Data Evaluation Report on the Acute Toxicity of the Sulfinic Acid Metabolite of Pyroxsulam (XDE-742) to Algae, *Pseudokirchneriella subcapitata*

PMRA Submission Number 2006-4727; ID 1283246 EPA MRID Number 469084-43 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE	Fresh water algae: 9.8.2
	EPA DP Barcode	D332116
	OECD Data Point	IIA 8.4
	EPA Guideline	850.5400 (123-2)

Test material:	**XDE sulfinic acid metabolite** (i.e. pyroxsulam sulfinic acid metabolite)	
Purity:	98%	
Common name:	Sulfinic acid Metabolite Pyroxsulam	
Chemical name:	3-pyridinesulfinic acid, 2-methoxy-4-(trifluoromethyl)-, lithium salt	
IUPAC:	2-methoxy-4-(trifluoromethyl) pyridine-3-sulfinic acid	
CAS name:	3-pyridinesulfinic acid, 2-methoxy-4-(trifluoromethyl)	
CAS No.:	Not listed	
Synonyms:	X11351479, TSN105138	

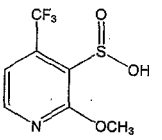
The metabolite is generally referred to as the sulfinic acid metabolite of pyroxsulam in this DER.

Note

Correspondence with Dow AgroSciences Australia Ltd confirmed that the study report's naming of the test substance as 3-pyridinesulfinic acid, 2-methoxy-3-(trifluoromethyl)-, lithium salt (in the Certificate of Analysis for Test/Reference/Control Substances, Appendix 2 of the study report) was an error, with the correct name being 3-pyridinesulfinic acid, 2-methoxy-4-(trifluoromethyl)-, lithium salt. Dow AgroSciences Australia Ltd advised that the lithium salt was used because of its being more stable in storage as that form rather than as the free acid.

With respect to testing the sulfinic, rather than sulfonic, acid metabolite, Dow AgroSciences Australia also advised that pyroxsulam was stable to hydrolysis with no metabolites being formed. However, the sulfinic acid was detected at greater than 10% (of the applied radioactivity) in an aqueous photolysis study (Byrne *et al.*, 2006) whereas the sulfonic acid was only detected as a minor metabolite (<10% of the applied radioactivity) in an aerobic soil degradation study (Yoder *et al.*, 2006). Consequently, the sulfonic acid was not subjected to the aquatic toxicity testing regime.

Chemical Structure:



(Chemical structure as given in Byrne *et al.*, 2006)

Primary Reviewer: Daryl Murphy D. Murphy 22/6/07 **Date:** 18 June 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland 22/6/08 **Date:** 19 June 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Émilie Larivière Émilie Rivière **Date:** 5 July 2007 05/07/08
Environmental Assessment Directorate, Pest Management Regulatory Agency, Health Canada

Christopher Salice C. J. Salice 4/9/08 **Date:** 12 September, 2007
Environmental Fate and Effects Division, US Environmental Protection Agency

Data Evaluation Report on the Acute Toxicity of the Sulfinic Acid Metabolite of Pyroxsulam (XDE-742) to Algae, *Pseudokirchneriella subcapitata*
PMRA Submission Number 2006-4727; ID 1283246 EPA MRID Number 469084-xx APVMA ATS 40362

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

CITATION: Hoberg, J. R. 2005. XDE-742 Sulfinic Acid Metabolite - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata.* Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6398 and Sponsor Protocol/Project No. 050110. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana 46268. 9 December 2005. Unpublished report.

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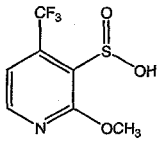

(Chemical structure as given in Byrne *et al.*, 2006)

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PMRA Submission Number 2006-4727; ID 1283246 EPA MRID Number 469084-43 APVMA ATS 40362

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

CITATION: Hoberg, J. R. 2005. XDE-742 Sulfinic Acid Metabolite - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6398 and Sponsor Protocol/Project No. 050110. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana 46268. 9 December 2005. Unpublished report.

EXECUTIVE SUMMARY:

The toxicity of the sulfinic acid metabolite of pyroxsulam (purity 98%) on the growth of the freshwater green alga, *Pseudokirchneriella subcapitata,* was investigated under static conditions for 96 hours. The experiment was carried out taking account of relevant OECD, European Communities and US EPA guidelines. Algae cultures were exposed to the sulfinic acid metabolite of pyroxsulam at nominal concentrations of 6.3, 13, 25, 50 and 100 mg/L. Mean measured concentrations were 6.4, 15, 25, 55 and 97 mg sulfinic acid metabolite of pyroxsulam/L. The growth and test medium used was Algal Assay Procedure (AAP) medium. Treatment groups were set in triplicate and the medium control group contained six replicates, with an initial cell density of approximately 10,000 cells/mL. Temperatures during the exposure period ranged from 24 to 26°C. The light intensity ranged from 7000 to 9100 lux. The pH values ranged from 6.9 to 7.1 at test initiation, and from 9.8 to 10.1 at test termination. Cell counts were conducted every 24 hours. The results were based on mean measured concentrations of sulfinic acid metabolite of pyroxsulam.

After 96 hours, inhibition of cell density relative to the control mean ranged from 8% at 15 mg sulfinic acid metabolite of pyroxsulam/L to 50% at 97 mg/L. Inhibition of biomass relative to controls at 72 hours ranged from -24% (i.e. growth stimulation occurred) at 15 mg sulfinic acid metabolite of pyroxsulam/L to 37% at 97 mg/L. Inhibition of mean specific growth rate relative to controls for the 0-72 hour period ranged from -6% at 15 mg sulfinic acid metabolite of pyroxsulam/L to 9% at 97 mg/L. The 96 hour NOEC for cell density and the 72 hour NOECs for total biomass and average growth rate were all set at 55 mg sulfinic acid metabolite of pyroxsulam/L. The 72-hour EC50 for mean specific growth rate (ErC50) and for biomass (area under the growth curve; EbC50) were both >97 mg sulfinic acid metabolite of pyroxsulam/L. The 96 hour cell density EC50 was 85 mg sulfinic acid metabolite of pyroxsulam/L. After 72 hours of exposure, cells exposed to mean measured concentrations of 55 and 97 mg sulfinic acid metabolite of pyroxsulam/L were recorded as "bloated". Cells exposed to all treatment levels tested were observed to be normal at 96 hours.

Deficiencies or deviations identified in the study, including an increase in the pH the controls of 3.2 units over the exposure period, are considered to have been of such a nature or degree as not to have adversely affected the study's conduct or outcomes.

