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TEXT SEARCHABLE DOCUMENT

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

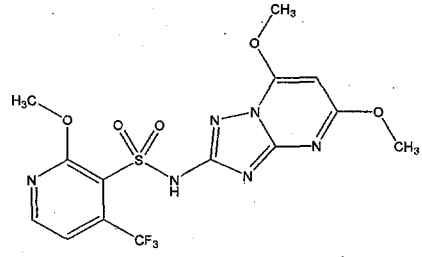
PMRA Submission Number 2006-4727; 1283263 EPA MRID Number 469084-44 APVMA ATS 40362

[DM1][DM2]

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:	X666742, XR-742	
Test Substance Number:	TSN103826	

Chemical structure:



Primary Reviewer: Daryl Murphy **Date:** 14 June 2007 22/02/08
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland **Date:** 14 June 2007 22/2/08
Australian Government Department of the Environment, Water, Heritage and the Arts

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

US EPA Reviewer: Christopher Salice **Date:** 24 July 2007 4/10/08
Environmental Fate and Effects Division, US Environmental Protection Agency

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

CITATION: Hancock, G. A. Sushynski, J. M. and Najar, J. R. 2005. Inhibition of Growth of the Aquatic Plant Duckweed, *Lemma Gibba*, Following One and Three Day Exposures to XDE-742. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID: 051169. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 28 November 2005. Unpublished report.

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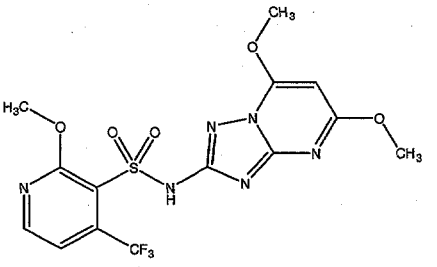

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EXECUTIVE SUMMARY:

In a 7 day acute toxicity study, freshwater floating aquatic vascular plants (duckweed, *Lemna gibba*) were exposed to pyroxsulam at nominal concentrations of 0 (medium and solvent controls), 1.00, 2.00, 4.00, 8.00, 16.00 and 32.0 µg pyroxsulam/L (and as initial measured concentrations, 0 and 0 (controls), 1.06, 2.21, 4.28, 15.9 and 31.2 µg pyroxsulam/L) under static conditions (without renewal) for one or three days followed by renewal with untreated medium for, respectively, six and four days for a total of 7 days growth in both situations. Growth medium was Modified (20X) Algal Assay Medium (AAM).

With the exception of the duration of exposure, the study generally conformed to procedures described by the OECD and US EPA (namely, OECD 221 "Lemna sp. Growth Inhibition Test" (draft, 2002) and Ecological Effects Test Guidelines. OPPTS 850.4400 Aquatic Plant Toxicity Test using Lemna sp., Tiers I and II. Draft April 1996)

The percentage growth inhibition was determined for frond number, mean specific growth rate and biomass (frond dry weight). For the frond count with one day's exposure, response relative to the solvent controls ranged from 10% to 45% inhibition of mean frond density. For the three day exposure, response relative to the pooled controls ranged from 15% to 79% inhibition of mean frond density.

Response relative to the solvent controls ranged from 4% to 23% inhibition of mean specific growth rate for the one day's exposure. For the three days' exposure, response relative to the pooled controls ranged from 6% to 55% inhibition of mean specific growth rate.

With biomass (as frond dry weight), response relative to the pooled controls ranged from 5% to 35% inhibition for the exposure of one day and, for the three day exposure, response relative to the pooled controls ranged from 17% to 67% inhibition of frond dry weight.

The 7 day NOECs based on frond number were, respectively, 1.06 and <1.06 µg pyroxsulam/L for the 1 and 3 day exposures respectively. The specific growth rate NOECs were, again respectively, 2.21 and 1.06 µg pyroxsulam/L while the equivalent biomass (frond dry weight) NOECs were 1.06 and <1.06 µg pyroxsulam/L.

The EC50 for frond numbers was >31.2 µg pyroxsulam/L, with 95% confidence intervals not calculable for a one day exposure period and 4.68 µg pyroxsulam/L with 95% confidence limits of 1.85 and 11.8 µg pyroxsulam/L for the three day exposure. The ErC50 (mean specific growth rate) was >31.2 µg pyroxsulam/L, with 95% confidence limits not calculable for the one day exposure and 17.2 µg pyroxsulam/L with 95% confidence limits of 8.31 and 35.4 µg pyroxsulam/L for the three days of exposure. The EbC50 (biomass, frond dry weight) was >31.2 µg pyroxsulam/L with 95% confidence limits undeterminable for the one day of exposure and 7.45 µg pyroxsulam/L, 95% confidence limits of 3.06 and 18.16 µg pyroxsulam/L, for the three days of exposure.

No abnormal observations were noted on duckweed fronds in the group of replicates that was exposed to pyroxsulam for one day followed by a six-day growth period in untreated medium at any observation period. For the fronds that were exposed to pyroxsulam for three days followed by a four-day growth period in untreated medium, duckweed fronds that were visually smaller than normal were noted in test levels ≥ 2.21 µg/L. Some of the fronds were noted as smaller than normal in the 2.21, 4.28, and 8.64 µg/L test levels on days 5 and 7. All fronds in the 15.9 and 31.2 µg/L test levels were noted as smaller than normal on days 5 and 7. The observation of smaller than normal fronds is consistent with the frond dry weight measurements.

The study was considered to meet the validity criteria set forth in the OECD Guideline 221 with respect to the validity criteria However, the significant deviation from these guidelines with respect the required exposure period of 7days results in the study being classed as invalid by the Australian Government Department of the

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Environment, Water, Heritage and the Arts with respect to compliance with the relevant OECD and US EPA OPPTS guidelines and the study's endpoints would not be used by the Australian Government Department of the Environment, Water, Heritage and the Arts in its aquatic risk assessment.

The US EPA stated that, because this study used exposure durations less than seven days, the guideline requirement for an acute toxicity study on *Lemna gibba* for pyroxsulam was not met. Significant differences between the medium and solvent control for the one-day exposure study compromises these results and therefore is classified as invalid. The three-day exposure study is scientifically sound and although the exposure duration does not adhere to guideline requirements, the study may be useful for risk assessment purposes and is classified as supplemental (three-day component only).

The PMRA does not have the same acceptability classification scheme as the Australian Government Department of the Environment, Water, Heritage and the Arts and the US EPA. Recognizing that results of the study could help determine whether pyroxsulam is phytocidal or phytostatic, the short exposure periods tested do not represent realistic environmental exposures. The study is of limited value to the PMRA, hence results would not be used in an aquatic risk assessment.

Results Synopsis

Test Organism:	Duckweed (*Lemna gibba*)
Test Type:	Static with one or three days of exposure to the test substance.

Day 7 frond number

One day of exposure to pyroxsulam followed by renewal with untreated medium for six days			
EC05	**0.44 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	0.55 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.21 to 2.1 µg pyroxsulam/L**
EC50:	**37.3 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	>31.2 (Linear interpolation)		**Not calculated**
NOEC:	**1.06 µg pyroxsulam/L**		
Probit Slope:	**0.85 (standard error 1.143) (Maximum likelihood probit only)**	95% C.I.:	**-0.154 to 1.86**
Three days of exposure to pyroxsulam followed by renewal with untreated medium for four days			
EC05	**0.26 µg pyroxsulam/L (Maximum likelihood probit)**		**1.9E-05 to 1.06 µg pyroxsulam/L**
	0.35 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.15 to 0.81 µg pyroxsulam/L**
EC50:	**4.4 µg pyroxsulam/L (Maximum likelihood probit)**		**1.17 to 11.7 µg pyroxsulam/L**
	3.7 µg pyroxsulam/L (Linear interpolation)		**3.0 to 4.2 µg pyroxsulam/L**
NOEC:	**<1.06 µg pyroxsulam/L**		
Probit Slope:	**1.35 (standard error 0.524) (Maximum likelihood probit only)**	95% C.I.:	**0.32 to 2.34**

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Specific growth rate over 7 days

One day of exposure to pyroxsulam followed by renewal with untreated medium for six days			
EC05	**1.52 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	2.28 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.00 to 3.62 µg pyroxsulam/L**
ErC50:	**319 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	>31.3 (Linear interpolation)		**Not calculated**
NOEC:	**2.21 µg pyroxsulam/L**		
Probit Slope:	**0.71 (standard error 0.556) (Maximum likelihood probit only)**	95% C.I.:	**-0.382 to 1.8**
Three days of exposure to pyroxsulam followed by renewal with untreated medium for four days			
EC05	**0.33 µg pyroxsulam/L (Maximum likelihood logit)**		**0.0051 to 1.30 µg pyroxsulam/L**
	0.92 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.367 to 1.68 µg pyroxsulam/L**
ErC50:	**17 µg pyroxsulam/L (Maximum likelihood logit)**		**9.6 to 57 µg pyroxsulam/L**
	15.7 µg pyroxsulam/L (Linear interpolation)		**9.4 to 32 µg pyroxsulam/L**
NOEC:	**1.06 µg pyroxsulam/L**		
Probit Slope:	**1.72 (standard error 0.536) (Maximum likelihood logit only)**	95% C.I.:	**0.0015 to 1.30**

Biomass (frond dry weight) over 7 days

One day of exposure to pyroxsulam followed by renewal with untreated medium for six days			
EC05	**0.366 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	0.996 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.029 to 2.32 µg pyroxsulam/L**
EbC50:	**98.2 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	>31.3 µg pyroxsulam/L (Linear interpolation)		**Not calculated**
NOEC:	**1.06 µg pyroxsulam/L**		
Probit Slope:	**0.68 (standard error 0.501) (Maximum likelihood probit only)**	95% C.I.:	**-0.304 to 1.7**
Three days of exposure to pyroxsulam followed by renewal with untreated medium for four days			
EC05	**0.099 µg pyroxsulam/L (Maximum likelihood logit)**	95% C.I.:	**0.00012 to 0.527 µg pyroxsulam/L**
	0.317 µg pyroxsulam/L (Linear interpolation)		**0.188 to 0.518 µg pyroxsulam/L**
EbC50:	**7.3 µg pyroxsulam/L**		**3.5 to 17.4 µg pyroxsulam/L**

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	(Maximum likelihood logit)		
	5.7 µg pyroxsulam/L (Linear interpolation)		**4.1 to 7.4 µg pyroxsulam/L**
NOEC:	**<1.06 µg pyroxsulam/L**		
Probit Slope:	**1.57 (standard error 0.492) (Maximum likelihood logit only)**	95% C.I.:	**0.609 to 2.54**

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, Water, Heritage and the Arts (EC50 <100 µg/L).

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
With the exception of the duration of exposure, the study generally conformed to procedures described by the Organisation for Economic Cooperation and Development (OECD), namely

- 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 (2006) 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.

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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:

During the study's 1 and 3 day exposure phases, the mean measured concentrations of pyroxsulam in the bulk dose solutions (1.0 to 32.0 µg pyroxsulam/L) ranged from 97.5 to 111% of target (nominal) concentrations, indicative of the pyroxsulam's being stable during the exposure.

In the spent test solutions analysed on days 1 and 3, the measured concentrations respectively ranged from 96.4 to 107 and 97.8 to 102% of nominal. These results indicate that nominal concentrations were maintained over the 1 and 3 days of exposure. The study report also stated that results from the analysis of the DMF-based dose stock solutions ranged from 83.5 to 91.6% of target (with the data not presented in the study report).

Actual concentrations are shown on page 17 of this DER.

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 given in the study report and the values recorded in the company's study profile template report (Dow Chemical Company study ID: 051169.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 1999 from USDA/ARS Beltsville Agricultural Research Center, Beltsville, Maryland.
Age of inoculum:	Fronds came from a 20 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 study report stated that a standard 7-day guideline study exposing *Lemna gibba* to pyroxsulam had determined an ErC50 (plus confidence interval) for growth rate of 3.88 (1.68-8.97) µg pyroxsulam/L and an EbC50 (plus confidence interval) for biomass (dry weight) of 3.82 (2.23-6.56) µg pyroxsulam/L (Hancock *et al.*, 2005). The test concentrations for the current study were set based on the results of the standard exposure study while also considering that the reduced exposure periods of one and three days may reduce the effect of the test material on the test organism. Therefore, target concentrations were set at 0 (medium and solvent controls), 1.00, 2.00, 4.00, 8.00, 16.0, and 32.0 µg pyroxsulam/L.

(b) Definitive Study

The definitive static exposure test was conducted between 23 August and 30 August 2005. The experimental design was modified from the standard guideline test to incorporate exposure periods of one and three days followed by growth periods in untreated medium of six and four days, respectively with no renewal of the exposure solutions. The total duration of the in-life phase was seven days in both exposure scenarios as in the standard OECD test.

The purpose of the study was to assess the effects of pyroxsulam on the growth of the aquatic plant duckweed, *Lemna gibba* L. G-3. The reasoning was that exposure periods of less than 7 days (i.e. the duration of the standard duckweed test according to OECD Guideline No.221) can occur in the environment due to run-off/drainage incidents. Consequently, the purpose of the study was to assess the inhibition of growth of the aquatic plant, duckweed, *Lemna gibba* L. G-3, following exposure to the herbicide active ingredient pyroxsulam for one and three-day exposure periods and subsequent six and four day growth periods respectively, in untreated medium.

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.

