cdot}2H_2O$	2.86 g/L 0.022 g/L	See below under C3.	**B** **B**	$CoCl_2{\cdot}6H_2O$ $CuCl_2{\cdot}2H_2O$	1.4 mg/L 0.012 mg/L	
C3.	2.5 mL of C2 in 500 mL of Sterile Deionised Water		0.0286 mg $CoCl_2{\cdot}6H_2O$ /L 0.00022 mg $CuCl_2{\cdot}2H_2O$ /L				0.028 mg $CoCl_2{\cdot}6H_2O$ /L 0.00024 mg $CuCl_2{\cdot}2H_2O$ /L
D.	$FeCl_3{\cdot}6H_2O$ $Na_2EDTA.2H_20$	0.16 g/L 0.30 g/L	0.0032 g/L 0.006 g/L	**B** **B**	$FeCl_3{\cdot}6H_2O$ $Na_2EDTA.2H_20$	0.16 g/L 0.30 g/L	0.0032 g/L 0.006 g/L

The 20X AAM and 20X AAP media are shown to contain the same ingredients at essentially the same concentrations in the made-up media.

The recipes for making up the 20X AAM and 20X AAP media were given as the following:

The study report stated that stock solutions of the 20X AAM were reported as prepared as follows:

A, B2, B3, B1: Add to 500 mL of sterile deionised water; C1 and C2 add to 1000 mL of sterile deionised H_2O and sterile filter through a 0.22 μm Millipore.
C1 and C3: Make 1:10 dilutions of original stocks with deionised sterile water at the time of medium preparation. Use this dilution as the stock for the preparation that follows.
For duckweed medium add 60 mL per 3 litres of sterile deionised water of each stock solution in the following order: (Swirl jug after each addition)
1. Stock A
2. Stock B2
3. Stock B3
4. Stock B1
5. Stock C1 (the 1:10 Stock C1 to sterile deionised water dilution)
6. Stock C3 (the 1:10 Stock C3 to sterile deionised water dilution)
7. Stock D (Prepare this $FeCl_3$ solution during medium prep. by adding the chemical to sterile deionised water.)

Measure pH immediately after it is made. It should be between 7.5 and 8.5. Store in refrigerator until use. For medium to be used in testing, a final pH adjustment to 7.5 ± 0.1 will be made.

OECD 221 states that the 20X AAP growth medium is prepared as follows:

Stock solutions are prepared in sterile distilled or deionised water.
Sterile stock solutions should be stored under cool and dark conditions. Under these conditions the stock solutions will have a shelf life of at least 6 – 8 weeks. Five nutrient stock solutions (A1, A2, A3, B and C) are prepared for 20X - AAP medium, using reagent grade chemicals. The 20 mL of each nutrient stock solution is added to approximately 850 mL deionised water to produce the growth medium. The pH is adjusted to 7.5 ± 0.1 with either 0.1 or 1 mol HCl or NaOH, and the volume adjusted to one litre with deionised water. The medium is then filtered through a 0.2 μm (approximate) membrane filter into a sterile container.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Frond number at 72 hours (3 days)

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

Conc-ug/L	1	2	3
D-Control	40.000	47.000	47.000
S-Control	43.000	43.000	39.000
0.335	51.000	44.000	39.000
0.681	49.000	41.000	38.000
1.34	36.000	33.000	34.000
2.81	26.000	27.000	24.000
5.23	15.000	21.000	23.000
10.3	16.000	19.000	16.000

			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	43.167	1.0000	43.167	39.000	47.000	7.810	6				43.167	0.0000
0.335	44.667	1.0347	44.667	39.000	51.000	13.495	3	-0.560	2.490	6.673	44.667	-0.0347
0.681	42.667	0.9884	42.667	38.000	49.000	13.327	3	0.187	2.490	6.673	42.667	0.0116
*1.34	34.333	0.7954	34.333	33.000	36.000	4.449	3	3.296	2.490	6.673	34.333	0.2046
*2.81	25.667	0.5946	25.667	24.000	27.000	5.951	3	6.530	2.490	6.673	25.667	0.4054
*5.23	19.667	0.4556	19.667	15.000	23.000	21.169	3	8.769	2.490	6.673	19.667	0.5444
*10.3	17.000	0.3938	17.000	16.000	19.000	10.189	3	9.764	2.490	6.673	17.000	0.6062