Based on the results of this study, the sulfinic acid metabolite of pyroxsulam would be classified as slightly toxic to *Pseudokirchneriella subcapitata* in accordance with the classification system of the Australian Government Department of the Environment, Water, Heritage and the Arts (10 < EC50 ≤ 100 mg/L).

This study is classified as acceptable and satisfies the guideline requirements for an acute toxicity study with the unicellular green alga, *Pseudokirchneriella subcapitata.*

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Results Synopsis

Test Organism Size/Age: *Pseudokirchneriella subcapitata*
Test Type: Static

Statistical Endpoint	Cell Density				Growth Rate (0-72 h)	Biomass (0-72 h)
	24 h	48 h	72 h	96 h		
NOEC (mg sulfinic acid metabolite of pyroxsulam/L)	Not reported	Not reported	Not reported	55	55	55
EC_{50} (mg sulfinic acid metabolite of pyroxsulam/L) (95% C.I. in brackets except where this parameter was not calculated)	44 (8.8-49)	>97	>97	85 (72-96)	>97	>97

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
The toxicity test was reported as performed according to the Springborn Smithers Laboratories protocol entitled "96-Hour Acute Toxicity Test with Freshwater Green Alga, *Pseudokirchneriella subcapitata*", Springborn Smithers Laboratories Protocol No.: 072505/Pss.-STA/Recovery/Dow. The methods described in the protocol were also stated to meet the requirements specified in OECD and EC guidelines, namely:

> Guideline for Testing of Chemicals. Alga, Growth Inhibition Test #201. Adopted 7 June 1984. Organization for Economic Cooperation and Development. Paris, France, and
>
> The Official Journal of the European Communities. 1992. Methods for the determination of Ecotoxicity. C.3. Algal Inhibition Test. L383A Volume 35, 29 December 1992.

In addition, the procedures had been modified based on the Study Sponsor's request to meet the primary objectives of the U.S. EPA FIFRA Subdivision J Guideline 123-2, namely:

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

The study report stated that the following deviation from the protocol occurred:

1. The protocol stated that the test solution temperature was to be within the range of 24 ± 1°C. Based on the continuous recording minimum/maximum thermometer, the maximum solution temperature of 26 °C was recorded between the 48 and 72 hour observation intervals. The solution temperature was within the acceptable range on the remaining days. A deviation of 1°C was not considered by the study author as substantial enough to affect the outcome of the test.

2. The protocol stated that the 72-hour control solution pH should not have increased by more than 1.5 units from test initiation. During this study, the initial control solution pH was 6.9. The 72-hour control solution pH was 10.0, which exceeded the initial value by 3.1 units. The study author considered the increase in solution pH was due to photosynthesis by the algae and could not be controlled. The study report also noted that the 72-hour mean control cell density (106 x 10^4 cells/mL) exceeded the required 16 times increase from the initial density (1.0 x 10^4

cells/mL). Additionally, the study report noted that the analytical data indicated that the test substance concentrations remained stable during the test and were not affected by the increase in solution pH. For these reasons, the study author considered the growth of the algal population had not been affected by the magnitude of the pH increase in the controls.

These deviations were not considered by the study author to have had a negative impact on the results or interpretation of the study.

COMPLIANCE:
The data and report for "XDE-742 Sulfinic Acid Metabolite - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*" were reported as produced and compiled in accordance with all pertinent OECD and U.S. EPA Good Laboratory Practice regulations, namely:

> OECD Good Laboratory Practice in the Testing of Chemicals. Paris, France. 1997.
>
> U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

with the following exception: routine dilution water contaminant screening analyses for pesticides, PCBs and toxic metals were conducted using standard U.S. EPA procedures by GeoLabs, Inc., Braintree, Massachusetts. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc.).

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

A. MATERIALS:

1. Test Material:	XDE-742 sulfinic acid metabolite (i.e. pyroxsulam sulfinic acid metabolite or sulfinic acid metabolite of pyroxsulam)
Description:	Solid
Lot No./Batch No.:	E1960-77
Purity:	98%.
Stability of Compound Under Test Conditions:	**Stable.** Test substance concentrations were measured at 0 hour (test initiation) and 96 hours (test termination). Measured concentrations closely approximated the desired nominal concentrations, decreased slightly between sampling intervals, but maintained the expected concentration gradient. Mean measured concentrations ranged from 97 to 110% of nominal concentrations over the 96 hour period.
Storage conditions of test chemicals:	Upon receipt at Springborn Smithers, the test substance (identified as SSL No. 113-71) was stored frozen (<-4°C) in the original container.
Physicochemical properties of sulfinic acid metabolite of pyroxsulam:	The study report stated that determination of stability and characterization, verification of the test substance identity, maintenance of records on the test substance, and archival of a sample of the test substance are the responsibility of the Study Sponsor. Consequently, the physicochemical

parameters for water solubility, vapour pressure, UV absorption, pKa and Kow were not presented in the study report. The study profile template (Hoberg, 2005a) stated that physicochemical properties were not available at the time of publication of the study profile template.

The study report also noted that concentrations were adjusted for the purity of the test substance and were presented as active ingredient (i.e. sulfinic acid metabolite of pyroxsulam).

2. Test organism:	Freshwater green alga
Species:	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Class:	Chlorophyceae
Strain:	1648
Source:	In-house stock cultures originally obtained from the University of Texas, Austin, Texas.
Age of inoculum:	3 days
Method of cultivation:	The stock cultures were maintained within the following conditions: a shaking rate of 100 ± 10 rpm, a temperature of 24 ± 2°C and continuous illumination at the surface of the medium with an intensity range of 7000 to 9100 lux. Lighting was supplied by fluorescent bulbs. Culture flasks were agitated continuously on an orbital shaker. Temperature was controlled using an environmental chamber. The culture was maintained under conditions equivalent to those used for testing.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study:

A preliminary range-finding exposure was conducted at Springborn Smithers at nominal pyroxsulam sulfinic acid metabolite concentrations of 10 and 100 mg/L, and a control. Three exposure vessels were established for each concentration and the control. Following 96 hours of exposure, cell densities in the 10 and 100 mg/L treatment levels averaged 183 and 159 x 10^4 cells/mL, respectively. The control averaged 184 x 10^4 cells/mL. Based on these results and consultation with the Study Sponsor, nominal pyroxsulam sulfinic acid metabolite concentrations of 6.3, 13, 25, 50 and 100 mg/L were selected for the definitive exposure.

b. Definitive Study

The experimental phase of the 96-hour acute toxicity test was conducted from 13 to 17 October 2005 at Springborn Smithers Laboratories, in Wareham, Massachusetts.