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Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period:	Axenic samples of the *L. gibba* were received in May of 1999 and a twenty-day-old subculture was used for the test.	See deviations/deficiency table on page 43 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 used in a test should be randomly selected from the culturing tank. Inocula should be taken from cultures which are less than 2 weeks old.
Culturing media and conditions: (same as test or not)	Stock cultures of the test organism were maintained axenically by weekly transfer into fresh medium. Typical culturing conditions were described as: **Conditions: Culture:** **Temperature (°C): 25 ± 2°C** **Light (lux): 5400 ± 1100** **Photoperiod: Continuous** **Medium: Modified (20X) AAM** **pH: ~7.5 to 8.5** **Aseptic Conditions: Axenic** **Culture Vessel: 500 mL Erlenmeyer flask** **Inoculation: Every seven days** **Culture Chamber: Environmental chamber**	Requirement considered met. Typical test conditions were described as: **Conditions: Test:** **Temperature (°C): 25 ± 2°C** **Light (lux): 6600 ± 990** **Photoperiod: Continuous** **Medium: Modified (20X) AAM** **pH: Adjusted to 7.5 prior to addition of test material.** **Aseptic Conditions: Axenic** **Culture Vessel: 270 mL borosilicate crystallizing dish with cover.** **Inoculation: Single** **Culture Environmental growth**

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Parameter	Details	Remarks *Criteria*
	Amount of Transfer: **Approximately five plants (15 fronds, three fronds/ plant)** Comparison of these culture conditions with the test parameters shown in the adjacent "Remarks" indicates that test conditions can be considered the same as the culture conditions.	**Chamber:** **chamber** **Amount of Transfer:** **Three plants, four fronds per plant.**
Health: (any toxicity observed)	No specific comment found in the test report but the stock cultures used were maintained axenically by weekly transfer into fresh medium.	Requirement considered met. OECD 221 recommends the 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 healthy duckweed. No phytotoxicity effects noted (Hancock, 2005). No specific requirements were identified in US EPA OPPTS 850.4400
Test system Static/static renewal	Static system used with no renewal of test solutions.	Requirements considered met. Static tests are acceptable according to OECD 211. US EPA OPPTS 850.4400 implies static tests are acceptable.
Renewal rate for static renewal:	Renewal of the test media did not take place. The study was designed to have static exposures of one and three days followed by renewal with untreated medium for six- and four-day growth periods, respectively (total duration of 7 days).	See deviations/deficiency table on page 43 of this report. OECD 221 allows for testing with and without renewal but requires a 7 day exposure to the test substance. US EPA OPPTS 850.4400 allows for static or static renewal tests with a 7 or 14 day exposure period. *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).*

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Parameter	Details	Remarks *Criteria*
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 with respectively 1 or 3 days exposure to the pyroxsulam containing solutions.	See deviations/deficiency table on page 43 of this report. The test specifically deviated from the OECD 221 and US EPA OPPTS 850.4400 which specify a 7 day exposure period. *EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel Material: (glass/polystyrene)	 Borosilicate crystallizing dish with cover	Requirement considered met. OECD 221 states glass beakers, crystallising dishes or glass Petri dishes of appropriate dimensions have all proved suitable. This guideline also states the test vessels must be covered and that crystallizing dishes are appropriate test vessels. US EPA OPPTS 850.4400 states test containers may be glass beakers or Erlenmeyer flasks.
Size:	270 mL	A minimum depth of 20 mm and minimum volume of 100 mL in each test vessel is advised by OECD 221. US EPA 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

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Parameter	Details	Remarks *Criteria*
Fill volume:	100 mL	OECD 221 advises there be a minimum fill volume of 100 mL while US EPA OPPTS 850.4400, as stated above, refers to vessels large enough to contain 150 mL of test solution or enough test solution to result in a volume to-vessel size ratio of 2:5.
Details of growth medium Name:	Modified 20X 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 49 of this DER.	See deviations/deficiency table on page 43 of this report. Hancock (2005) states that the study report refers to the 20X AAP medium as 20X AAM. 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 does state 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.*
pH (in the bulk exposure solutions and spent solutions) at days 0, 1, 3 and 7 (i.e. at test initiation, during and at the end of the test):	In the bulk media control, the pH values reported for days 0, 3 and 7 were summarised as: pH values (days 0, 1, 3, and 7): Minimum Maximum	See deviations/deficiency table on page 43 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

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<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td>
Bulk test solutions, range 7.5 7.8
Spent test solutions, range 8.4 8.9

The pH values recorded at specific times and in specific test solutions were:
<table>
<tr><th></th><th>Day 0</th><th>Day 1</th><th>Day 3</th><th>Day 7</th></tr>
<tr><td colspan="5">Media control#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td>-**</td></tr>
<tr><td>1, 2, 3*</td><td>-</td><td>8.4</td><td>-</td><td>8.9</td></tr>
<tr><td>4, 5, 6*</td><td>-</td><td>-</td><td>8.5</td><td>8.9</td></tr>
<tr><td colspan="5">Solvent (DMF) control#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td></td></tr>
<tr><td>7, 8, 9*</td><td>-</td><td>8.4</td><td>-</td><td>8.9</td></tr>
<tr><td>10, 11, 12*</td><td>-</td><td>-</td><td>8.6</td><td>8.9</td></tr>
<tr><td colspan="5">1.00/1.06#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td>-</td></tr>
<tr><td>13, 14, 15*</td><td>-</td><td>-</td><td>8.4</td><td>8.8</td></tr>
<tr><td>16, 17, 18*</td><td>-</td><td></td><td></td><td>8.9</td></tr>
<tr><td colspan="5">2.00/2.21#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td>-</td></tr>
<tr><td>19, 20, 21*</td><td>-</td><td>8.5</td><td>-</td><td>8.8</td></tr>
<tr><td>22, 23, 24*</td><td>-</td><td>-</td><td>8.6</td><td>8.7</td></tr>
<tr><td colspan="5">4.00/4.28#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td>-</td></tr>
<tr><td>25, 26, 27*</td><td>-</td><td>8.4</td><td>-</td><td>8.8</td></tr>
<tr><td>28, 29, 30*</td><td>-</td><td>-</td><td>8.6</td><td>8.6</td></tr>
<tr><td colspan="5">8.00/8.64#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td>-</td></tr>
<tr><td>31, 32, 33*</td><td>-</td><td>8.5</td><td>-</td><td>8.6</td></tr>
<tr><td>34, 35, 36*</td><td>-</td><td>-</td><td>8.6</td><td>8.5</td></tr>
<tr><td colspan="5">16.0/15.9#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td>-</td></tr>
<tr><td>37, 38, 39*</td><td>-</td><td>8.5</td><td>-</td><td>8.7</td></tr>
<tr><td>40, 41, 42*</td><td>-</td><td>-</td><td>8.6</td><td>8.5</td></tr>
<tr><td colspan="5">32.0/31.2#</td></tr>
<tr><td>Bulk</td><td>7.8</td><td>7.6</td><td>7.5</td><td>-</td></tr>
<tr><td>43, 44, 45*</td><td>-</td><td>8.5</td><td>-</td><td>8.7</td></tr>
<tr><td>46, 47, 48*</td><td>-</td><td>-</td><td>8.6</td><td>8.6</td></tr>
</table>
 # Nominal concentrations and initial measured concentrations, expressed as µg pyroxsulam/L.
*** Spent replicates. ** Not applicable.**
</td><td>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 control bulk medium having an increase in pH from 7.5 to 7.8 at day 0 is not known.</td></tr>
<tr><td>Chelator used:</td><td>Na_2EDTA.

A comparison of the 20X AAM and 20X AAP growth media is given on page 49 of this DER.</td><td>Requirement considered met.

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

US EPA OPPTS 850.4400 observes that chelating agents, such as EDTA, are present in the 20X-AAP medium to ensure that trace nutrients will be available to the *Lemna*</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
		fronds and that M-Hoagland's medium (which contains no EDTA) should be used for test solution preparation if it suspected that the chelator will interact with the test chemical. *Chelators are not recommended (US EPA).*
Carbon source:	Not identified. Stated to be ambient carbon dioxide by Hancock (2005)	Requirement considered met on the basis of satisfactory growth in the controls. OECD 221 and US EPA OPPTS 850.4400 do not refer to a "carbon source".
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Although the 20X 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. (see Attachment 1, page 49 of this DER for details on the composition of the 20X AAM medium).	Requirement considered met.
Dilution water 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 twenty-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 prior to the addition of the pyroxsulam.	Requirement considered met. OECD 221 and US EPA OPPTS 850.4400 state that if 20X-AAP medium is used, the

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Parameter	Details	Remarks *Criteria*
		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: Particulate matter: Metals: Pesticides: Chlorine: Water pretreatment (if any): Intervals of water quality measurement	Not reported. Not reported Not reported Not reported Not reported. Deionisation 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 twenty-day-old subculture was used for the test with the controls growing satisfactorily, the water used is considered to have been acceptable.
Indicate how the test material is added to the medium (added directly or used stock solution)	Test solutions were prepared from concentrated stock solutions. Stock solutions were prepared as serial dilutions from a primary stock solution. A 320 µg pyroxsulam/mL solution was prepared by dissolving 81.63 mg pyroxsulam (corrected for percent active ingredient) in 250 mL of dimethylformamide (DMF). The 160 µg pyroxsulam/mL stock solution was prepared by diluting 50 mL of the 320 µg pyroxsulam/mL stock solution with 50 mL DMF. Subsequent stock solutions (80.0, 40.0, 20.0 and 10.0 µg pyroxsulam/mL) were prepared similarly as serial dilutions of the next highest concentration stock solution. The 32.0, 16.0, 8.00, 4.00, 2.00 and 1.00 µg pyroxsulam/L exposure solutions were prepared using the 320, 160, 80.0, 40.0, 20.0 and 10.0 µg pyroxsulam/mL DMF stock solutions, respectively. Exposure solutions were prepared by injecting 100 µL of each corresponding DMF stock	Requirements considered met. The primary stock solution was made up taking into account the 98% purity of the pyroxsulam.

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Parameter	Details	Remarks *Criteria*
	solution into 1 L of 20X AAM. The solvent control solution was prepared by injecting 100 µL of DMF into 1 L of 20X AAM. This allowed for a consistent DMF concentration in the solvent control and exposure solutions of 0.100 mL/L. On day 1, half of the replicates at each dose level and control were renewed with medium without test material. On day 3, the remaining replicates at each dose level and control were renewed with medium without test material. The growth period was carried out until day 7 for both sets of replicates.	
Aeration or agitation	Agitation and aeration were 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 this duckweed exposure test.	Requirements considered met.
Number of replicates Control:	Six replicates were inoculated and set for the medium controls. Three replicates at each level (including control and solvent control replicates) were exposed for one day to medium containing pyroxsulam. The three remaining replicates at each level were exposed for three days to medium containing test material. After three days, the treated medium in each replicate was replaced with fresh medium without test material for an	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.

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Parameter	Details	Remarks *Criteria*
	additional four-day growth period. Three replicates in each control group were also treated the same way.	
Solvent control:	Six, see above.	Requirement considered met.
Treatments:	Six, see above.	Requirement considered met.
Number of plants/replicate	Each test vessel was inoculated with three plants (with four fronds per plant).	Requirement considered met. OECD states that each test vessel should contain a total of 9 to 12 fronds. The number of fronds and colonies should be the same in each test vessel. 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.*
<u>Test concentrations</u> Nominal:	0 (control, 20X AAM medium), 0 (DMF solvent control), 1.00, 2.00, 4.00, 8.00, 16.0 and 32.0 µg pyroxsulam/L 20X AAM Nominal concentrations were in the 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.

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<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td></td><td>US EPA OPPTS 850.4400 refers to use of at least five concentrations of chemical, excluding controls, for use 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.</td></tr>
<tr><td>Measured:</td><td><u>Mean</u> measured concentrations (based on the mean of the <u>bulk dose measured</u> concentrations for analysis on days 0, 3 and 5) were:

<table>
<tr><th>Nominal pyroxsulam value,µg/L</th><th colspan="3">Corrected concentrations[a] (µg/L) (% of nominal)</th></tr>
<tr><th></th><th>Day 0 bulk dose solutions</th><th>Day 1 spent test solutions[b]</th><th>Day 3 spent test solutions</th></tr>
<tr><td>Control, 20X AAM</td><td><LLQ[c]</td><td><LLQ</td><td><LLQ</td></tr>
<tr><td>Solvent (DMF) control</td><td><LLQ</td><td><LLQ</td><td><LLQ</td></tr>
<tr><td>1.00</td><td>1.06[d] (106%)</td><td>0.964 (96.4%)</td><td>0.978 (97.8%)</td></tr>
<tr><td>2.00</td><td>2.21 (111%)</td><td>1.94 (97.0%)</td><td>2.00 (100%)</td></tr>
<tr><td>4.00</td><td>4.28 (107%)</td><td>4.01 (100%)</td><td>4.01 (100%)</td></tr>
<tr><td>8.00</td><td>8.64 (108%)</td><td>8.54 (107%)</td><td>8.19 (102%)</td></tr>
<tr><td>16.0</td><td>15.9 (99.4%)</td><td>15.6 (97.5%)</td><td>16.1 (101%)</td></tr>
<tr><td>32.0</td><td>31.2[d] (97.5%)</td><td>31.3 (97.8%)</td><td>31.3 (97.8%)</td></tr>
</table>
a. The value for the 1.00, 2.00, 4.00, and 8.00 µg/mL concentrations were corrected for spike recovery, however 16.0 and 32.0 µg/mL concentrations were not corrected for a recovery, as this was not needed.
b. Spent Test Solutions = composite of three spent test solutions to provide one spent exposure solution per dose level.
c. <LLQ = Less than Lowest Level Quantified; LLQ = 0.152 µg pyroxsulam/L 20xAAM.
d. This value represents the mean of 4 injections (4 samples, 1 injection/sample). Four replicate samples were collected on day 0 of the study to determine method precision.</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 in the 20X Algal Assay Medium/

OECD 221 refers to the situation in which a preliminary stability test shows that the test substance concentration cannot be</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
		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. 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."
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 day 0 of the study immediately following preparation. On days 1 and 3, the spent test study solutions at each dose level were pooled to provide one composite exposure sample per dose level for analytical confirmation. Pyroxsulam solutions extracted from the solutions were vortex mixed and analysed using high performance liquid chromatography with mass spectrometric detection (HPLC/MS).	Requirement considered met. To assess analytical method precision and solution homogeneity, three additional samples were collected on day 0 from the 1.00 and 32.0 µg/L bulk dose solutions. These additional samples were collected, extracted or diluted, and analysed along with the other day 0 samples. Assessment of extraction efficiency yielded average recovery values of 88.7%, 94.7% and 95.5% for days 0, 1 and 3, respectively, which were used to adjust the analysed

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Parameter	Details	Remarks *Criteria*
		concentrations of the extracted test solutions for method recovery on each analysis day. A secondary stock solution containing a nominal concentration of 2.03 µg pyroxsulam/mL acetonitrile was used to prepare analytical standards over a concentration range of 1.52 to 115 µg analyte/L diluent. The range encompassed the expected sample concentrations. Analytical standards were analysed with each set of samples to define the detector response. Since the mass spectrum response profile could not be adequately defined by a linear regression throughout the concentration range of interest, the detector response was mathematically defined by generating a power curve equation of peak area ratios (PAR) versus pyroxsulam concentrations (using Analyst software). Concentrations in the samples were calculated by application of the power curve equation to the PAR value derived for each analysis and multiplying by the appropriate dilution factor and accounting for method recovery as needed. 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 0.152 µg/L 20X AAM, which was the concentration of the lowest standard quantified times the lowest dilution factor. Typical chromatograms of a control, a standard, and a sample were presented.
Limit of Quantitation:	The lowest level quantified was set at 0.152 µg pyroxsulam/L 20 X AAM.	
Limit of Detection:	Not reported.	
<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

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Parameter	Details	Remarks *Criteria*
		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 6440 ± 233 lux with a range of 6150–7000 lux.	Requirement considered met. OECD 221 refers use of light of an intensity equivalent to 6500-10000 lux and to 85-135 $\mu E/m^2/s$ when measured in a photosynthetically active radiation (400-700 nm) US EPA OPPTS 850.4400 states that a light intensity in the range of 4,200 and 6,700 lux should be used. *EPA light: 5.0 Klux (15%)*
Reference chemical (if used) Name: Concentrations:	No reference chemical mentioned.	See deviations/deficiency table on page 43 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 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