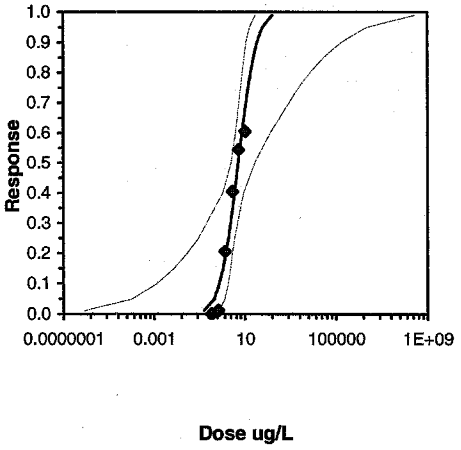
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96678	0.884	0.23571	-0.3259
Bartlett's Test indicates equal variances (p = 0.36)	6.56961	16.8119		
The control means are not significantly different (p = 0.33)	1.11631	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.681	1.34	0.95527		6.67272	0.15458	460.299	14.3627	2.3E-08	6, 17
Treatments vs Pooled Controls										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.541402	0.622036	0.322211	2.76059	0	0.87029	9.48773	0.93	0.71001	0.64876	6
Intercept	3.905583	0.423974	3.074595	4.73657							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	0.158771	8.52E-07	0.70274
EC05	3.355	0.439439	0.000107	1.28747
EC10	3.718	0.756126	0.001382	1.81023
EC15	3.964	1.090479	0.007637	2.31696
EC20	4.158	1.458826	0.029127	2.87568
EC25	4.326	1.872543	0.089358	3.55756
EC40	4.747	3.512791	1.047423	8.74499
EC50	5.000	5.128777	2.480256	27.8891
EC60	5.253	7.488163	3.951156	132.208
EC75	5.674	14.04739	6.53683	2302.55
EC80	5.842	18.03118	7.739136	7381.2
EC85	6.036	24.12184	9.333435	28970.6
EC90	6.282	34.7883	11.70917	163306
EC95	6.645	59.85894	16.21361	2141804
EC99	7.326	165.6745	29.34827	2.7E+08



EC50 values etc. are reported as μg pyroxsulam/L.

The 1.34 to 10.3 μg/L means for frond numbers at 72 hours (3 days) were identified as statistically significantly less than the control mean at that time (Dunnett's test). The study report did not report on whether the 72 hour frond counts means were statistically significantly reduced compared to the control.

Frond number at 120 hours (5 days)

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

Conc-ug/L	1	2	3
D-Control	91.000	104.000	96.000
S-Control	88.000	97.000	86.000
0.335	96.000	97.000	85.000
0.681	100.000	93.000	90.000
1.34	69.000	65.000	66.000
2.81	42.000	40.000	39.000
5.23	23.000	24.000	25.000
10.3	20.000	20.000	18.000

			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	93.667	1.0000	93.667	86.000	104.000	7.103	6				93.667	0.0000
0.335	92.667	0.9893	92.667	85.000	97.000	7.185	3	0.299	2.490	8.332	92.667	0.0107
0.681	94.333	1.0071	94.333	90.000	100.000	5.440	3	-0.199	2.490	8.332	94.333	-0.0071
*1.34	66.667	0.7117	66.667	65.000	69.000	3.122	3	8.069	2.490	8.332	66.667	0.2883
*2.81	40.333	0.4306	40.333	39.000	42.000	3.787	3	15.939	2.490	8.332	40.333	0.5694
*5.23	24.000	0.2562	24.000	23.000	25.000	4.167	3	20.821	2.490	8.332	24.000	0.7438
*10.3	19.333	0.2064	19.333	18.000	20.000	5.973	3	22.215	2.490	8.332	19.333	0.7936