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

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	The inoculum used to initiate the toxicity test with pyroxsulam sulfinic acid metabolite was taken from a stock culture that had been transferred to fresh medium three days before testing.	Parameter considered met. OECD 201 states that an inoculum culture in the test medium is prepared 2-4 days before start of the test with the inoculum culture incubated under the same conditions as the test cultures. US EPA OPPTS 850.5400 states that the test begins when algae (inocula) from 3 to 7 day–old stock cultures are placed in the test chambers containing test solutions having the appropriate concentrations of the test substance. This guideline also states that toxicity testing should not be performed until algal cultures are shown to be actively growing (i.e. capable of logarithmic growth within the test period) in at least two subcultures lasting 7 days each prior to the start of the definitive test. *EPA recommends two week acclimation period.* This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements. *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td>Culturing media and conditions: (same as test or not)</td><td>Culturing medium was the same as the test medium (AAP medium in both cases). Conditions of culturing and testing considered equivalent (see following comparisons).

<table>
<tr><th>Parameter</th><th>Culture</th></tr>
<tr><td>Temperature:</td><td>24 ± 2°C</td></tr>
<tr><td>Light (lux):</td><td>7000 to 9100 lux</td></tr>
<tr><td>Photoperiod:</td><td>Continuous (24 hours light/day)</td></tr>
<tr><td>Medium:</td><td>AAP</td></tr>
<tr><td>pH range:</td><td>7.5 ± 0.1</td></tr>
<tr><td>Culture Volume:</td><td>50 mL</td></tr>
<tr><td>Culture Vessel:</td><td>125 mL glass flask</td></tr>
<tr><td>Culture Vessel Cap:</td><td>stainless steel caps which permitted gas exchange</td></tr>
<tr><td>Agitation</td><td>Continuous (100 rpm) on an orbital shaker</td></tr>
<tr><td>Growth conducted in:</td><td>Environmental chamber</td></tr>
</table>
</td><td>Parameter considered met.

Comparison of the culturing and test conditions indicates they were equivalent.

<table>
<tr><th>Parameter</th><th>Test</th></tr>
<tr><td>Temperature:</td><td>24 ± 1°C (measured 24-26°C)</td></tr>
<tr><td>Light (lux):</td><td>7000 to 9100 lux)</td></tr>
<tr><td>Photoperiod:</td><td>Continuous (24 hours light/day)</td></tr>
<tr><td>Medium:</td><td>AAP</td></tr>
<tr><td>pH range:</td><td>7.5 ± 0.1</td></tr>
<tr><td>Culture Volume:</td><td>100 mL</td></tr>
<tr><td>Culture Vessel:</td><td>250-mL flask</td></tr>
<tr><td>Culture Vessel Cap:</td><td>stainless steel caps which permitted gas exchange</td></tr>
<tr><td>Agitation</td><td>Continuous (100 rpm)</td></tr>
<tr><td>Growth conducted in:</td><td>Environmental chamber</td></tr>
</table>
</td></tr>
<tr><td>Health: (any mortality observed)</td><td>Observations of the health of the algal cells were made at each 24-hour interval. The satisfactory growth of the controls indicates the algal health was acceptable at the start of the study.</td><td>Parameter considered met.

OECD 201 states microscopic observation should be performed to verify a normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae (as may be caused by the exposure to the test substance) at the end of the test.

US EPA OPPTS 850.5400 states that any unusual cell shapes, color differences, differences in chloroplast morphology, flocculation, adherence of algae to test containers, or aggregation of algal cells at the test end are to be noted.

Note that while cells were stated to be observed at 24 hour intervals, only the results at 96 hours were reported in the study report.</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
Test system Static/static renewal Renewal rate for static renewal	Static N/A (not applicable, no renewal occurred)	Test system is acceptable. Parameter considered met. OECD 201 does not specifically refer to the terminology "static" tests but can be interpreted as referring to these conditions as no mention is made of renewal of test solutions. US EPA OPPTS 850.5400 indicates static tests are acceptable
Incubation facility	An environmental chamber designed to maintain the test conditions specified in the protocol	Incubation facility is acceptable. Parameter considered met. OECD 201 refers to use of a cabinet or chamber, in which the chosen incubation temperature can be maintained at ± 2°C. US EPA OPPTS 850.5400 refers to use of a growth chamber or controlled environment room that can hold the test containers and maintain the necessary growth parameters (e.g. temperature, lighting).
Duration of the test	96-hours	Parameter considered met. Test duration is acceptable. OECD 201 (2006) refers to the test normally being for 72 hours but with shorter or longer periods allowed provided that guideline's validity criteria are met. US EPA OPPTS 850.5400 refers to cell counts at 24, 48, 72 and 96 hours. *EPA requires: 96-120 hours* *OECD: 72 h*

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Flasks 250 mL 100 mL	Parameter considered met. OECD 201 states that the test vessels will normally be glass flasks of dimensions that allow a sufficient volume of culture for measurements during the test and a sufficient mass transfer of CO_2 from the atmosphere. US EPA OPPTS 850.5400 states Erlenmeyer flasks should be used for test containers and may be of any volume between 125 and 500 mL as long as the same size is used throughout a test and the test solution volume does not exceed 50 percent of the flask volume. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name	Algal Assay Procedure (AAP) medium. Medium details provided in the study report were considered equivalent to the AAP medium composition recorded in OECD 201 with the following exception: The test medium contained sodium selenate at 1.88 µg/L. The study report noted this was an additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.	See deviations/deficiencies table, page 33 of this DER with respect to use of sodium selenate. OECD 201 refers to AAP medium and provides a comparison (Annex 3 of the Guideline) of the US EPA AAP medium and the OECD 201 medium. The guideline identifies both as suitable growth media. US EPA OPPTS 850.5400 does not specifically refer to media composition, instead referring to other sources for this information. *EPA recommends 20-AAP medium and no chelators.* This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements which allow use of chelating agents (the AAP medium used contains sodium EDTA as a chelating agent).

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td>pH at test initiation and at test termination:</td><td>
<table>
<tr><th>Time:</th><th>0 h</th><th>96 h</th></tr>
<tr><td>Control</td><td>6.9</td><td>10.1</td></tr>
<tr><td>6.3*</td><td>7.0</td><td>10.0</td></tr>
<tr><td>13</td><td>7.1</td><td>10.1</td></tr>
<tr><td>25</td><td>7.1</td><td>10.0</td></tr>
<tr><td>50</td><td>7.1</td><td>9.9</td></tr>
<tr><td>100</td><td>7.1</td><td>9.8</td></tr>
</table>
* mg pyroxsulam sulfinic acid metabolite/L (nominal).