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Parameter	Details	Remarks *Criteria*
		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, 1, 3, 5 and 7 in each replicate. At test termination, frond dry weights were determined for each control and test treatment. pH, temperature, and analyte concentrations were determined either continuously or at defined intervals during the study. Light intensity was measured at test initiation. Fronds were examined for abnormalities over the exposure and post-exposure periods.	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

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		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 the edge of the parent frond counted. Dry weight (at least 48 hours at 60°C).	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 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, 1, 3, 5 and 7. On day 0, an initial pH was taken from a sample of each bulk test solution. A final pH of spent exposure solutions was also taken on days 1 and 3 from a pooled sample of the three replicates at each level that were renewed with fresh medium without test material. On days 1	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

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	and 3, a pH measurement was also taken from the bulk preparation of the appropriate vessels. Since replicate groups at each test level were transferred to fresh, untreated medium on different days (days 1 or 3), the final pH measurement taken at test termination was taken using pooled samples by level from these groupings. Light intensity was measured at test initiation. Pyroxsulam determinations in bulk dose solutions were made on day 0 with analyses of the spent test solutions made on days 1 and 3. Temperature was monitored continuously during the test.	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. **The results (study endpoints) of the study were evaluated based on the initial measured concentrations of pyroxsulam from the day 0 bulk solutions.**	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

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		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, 204 and 171 for the one day exposure and 214 and 184 in the three day exposure. These values represent, respectively, a 17 and a 14.2 increase over 7 days of the initial frond number (12) in the control and solvent control replicates for the one day exposure and 17.8 and 15.3 for the three day exposure. The mean specific growth rates for the control and solvent control were reported as, respectively, 0.404 and 0.379 day^{-1} for the one day exposure and 0.412 and 0.390 for the three day exposure period. These criteria meet the OECD 221 requirements for growth and show that exponential growth occurred in the control.	Requirement considered met. OECD 221 states, "For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}". No specific requirements were identified in US EPA OPPTS 850.4400.
Water quality was acceptable (Yes/No)	Not specifically recorded in the test report but the successful control growth indicates the quality was acceptable.	Requirement considered met.
Were raw data included?	No. Tabulated results for duckweed growth data (specific growth rate, frond counts, dry weight and % 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	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

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	Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.	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, 1, 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 day 0 analysis of bulk dose solutions yielded percent of target values ranging from 97.5 to 111%. The exposure test solution concentrations measured on days 1 and 3 had percent of target values ranging from 96.4 to 107%. Results from the analysis of the DMF-based dose stock solutions ranged from 83.5 to 91.6% of target. The analyzed concentrations of the dose stock solutions provide further indication that the test solutions were, indeed, dosed at their intended concentrations. As a result, biological results were based on initial measured pyroxsulam concentrations.

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Frond counts and related inhibition

One day exposure
Mean frond counts after a one-day exposure to pyroxsulam and a subsequent six-day growth period in untreated medium were 204, 171, 154, 150, 137, 113, 105 and 94 fronds for the medium control, solvent control, 1.06, 2.21, 4.28, 8.64, 15.9 and 31.2 µg/L test levels, respectively. A t-test comparison of the control groups indicated that they were significantly different so the statistical comparisons were made versus the solvent control group (the US EPA noted that a significant difference between controls is typically interpreted by EPA as a serious deficiency that can invalidate the study). Response relative to the solvent controls ranged from 10% to 45% inhibition of mean frond density. No statistical determination of the EC50 was conducted because no effect greater than 50% was observed.

The effect of a one day exposure to various pyroxsulam concentrations on frond numbers of *Lemna gibba* are shown in Table 3 which summarizes the study report's findings. The reported means, standard deviations and percentage inhibition values were confirmed by the reviewer as correct.

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Table 3. Effect of pyroxsulam on frond number of the freshwater duckweed (*Lemna gibba*) as given in the study report (Hancock *et al.*, 2005). Replicate values for the number of fronds exposed to pyroxsulam for one day.

Treatment (nominal and measured concentration[a]), µg pyroxsulam/L	Replicate Number	Frond counts at day: 0	1	3	5	7	Day 7 % inhibition from the solvent control
0 (Control)/<LLQ[b]	**1**	**12**	**16**	**41**	**93**	**217**	
	2	**12**	**16**	**36**	**79**	**181**	
	3	**12**	**20**	**46**	**107**	**215**	
	Mean (sd)	12 (0)	17 (2)	41 (5)	93 (14)	204 (20)	Not applicable
0 (Solvent (DMF) Control)	**7**	**12**	**18**	**40**	**82**	**175**	
	8	**12**	**15**	**37**	**74**	**167**	
	9	**12**	**14**	**34**	**75**	**170**	
	Mean (sd)	12 (0)	16 (2)	37 (3)	77 (4)	171 (4)	Not applicable
1.00/1.06	**13**	**12**	**15**	**37**	**87**	**172**	
	14	**12**	**15**	**35**	**81**	**151**	
	15	**12**	**14**	**31**	**79**	**140**	
	Mean (sd)	12 (0)	15 (1)	34 (3)	82 (4)	154 (16)	10
2.00/2.21	**19**	**12**	**19**	**35**	**79**	**158**	
	20	**12**	**16**	**30**	**68**	**145**	
	21	**12**	**15**	**31**	**68**	**147**	
	Mean (sd)	12 (0)	17 (2)	32 (3)	72 (6)	150* (7)	12
4.00/4.28	**25**	**12**	**15**	**32**	**69**	**130**	
	26	**12**	**16**	**29**	**68**	**134**	
	27	**12**	**17**	**29**	**70**	**146**	
	Mean (sd)	12 (0)	16 (1)	30 (2)	69 (1)	137* (8)	20
8.00/8.64	**31**	**12**	**15**	**34**	**68**	**126**	
	32	**12**	**14**	**23**	**53**	**110**	
	33	**12**	**14**	**25**	**53**	**103**	
	Mean (sd)	12 (0)	14 (1)	27 (6)	58 (9)	113* (12)	34
16.0/15.9	**37**	**12**	**14**	**25**	**58**	**110**	
	38	**12**	**16**	**25**	**59**	**104**	
	39	**12**	**14**	**30**	**59**	**100**	
	Mean (sd)	12 (0)	15 (1)	27 (3)	59 (1)	105* (5)	39
32.0/31.2	**43**	**12**	**14**	**21**	**44**	**102**	
	44	**12**	**14**	**20**	**44**	**81**	
	45	**12**	**12**	**25**	**55**	**99**	
	Mean (sd)	12 (0)	13 (1)	22 (3)	48 (6)	96* (11)	45

a. Initial measured concentrations based on day 0 bulk dose solutions. b. <LLQ = Less than Lowest Level Quantified = 0.152 mg XDE-742/L. c. sd = standard deviation. * Significant difference from the controls; $p \leq 0.05$, one-tailed Dunnett's t-test.

Three day exposure

Mean frond counts after a three-day exposure to pyroxsulam and a subsequent four-day growth period in untreated medium were 214, 184, 169, 140, 83, 59, 49 and 42 frond for the medium control, solvent control, 1.06, 2.21, 4.28, 8.64, 15.9 and 31.2 µg/L test levels, respectively. A t-test comparison of the control groups indicated that they were not significantly different so the statistical comparisons were made versus the pooled control group. Response relative to the pooled controls ranged from 15% to 79% inhibition of mean frond density.

The effect of a three day exposure to various pyroxsulam concentrations on frond numbers of *Lemna gibba* are shown in Table 4 which summarizes the study report's findings. The reported means, standard deviations and percentage inhibition values were confirmed by the reviewer as correct.

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Table 4. Effect of pyroxsulam on frond number of the freshwater duckweed (*Lemna gibba*) as given in the study report (Hancock *et al.*, 2005). Replicate values for the number of fronds exposed to pyroxsulam for three days.

Treatment (nominal and measured concentration[a]), µg pyroxsulam/L	Replicate Number	Frond counts at day: 0	1	3	5	7	Day 7 % inhibition from the pooled control
0 (Control)/<LLQ[b]	**4**	**12**	**19**	**41**	**95**	**231**	
	5	**12**	**19**	**36**	**89**	**213**	
	6	**12**	**17**	**37**	**86**	**199**	
	Mean (sd[c])	12 (0)	18 (1)	38 (3)	90 (5)	214 (16)	Not applicable
0 (Solvent (DMF) Control)	**10**	**12**	**15**	**33**	**81**	**168**	
	11	**12**	**17**	**36**	**96**	**182**	
	12	**12**	**20**	**43**	**102**	**202**	
	Mean (sd)	12 (0)	17 (3)	35 (5)	93 (11)	184 (17)	Not applicable
Pooled control	Mean (sd)	12 (0)	18 (2)	38 (4)	92 (8)	199 (22)	Not applicable
1.00/1.06	**16**	**12**	**17**	**34**	**102**	**173**	
	17	**12**	**14**	**31**	**87**	**173**	
	18	**12**	**15**	**29**	**81**	**160**	
	Mean (sd)	12 (0)	15 (2)	31 (3)	90 (11)	169* (8)	15
2.00/2.21	**22**	**12**	**15**	**24**	**57**	**123**	
	23	**12**	**16**	**26**	**71**	**141**	
	24	**12**	**17**	**27**	**84**	**155**	
	Mean (sd)	12 (0)	16 (1)	29 (6)	71 (14)	140* (16)	30
4.00/4.28	**28**	**12**	**15**	**21**	**42**	**85**	
	29	**12**	**16**	**23**	**36**	**79**	
	30	**12**	**15**	**23**	**47**	**86**	
	Mean (sd)	12 (0)	15 (1)	22 (1)	42 (6)	83* (4)	58
8.00/8.64	**34**	**12**	**15**	**17**	**27**	**55**	
	35	**12**	**12**	**16**	**28**	**58**	
	36	**12**	**14**	**17**	**31**	**63**	
	Mean (sd)	12 (0)	14 (2)	17 (1)	29 (2)	59* (4)	70
16.0/15.9	**40**	**12**	**14**	**18**	**32**	**60**	
	41	**12**	**13**	**16**	**23**	**38**	
	42	**12**	**13**	**15**	**22**	**50**	
	Mean (sd)	12 (0)	13 (1)	16 (2)	26 (6)	49* (11)	75
32.0/31.2	**46**	**12**	**12**	**13**	**21**	**38**	
	47	**12**	**15**	**16**	**23**	**47**	
	48	**12**	**13**	**13**	**16**	**41**	
	Mean (sd)	12 (0)	13 (2)	14 (2)	20 (4)	42* (5)	79

a. Initial measured concentrations based on day 0 bulk dose solutions. b. <LLQ = Less than Lowest Level Quantified = 0.152 mg XDE-742/L. c. sd = standard deviation. * Significant difference from the controls; $p \leq 0.05$, one-tailed Dunnett's t-test.

Frond health following exposure of duckweed to pyroxsulam for one and three days

No abnormal observations were noted on duckweed fronds in the group of replicates that was exposed to pyroxsulam for one day followed by a six-day growth period in untreated medium at any observation period. For the fronds that were exposed to pyroxsulam for three days followed by a four-day growth period in untreated medium, duckweed fronds that were visually smaller than normal were noted in test levels ≥ 2.21 µg/L. Some of the fronds were noted as smaller than normal in the 2.21, 4.28 and 8.64 µg/L test levels of days 5 and 7. All fronds in the 15.9 and 31.2 µg/L test levels were noted as smaller than normal on days 5 and 7. The observation of smaller than normal fronds was considered by the study report consistent with the frond dry weight measurements.

Mean specific growth rate and related inhibition

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One day exposure

Mean specific growth rates after a one-day exposure to pyroxsulam and a subsequent six day growth period in untreated medium were 0.404, 0.379, 0.364, 0.361, 0.347, 0.320, 0.309 and 0.293 day^{-1} for the medium control, solvent control, 1.06, 2.21, 4.28, 8.64, 15.9, and 31.2 µg/L test levels, respectively. A t-test comparison of the control group indicated that they were significantly different so the statistical comparisons were made versus the solvent control group. Response relative to the solvent controls ranged from 4% to 23% inhibition of mean specific growth rate. No statistical determination of the ErC50 was conducted since no effect greater than 50% was observed.

Mean specific growth rates after a three-day exposure and a subsequent four-day growth period in untreated medium were 0.412, 0.390, 0.377, 0.350, 0.277, 0.226, 0.199 and 0.178 day^{-1} for the medium control, solvent control, 1.06, 2.21, 4.28, 8.64, 15.9 and 31.2 •g/L test levels, respectively. A t-test comparison of the control groups indicated that they were not significantly different so the statistical comparisons were made versus the pooled controls. Response relative to the pooled controls ranged from 6% to 55% inhibition of mean specific growth rate.

The effect of a one and three day exposure to various pyroxsulam concentrations on frond numbers of *Lemna gibba* are shown in Table 5 which presents the study report's calculated specific growth rates for each replicate. The reported replicate specific growth rates and associated means, standard deviations and percentage inhibition values were recalculated by the reviewer and confirmed as correct.

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Table 5. Effect of pyroxsulam on specific growth rates (as day^{-1}) of the freshwater duckweed (*Lemna gibba*) following one or three days exposure and as given in the study report (Hancock *et al.*, 2005). Replicate values for the specific growth rates and associated mean, standard deviation and percentage inhibition results are shown.

Treatment (nominal and measured concentration[a]), µg pyroxsulam/L	Replicate	7 day specific growth rate (day^{-1}) after exposure to pyroxsulam for one day	Day 7 % inhibition from the solvent control	Replicate	7 day specific growth rate (day^{-1}) after an exposure to pyroxsulam for three days.	Day 7 % inhibition from the pooled control
0 (Control) (<LLQ)	**1**	**0.4136**		**4**	**0.4225**	
	2	**0.3877**		**5**	**0.4109**	
	3	**0.4123**		**6**	**0.4012**	
	Mean (sd)	0.4045 (0.0146)	Not applicable		0.4115 (0.0107)	Not applicable
0 (Solvent (DMF) Control) (<LLQ)	**7**	**0.3828**		**10**	**0.3770**	
	8	**0.3762**		**11**	**0.3884**	
	9	**0.3787**		**12**	**0.4033**	
	Mean (sd)	0.3792 (0.0034)	Not applicable		0.3896 (0.0132	Not applicable
Pooled control	Mean (sd)	Not determined by study report	Not applicable	Mean (sd)	0.4006 (0.0161)	Not applicable
1.00/1.06	**13**	**0.3804**		**16**	**0.3812**	
	14	**0.3618**		**17**	**0.3812**	
	15	**0.3510**		**18**	**0.3700**	
	Mean (sd)	0.3644 (0.0149	4	Mean (sd)	0.3775 (0.0064)	6
2.00/2.21	**19**	**0.3682**		**22**	**0.3325**	
	20	**0.3560**		**23**	**0.3520**	
	21	**0.3579**		**24**	**0.3655**	
	Mean (sd)	0.3607 (0.0066)	5	Mean (sd)	0.3500* (0.0166)	13
4.00/4.28	**25**	**0.3404**		**28**	**0.2797**	
	26	**0.3447**		**29**	**0.2692**	
	27	**0.3570**		**30**	**0.2813**	
	Mean (sd)	0.3473* (0.0086)	8	Mean (sd)	0.2768* (0.0066)	31
8.00/8.64	**31**	**0.3359**		**34**	**0.2175**	
	32	**0.3165**		**35**	**0.2251**	
	33	**0.3071**		**36**	**0.2369**	
	Mean (sd)	0.3198* (0.0147)	16	Mean (sd)	0.2265* (0.0098)	43
16.0/15.9	**37**	**0.3165**		**40**	**0.2299**	
	38	**0.3085**		**41**	**0.1647**	
	39	**0.3029**		**42**	**0.2039**	
	Mean (sd)	0.3093* (0.0068)	18	Mean (sd)	0.1995* (0.0328)	50
32.0/31.2	**43**	**0.3057**		**46**	**0.1647**	
	44	**0.2728**		**47**	**0.1950**	
	45	**0.3015**		**48**	**0.1755**	
	Mean (sd)	0.2933* (0.0179)	23	Mean (sd)	0.1783* (0.0154)	55

a. Initial measured concentrations based on day 0 bulk dose solutions. b. <LLQ = Less than Lowest Level Quantified = 0.152 mg XDE-742/L. c. sd = standard deviation. * Significant differences between the controls and pyroxsulam containing replicates demonstrated ($p \leq 0.05$, one-tailed Dunnett's t-test).