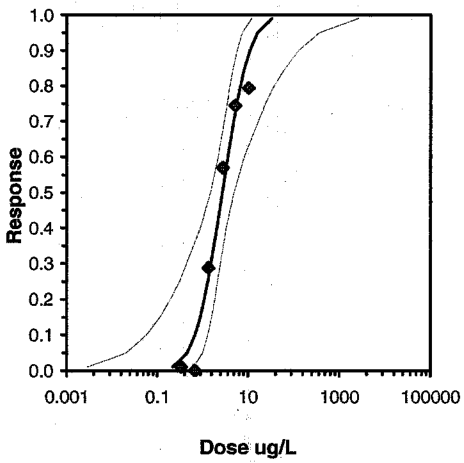
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96595	0.884	0.19426	0.98914
Bartlett's Test indicates equal variances (p = 0.05)	12.4044	16.8119		
The control means are not significantly different (p = 0.26)	1.31306	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.681	1.34	0.95527		8.33167	0.08895	3822.19	22.3922	3.1E-14	6, 17
Treatments vs Pooled Controls										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.139586	0.688454	0.790216	3.48896	0	1.22797	9.48773	0.87	0.43368	0.46738	4
Intercept	4.072099	0.369839	3.347214	4.79698							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	0.222021	0.00289	0.62474
EC05	3.355	0.462286	0.020506	1.00567
EC10	3.718	0.683456	0.057677	1.30982
EC15	3.964	0.889763	0.114915	1.57866
EC20	4.158	1.0973	0.197085	1.84662
EC25	4.326	1.313528	0.310071	2.13292
EC40	4.747	2.066679	0.893607	3.33382
EC50	5.000	2.714454	1.499913	4.91182
EC60	5.253	3.565265	2.210063	8.24371
EC75	5.674	5.609517	3.454654	23.7562
EC80	5.842	6.714898	3.990311	37.3747
EC85	6.036	8.281152	4.667672	64.0991
EC90	6.282	10.78087	5.625736	127.708
EC95	6.645	15.93875	7.327172	359.205
EC99	7.326	33.18724	11.79501	2548.74



EC50 values etc. are reported as µg pyroxsulam/L.

The 1.34 to 10.3 µg/L means for frond numbers at 120 hours (5 days) were identified as statistically significantly less than the control mean at that time (Dunnett's test). The study report did not report on whether the 72 hour frond counts means were statistically significantly reduced compared to the control.

Frond number at 168 hours (7 days)