The control pH increased by 3.2 units over the 96 hours of the exposure period.

pH was measured at test initiation and at the termination of the 96-hour exposure period. Measurements at test initiation were conducted on the test solution remaining after the individual test flasks had been filled. At test termination, after cell counts were completed, the three replicate solutions for each treatment and the six controls were respectively composited for pH measurements.</td><td>See deviations/deficiencies table, page 33 of this DER with respect to the pH change observed in the controls over 96 hours and the initial control pH value.

The initial control pH of 6.9 is unexpected as the medium was at pH 7.5.

The changes in the control pH over the 96 hours are greater than recommended by the OECD 201 (2006) which recommends that the (control) medium pH should not increase by greater that 1.5 pH units during the test.

US EPA 850.5400 states that the initial pH of the nutrient medium is to be 7.4 to 7.6 and notes that if the test chemical is highly acidic and reduces the pH of the test solution below 5.0 at the first measurement, appropriate adjustments to pH should be considered, This was not the situation in the study under assessment.

This increase in pH is considered a common occurrence, especially when there is no inhibition and was reported as due to photosynthesis by a rapidly growing algal population and not to be controllable.

OECD recommends the medium pH after equilibration with air be ~8 with less than 0 .001 mmol/L chelator, if used. This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements with respect to medium pH and specified concentrations of chelating agents.</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
Chelator used:	Yes, as required for AAP media $Na_2EDTA \cdot 2H_2O$ at 300 µg/L	Parameter considered met. The presence of EDTA as a chelator is considered acceptable on the basis of its permitted presence in both the US EPA AAP medium and the OECD TG 201 medium. *EPA recommends 20X-AAP and no chelators.* This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements which advises on the media to use and allows use of chelating agents.
Carbon source:	Not identified.	Parameter considered met. OECD 201 and US EPA OPPTS 850.5400 do not make specific reference to a carbon source.
Salinity (for marine algae):	N/A as a freshwater alga was used.	Parameter is not relevant for a freshwater alga.
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	The medium used was standard AAP medium modified by addition of sodium selenate. Yes, full details of the medium's composition were provided.	AAP is a standard medium.
Dilution water source/type:	Source not identified in study report. The water used to make up the AAP medium was sterile and deionised.	Dilution water parameters considered met.

Parameter	Details	Remarks *Criteria*
pH:	The pH of the culture medium was adjusted to pH 7.5 ± 0.1	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Salinity (for marine algae):	N/A for a freshwater alga.	*EPA salinity: 30-35 ppt.*
Water pretreatment (if any): Intervals of water quality measurement.	Sterile, deionised water was used to prepare the AAP growth medium. Intervals of water quality measurement not referred to.	No specific requirement identified in the guidelines.
Total Organic Carbon:	A representative sample of AAP medium was analysed monthly for total organic carbon (TOC) concentration. The TOC concentration of the sample collected in October 2005 was 0.46 mg/L.	No specific requirements identified for these parameters (TOC, particulate matter, metal and pesticides and chlorine levels) in OECD 201 or US EPA OPPTS 850.5400 other than OECD 201 refers to use of deionised water to prepare the growth media while the US EPA guideline refers to use of water of sufficient quality (e.g. ASTM Type I water) to prepare the nutrient medium. The successful maintenance of the algae and their acceptable growth in the controls indicate the dilution water was of acceptable quality.

Parameter	Details	Remarks *Criteria*
Particulate matter and metals and pesticides:	Representative samples of the dilution water source used in the preparation of the culture medium were reported analysed periodically for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were reported as having been detected at concentrations that were considered toxic in any of the water samples analysed in agreement with ASTM guidelines (2002). Reference made in the study report to "routine dilution water contaminant screening for pesticides, PCBs and toxic metals ...".	No specific requirement identified in the guidelines.
Chlorine:	No details provided in study report.	No specific requirement identified in the guidelines. *EPA is against the use of dechlorinated water.* Sterile, deionised water was used to prepare the AAP medium.
Indicate how the test material is added to the medium (added directly or used stock solution)	A 100 mg sulfinic acid metabolite of pyroxsulam/L stock solution was prepared prior to test initiation by placing 0.2041 g of the sulfinic acid metabolite of pyroxsulam (0.2000 g as active ingredient) in a 2000-mL volumetric flask and bringing it to volume with AAP medium. The resulting stock solution was observed to be clear and colourless with no visible undissolved test substance. Test solutions were prepared from the 100 mg/L stock solution by serial dilutions. All resulting test solutions were clear and colourless, with no visible undissolved material reported.	Parameter considered met. Note that the nominal test concentrations were adjusted for the 98% purity of the test substance.

Parameter	Details	Remarks *Criteria*
Aeration or agitation	Continuous shaking (orbital shaker) at approximately 100 revolutions/minute (rpm).	Parameter considered met. OECD 201 states that during the test it is necessary to keep the algae in suspension and to facilitate transfer of CO_2. To this end constant shaking or stirring should be used and reference is made to an orbital or reciprocate shaker table being used at ~150 rpm. The 100 rpm rate used in the study is considered not to be too dissimilar to this recommended rate. US EPA OPPTS 850.5400 states that test containers should be placed on a rotary shaking apparatus and oscillated at approximately 100 cycles/min for *Selenastrum*.
Initial cell density	Approximately 10,000 cells/mL (for each replicate).	Parameter considered met. Initial cell density considered acceptable. OECD 201 recommends an initial cell concentration for *Pseudokirchneriella subcapitata*: of 5 x 10^3 - 10^4 cells/mL. US EPA OPPTS 850.5400 states that each test chamber in the definitive study should contain equal volumes of test solution and approximately 1 x 10^4 *Selenastrum* cells per millilitre of test solution. *EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treatments:	6 replicates inoculated with algae. N/A 3 replicates/treatment level inoculated with algae. One extra replicate was prepared at 25 mg/L without algae to allow determination of the effect of the algal cells on uptake/degradation of the sulfinic acid metabolite of pyroxsulam.	Parameter requirements considered met. The numbers of replicates used are acceptable. OECD 201 states that the test design should include three replicates at each test concentration and that the number of control replicates must be at least three, and ideally should be twice the number of replicates used for each test concentration. US EPA 850.5400 states that a minimum of three replicates is required for each concentration of test chemical and control. A solvent control was not used. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal:	Nominal concentrations were 0 (control), 6.3 13, 25, 50 and 100 mg sulfinic acid metabolite of pyroxsulam/L The nominal test concentrations were in the ratios of 1:92 to 1:2.06.	Nominal and measured test concentrations parameter considered met. The mean measured concentrations were 96-100% of nominal. There was no affect from algal cells on measured concentration of the test compound. OECD 201 states that, for the final definitive test, at least five concentrations, arranged in a geometric series with a factor not exceeding 3.2, should be selected. The OECD guideline also states that, the concentration series should preferably cover the range causing 5-75 % inhibition