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Mean frond dry weight and related inhibition
One day exposure

Mean frond dry weights after a one-day exposure and a subsequent six-day growth period in untreated medium were 25.30, 22.69, 22.72, 20.64, 19.89, 16.85, 17.23 and 15.62 mg for the medium control, solvent control, 1.06, 2.21, 4.28, 8.64, 15.9 and 31.2 •g/L test levels, respectively. A t-test comparison of the control groups indicated that they were not significantly different so the statistical comparisons were made versus the pooled controls. Response relative to the pooled controls ranged from 5% to 35% inhibition of frond dry weight. No statistical determination of the EbC50 was conducted since no effect greater than 50% was observed.

Mean frond dry weights after a three-day exposure and a subsequent four-day growth period in untreated medium were 27.42, 26.22, 22.33, 19.48, 14.39, 11.39, 9.79 and 8.93 mg for the medium control, solvent control, 1.06, 2.21, 4.28, 8.64, 15.9 and 31.2 µg/L test levels, respectively. A t-test comparison of the control groups indicated that they were not significantly different so the statistical comparisons were made versus the pooled controls.

The effect of a one and three day exposure to various pyroxsulam concentrations on frond dry weight of *Lemna gibba* are shown in Table 6 which presents the study report's dry frond weight for each replicate. The reported replicate dry weights and associated means, standard deviations and percentage inhibition values were recalculated by the reviewer and confirmed as correct.

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Table 6. Effect of pyroxsulam on frond dry weight (as mg) of the freshwater duckweed (*Lemna gibba*) following one or three days exposure and as given in the study report (Hancock *et al.*, 2005). Replicate values for the frond dry weights and associated mean, standard deviation and percentage inhibition results are shown.

Treatment (nominal and measured concentration[a]), µg pyroxsulam/L	Replicate	7 day frond dry weight (mg) after exposure to pyroxsulam for one day	Day 7 % inhibition from the pooled control	Replicate	7 frond dry weight (mg) after an exposure to pyroxsulam for three days.	Day 7 % inhibition from the pooled control
0 (Control) (<LLQ)	**1**	**26.31**		**4**	**28.62**	
	2	**22.72**		**5**	**27.63**	
	3	**26.87**		**6**	**26.01**	
	Mean (sd)	25.3 (2.25)	Not applicable		27.42 (1.32)	Not applicable
0 (Solvent (DMF) Control) (<LLQ)	**7**	**23.95**		**10**	**24.87**	
	8	**20.97**		**11**	**25.15**	
	9	**23.16**		**12**	**28.64**	
	Mean (sd)	22.69 (1.54)	Not applicable		26.22 (2.10)	Not applicable
Pooled control	Mean (sd)	24.0 (2.24)	Not applicable	Mean (sd)	26.82 (1.70)	Not applicable
1.00/1.06	**13**	**24.48**		**16**	**23.66**	
	14	**21.97**		**17**	**21.89**	
	15	**21.71**		**18**	**21.45**	
	Mean (sd)	22.72 (1.53)	5	Mean (sd)	22.33* (1.17)	17
2.00/2.21	**19**	**22.42**		**22**	**18.93**	
	20	**19.34**		**23**	**17.22**	
	21	**20.15**		**24**	**22.29**	
	Mean (sd)	20.64* (1.60)	14	Mean (sd)	19.48* (2.58)	27
4.00/4.28	**25**	**19.7**		**28**	**14.17**	
	26	**19.66**		**29**	**14.23**	
	27	**20.32**		**30**	**14.76**	
	Mean (sd)	19.89* (0.37)	17	Mean (sd)	14.39* (0.32)	46
8.00/8.64	**31**	**18.58**		**34**	**10.84**	
	32	**15.95**		**35**	**11.09**	
	33	**16.02**		**36**	**12.23**	
	Mean (sd)	16.85* (1.50)	30	Mean (sd)	11.39* (0.74)	58
16.0/15.9	**37**	**16.7**		**40**	**11.68**	
	38	**17.25**		**41**	**9**	
	39	**17.73**		**42**	**8.69**	
	Mean (sd)	17.23* (0.52)	28	Mean (sd)	9.79* (1.64)	63
32.0/31.2	**43**	**16.18**		**46**	**8.71**	
	44	**14.22**		**47**	**9.86**	
	45	**16.45**		**48**	**8.22**	
	Mean (sd)	15.62* (1.22)	35	Mean (sd)	8.93* (0.84)	67

a. Initial measured concentrations based on day 0 bulk dose solutions. b. <LLQ = Less than Lowest Level Quantified = 0.152 mg XDE-742/L. c. sd = standard deviation. * Significant differences between the controls and pyroxsulam containing replicates demonstrated ($p \leq 0.05$, one-tailed Dunnett's t-test).

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STATISTICAL ENDPOINT VALUES REPORTED IN THE STUDY REPORT

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

Table 7. 7 Day statistical endpoint values (NOEC, LOEC and EC50 values for duckweed exposed to various pyroxsulam concentrations for 1 or 3 days in a static test without renewal) as reported by Hancock *et al.*, 2005.

7 day Statistical Endpoint	Frond No.	Mean specific growth rate (per day)	Biomass (frond dry weight)
One day's exposure to pyroxsulam containing solutions			
NOEC (µg pyroxsulam/L)	1.06	2.21	1.06
LOEC (µg pyroxsulam/L)	Not reported	Not reported	Not reported
EC50 (µg pyroxsulam/L) (95% C.I.)	EC50 >31.2 95% confidence limits not calculable	ErC50 >31.2 95% confidence limits not calculable	EbC50 >31.2 95% confidence limits not calculable
Three days' exposure to pyroxsulam containing solutions			
NOEC (µg pyroxsulam/L)	< 1.06	1.06	<1.06
LOEC (µg pyroxsulam/L)	Not reported	Not reported	Not reported
EC50 (µg pyroxsulam/L) (95% C.I.)	EC50 4.68 (1.85, 11.8)	ErC50 17.2 (8.31, 35.4)	EbC50 7.45 (3.06, 18.16)
Reference chemical **NOEC** **IC50/EC50**	No reference chemical used.		

Note: bracketed values are 95% confidence limits.

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 (or less understood).

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

$T_d = \ln 2/\mu$

where μ is the average specific growth rate

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

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

where:

- μ_{i-j}: average specific growth rate from time i to j
- Ni : measurement variable in the test or control vessel at time i
- Nj : measurement variable in the test or control vessel at time j

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- 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 8.

Table 8. Reviewer calculated control doubling time for frond numbers in *Lemna gibba*

Sample	Reported mean specific growth rate, per day	Td (doubling time), days (= ln 2/μ)
One day's exposure to pyroxsulam containing solutions		
Control	**0.404**	**1.72**
Solvent control	**0.379**	**1.83**
Pooled control	**Not determined**	-
Three days' exposure to pyroxsulam containing solutions		
Control	**0.412**	**1.68**
Solvent control	**0.390**	**1.78**
Pooled control	**0.401**	**1.73**

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 divided by the initial frond number (12) results are shown in Table 9 and show that this OECD 211 criterion was met.

Table 9. Day seven frond counts in the controls and the calculated increase in those numbers over 7 days.

Sample	Mean frond count at day 7	Increase in frond number from time 0 (= day 7 count/12)
One day's exposure to pyroxsulam containing solutions		
Control	**204**	**17.0**
Solvent control	**171**	**14.2**
Pooled control	**Not reported or calculated**	-
Three days' exposure to pyroxsulam containing solutions		
Control	**214**	**17.8**
Solvent control	**184**	**15.3**
Pooled control	**199**	**16.6**

Note: Initial frond number = 12.

B. <u>REPORTED STATISTICS</u>:

Because results from the chemical analysis of the bulk exposure solutions for pyroxsulam had yielded percent of target values ranging from 97.5-111%, biological results (frond numbers, mean specific growth rate and biomass) in the study report were based on initial measured pyroxsulam concentrations.

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.

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A t-test was conducted to compare the medium controls and solvent controls for each endpoint ($\alpha = 0.05$). If the t-test was significant, indicating that there was a difference between the controls, then the solvent control was used for further comparisons. If the t-test was not significant, indicating that there was not a statistical difference between the controls, then the controls were pooled and used for further comparisons.

The study report stated that EC50 values for frond number (the concentration estimated to limit frond growth to 50% of that observed in the control population) were determined at 7 days. The first step in the EC50 estimations was to fit a line of the response variable (linear part of the curve) on the concentration and the common log of the concentration using least squares estimation. Once the equations for the line were determined the EC50 estimates, with their confidence intervals, were calculated using the method of inverse estimation. The line with the highest R^2 was then reported if the estimates were consistent with the observed data. In the event that there was not an effect greater than 50%, the EC50 value was empirically determined to be greater than the highest concentration tested.

The ErC50 values (the concentration estimated to inhibit the growth rate to 50% relative to the control) were calculated for the 0 to 7 period by two methods, the study report stated. First, 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. Second, the growth was rate was regressed against the concentration. The ErC50 values were determined by inverse estimation from the regression equations. The line with the highest R^2 was then reported if the estimates were consistent with the observed data. In the event that there was not an effect greater than 50%, the ErC50 value was empirically determined to be greater than the highest concentration tested.

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 values (the concentration that inhibited the frond dry weight of this species to 50% of the test population compared to the control population) were determined at 7 days. The first step in the EbC50 estimation was to regress percent inhibition of biomass, compared to the control, against the natural logarithm of the concentration and to regress the dry weight against concentration. Once the equations for the line were determined, the EbC50 estimates, with their confidence intervals, were calculated using the method of inverse estimation. The line with the highest R^2 was then reported if the estimates were consistent with the observed data. In the event that there was not an effect greater than 50%, the EbC50 value was empirically determined to be greater than the highest concentration tested.

The data were tested for normality using Shapiro-Wilk's Test and for homogeneity of variance using Bartlett's test. The data for frond number, growth rate and biomass (dry weight) 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

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growth rates and biomass (as dry weight) was performed. Toxicity endpoints are expressed as mean measured concentrations.

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
One day exposure to pyroxsulam

The ToxCalc analysis used the untransformed day 7 frond counts with the means of the dilution and solvent controls frond counts identified as significantly different (p = 0.05) and therefore not pooled. Treatment means were compared to the mean of the solvent control using Dunnett's test. Shapiro Wilk's and Bartlett's tests respectively confirmed normality and equality of variance of the untransformed data.

The results of these frond analyses are shown in Table 10 with the ToxCalc results presented on pages 51 and 52 of this DER. The table also shows the study report's EC50 and NOEC values for the effect of the one day exposure on frond number. The reviewer's statistical examination has verified the study report's results for the effect of pyroxsulam on duckweed frond numbers following an exposure period of one day.

Table 10. Reviewer calculated 7 day EC50 and NOEC values for *Lemna gibba* frond counts after a one day exposure to pyroxsulam with the results based on comparison of the treatment means with the solvent control mean. EC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L and based on initial measured pyroxsulam concentrations. The study report's results for the one day exposure are also shown.

Exposure period	7 day EC50	95% Confidence limits	7 days NOEC	Mean measured concentrations which had statistically significantly lower mean frond counts compare to the mean of the solvent control (Dunnett's test)
One day	**37.3 (Maximum likelihood probit)**	**Not determined**	**1.06**	**≥2.21**
	>31.2 (Linear interpolation)	**Not determined**	**1.06**	**≥2.21**
Study report's results	**>31.2**	**Not determined**	**1.06**	**≥2.21**

Three day exposure to pyroxsulam
The ToxCalc analysis used the untransformed day 7 frond counts with the means of the dilution and solvent controls frond counts identified as not significantly different (p = 0.05) and therefore pooled. Treatment means were compared to the pooled control means using Dunnett's test. Shapiro Wilk's and Bartlett's tests respectively confirmed normality and equality of variance of the untransformed data.

The results of these frond analyses are shown in Table 11 with the ToxCalc results presented on pages 53 and 54 of this DER. The table also shows the study report's EC50 and NOEC values for the effect of the three day exposure on frond number. The reviewer's statistical examination has verified the study report's results for the effect of pyroxsulam on duckweed frond numbers following an exposure period of three days.

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Table 11. Reviewer calculated 7 day EC50 and NOEC values for *Lemna gibba* frond counts after a three day exposure to pyroxsulam with the results based on comparison of the treatment means with the pooled control means. EC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L and based on initial measured pyroxsulam concentrations. The study report's results for the three days exposure are also shown.

Exposure period = 3 days	7 day EC50	95% Confidence limits	7 day NOEC	Mean measured concentrations which had statistically significantly lower mean frond counts compare to the mean of the pooled controls (Dunnett's test)
Reviewer calculated results	**4.4 (Maximum likelihood probit)**	**1.2-12**	**<1.06**	**≥1.06**
	3.7 (Linear interpolation)	**3.0-4.2**	**<1.06**	**≥1.06**
Study report's results	**4.68**	**1.85-11.8**	**< 1.06**	**≥1.06**

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 reviewer 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.

One day exposure to pyroxsulam

The ToxCalc analysis used the untransformed day 7 specific growth rates for each replicate with the means of the dilution and solvent controls specific growth rates identified as significantly different ($p = 0.04$) and therefore not pooled. Treatment means were compared to the mean of the solvent control using Dunnett's test. Shapiro Wilk's and Bartlett's tests respectively confirmed normality and equality of variance of the untransformed data.

The results of these specific growth rate analyses are shown in Table 12 with the ToxCalc results presented on pages 55 and 56 of this DER. The table also shows the study report's ErC50 and NOEC values for the effect of the one day exposure on specific growth rates. The reviewer's statistical examination has verified the study report's results for the effect of pyroxsulam on duckweed specific growth rate following an exposure period of one day.