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

```
  Conc-ug/L      1        2        3
  D-Control   186.00   218.00   206.00
  S-Control   190.00   187.00   185.00
      0.335   222.00   220.00   198.00
      0.681   216.00   205.00   194.00
       1.34   167.00   151.00   186.00
       2.81    73.00    65.00    68.00
       5.23    30.00    30.00    30.00
       10.3    21.00    22.00    22.00
                                  Transform: Untransformed                       1-Tailed
  Conc-ug/L    Mean   N-Mean    Mean     Min      Max     CV%    N     t-Stat  Critical    MSD     Mean   N-Mean
     Pooled  195.33   1.0000  195.33  185.00   218.00   6.942    6                               195.33   0.0000
      0.335  213.33   1.0922  213.33  198.00   222.00   6.242    3     -2.257     2.490   19.86   213.33  -0.0922
      0.681  205.00   1.0495  205.00  194.00   216.00   5.366    3     -1.212     2.490   19.86   205.00  -0.0495
      *1.34  168.00   0.8601  168.00  151.00   186.00  10.429    3      3.427     2.490   19.86   168.00   0.1399
      *2.81   68.67   0.3515   68.67   65.00    73.00   5.886    3     15.880     2.490   19.86    68.67   0.6485
      *5.23   30.00   0.1536   30.00   30.00    30.00   0.000    3     20.727     2.490   19.86    30.00   0.8464
      *10.3   21.67   0.1109   21.67   21.00    22.00   2.665    3     21.772     2.490   19.86    21.67   0.8891
Auxiliary Tests                                            Statistic           Critical            Skew     Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)  0.95926            0.884           0.44218  0.28428
Equality of variance cannot be confirmed
The control means are not significantly different (p = 0.17)  1.69388          2.77645
Hypothesis Test (1-tail, 0.05)  NOEC    LOEC    ChV      TU     MSDu     MSDp      MSB      MSE    F-Prob     df
Dunnett's Test                  0.681   1.34   0.95527         19.8619  0.10168  23818.7  127.255  1.4E-14   6, 17
Treatments vs Pooled Controls
                                            Maximum Likelihood-Probit
Parameter      Value       SE     95% Fiducial Limits     Control   Chi-Sq  Critical  P-value     Mu     Sigma    Iter
Slope        3.80806  1.14335    1.5671  6.04902            0    2.53479  9.48773    0.64   0.38499  0.2626     6
Intercept    3.53393  0.50721    2.5398  4.52806
TSCR
Point     Probits     ug/L    95% Fiducial Limits
EC01        2.674  0.59441    0.0727  1.05438
EC05        3.355  0.89753   0.19497  1.38703
EC10        3.718  1.11803   0.32807  1.61426
EC15        3.964  1.29665   0.46407  1.79594
EC20        4.158  1.45875   0.60883  1.96292
EC25        4.326  1.61387   0.76502  2.12817
EC40        4.747  2.08191   1.31131  2.70603
EC50        5.000  2.42656   1.72655  3.28403
EC60        5.253  2.82826   2.14904  4.21591
EC75        5.674  3.64848   2.79618  7.06208
EC80        5.842  4.03646   3.04447   8.8362
EC85        6.036  4.54107   3.33826  11.5552
EC90        6.282  5.26656   3.72334  16.3043
EC95        6.645   6.5604   4.34244  27.3773
EC99        7.326  9.90584   5.72264  73.2937
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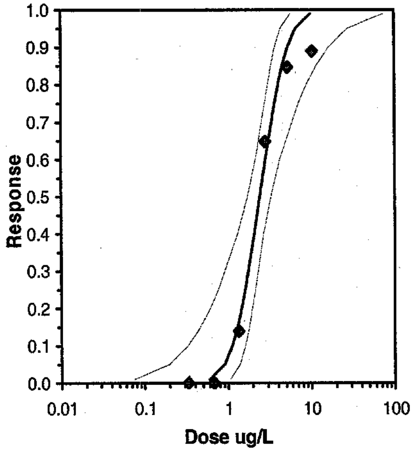


Note that equality of variance could not be achieve by arcsine square root, reciprocal or log transformations. EC50 values etc. are reported as µg pyroxsulam/L.

The 1.34 to 10.3 µg/L means for frond numbers at 72 hours (3 days) were identified as statistically significantly less than the control mean at that time (Dunnett's test). The study report similarly identified the means determined for these concentrations as statistically significantly reduced compared to the control (Dunnett's test).

Specific growth rate at 168 hours (7 days)

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

Conc-ug/L	1	2	3
D-Control	0.3915	0.4142	0.4061
S-Control	0.3946	0.3923	0.3908
0.335	0.4168	0.4155	0.4005
0.681	0.4129	0.4054	0.3976
1.34	0.3762	0.3618	0.3915
2.81	0.2579	0.2414	0.2478
5.23	0.1309	0.1309	0.1309
10.3	0.0799	0.0866	0.0866

			Transform: Log						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	0.3983	1.0000	-0.3999	-0.4081	-0.3828	-2.610	6				0.3983	0.0000
0.335	0.4109	1.0318	-0.3863	-0.3974	-0.3800	-2.502	3	-1.536	2.490	0.0221	0.4109	-0.0318
0.681	0.4053	1.0177	-0.3923	-0.4006	-0.3841	-2.097	3	-0.863	2.490	0.0221	0.4053	-0.0177
*1.34	0.3765	0.9453	-0.4245	-0.4416	-0.4072	-4.047	3	2.763	2.490	0.0221	0.3765	0.0547