Parameter	Details	Remarks *Criteria*
		of algal growth rate. US EPA OPPTS 850.5400 states algae should be exposed to five or more concentrations of the test chemical in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, and 64 mg/L). The nominal concentration ratios of 1:1.92 to 1:2.06 are considered sufficiently close to meet this criterion. *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.* These template requirements are noted but are not considered further in the light of the OECD and US EPA OPPTS having equivalent requirements.

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td>Measured:</td><td>Mean measured concentrations at 0 and 96 hours were as follows:

<table>
<tr><th>Nominal Conc.[a]</th><th>0 h</th><th>96 h</th><th>Mean</th><th>% of nominal.</th></tr>
<tr><td>Control</td><td><0.25</td><td><0.33</td><td>NA[b]</td><td>NA</td></tr>
<tr><td>6.3</td><td>6.5</td><td>6.2</td><td>6.4</td><td>100</td></tr>
<tr><td>13</td><td>16</td><td>14</td><td>15</td><td>110</td></tr>
<tr><td>25</td><td>26</td><td>25/ 24[c]</td><td>25</td><td>100</td></tr>
<tr><td>50</td><td>59</td><td>51</td><td>55</td><td>110</td></tr>
<tr><td>100</td><td>93</td><td>101</td><td>97</td><td>97</td></tr>
</table>
a. as mg sulfinic acid metabolite of pyroxsulam/L.
b. NA = not applicable.
c. Result of the additional sample without algae present to determine biological uptake or degradation.

The mean (measured concentrations) and % of nominal results are based on actual analytical (unrounded results) and not the rounded values shown in the table.

In the quality control samples (5.00, 25.0 and 100 mg sulfinic acid metabolite of pyroxsulam/L), time 0 recoveries were 83.9 (25.0 mg/L) to 93.9% (5.00 mg/L) of nominal and, at 96 hours, 99.2 to 100% of nominal.</td><td></td></tr>
<tr><td>Solvent (type, percentage, if used)</td><td>N/A; a solvent was not used</td><td>The parameter is not relevant as a solvent was not used.

OECD 201 and US EPA OPPTS 850.5400 allow, but do not require, the use of solvents.</td></tr>
<tr><td>Method and interval of analytical verification</td><td>All exposure solutions and QC samples were analysed for sulfinic acid metabolite of pyroxsulam using high performance liquid chromatographic system equipped with LC/MS/MS detection (HPLC/UV) based on methodology validated at Springborn Smithers. Validation was by fortification of 20X AAP medium at nominal pyroxsulam sulfinic acid</td><td>Parameter considered met.

Defined limits for acceptance of quality control sample performance in subsequent studies with sulfinic acid metabolite of pyroxsulam were set at 70 to 120%.

Conditions and procedures used throughout the analysis of exposure solutions and QC</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
Limit of quantitation: Limit of detection:	metabolite concentrations of 1.00, 25.0 and 100 mg/L. Recoveries averaged 101% ± 5.76%. The limit of quantitation was 0.186 mg sulfinic acid metabolite of pyroxsulam/L. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 70 to 120%. Test solutions were analyzed for the presence of pyroxsulam at 0 and 96 hours. Limit of detection not reported.	samples during this study were similar to those used in the method validation study. Representative chromatograms from the analysis of a calibration standard, recovery sample and a control sample are presented as was a typical linear regression analysis for sulfinic acid metabolite of pyroxsulam (response versus concentration) which had an r^2 value of 0.99668.
Test conditions		The test conditions meet US EPA and OECD Guidelines with the exception of the test temperature (see below).
Temperature:	24 to 26°C	See deviations/deficiencies table, page 33 of this DER with respect to the temperature being >24°C. OECD 201 states the cultures should be maintained at a temperature in the range of 21 to 24°C, controlled at ± 2°C. The 1984 OECD guideline set the range as 21 to 25°C. US EPA OPPTS 850.5400 states the test temperature is to be 24°C for *Selenastrum* and that excursions from the test temperature should be no greater than ± 2°C. *EPA temperature: Skeletonema: 20°C, Others: 24-25°C.* *OECD recommended the temperature be in the range of 21 to 25°C maintained at ± 2°C.* These template requirements are noted but not considered further in the light of OECD and US EPA OPPTS having equivalent requirements.

Parameter	Details	Remarks *Criteria*
Photoperiod:	Continuous	Photoperiod requirement considered met. OECD 201 refers to use of continuous light while US EPA OPPTS 850.5400 refers to test chambers containing *Selenastrum*, *Navicula*, and *Anabaena* being illuminated continuously. *EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous. OECD recommends and continuous uniform illumination.*
Light intensity and quality:	7000-9100 lux The photolytically active radiation (PAR) of the test area measured at test initiation ranged from 115 to 137 µE/m2/s.	See deviations/deficiencies table, page 33 of this DER with respect to meeting OECD and US EPA requirements. OECD 201 (2006) refers to light intensity at the level of the test solutions from the range of 60-120 $\mu E \cdot m^{-2} s^{-1}$, which it states is equivalent to a range of 4440-8880 lux. US EPA light intensity requirement not met (US EPA OPPTS 850.5400 states fluorescent lights providing 4.3 Klux are to be used for *Selenastrum.* *OECD states approximately 8000 Lux measured with a spherical collector.* *EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* These template requirements are noted but not considered further in the light of the OECD and US EPA OPPTS having specific requirements.
Reference chemical (if used) name: concentrations:	 N/A N/A	Not relevant as a reference chemical was not used. OECD 201 notes that a reference substance may be tested as a means of checking test procedures and that this should be done at least twice a year. US EPA OPPTS 850.5400 also states that positive controls

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td></td><td>using zinc chloride as a reference chemical should also be run periodically.