Table 12. Reviewer calculated ErC50 and NOEC values determined from the specific growth rates (as day^{-1}) for *Lemna gibba* after a one day exposure to pyroxsulam with the results based on comparison of the treatment means with the solvent control mean. Seven day ErC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L. Equivalent study report values are also shown.

Exposure period = one day	7 day ErC50	95% Confidence limits	7 day NOEC	Mean measured concentrations which had statistically significantly lower mean specific growth rates compared to the mean of the solvent control
Reviewer calculated results	**318 (Maximum likelihood probit)**	**Not calculated**	**2.21**	**≥4.28**
	>31.3 (Linear interpolation)	**Not calculated**	**2.21**	**≥4.28**
Study report's results	**>31.2**	**Not calculated**	**2.21**	**≥4.28**

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The 7 day ErC50 of 318 µg/L appears anomalous but is the value determined by the maximum likelihood probit analysis of the one day exposure data. The 1 day ErC50 is taken as >31.2 µg pyroxsulam/L.

Three day exposure to pyroxsulam

The ToxCalc analysis used the untransformed day 7 specific growth rates for each replicate with the means of the dilution and solvent controls identified as not significantly different (p = 0.09) and therefore pooled. Treatment means were compared to the pooled control means using Dunnett's test. Shapiro Wilk's and Bartlett's tests respectively confirmed normality and equality of variance of the untransformed data.

The results of these frond analyses are shown in Table 13 with the ToxCalc results presented on pages 57 and 57 of this DER. The table also shows the study report's EC50 and NOEC values for the effect of the three day exposure on frond number. The reviewer's statistical examination has verified the study report's results for the effect of pyroxsulam on duckweed frond numbers following an exposure period of three days.

Table 13. Reviewer calculated 7 day EC50 and NOEC values for *Lemna gibba* specific growth rates after a three day exposure to pyroxsulam with the results based on comparison of the treatment means with the pooled control mean. EC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L and based on initial measured pyroxsulam concentrations. The study report's results for the three days exposure are also shown.

Exposure period = 3 days	7 day ErC50	95% Confidence limits	7 day NOEC	Mean measured concentrations which had statistically significantly lower mean specific growth rates compare to the mean of the pooled controls (Dunnett's test)
Reviewer calculated results	**17.0 (Maximum likelihood probit)**	**9.6-57**	**1.06**	**≥2.21**
	15.7 (Linear interpolation)	**9.5-33**	**1.06**	**≥2.21**
Study report's results	**17.2**	**8.31-35.4**	**1.06**	**≥2.21**

Verification of biomass (frond dry weight) statistics

Verification of the study report's biomass (frond dry weight) statistics was based on a ToxCalc analysis of the reported frond dry weight replicate values.

One day exposure to pyroxsulam

The ToxCalc analysis used the untransformed day 7 frond dry weights with the means of the dilution and solvent controls frond counts identified as not significantly different (p = 0.17) and therefore pooled. Treatment means were compared to the pooled control means using Dunnett's test. Shapiro Wilk's and Bartlett's tests respectively confirmed normality and equality of variance of the untransformed data.

The results of these frond dry weight analyses are shown in Table 14 with the ToxCalc results presented on pages 59 and 60 of this DER. The table also shows the study report's ErC50 and NOEC values for the effect of the one day exposure on biomass. The reviewer's statistical examination has verified the study report's results for the effect of pyroxsulam on duckweed specific growth rate following an exposure period of one day.

Table 14. Reviewer calculated 7 day EC50 and NOEC values for *Lemna gibba* biomass (frond dry weights) after a one day exposure to pyroxsulam with the results based on comparison of the treatment means with the pooled control mean. EC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L and based on initial measured pyroxsulam concentrations. The study report's results for the one day exposure are also shown.

Exposure period = 1day	7 day EbC50	95% Confidence limits	7 day NOEC	Mean measured concentrations which had statistically significantly lower mean dry frond weights compare to the mean of the pooled controls (Dunnett's test)
Reviewer calculated results	**98.2 (Maximum likelihood probit)**	**Not calculated**	**1.06**	**≥2.21**
	>31.3 (Linear interpolation)	**Not calculated**	**1.06**	**≥2.21**
Study report's results	**>31.2**	**Not calculated**	**1.06**	**≥2.21**

The 1 day EbC50 is taken as >31.2 µg pyroxsulam/L.

Three day exposure to pyroxsulam

The ToxCalc analysis used the untransformed day 7 frond dry weights with the means of the dilution and solvent controls frond counts identified as not significantly different (p = 0.45) and therefore pooled. Treatment means were compared to the pooled control means using Dunnett's test. Shapiro Wilk's and Bartlett's tests respectively confirmed normality and equality of variance of the untransformed data.

The results of these frond dry weight analyses are shown in Table 15 with the ToxCalc results presented on pages 61 and 62 of this DER. The table also shows the study report's ErC50 and NOEC values for the effect of the one day exposure on biomass. The reviewer's statistical examination has verified the study report's results for the effect of pyroxsulam on duckweed specific growth rate following an exposure period of one day.

Table 15. Reviewer calculated 7 day EC50 and NOEC values for *Lemna gibba* biomass (frond dry weights) after a three day exposure to pyroxsulam with the results based on comparison of the treatment means with the pooled control mean. EC50, 95% confidence limits and NOEC values are as µg pyroxsulam/L and based on initial measured pyroxsulam concentrations. The study report's results for the one day exposure are also shown.

Exposure period = 3 days	7 day EbC50	95% Confidence limits	7 day NOEC	Mean measured concentrations which had statistically significantly lower mean dry frond weights compare to the mean of the pooled controls (Dunnett's test)
Reviewer calculated results	**7.3 (Maximum likelihood logit)**	**3.5-17.4**	**<1.06**	**≥1.06**
	5.7 (Linear interpolation)	**4.1-7.4**	**<1.06**	**≥1.06**
Study report's results	**7.45**	**3.06-18.16**	**<1.06**	**≥1.06**

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

Day 7 frond number

One day of exposure to pyroxsulam followed by renewal with untreated medium for six days

EC05	**0.44 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	0.55 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.21 to 2.1 µg pyroxsulam/L**
EC50:	**37.3 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	>31.2 (Linear interpolation)		**Not calculated**
NOEC:	**1.06 µg pyroxsulam/L**		
Probit Slope:	**0.85 (standard error 1.143) (Maximum likelihood probit only)**	95% C.I.:	**-0.154 to 1.86**

Three days of exposure to pyroxsulam followed by renewal with untreated medium for six days

EC05	**0.26 µg pyroxsulam/L (Maximum likelihood probit)**		**1.9E-05 to 1.06 µg pyroxsulam/L**
	0.35 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.15 to 0.81 µg pyroxsulam/L**
EC50:	**4.4 µg pyroxsulam/L (Maximum likelihood probit)**		**1.17 to 11.7 µg pyroxsulam/L**
	3.7 µg pyroxsulam/L (Linear interpolation)		**3.0 to 4.2 µg pyroxsulam/L**
NOEC:	**<1.06 µg pyroxsulam/L**		
Probit Slope:	**1.35 (standard error 0.524) (Maximum likelihood probit only)**	95% C.I.:	**0.32 to 2.34**

Specific growth rate over 7 days

One day of exposure to pyroxsulam followed by renewal with untreated medium for six days

EC05	**1.52 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	2.28 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.00 to 3.62 µg pyroxsulam/L**
ErC50:	**318 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	>31.3 (Linear interpolation)		**Not calculated**
NOEC:	**2.21 µg pyroxsulam/L**		
Probit Slope:	**0.71 (standard error 0.556) (Maximum likelihood probit only)**	95% C.I.:	**-0.382 to 1.8**

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Three days of exposure to pyroxsulam followed by renewal with untreated medium for six days

EC05	**0.33 µg pyroxsulam/L (Maximum likelihood logit)**		**0.0051 to 1.30 µg pyroxsulam/L**
	0.92 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.367 to 1.68 µg pyroxsulam/L**
ErC50:	**17 µg pyroxsulam/L (Maximum likelihood logit)**		**9.6 to 57 µg pyroxsulam/L**
	15.7 µg pyroxsulam/L (Linear interpolation)		**9.5 to 33µg pyroxsulam/L**
NOEC:	**1.06 µg pyroxsulam/L**		
Probit Slope:	**1.72 (standard error 0.536) (Maximum likelihood logit only)**	95% C.I.:	**0.0015 to 1.30**

Biomass (frond dry weight) over 7 days

One day of exposure to pyroxsulam followed by renewal with untreated medium for six days

EC05	**0.366 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	0.996 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.029 to 2.32 µg pyroxsulam/L**
EbC50:	**98.2 µg pyroxsulam/L (Maximum likelihood probit)**		**Not calculated**
	>31.3 µg pyroxsulam/L (Linear interpolation)		**Not calculated**
NOEC:	**1.06 µg pyroxsulam/L**		
Probit Slope:	**0.68 (standard error 0.501) (Maximum likelihood probit only)**	95% C.I.:	**-0.304 to 1.7**

Three days of exposure to pyroxsulam followed by renewal with untreated medium for six days

EC05	**0.099 µg pyroxsulam/L (Maximum likelihood logit)**		**0.00012 to 0.527 µg pyroxsulam/L**
	0.317 µg pyroxsulam/L (Linear interpolation)	95% C.I.:	**0.188 to 0.518 µg pyroxsulam/L**
EbC50:	**7.3 µg pyroxsulam/L (Maximum likelihood logit)**		**3.5 to 17.4 µg pyroxsulam/L**
	5.7 µg pyroxsulam/L (Linear interpolation)		**4.1 to 7.4 µg pyroxsulam/L**
NOEC:	**<1.06 µg pyroxsulam/L**		
Probit Slope:	**1.57 (standard error 0.492) (Maximum likelihood logit only)**	95% C.I.:	**0.609 to 2.54**

These EC50 values from a one day exposure to pyroxsulam classify pyroxsulam as, at worst, very highly toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment, Water, Heritage and the Arts (EC50 <100 µg/L).

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Similarly, the EC50 values from the three days of exposure classify pyroxsulam as very highly toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment, Water, Heritage and the Arts (EC50 <100 µg/L).

D. STUDY DEFICIENCIES:

Table 16 summarises deficiencies and deviations from the OECD 221 and US EPA OPPTS 850.4400 Guidelines.

Table 16. 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 twenty-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.
Renewal rate for static renewal:	Static exposures of one and three days followed by renewal with untreated medium for six- and four-day growth periods, respectively (total duration of 7 days).	OECD 221 allows for testing with and without renewal but requires a 7 day exposure to the test substance.	US EPA OPPTS 850.4400 allows for static or static renewal tests with a 7 or 14 day exposure period.
Duration of the test	7 days with respectively 1 or 3 days exposure to the pyroxsulam containing solutions.	The test specifically deviated from the OECD 221 which specifies a 7 day exposure period.	The test specifically deviated from US EPA OPPTS 850.4400 which specifies a 7 (or 14) day exposure period.
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 AMM 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 20 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.

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The use of a one or three day's exposure is a significant deviation from the OECD 221 and US EPA OPPTS 850.4400 guidelines. While the reason for doing this is adequately explained in the study report, compliance with the guidelines' requirements has not occurred and the Australian Government Department of the Environment, Water, Heritage and the Arts considers this test invalid in line with US EPA guidelines (see http://www.ipmcenters.org/Ecotox/DatabaseGuidance.pdf).

Recognizing that results of the study could be used to determine whether pyroxsulam is phytocidal or phytostatic, the short exposure periods tested do not represent a realistic environmental exposure. The study is of limited value to the PMRA, hence results would not be used in an aquatic risk assessment.

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 49 of this DER). Consequently, the use of 20X AAM is not considered to have adversely affected the study or its outcomes. Consequently use of 20X AAM is not considered a significant deficiency.

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. The absence of results from a reference chemical is taken as a minor deficiency.

E. REVIEWER'S COMMENTS:

Except in relation to the reduction in exposure times from 7 to 1 or 3 days, the study is considered to have been conducted following the requirements of OECD 221 and US EPA OPPTS 850.4400 and to have yielded reliable results. The OECD 221 validity requirements with respect to doubling time of frond numbers in the controls being less than 2.5 days and there being an approximately 7-fold increase in frond numbers in seven days are considered met. The deficiencies/deviations found with respect to the three day exposure are not considered to have adversely affected either the study's conduct or its results, however, the reduction in exposure time means that use of the ecotoxicological endpoints derived from the study are not suitable for risk assessment. With respect to the one day exposure, the differences identified between the medium and solvent controls compromise the results from that exposure period.

F. CONCLUSIONS:

The static exposure of duckweed to pyroxsulam for one or three days followed by renewal with medium without pyroxsulam for, respectively, six or four days, is considered to have been satisfactorily conducted. However, the minimum exposure results in the study being classed as invalid with respect to compliance with the relevant OECD and US EPA OPPTS guidelines and the study's endpoints would not be used by the Australian Government Department of the Environment, Water, Heritage and the Arts in its aquatic risk assessment.

The PMRA does not have the same acceptability classification scheme as the Australian Government Department of the Environment, Water, Heritage and the Arts and the US EPA. Recognizing that results of the study could help

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determine whether pyroxsulam is phytocidal or phytostatic, the short exposure periods tested do not represent realistic environmental exposures. The study is of limited value to the PMRA, hence results would not be used in an aquatic risk assessment.

The US EPA notes that, because this study used exposure durations less than seven days, the guideline requirement for an acute toxicity study on *Lemna gibba* for pyroxsulam was not met. Significant differences between the medium and solvent control for the one-day exposure study compromises these results and therefore is classified as invalid. The three-day exposure study is scientifically sound and although the exposure duration does not adhere to guideline requirements, the study may be useful for risk assessment purposes and is classified as supplemental (three-day component only).

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 the fronds exposed to pyroxsulam for one day, the mean numbers of fronds after a further 6 days growth in untreated medium were statistically significantly less from the solvent control's mean at mean measured concentrations of ≥2.21 mg pyroxsulam/L. For growth rate, the control results were pooled and mean specific growth rates for pyroxsulam concentrations of ≥4.28 mg/L were statistically significantly less than the pooled control mean. For frond dry weight, the control means were again pooled and means from exposure to concentrations of ≥2.21 mg pyroxsulam/L were identified as statistically significantly less than the pooled control's mean value.

For fronds exposed to pyroxsulam concentrations for 3 days followed by 4 days in untreated medium, control results were again pooled and mean frond counts from duckweed exposed to ≥1.06 mg pyroxsulam/L (the lowest concentration tested) were identified as statistically significantly less than the pooled control mean. For specific growth rates after 3 days exposure, controls were pooled and mean specific growth rates for concentrations of ≥2.21 mg pyroxsulam/L were identified as statistically significantly lower than the pooled control mean. For frond dried weight, control results were again pooled and mean frond dry weight results from duckweed exposed to ≥1.06 mg pyroxsulam/L identified as statistically significantly less than the pooled control mean's value.