*2.81	0.2490	0.6253	-0.6039	-0.6173	-0.5885	-2.406	3	22.969	2.490	0.0221	0.2490	0.3747
*5.23	0.1309	0.3287	-0.8831	-0.8831	-0.8831	0.000	3	54.404	2.490	0.0221	0.1309	0.6713
*10.3	0.0844	0.2119	-1.0741	-1.0972	-1.0625	-1.864	3	75.914	2.490	0.0221	0.0844	0.7881

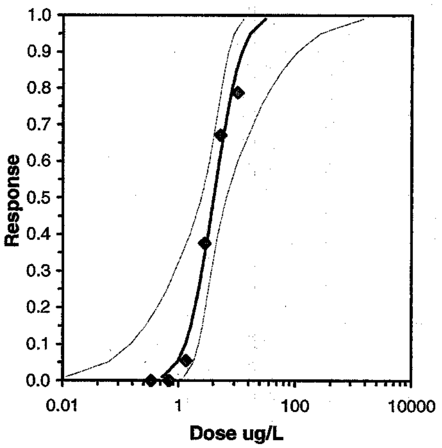
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97379	0.884	-0.1697	-0.4731
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.17)	1.69657	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.681	1.34	0.95527		0.01977	0.04964	0.24881	0.00016	2.2E-22	6, 17
Treatments vs Pooled Controls										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.64385	0.87946	0.9201	4.3676	0	0.80963	9.48773	0.94	0.59833	0.37824	5
Intercept	3.41811	0.56709	2.30663	4.5296							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	0.5229	0.0113	1.222
EC05	3.355	0.94664	0.06094	1.78723
EC10	3.718	1.29897	0.14833	2.20725
EC15	3.964	1.60809	0.26849	2.56259
EC20	4.158	1.90544	0.42729	2.90548
EC25	4.326	2.20399	0.63144	3.26224
EC40	4.747	3.18055	1.57097	4.69702
EC50	5.000	3.96577	2.43941	6.51717
EC60	5.253	4.94484	3.35936	10.1962
EC75	5.674	7.13581	4.80882	25.515
EC80	5.842	8.25389	5.39428	37.7409
EC85	6.036	9.78011	6.11197	60.1033
EC90	6.282	12.1076	7.0923	108.847
EC95	6.645	16.6139	8.75544	265.069
EC99	7.326	30.0771	12.8008	1429.25



Note that equality of variance could not be achieved by use of untransformed data or by arcsine square root, reciprocal or log transformations. EC50 values etc. are reported as µg pyroxsulam/L.

The 1.34 to 10.3 µg/L means for specific growth rate after 7 days were identified as statistically significantly less than the control mean at that time (Dunnett's test). The study report similarly identified the means determined for these concentrations as statistically significantly reduced compared to the control mean (Dunnett's test).

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-ug/L	1	2	3
D-Control	21.640	26.250	22.350
S-Control	22.010	21.960	21.150
0.335	25.370	25.000	22.520
0.681	22.140	23.280	21.930
1.34	16.950	16.860	17.850
2.81	11.360	12.010	12.400
5.23	7.520	8.240	9.000
10.3	6.960	7.100	7.210

			Transform: Log						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	22.560	1.0000	1.3522	1.3253	1.4191	2.497	6				22.560	0.0000
0.335	24.297	1.0770	1.3849	1.3526	1.4043	2.037	3	-1.745	2.490	0.0467	24.297	-0.0770
0.681	22.450	0.9951	1.3511	1.3410	1.3670	1.032	3	0.061	2.490	0.0467	22.450	0.0049
*1.34	17.220	0.7633	1.2359	1.2269	1.2516	1.108	3	6.201	2.490	0.0467	17.220	0.2367
*2.81	11.923	0.5285	1.0761	1.0554	1.0934	1.789	3	14.720	2.490	0.0467	11.923	0.4715
*5.23	8.253	0.3658	0.9155	0.8762	0.9542	4.262	3	23.285	2.490	0.0467	8.253	0.6342
*10.3	7.090	0.3143	0.8506	0.8426	0.8579	0.903	3	26.743	2.490	0.0467	7.090	0.6857

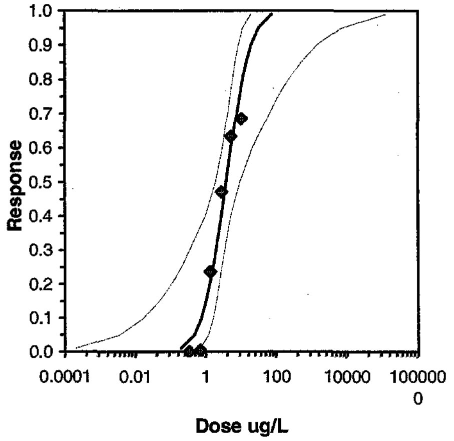
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93718	0.884	0.99442	2.20556
Bartlett's Test indicates equal variances (p = 0.40)	6.17492	16.8119		
The control means are not significantly different (p = 0.30)	1.18194	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.681	1.34	0.95527		2.29416	0.10196	0.1611	0.0007	2.7E-15	6, 17
Treatments vs Pooled Controls										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.79904	0.64654	0.53182	3.06626	0	1.42762	9.48773	0.84	0.58192	0.55585	6
Intercept	3.9531	0.40938	3.15072	4.75548							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	0.19445	0.00019	0.68552
EC05	3.355	0.46519	0.00358	1.17967
EC10	3.718	0.74057	0.01674	1.59688
EC15	3.964	1.01349	0.04688	1.98158
EC20	4.158	1.30048	0.10495	2.38157
EC25	4.326	1.61067	0.20637	2.83113
EC40	4.747	2.7612	0.97231	5.1054
EC50	5.000	3.81876	1.93336	9.30089
EC60	5.253	5.28136	3.03398	21.4698
EC75	5.674	9.05397	4.94533	111.914
EC80	5.842	11.2135	5.79544	223.235
EC85	6.036	14.3889	6.89599	504.777
EC90	6.282	19.6914	8.49469	1423.62
EC95	6.645	31.3486	11.4321	6699.32
EC99	7.326	74.9945	19.5795	124753



Note that untransformed data had a non-normal distribution (Shapiro-Wilk's Test indicated a non-normal distribution (p <= 0.01)). A log transformation resulted in normality of distribution and equal variances being achieved.

The 1.34 to 10.3 μg/L means for frond dry weight after 7 days were *identified as statistically* significantly less than the control mean at that time (Dunnett's test). The study report similarly identified the means determined for these concentrations as statistically significantly reduced compared to the control mean (Dunnett's test).