While it is most probable that testing with a reference chemical had been conducted with satisfactory results and it is only an oversight that the relevant results were not provided, inclusion of such results would have added value to the test report.</td></tr>
<tr><td>Other parameters, if any</td><td>Conductivity of the test solutions was determined at 0 and 96 hours. The reported results were:

<table>
<tr><th rowspan="2">Concen-tration*</th><th colspan="2">Conductivity (µmhos/cm)</th></tr>
<tr><th>0 h</th><th>96 h</th></tr>
<tr><td>Control</td><td>80</td><td>90</td></tr>
<tr><td>6.3</td><td>80</td><td>100</td></tr>
<tr><td>13</td><td>90</td><td>110</td></tr>
<tr><td>25</td><td>90</td><td>110</td></tr>
<tr><td>50</td><td>100</td><td>120</td></tr>
<tr><td>100</td><td>130</td><td>140</td></tr>
</table>
* mg sulfinic acid metabolite of pyroxsulam/L.

Conductivity was measured at test initiation and at the termination of the 96-hour exposure period. Measurements at test initiation were conducted on the test solution remaining after the individual test flasks had been filled. At test termination, after cell counts were completed, the three replicate solutions for each treatment and the six controls were respectively composited for conductivity measurements.

Observations of the health of the algal cells were made at each 24-hour interval.</td><td>Acceptable.</td></tr>
</table>

2. Observations:

Table 2. Observation parameters

Parameters	Details	Remarks/ Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density and biomass (area under the growth curve), growth rate (per day). Cell appearance was observed at 24, 48, 72 and 96 hours. pH, temperature, light intensity and concentrations of the sulfinic acid metabolite of pyroxsulam in the test solutions were also determined over the course of the study.	The parameters determined are acceptable and their requirements are considered met. OECD 201 refers to growth and growth inhibition being quantified from measurements of the algal biomass as a function of time. US EPA OPPTS 850.5400 refers to enumeration of the algal cells to determine inhibition or stimulation of growth and the pattern of growth in test containers compared to controls. *EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks/ Criteria
Measurement technique for cell density and other end points	Single cell counts were conducted using a haemocytometer and a compound microscope. Appropriate instrumental techniques were used for physico-chemical parameters listed above.	Observation intervals considered appropriate and the parameters met. OECD 201 refers to algal biomass in each flask being determined daily. US EPA OPPTS 850.5400 states that at the end of 96 h, and, if possible, at the end of 24, 48, and 72 h, the algal growth response (number or weight of algal cells per millilitre) in all test containers and controls is to be determined by an indirect (spectrophotometry, electronic cell counters, dry weight, etc.) or a direct (actual microscopic cell count of at least 400 cells per flask) method. Indirect methods are to be calibrated by a direct microscopic count or data should be presented that relate electronic counts with microscopic counts. *EPA recommends the measurement technique of cell counts or chlorophyll a.* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (Note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	0, 24, 48, 72 and 96 hours	Observation intervals considered appropriate. OECD 201 refers to algal biomass in each flask being determined daily. US EPA OPPTS 850.5400 states that at the end of 96 h, and, if possible, at the end of 24, 48, and 72 h, the algal growth response (number or weight of algal cells per millilitre) in all test containers and controls is to be determined. *EPA and OECD: every 24 hours.*
Other observations, if any	At test termination observation of the cells at each test concentration were made.	Requirement considered met. Observation made is appropriate

Parameters	Details	Remarks/ Criteria
Indicate whether there was an exponential growth in the control	Yes. The mean control 72-hour cell growth was 106 x 10^4 cells/mL. This represents an approximate 100-fold increase in cell numbers from the original 10,000 cells/mL. At 96 hours, the mean control cell density was ~315 X 10^4 cells/mL, i.e. ~3.15 X 10^6 cells/mL. The 0 to 72 hour growth rate in the control averaged 1.58 $days^{-1}$.	Parameter is considered met. OECD 201 requires, *inter alia,* that biomass in the control cultures should have increased by a factor of at least 16 within the 72 hour test period (note that cell count has been used as the measure of biomass in this situation). OECD 201 also states that the desired increase in biomass corresponds to a specific growth rate of 0.92 day^{-1}. Note that OECD 201 states that *P. subcapitata* is expected to have a growth rate of 1.5 to 1.7 in light intensity of approximately 70 $\mu E/m^2/sec$ at 21°C when grown in OECD medium. US EPA OPPTS 850.5400 states that algal growth in controls should reach the logarithmic growth phase by 96 h (at which time the number of algal cells should be approximately 3.5 X 10^6/mL for *Selenastrum.* The mean measured value of ~3.15 X 10^6 cells/mL is considered to meet this requirement *EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.* These template requirements are noted but not considered further in the light of the OECD and US EPA OPPTS having specific requirements.
Water quality was acceptable? (Yes/No)	Yes	Parameter considered met on basis of successful growth of the controls and details provided on the medium's preparation from sterile, deionised water.
Were raw data included?	As laboratory data, no. Individual replicate data were presented. The study report notes that all data generated are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.	Parameter considered met. While raw data were not submitted, the tabulated results presented provided individual replicate data which were sufficient to allow statistical analysis by the reviewer. OECD 201 lists the results which must be presented in the test report. These are not

Parameters	Details	Remarks/ Criteria
		considered to necessarily include raw, i.e. laboratory data. The tabulated data presented in the study report are considered to have complied with the OECD requirement. While US EPA OPPTS 850.5400 states that the sponsor must submit to the EPA all data developed by the test including those that are suggestive or predictive of acute phytotoxicity, advice from the US EPA was that, because the tabulated results presented in the study report were sufficient to allow statistical analysis, the guideline would be considered met .

II. RESULTS and DISCUSSION:

INHIBITORY EFFECTS:

After 96 hours, inhibition of cell density relative to the control mean ranged from 8% at 15 mg sulfinic acid metabolite of pyroxsulam/L to 50% at 97 mg/L. Inhibition of biomass relative to controls at 72 hours ranged from -24% (i.e. growth stimulation occurred) at 15 mg sulfinic acid metabolite of pyroxsulam/L to 37% at 97 mg/L. Inhibition of mean specific growth rate relative to controls for the 0-72 hour period ranged from -6% at 15 mg sulfinic acid metabolite of pyroxsulam/L to 9% at 97 mg/L.

The 96 hour NOEC for cell density and the 72 hour NOECs for total biomass and average growth rate were all set at 55 mg sulfinic acid metabolite of pyroxsulam/L.

There was a major change in control pH - from 6.9 at time 0 to 10.1 after 96 hours. Increases in pH were also seen in the test solutions (pH values at time 0 were 7.0 or 7.1 and, after 96 hours, 9.8 to 10.1).