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The toxicity EC50 an NOEC endpoints from the study report and as calculated by the reviewer were as follows:

1 and 3 day duckweed growth endpoints, as µg pyroxsulam/L with 95% confidence limits shown in brackets:			
		Study report	As calculated by the reviewer*
Exposure to pyroxsulam for one day, followed by six days in medium containing no pyroxsulam:			
Frond number	EC50	**>31.2 (Not determinable)**	**>31.2 (Not determinable)**
	NOEC	**1.06**	**1.06**
Mean specific growth rate (day^{-1})	ErC50	**>31.2 (Not determinable)**	**>31.3 (Not determinable)**
	NOEC	**2.21**	**2.21**
Biomass (frond dry weight)	EbC50	**>31.2 (Not determinable)**	**>31.3 (Not determinable)**
	NOEC	**1.06**	**1.06**
Exposure to pyroxsulam for three days, followed by four days in medium containing no pyroxsulam:			
Frond number	EC50	**4.68 (1.85-11.8)**	**4.4 (1.17-.11.7) by maximum likelihood probit** **3.7 (3.0-4.2) by linear interpolation**
	NOEC	**<1.06**	**<1.06**
Mean specific growth rate (day^{-1})	ErC50	**17.2 (8.31-35.4)**	**17.0 (9.6-57) by maximum likelihood logic** **15.7 (9.5-33) by linear interpolation**
	NOEC	**1.06**	**1.06**
Biomass (frond dry weight)	EbC50	**7.45 (3.06-18.16**	**7.3 (3.5-17.4 by, maximum likelihood logit** **5.7 (4.1-7.4) by linear interpolation**
	NOEC	**<1.06**	**<1.06**

*** Reviewer calculated EC50 results are based on linear interpolation unless stated otherwise.**

The study report and reviewer calculated EC50 values from a one day exposure to pyroxsulam classify pyroxsulam as, at worst, very highly toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment, Water, Heritage and the Arts (EC50 <100 µg/L).

Similarly, the study report and reviewer calculated EC50 values from the three days of exposure classify pyroxsulam as very highly toxic to the duckweed *Lemna gibba* according to the classification scheme of the Australian Government Department of the Environment, Water, Heritage and the Arts (EC50 <100 µg/L).

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Approved 04/01/01 C.K.

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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.	**$NaNO_3$**	**12.75 g/500 mL**	**0.51 g/L**	A1	**$NaNO_3$**	**26 g/L**	**0.52 g/L**
	$MgCl_2 \cdot 6H_2O$	**6.08 g/500 mL**	**0.24 g/L**		**$MgCl_2 \cdot 6H_2O$**	**12 g/L**	**0.24 g/L**
	$CaCl_2 \cdot 2H_2O$	**2.20 g/500 mL**	**0.088 g/L**		**$CaCl_2 \cdot 2H_2O$**	**4.4 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**	**1.86 g/L**	**0.0037 g/L**	B	**H_3BO_3**	**0.19 g/L**	**0.0038 g/L**
	$MnCl_2 \cdot 4H_2O$	**4.16 g/L**	**0.0083 g/L**	B	**$MnCl_2 \cdot 4H_2O$**	**0.42 g/L**	**0.0084 g/L**
	$ZnCl_2$	**0.0327 g/L**	**0.065 mg/L**	B	**$ZnCl_2$**	**3.3 mg/L**	**0.066 mg/L**
	$Na_2MoO_4 \cdot 2H_2O$	**0.0726 g/L**	**0.145 mg/L**	B	**$Na_2MoO_4 \cdot 2H_2O$**	**7.3 mg/L**	**0.146 mg/L**
C2.	**$CoCl_2 \cdot 6H_2O$**	**2.86 g/L**	**See below**	B	**$CoCl_2 \cdot 6H_2O$**	**1.4 mg/L**	
	$CuCl_2 \cdot 2H_2O$	**0.022 g/L**	**under C3.**	B	**$CuCl_2 \cdot 2H_2O$**	**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**				**0.028 mg $CoCl_2 \cdot 6H_2O$ /L**
			0.00022 mg $CuCl_2 \cdot 2H_2O$ /L				**0.00024 mg $CuCl_2 \cdot 2H_2O$ /L**
D.	**$FeCl_3 \cdot 6H_2O$**	**0.16 g/L**	**0.0032 g/L**	B	**$FeCl_3 \cdot 6H_2O$**	**0.16 g/L**	**0.0032 g/L**
	$Na_2EDTA.2H_20$	**0.30 g/L**	**0.006 g/L**	B	**$Na_2EDTA.2H_20$**	**0.30 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:

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.
(Information from the study report)

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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.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Frond number at 7 days after one day of exposure to pyroxsulam (1)

The ToxCalc calculations for the frond counts (untransformed) following one day of exposure of duckweed to pyroxsulam were as follows with frond count numbers at 7 days (168 hours) also shown. EC50 values etc. are reported as µg pyroxsulam/L. Maximum likelihood probit was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	217.00	181.00	215.00
S-Control	175.00	167.00	170.00
1.06	172.00	151.00	140.00
2.21	158.00	145.00	147.00
4.28	130.00	134.00	146.00
8.64	126.00	110.00	103.00
15.9	110.00	104.00	100.00
31.2	102.00	81.00	99.00

			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	204.33	1.1973	204.33	181.00	217.00	9.901	3					
S-Control	170.67	1.0000	170.67	167.00	175.00	2.368	3	*			170.67	0.0000
1.06	154.33	0.9043	154.33	140.00	172.00	10.535	3	2.011	2.530	20.54	154.33	0.0957
*2.21	150.00	0.8789	150.00	145.00	158.00	4.667	3	2.545	2.530	20.54	150.00	0.1211
*4.28	136.67	0.8008	136.67	130.00	146.00	6.093	3	4.187	2.530	20.54	136.67	0.1992
*8.64	113.00	0.6621	113.00	103.00	126.00	10.433	3	7.102	2.530	20.54	113.00	0.3379
*15.9	104.67	0.6133	104.67	100.00	110.00	4.809	3	8.128	2.530	20.54	104.67	0.3867
*31.2	94.00	0.5508	94.00	81.00	102.00	12.083	3	9.442	2.530	20.54	94.00	0.4492

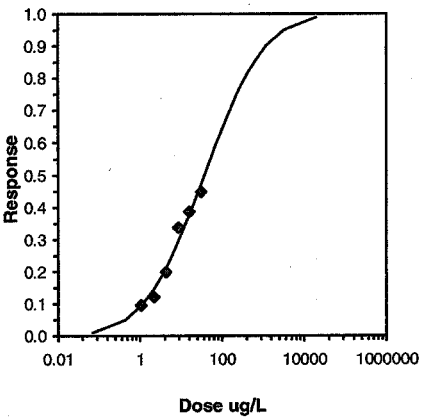
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96923	0.873	0.29562	-0.2973
Bartlett's Test indicates equal variances (p = 0.62)	4.42097	16.8119		
The control means are significantly different (p = 0.05)	2.82635	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1.06	2.21	1.53056		20.5439	0.12037	2445.86	98.9048	1.1E-06	6, 14
Treatments vs S-Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.852932	0.513736	-0.15399	1.85985	0	0.12501	9.48773	1	1.5713	1.17243	3
Intercept	3.659791	0.563635	2.555066	4.76452							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits
EC01	2.674	0.069795	
EC05	3.355	0.439366	
EC10	3.718	1.171579	
EC15	3.964	2.270672	
EC20	4.158	3.841998	
EC25	4.326	6.032626	
EC40	4.747	18.80464	
EC50	5.000	37.26454	
EC60	5.253	73.84592	
EC75	5.674	230.1892	
EC80	5.842	361.4384	
EC85	6.036	611.5574	
EC90	6.282	1185.277	
EC95	6.645	3160.565	
EC99	7.326	19896.04	



The 2.21 to 31.2 µg/L means for frond numbers 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).

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Frond number at 7 days after one day of exposure to pyroxsulam (2)

The ToxCalc calculations for the frond counts (untransformed) following one day of exposure of duckweed to pyroxsulam were as follows with frond count numbers at 7 days (168 hours) also shown. EC50 values etc. are reported as µg pyroxsulam/L. Linear interpolation was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	217.00	181.00	215.00
S-Control	175.00	167.00	170.00
1.06	172.00	151.00	140.00
2.21	158.00	145.00	147.00
4.28	130.00	134.00	146.00
8.64	126.00	110.00	103.00
15.9	110.00	104.00	100.00
31.2	102.00	81.00	99.00

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	204.33	1.1973	204.33	181.00	217.00	9.901	3				170.67	1.0000
S-Control	170.67	1.0000	170.67	167.00	175.00	2.368	3	*			170.67	1.0000
1.06	154.33	0.9043	154.33	140.00	172.00	10.535	3	2.011	2.530	20.54	154.33	0.9043
*2.21	150.00	0.8789	150.00	145.00	158.00	4.667	3	2.545	2.530	20.54	150.00	0.8789
*4.28	136.67	0.8008	136.67	130.00	146.00	6.093	3	4.187	2.530	20.54	136.67	0.8008
*8.64	113.00	0.6621	113.00	103.00	126.00	10.433	3	7.102	2.530	20.54	113.00	0.6621
*15.9	104.67	0.6133	104.67	100.00	110.00	4.809	3	8.128	2.530	20.54	104.67	0.6133
*31.2	94.00	0.5508	94.00	81.00	102.00	12.083	3	9.442	2.530	20.54	94.00	0.5508

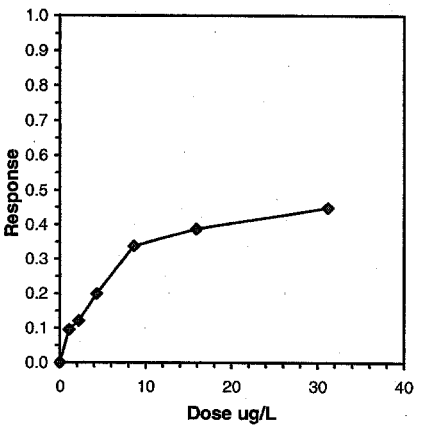
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96923	0.873	0.29562	-0.2973
Bartlett's Test indicates equal variances (p = 0.62)	4.42097	16.8119		
The control means are significantly different (p = 0.05)	2.82635	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs S-Control	1.06	2.21	1.53056		20.5439	0.12037	2445.86	98.9048	1.1E-06	6, 14

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	0.554	0.351	0.207	2.129	1.0628
IC10	1.255	0.535	0.326	2.980	0.3867
IC15	2.976	0.645	0.000	5.038	-0.2533
IC20	4.305	0.574	2.864	6.100	0.5143
IC25	5.877	0.653	4.141	8.145	0.4157
IC40	19.151				
IC50	>31.2				

* indicates IC estimate less than the lowest concentration



The 2.21 to 31.2 µg/L means for frond numbers 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).

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Frond number at 7 days after three days of exposure to pyroxsulam (1)

The ToxCalc calculations for the untransformed frond counts following one day of exposure of duckweed to pyroxsulam were as follows with frond count numbers at 7 days (168 hours) also shown. EC50 values etc. are reported as µg pyroxsulam/L. Maximum likelihood probit was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	231.00	213.00	199.00
S-Control	168.00	182.00	202.00
1.06	173.00	173.00	160.00
2.21	123.00	141.00	155.00
4.28	85.00	79.00	86.00
8.64	55.00	58.00	63.00
15.9	60.00	38.00	50.00
31.3	38.00	47.00	41.00

			Transform: Untransformed					1-Tailed				
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	199.17	1.0000	199.17	168.00	231.00	11.179	6				199.17	0.0000
*1.06	168.67	0.8469	168.67	160.00	173.00	4.450	3	3.027	2.490	25.09	168.67	0.1531
*2.21	139.67	0.7013	139.67	123.00	155.00	11.486	3	5.905	2.490	25.09	139.67	0.2987
*4.28	83.33	0.4184	83.33	79.00	86.00	4.543	3	11.495	2.490	25.09	83.33	0.5816
*8.64	58.67	0.2946	58.67	55.00	63.00	6.889	3	13.943	2.490	25.09	58.67	0.7054
*15.9	49.33	0.2477	49.33	38.00	60.00	22.328	3	14.870	2.490	25.09	49.33	0.7523
*31.3	42.00	0.2109	42.00	38.00	47.00	10.911	3	15.597	2.490	25.09	42.00	0.7891

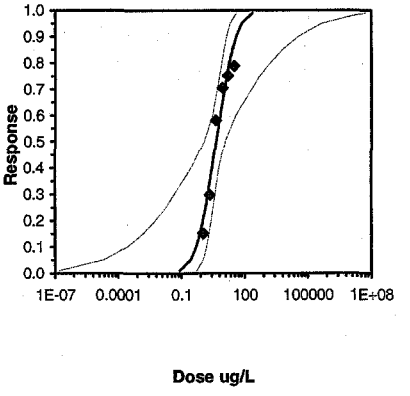
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94236	0.884	-0.0548	2.23954
Bartlett's Test indicates equal variances (p = 0.07)	11.5271	16.8119		
The control means are not significantly different (p = 0.09)	2.24162	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<1.06	1.06			25.0903	0.12598	15712	203.069	2.1E-11	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	1.347712	0.524152	0.320374	2.37505	0	0.7387	9.48773	0.95	0.64376	0.742	3
Intercept	4.132394	0.426294	3.296859	4.96793							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	0.082721	1.48E-07	0.53076
EC05	3.355	0.265023	1.92E-05	1.06215
EC10	3.718	0.493007	0.000253	1.55763
EC15	3.964	0.749428	0.001427	2.0383
EC20	4.158	1.045395	0.005585	2.55176
EC25	4.326	1.390887	0.017771	3.13466
EC40	4.747	2.856136	0.287588	6.0116
EC50	5.000	4.403136	1.167379	11.6951
EC60	5.253	6.788053	2.947657	36.576
EC75	5.674	13.93902	6.527776	512.58
EC80	5.842	18.54572	8.160885	1602.64
EC85	6.036	25.86986	10.33867	6197.43
EC90	6.282	39.32522	13.64476	34674.9
EC95	6.645	73.15445	20.14308	454795
EC99	7.326	234.3736	40.52564	5.9E+07



The 1.06 to 31.2 µg/L means for frond numbers 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).

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Frond number at 7 days after three days of exposure to pyroxsulam (2)

The ToxCalc calculations for the untransformed frond counts following one day of exposure of duckweed to pyroxsulam were as follows with frond count numbers at 7 days (168 hours) also shown. EC50 values etc. are reported as µg pyroxsulam/L. Linear interpolation was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	231.00	213.00	199.00
S-Control	168.00	182.00	202.00
1.06	173.00	173.00	160.00
2.21	123.00	141.00	155.00
4.28	85.00	79.00	86.00
8.64	55.00	58.00	63.00
15.9	60.00	38.00	50.00
31.2	38.00	47.00	41.00

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	199.17	1.0000	199.17	168.00	231.00	11.179	6				199.17	1.0000
*1.06	168.67	0.8469	168.67	160.00	173.00	4.450	3	3.027	2.490	25.09	168.67	0.8469
*2.21	139.67	0.7013	139.67	123.00	155.00	11.486	3	5.905	2.490	25.09	139.67	0.7013
*4.28	83.33	0.4184	83.33	79.00	86.00	4.543	3	11.495	2.490	25.09	83.33	0.4184
*8.64	58.67	0.2946	58.67	55.00	63.00	6.889	3	13.943	2.490	25.09	58.67	0.2946
*15.9	49.33	0.2477	49.33	38.00	60.00	22.328	3	14.870	2.490	25.09	49.33	0.2477
*31.2	42.00	0.2109	42.00	38.00	47.00	10.911	3	15.597	2.490	25.09	42.00	0.2109

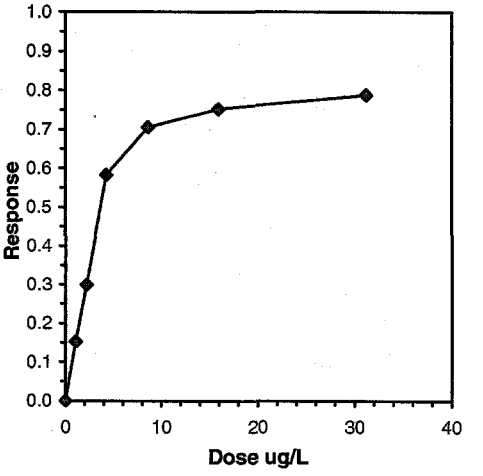
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94236	0.884	-0.0548	2.23954
Bartlett's Test indicates equal variances (p = 0.07)	11.5271	16.8119		
The control means are not significantly different (p = 0.09)	2.24162	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<1.06	1.06			25.0903	0.12598	15712	203.069	2.1E-11	6, 17
Treatments vs Pooled Controls										

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	0.3461	0.1150	0.1530	0.8142	1.6037
IC10*	0.6922	0.2011	0.3060	1.4576	0.9485
IC15*	1.0383	0.2446	0.4591	1.8529	0.6305
IC20	1.4301	0.2952	0.5847	2.3697	0.3740
IC25	1.8250	0.3085	0.8913	2.7803	0.0962
IC40	2.9510	0.2590	1.9237	3.5712	-0.5177
IC50	3.6829	0.1818	2.9928	4.1664	-0.4610

* indicates IC estimate less than the lowest concentration



The 1.06 to 31.2 µg/L means for frond numbers 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).

US EPA ARCHIVE DOCUMENT

Specific growth rate at 7 days (168 hours) after a one day exposure to pyroxsulam (1)

The ToxCalc calculations for the specific growth rates following one day of duckweed exposure to pyroxsulam were as follows with the 1 to 7 days specific growth rates (days^{-1}) also shown. EC50 values etc. are reported as µg pyroxsulam/L. Maximum likelihood probit was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	0.4136	0.3877	0.4123
S-Control	0.3828	0.3762	0.3787
1.06	0.3804	0.3618	0.3510
2.21	0.3682	0.3560	0.3579
4.28	0.3404	0.3447	0.3570
8.64	0.3359	0.3165	0.3071
15.9	0.3165	0.3085	0.3029
31.3	0.3057	0.2728	0.3015

			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.4045	1.0666	0.4045	0.3877	0.4136	3.608	3					
S-Control	0.3792	1.0000	0.3792	0.3762	0.3828	0.889	3	*			0.3792	0.0000
1.06	0.3644	0.9608	0.3644	0.3510	0.3804	4.083	3	1.577	2.530	0.0239	0.3644	0.0392
2.21	0.3607	0.9512	0.3607	0.3560	0.3682	1.826	3	1.964	2.530	0.0239	0.3607	0.0488
*4.28	0.3473	0.9159	0.3473	0.3404	0.3570	2.476	3	3.382	2.530	0.0239	0.3473	0.0841
*8.64	0.3198	0.8434	0.3198	0.3071	0.3359	4.590	3	6.299	2.530	0.0239	0.3198	0.1566
*15.9	0.3093	0.8156	0.3093	0.3029	0.3165	2.213	3	7.418	2.530	0.0239	0.3093	0.1844
*31.3	0.2933	0.7735	0.2933	0.2728	0.3057	6.106	3	9.113	2.530	0.0239	0.2933	0.2265

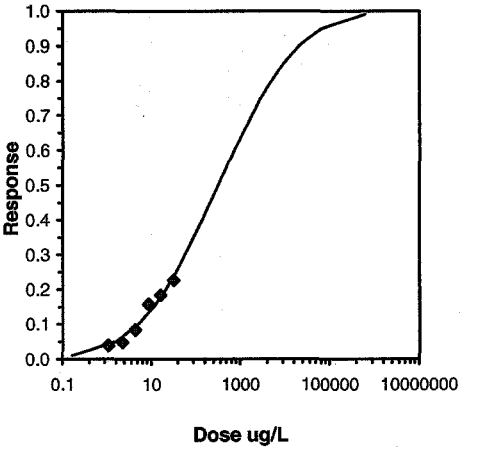
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96531	0.873	-0.0942	-0.2824
Bartlett's Test indicates equal variances (p = 0.47)	5.63377	16.8119		
The control means are significantly different (p = 0.04)	2.92116	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs S-Control	2.21	4.28	3.07552		0.02385	0.06289	0.00307	0.00013	1.7E-06	6, 14

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.708633	0.556424	-0.38196	1.79922	0	0.07814	9.48773	1	2.50297	1.41117	3
Intercept	3.226316	0.659629	1.933442	4.51919							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits
EC01	2.674	0.165997	
EC05	3.355	1.519854	
EC10	3.718	4.948594	
EC15	3.964	10.9745	
EC20	4.158	20.66794	
EC25	4.326	35.57523	
EC40	4.747	139.7818	
EC50	5.000	318.3946	
EC60	5.253	725.2386	
EC75	5.674	2849.598	
EC80	5.842	4904.945	
EC85	6.036	9237.337	
EC90	6.282	20485.63	
EC95	6.645	66700.54	
EC99	7.326	610706.4	



The 4.28 to 31.2 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).

US EPA ARCHIVE DOCUMENT

Specific growth rate at 7 days (168 hours) after a one day exposure to pyroxsulam (2)

The ToxCalc calculations for the specific growth rates following one day of duckweed exposure to pyroxsulam were as follows with the 1 to 7 days specific growth rates ($days^{-1}$) data also shown. EC50 values etc. are reported as µg pyroxsulam/L. Linear interpolation was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	0.4136	0.3877	0.4123
S-Control	0.3828	0.3762	0.3787
1.06	0.3804	0.3618	0.3510
2.21	0.3682	0.3560	0.3579
4.28	0.3404	0.3447	0.3570
8.64	0.3359	0.3165	0.3071
15.9	0.3165	0.3085	0.3029
31.3	0.3057	0.2728	0.3015

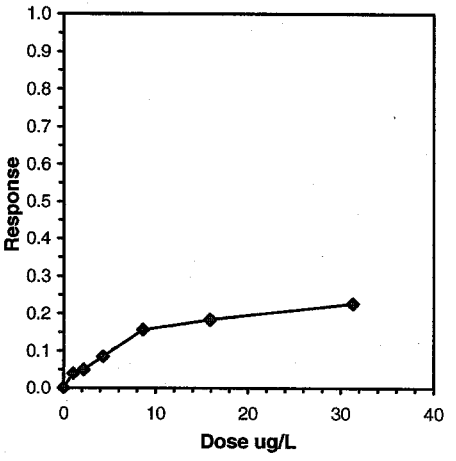
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.4045	1.0666	0.4045	0.3877	0.4136	3.608	3					
S-Control	0.3792	1.0000	0.3792	0.3762	0.3828	0.889	3	*			0.3792	1.0000
1.06	0.3644	0.9608	0.3644	0.3510	0.3804	4.083	3	1.577	2.530	0.0239	0.3644	0.9608
2.21	0.3607	0.9512	0.3607	0.3560	0.3682	1.826	3	1.964	2.530	0.0239	0.3607	0.9512
*4.28	0.3473	0.9159	0.3473	0.3404	0.3570	2.476	3	3.382	2.530	0.0239	0.3473	0.9159
*8.64	0.3198	0.8434	0.3198	0.3071	0.3359	4.590	3	6.299	2.530	0.0239	0.3198	0.8434
*15.9	0.3093	0.8156	0.3093	0.3029	0.3165	2.213	3	7.418	2.530	0.0239	0.3093	0.8156
*31.3	0.2933	0.7735	0.2933	0.2728	0.3057	6.106	3	9.113	2.530	0.0239	0.2933	0.7735

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96531	0.873	-0.0942	-0.2824
Bartlett's Test indicates equal variances (p = 0.47)	5.63377	16.8119		
The control means are significantly different (p = 0.04)	2.92116	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs S-Control	2.21	4.28	3.07552		0.02385	0.06289	0.00307	0.00013	1.7E-06	6, 14

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05	2.279	0.747	0.000	3.654	-0.1092
IC10	5.237	0.621	3.549	7.125	0.0663
IC15	8.243	1.532	6.205	14.615	1.1155
IC20	21.600				
IC25	>31.3				
IC40	>31.3				
IC50	>31.3				



The 4.28 to 31.2 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).

US EPA ARCHIVE DOCUMENT

Specific growth rate at 7 days (168 hours) after a three day exposure to pyroxsulam (1)

The ToxCalc calculations for the specific growth rates (untransformed data) following a three day exposure of duckweed to pyroxsulam were as follows with the 1 to 7 days specific growth rates ($days^{-1}$) data also shown. EC50 values etc. are reported as µg pyroxsulam/L. Maximum likelihood logit used to determine EC50 and 95% confidence limits. Maximum likelihood probit results from the ToxCalc program gave an ErC50 of 17.0 µg pyroxsulam/L, equivalent to the logit ErC50 value but with wider 95% confidence limits (6.12 to 5E+08). The NOEC was 1.06 µg pyroxsulam/L (ToxCalc maximum likelihood probit results not presented in this DER).

Conc-ug/L	1	2	3
D-Control	0.4225	0.4109	0.4012
S-Control	0.3770	0.3884	0.4033
1.06	0.3812	0.3812	0.3700
2.21	0.3325	0.3520	0.3655
4.28	0.2797	0.2692	0.2814
8.64	0.2175	0.2251	0.2369
15.9	0.2299	0.1647	0.2039
31.3	0.1647	0.1950	0.1752

			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	0.4006	1.0000	0.4006	0.3770	0.4225	4.023	6				0.4006	0.0000
1.06	0.3775	0.9424	0.3775	0.3700	0.3812	1.707	3	1.934	2.490	0.0297	0.3775	0.0576
*2.21	0.3500	0.8737	0.3500	0.3325	0.3655	4.745	3	4.238	2.490	0.0297	0.3500	0.1263
*4.28	0.2768	0.6909	0.2768	0.2692	0.2814	2.376	3	10.374	2.490	0.0297	0.2768	0.3091
*8.64	0.2265	0.5654	0.2265	0.2175	0.2369	4.316	3	14.586	2.490	0.0297	0.2265	0.4346
*15.9	0.1995	0.4980	0.1995	0.1647	0.2299	16.465	3	16.848	2.490	0.0297	0.1995	0.5020
*31.3	0.1783	0.4451	0.1783	0.1647	0.1950	8.644	3	18.623	2.490	0.0297	0.1783	0.5549

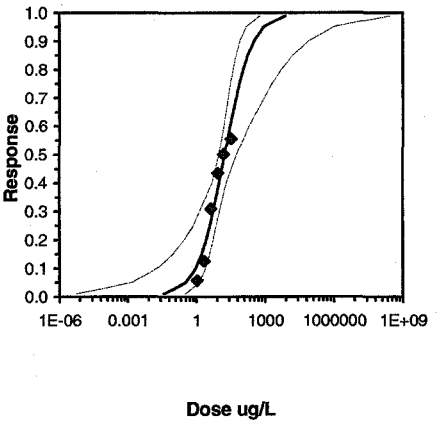
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98221	0.884	-0.2615	0.62819
Bartlett's Test indicates equal variances (p = 0.34)	6.84309	16.8119		
The control means are not significantly different (p = 0.09)	2.2391	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs Pooled Controls	1.06	2.21	1.53056		0.02972	0.07419	0.02979	0.00028	1.8E-12	6, 17

Maximum Likelihood-Logit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.723231	0.536015	0.672642	2.77382	0	0.31829	9.48773	0.99			8
Intercept	-2.12128	0.577934	-3.25403	-0.9885							
TSCR											

Point	Logits	ug/L	95% Fiducial Limits	
EC01	-4.595	0.036679	5.52E-06	0.32133
EC05	-2.944	0.332903	0.001524	1.3038
EC10	-2.197	0.903504	0.019071	2.50104
EC15	-1.735	1.67646	0.089709	3.80371
EC20	-1.386	2.670037	0.28253	5.31332
EC25	-1.099	3.921576	0.710389	7.18352
EC40	-0.405	9.90142	4.796994	20.2879
EC50	0.000	17.02123	9.607353	56.8294
EC60	0.405	29.26068	15.54395	197.054
EC75	1.099	73.87904	30.39241	1922.01
EC80	1.386	108.5087	39.36231	5044.78
EC85	1.735	172.818	53.47359	16337
EC90	2.197	320.6649	79.77883	78338.5
EC95	2.944	870.2895	150.8536	994381
EC99	4.595	7898.834	605.5142	2.8E+08



The 4.28

The 2.21 to 31.2 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).