The reduction of cell density, biomass and growth rate were the only phytotoxic effects reported.

The effects of the sulfinic acid metabolite of pyroxsulam on the growth of *Pseudokirchneriella subcapitata* under the test conditions are shown in Table 3 by the cell density counts at 24, 48, 72 and 96 hours and the % inhibition after 96 hours.

Table 3. Effect of sulfinic acid metabolite of pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*) – Mean cell density over 96 hours shown.

Treatment (mean measured concentration (mg ADTP metabolite of pyroxsulam/L)	Initial cell density, cells/mL	Mean cell density ($x10^4$) and standard deviation in brackets at 24, 48, 72 and 96 hours				
		24 hours	48 hours	72 hours	96 hours	Percent inhibition[1] at 96 hours
Negative control	$1 X 10^4$	4.04 (1.77)	25.46 (7.57)	106.00 (22.61)	315.58 (75.35)	NA
6.4	$1 X 10^4$	2.25 (0.87)	23.17 (7.42)	91.25 (8.71)	236.83 (52.02)	25
15	$1 X 10^4$	2.25 (0.66)	30.42 (9.75)	139.19 (43.33)	291.83 (49.04)	8
25	$1 X 10^4$	1.67 (0.52)	34.50 (9.69)	116.50 (9.78)	234.78 (42.30)	26
55	$1 X 10^4$	2.33 (0.63)	20.17 (2.93)	90.67 (18.64)	240.67 (30.90)	24
97	$1 X 10^4$	1.25 (0.75)	17.00 (4.63)	70.17 (23.63)	157.22 (65.02)[2]	50
Reference chemical (if used)	NA. A reference chemical was not reported used.					

1. Relative to control. Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table. 2. Significantly reduced compared to the control, based on Williams' Test. NA = not applicable.

The 96 hour exposure mean of 157.22 X 10^4 cells/mL from exposure to 97 mg sulfinic acid metabolite of pyroxsulam/L was statistically significantly reduced from the mean control count at that time of 315.58 X 10^4 cell/mL (William's Test).

Table 4. Effect of sulfinic acid metabolite of pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*) – Mean specific growth rates and biomass (area under the growth curve) shown.

Treatment measured concentrations (mg sulfinic acid metabolite of pyroxsulam/L)	Mean Specific Growth Rate per day		Mean Area Under the Growth Curve (Biomass)	
	0-72 hours	Percent Inhibition[1]	0-72 hours	Percent Inhibition[1]
Negative control	1.58	NA	71.06	NA
6.4	1.54	3	60.80	14
15	1.67	-6	87.82	-24
25	1.62	-3	82.00	-15
55	1.53	3	57.62	19
97	1.44[2]	9	44.85[2]	37

1. Relative to control. 2. Significantly reduced compared to the control, based on Williams' Test. NA = not applicable.

The 0-72 hour exposure specific growth rate and biomass (area under the curve) mean were statistically significantly reduced from the respective control means (William's Test).

The OECD 201 guideline states that the concentration series should preferably cover the range causing 5-75 % inhibition of algal growth rate. Based on the % inhibition in mean specific growth rate shown in Table 4, this requirement is not considered to have been fully complied with.

The study's reported statistical endpoints are as shown in Table 5.

Table 5. Statistical endpoint values. EC50 and NOEC results expressed as mg sulfinic acid metabolite of pyroxsulam/L

Statistical Endpoint	Cell Density				Growth Rate (0-72 h)	Biomass (0-72 h)
	24 h	48 h	72 h	96 h		
NOEC (mg sulfinic acid metabolite of pyroxsulam/L)	Not reported	Not reported	Not reported	55	55	55
EC_{50} (mg sulfinic acid metabolite of pyroxsulam/L) (95% C.I. in brackets except where this parameter was not calculated)	44 (8.8-49)	>97	>97	85 (72-96)	>97	>97
Reference chemical, if used	No reference chemical used.					

Validity of test

OECD 201 (2006) requires that, for the test to be valid, the following performance criteria should be met:

- the biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period;
- the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures (See Annex 1 under "coefficient of variation") must not exceed 35%; and
- the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%.

In contrast, OECD 201 (1984), the guideline version the study followed, requires only that the cell concentration in the control cultures should have increased by a factor of at least 16 within three days.

US EPA OPPTS 850.5400 states that algal growth in controls should reach the logarithmic growth phase by 96 h, at which time the number of algal cells should be approximately 1.5 X 10^6/mL for *Skeletonema* or 3.5 X 10^6/mL for *Selenastrum*. No reference to coefficient of variation requirements was identified in this US EPA guideline.

With respect to exponential growth, this requirement is considered to have been met (see Table 2, page 23 of this DER under the parameter "Indicate whether there was an exponential growth in the control") because the mean control 72 hour cell growth was ~106 x 10^4 cells/mL. This represents an approximate 100-fold increase in cell numbers from the original 10,000 cells/mL. At 96 hours, the mean control cell density was ~315 X 10^4 cells/mL, i.e. ~3.15 X 10^6 cells/mL. This value is considered to meet the US EPA OPPTS 850.5400 requirement that the cell count at that time should be approximately 3.5 X 10^6 cells/mL for *Pseudokirchneriella subcapitata* at 96 hours, being ~90% of the recommended US EPA value.

The 0 to 72 hour growth rate in the control averaged 1.58 $days^{-1}$ with this value meeting the OECD 201 statement that the desired increase in biomass is shown by a specific growth rate of 0.92 day^{-1}.

The 0-24, 24-48 and 48-72 hour control replicate growth rates were calculated from the initial (10,000 cells/mL), 24, 48 and 72 hour cell density counts using the formula shown under "Reported Statistics" on page 28 of this DER. The values and calculated statistics, including the overall mean % coefficient of variation (%CV) are as shown in Table 6.

Table 6. Reviewer calculated growth rates (as day^{-1}) for the 0-24, 24-48, 48-72 and 0-72 hour periods in the control replicates and associated means, standard deviations and percentage coefficients of variation. Study report results are shown for the 0-24 and 0-72 hour replicates and for the percentage coefficients of variation.