Specific growth rate at 7 days (168 hours) after a three day exposure to pyroxsulam (2)

US EPA ARCHIVE DOCUMENT

The ToxCalc calculations for the specific growth rates (untransformed data) following a three day exposure of duckweed to pyroxsulam were as follows with the 1 to 7 days specific growth rates ($days^{-1}$) data also shown. EC50 values etc. are reported as µg pyroxsulam/L. Linear interpolation was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	0.4225	0.4109	0.4012
S-Control	0.3770	0.3884	0.4033
1.06	0.3812	0.3812	0.3700
2.21	0.3325	0.3520	0.3655
4.28	0.2797	0.2692	0.2814
8.64	0.2175	0.2251	0.2369
15.9	0.2299	0.1647	0.2039
31.2	0.1647	0.1950	0.1752

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	0.4006	1.0000	0.4006	0.3770	0.4225	4.023	6				0.4006	1.0000
1.06	0.3775	0.9424	0.3775	0.3700	0.3812	1.707	3	1.934	2.490	0.0297	0.3775	0.9424
*2.21	0.3500	0.8737	0.3500	0.3325	0.3655	4.745	3	4.238	2.490	0.0297	0.3500	0.8737
*4.28	0.2768	0.6909	0.2768	0.2692	0.2814	2.376	3	10.374	2.490	0.0297	0.2768	0.6909
*8.64	0.2265	0.5654	0.2265	0.2175	0.2369	4.316	3	14.586	2.490	0.0297	0.2265	0.5654
*15.9	0.1995	0.4980	0.1995	0.1647	0.2299	16.465	3	16.848	2.490	0.0297	0.1995	0.4980
*31.2	0.1783	0.4451	0.1783	0.1647	0.1950	8.644	3	18.623	2.490	0.0297	0.1783	0.4451

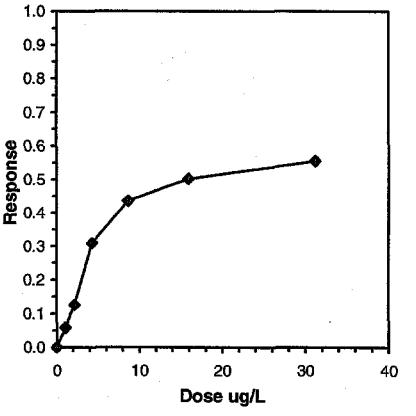
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98221	0.884	-0.2615	0.62819
Bartlett's Test indicates equal variances (p = 0.34)	6.84309	16.8119		
The control means are not significantly different (p = 0.09)	2.2391	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs Pooled Controls	1.06	2.21	1.53056		0.02972	0.07419	0.02979	0.00028	1.8E-12	6, 17

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	0.920	0.228	0.364	1.677	0.5377
IC10	1.770	0.295	0.925	2.684	0.1858
IC15	2.479	0.235	1.639	3.045	-0.3314
IC20	3.045	0.194	2.322	3.537	-0.4883
IC25	3.611	0.158	3.060	4.037	-0.2956
IC40	7.438	0.417	6.337	8.883	0.5705
IC50	15.686	4.399	9.504	32.721	0.6536

* indicates IC estimate less than the lowest concentration



The 2.21 to 31.2 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).

US EPA ARCHIVE DOCUMENT

Biomass (Frond dry weight, as mg) at 7 days (168 hours) after one day of exposure to pyroxsulam (1)

The ToxCalc calculations for the biomass (untransformed frond dry weights) following a one day exposure of duckweed to pyroxsulam were as follows with the 1 to 7 days biomass values (as mg) data also shown. EC50 values etc. are reported as µg pyroxsulam/L. Maximum likelihood probit was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	26.310	22.720	26.870
S-Control	23.950	20.970	23.160
1.06	24.480	21.970	21.710
2.21	22.420	19.340	20.150
4.28	19.700	19.660	20.320
8.64	18.580	15.950	16.020
15.9	16.700	17.250	17.730
31.3	16.180	14.220	16.450

			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	23.997	1.0000	23.997	20.970	26.870	9.337	6				23.997	0.0000
1.06	22.720	0.9468	22.720	21.710	24.480	6.733	3	1.133	2.490	2.805	22.720	0.0532
*2.21	20.637	0.8600	20.637	19.340	22.420	7.737	3	2.983	2.490	2.805	20.637	0.1400
*4.28	19.893	0.8290	19.893	19.660	20.320	1.860	3	3.643	2.490	2.805	19.893	0.1710
*8.64	16.850	0.7022	16.850	15.950	18.580	8.894	3	6.345	2.490	2.805	16.850	0.2978
*15.9	17.227	0.7179	17.227	16.700	17.730	2.992	3	6.010	2.490	2.805	17.227	0.2821
*31.3	15.617	0.6508	15.617	14.220	16.450	7.793	3	7.440	2.490	2.805	15.617	0.3492

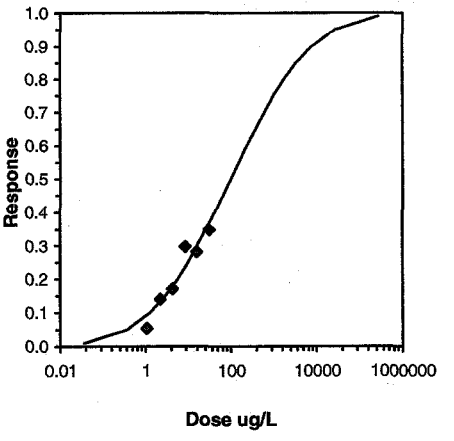
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96044	0.884	0.27761	0.13586
Bartlett's Test indicates equal variances (p = 0.31)	7.16473	16.8119		
The control means are not significantly different (p = 0.17)	1.65367	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs Pooled Controls	1.06	2.21	1.53056		2.80474	0.11688	38.2398	2.53755	5.8E-06	6, 17

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.677189	0.500867	-0.30451	1.65889	0	0.24452	9.48773	0.99	1.9919	1.47669	3
Intercept	3.651108	0.552999	2.567229	4.73499							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits
EC01	2.674	0.036025	
EC05	3.355	0.365557	
EC10	3.718	1.257305	
EC15	3.964	2.893374	
EC20	4.158	5.611541	
EC25	4.326	9.905669	
EC40	4.747	41.47456	
EC50	5.000	98.1516	
EC60	5.253	232.2807	
EC75	5.674	972.5478	
EC80	5.842	1716.772	
EC85	6.036	3329.587	
EC90	6.282	7662.209	
EC95	6.645	26353.56	
EC99	7.326	267421.9	



The 2.21 to 31.2 µg/L means for biomass 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).

US EPA ARCHIVE DOCUMENT

Biomass (Frond dry weight, as mg) at 7 days (168 hours) after one day of exposure to pyroxsulam (2)

The ToxCalc calculations for the biomass (untransformed frond dry weights) following a one day exposure of duckweed to pyroxsulam were as follows with the 1 to 7 days biomass values (as mg) data also shown. EC50 values etc. are reported as µg pyroxsulam/L. Linear interpolation was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	26.310	22.720	26.870
S-Control	23.950	20.970	23.160
1.06	24.480	21.970	21.710
2.21	22.420	19.340	20.150
4.28	19.700	19.660	20.320
8.64	18.580	15.950	16.020
15.9	16.700	17.250	17.730
31.3	16.180	14.220	16.450

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	23.997	1.0000	23.997	20.970	26.870	9.337	6				23.997	1.0000
1.06	22.720	0.9468	22.720	21.710	24.480	6.733	3	1.133	2.490	2.805	22.720	0.9468
*2.21	20.637	0.8600	20.637	19.340	22.420	7.737	3	2.983	2.490	2.805	20.637	0.8600
*4.28	19.893	0.8290	19.893	19.660	20.320	1.860	3	3.643	2.490	2.805	19.893	0.8290
*8.64	16.850	0.7022	16.850	15.950	18.580	8.894	3	6.345	2.490	2.805	17.038	0.7100
*15.9	17.227	0.7179	17.227	16.700	17.730	2.992	3	6.010	2.490	2.805	17.038	0.7100
*31.3	15.617	0.6508	15.617	14.220	16.450	7.793	3	7.440	2.490	2.805	15.617	0.6508

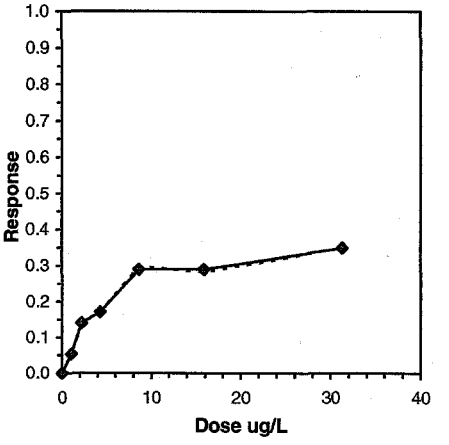
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96044	0.884	0.27761	0.13586
Bartlett's Test indicates equal variances (p = 0.31)	7.16473	16.8119		
The control means are not significantly different (p = 0.17)	1.65367	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1.06	2.21	1.53056		2.80474	0.11688	38.2398	2.53755	5.8E-06	6, 17
Treatments vs Pooled Controls										

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	0.9962	0.4446	0.0293	2.3254	0.9349
IC10	1.6799	0.6612	0.2462	4.4217	1.3674
IC15	2.8769	1.2261	0.3435	7.2440	0.6339
IC20	5.3429	1.4180	0.0009	9.0496	0.5120
IC25	7.1752	3.0491	4.2963	24.6291	2.9728
IC40	>31.3				
IC50	>31.3				

* indicates IC estimate less than the lowest concentration



The 2.21 to 31.2 µg/L means for biomass 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).

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Biomass (Frond dry weight, as mg) at 7 days (168 hours) after three days of exposure to pyroxsulam (1)

The ToxCalc calculations for the biomass (untransformed frond dry weights) following a three day exposure of duckweed to pyroxsulam were as follows with the 1 to 7 days biomass values (as mg) data also shown. EC50 values etc. are reported as µg pyroxsulam/L. Maximum likelihood logit was used to determine EC50 and 95% confidence limits. Maximum likelihood probit results from the ToxCalc program gave an EbC50 of 7.36 µg pyroxsulam/L, equivalent to the logit ErC50 value but with wider 95% confidence limits (0.478 to 7466). The NOEC was <1.06 µg pyroxsulam/L (ToxCalc maximum likelihood probit results not presented in this DER).

Conc-ug/L	1	2	3
D-Control	28.620	27.630	26.010
S-Control	24.870	25.150	28.640
1.06	23.660	21.890	21.450
2.21	18.930	17.220	22.290
4.28	14.170	14.230	14.760
8.64	10.840	11.090	12.230
15.9	11.680	9.000	8.690
31.3	8.710	9.860	8.220

			Transform: Untransformed						1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	26.820	1.0000	26.820	24.870	28.640	6.340	6				26.820	0.0000
*1.06	22.333	0.8327	22.333	21.450	23.660	5.238	3	4.209	2.490	2.654	22.333	0.1673
*2.21	19.480	0.7263	19.480	17.220	22.290	13.241	3	6.886	2.490	2.654	19.480	0.2737
*4.28	14.387	0.5364	14.387	14.170	14.760	2.257	3	11.664	2.490	2.654	14.387	0.4636
*8.64	11.387	0.4246	11.387	10.840	12.230	6.507	3	14.479	2.490	2.654	11.387	0.5754
*15.9	9.790	0.3650	9.790	8.690	11.680	16.794	3	15.977	2.490	2.654	9.790	0.6350
*31.3	8.930	0.3330	8.930	8.220	9.860	9.427	3	16.783	2.490	2.654	8.930	0.6670

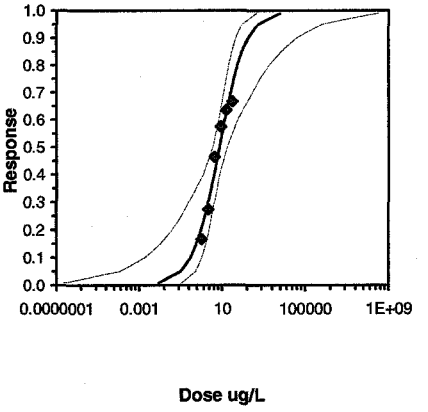
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96256	0.884	0.38429	-0.3711
Bartlett's Test indicates equal variances (p = 0.29)	7.34528	16.8119		
The control means are not significantly different (p = 0.45)	0.83825	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs Pooled Controls	<1.06	1.06			2.65419	0.09896	190.48	2.27245	1.1E-11	6, 17

Maximum Likelihood-Logit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.574337	0.492638	0.608767	2.53991	0	0.22092	9.48773	0.99			7
Intercept	-1.36243	0.459066	-2.2622	-0.4627							
TSCR											

Point	Logits	ug/L	95% Fiducial Limits	
EC01	-4.595	0.008844	2.34E-07	0.11476
EC05	-2.944	0.098885	0.000117	0.52661
EC10	-2.197	0.294951	0.001925	1.06376
EC15	-1.735	0.580234	0.010785	1.66198
EC20	-1.386	0.965703	0.039021	2.35193
EC25	-1.099	1.470876	0.111324	3.17662
EC40	-0.405	4.05373	1.196345	7.62446
EC50	0.000	7.335021	3.51565	17.3676
EC60	0.405	13.27236	7.089548	57.6512
EC75	1.099	36.57857	15.98549	659.495
EC80	1.386	55.71331	21.43296	1895.33
EC85	1.735	92.72553	30.18166	6891.43
EC90	2.197	182.4114	46.99046	38736.4
EC95	2.944	544.0936	94.6678	639056
EC99	4.595	6083.691	433.5331	3.2E+08



The 1.06 to 31.2 µg/L means for biomass 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).

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Biomass (Frond dry weight, as mg) at 7 days (168 hours) after three days of exposure to pyroxsulam (2)

The ToxCalc calculations for the biomass (untransformed frond dry weights) following a three day exposure of duckweed to pyroxsulam were as follows with the 1 to 7 days biomass values (as mg) data also shown. EC50 values etc. are reported as µg pyroxsulam/L. Linear interpolation was used to determine EC50 and 95% confidence limits.

Conc-ug/L	1	2	3
D-Control	28.620	27.630	26.010
S-Control	24.870	25.150	28.640
1.06	23.660	21.890	21.450
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8.64	10.840	11.090	12.230
15.9	11.680	9.000	8.690
31.2	8.710	9.860	8.220

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	26.820	1.0000	26.820	24.870	28.640	6.340	6				26.820	1.0000
*1.06	22.333	0.8327	22.333	21.450	23.660	5.238	3	4.209	2.490	2.654	22.333	0.8327
*2.21	19.480	0.7263	19.480	17.220	22.290	13.241	3	6.886	2.490	2.654	19.480	0.7263
*4.28	14.387	0.5364	14.387	14.170	14.760	2.257	3	11.664	2.490	2.654	14.387	0.5364
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*15.9	9.790	0.3650	9.790	8.690	11.680	16.794	3	15.977	2.490	2.654	9.790	0.3650
*31.2	8.930	0.3330	8.930	8.220	9.860	9.427	3	16.783	2.490	2.654	8.930	0.3330

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96256	0.884	0.38429	-0.3711
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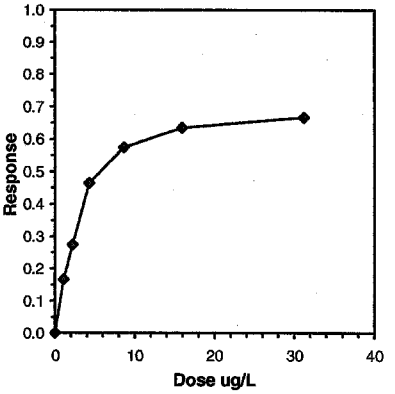
Dunnett's Test Treatments vs Pooled Controls	<1.06	1.06			2.65419	0.09896	190.48	2.27245	1.1E-11	6, 17

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	0.3168	0.0595	0.1883	0.5179	1.0891
IC10*	0.6336	0.1181	0.3767	1.0357	1.0297
IC15*	0.9505	0.2002	0.5650	1.6156	1.8363
IC20	1.4136	0.3366	0.6655	2.7005	0.8941
IC25	1.9541	0.3637	0.9073	3.0357	0.1901
IC40	3.5869	0.2662	2.4359	4.1385	-1.0067
IC50	5.6994	0.5245	4.1361	7.4003	0.0958

* indicates IC estimate less than the lowest concentration



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