Reviewer and study report's growth rates (day^{-1}) for the control replicates and associated means, standard deviations and % coefficients of variation (%CV)						
Replicate	0-24 h		24-48 h, Reviewer	48-72 h, Reviewer	0-72 hours	
	Reviewer	Study report			Reviewer	Study report
1	1.39	1.52	1.62	1.69	1.57	1.60
2	1.87	2.05	1.42	1.43	1.57	1.61
3	1.25	1.37	2.37	1.16	1.59	1.63
4	1.66	1.82	1.71	1.51	1.63	1.66
5	1.32	1.45	1.82	1.05	1.40	1.43
6	0.22	0.24	2.53	1.81	1.52	1.56
Mean, standard deviation and %CV determined from replicate results :						
Mean	1.29	1.41	1.91	1.44	1.55	1.58
Standard deviation	0.57	0.62	0.44	0.30	0.08	0.08
%CV	44.3	44	23.0	20.5	5.2	5.1

The %CV value for the 0-24 hour mean growth rate was calculated as 44% (the same result as reported in the study report), which exceeds the OECD 201 (2006) edition requirement of the %CV not exceeding 35%. The 24-48 and 48-72 hour growth rate values do not exceed the 35% value set by the 2006 OECD 201 guideline. Because the %CV value of for the 0-24 hour period exceeded the 35% limit set by the 2006 OECD 201 guideline, it is identified as a deficiency with respect to that guideline (see the deviations table on page 33 of this DER).

The 0-72 h %CV was calculated by the reviewer as 5.2% (mean 1.55, standard deviation 0.08, see page 44 for the data and ToxCalc determinations) which meets the OECD 201 limit of 7% for *Pseudokirchneriella subcapitata*. The study report's %CV for the 0-72 hour period was 5.1%.

B. REPORTED STATISTICS:

The study report stated that, for determination of EC50 and NOEC values, the cell density in each test flask was calculated for each daily interval by dividing the number of cells counted by the number of fields examined. Means and standard deviations for cell density for each treatment and the control were calculated from individual replicate values.

The growth rate (μ) for each replicate flask was calculated for the period from test initiation to each observation time using the following equation

$$\mu = \frac{\ln X_t - \ln X_0}{t_t - t_0}$$

where:

μ = specific growth rate ($days^{-1}$)
ln = natural logarithm
X_0 = initial cell density in cells/mL
X_t = cell density at the specified time interval in cells/mL
t_0 = time of test initiation
t_t = time of observation interval in days (i.e., 1, 2, 3)

The biomass (area under the growth curve) for each replicate vessel was calculated for the exposure period between 0 and 72 hours using the following equation:

$$A = \frac{N_1 - N_0}{2} x\, t_1 + \frac{N_1 + N_2 - 2N_0}{2} x\,(t_2 - t_1) + \ldots + \frac{N_{n-1} + N_n - 2N_0}{2} x\,(t_n - t_{n-1})$$

where:

A = area under the growth curve (units: x 10^4 cells•days/mL)
N_0 = calculated number of cells/mL at time t_0
N_1 = measured number of cells/mL at t_1
N_n = measured number of cells/mL at time t_n
t_1 = time of first measurement after beginning of test
t_n = time of n^{th} measurement after beginning of test
n = number of measurements taken after test initiation

The EC25 and EC50 values (the concentration of test substance which reduced cell density, total biomass and average growth rate by 25 and 50%, respectively, relative to the control) were calculated for the 24-, 48-, 72- and 96-hour observation intervals for cell density and EC50 values for the 72-hour observation interval for total biomass, denoted as EbC50, and average growth rate, denoted as ErC50. The EC50 values and their 95% confidence intervals were determined by linear regression of response (percent reduction of cell density, total biomass and average growth rate as compared with the control) versus the mean measured concentration (Norberg-King, 1993). TOXSTAT® version 3.5 (Gulley *et al.*, 1996), was used to assist in these computations. If less than the designated percent inhibition was observed for the noted parameter, the EC value was empirically estimated to be greater than the highest concentration tested.

Based on the results of statistical analysis performed for 96-hour cell density and 72-hour total biomass and average growth rate data, the No-Observed-Effect Concentration (NOEC), the highest test concentration which demonstrated no statistically adverse effect ($p \leq 0.05$) for each parameter when compared to the control data, was determined. The data were first checked for normality using Shapiro-Wilks' Test (Weber, et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). If the data sets passed the test for homogeneity and normality, Williams' Test (Williams, 1971, 1972) was used to determine the NOEC. If the data did not pass the tests for homogeneity and normality, then Kruskal-Wallis' Test was used to determine the NOEC. All statistical determinations were made at the 95% level of certainty, except in the case of Shapiro-Wilks' and Bartlett's Tests, where the 99% level of certainty was applied.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s):

Cell counts
Replicate data for cell density 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 cell counts, mean specific growth rates and mean biomass results of the sulfinic acid metabolite of pyroxsulam exposed algae and the mean of the controls by Bonferroni's t test. All NOEC values were determined using the ToxCalc package.

The ToxCalc results for the 24, 48, 72 and 96 hour cell counts are respectively given on pages 38, 40, 41 and 42 of this DER (page 43 shows the results of analysis of the 96 hour cell counts using William's test for the hypothesis testing).

0-72 Hour growth rate
Using the cell density data presented in the study report and the formula for calculation of growth rate presented above, the 72 hours specific growth rate values for control and test replicates presented in the study report were recalculated and shown to be equivalent to those given in the study report.

The reviewer calculated and study report growth rates over 0 to 72 hours were as shown in Table 7.

The ToxCalc analysis of the study report and reviewer calculated 0-72 hour specific growth rates are shown on, respectively, pages 44 and 45 of this DER. The ToxCalc analysis identified no statistically significant differences between the control mean's specific growth rate and the means of the replicates containing the various sulfinic acid metabolite of pyroxsulam concentrations when using Bonferroni's test for comparison of the means. When William's test was used for this purpose, the 97 mg/L mean (1.437 day^{-1}) was identified as statistically significantly lower than the control mean (1.582 day^{-1}) based on the study report's specific growth rates and, for the reviewer calculated results, respectively 1.404 and 1.547 day^{-1} respectively.

Table 7. Comparison of reviewer calculated and study report 0-72 hour specific growth rates (as day^{-1}).

Mean measured concentration as mg sulfinic acid metabolite of pyroxsulam/L	Replicate	Reviewer calculated specific growth rates (day^{-1})	Specific growth rates reported in the study report (day^{-1}) as rounded values.
Control	A	1.57	1.60
	B	1.57	1.61
	C	1.59	1.63
	D	1.63	1.66
	E	1.40	1.43
	F	1.52	1.56
6.4	A	1.53	1.56
	B	1.47	1.5
	C	1.51	1.55
15	A	1.71	1.74
	B	1.50	1.53
	C	1.70	1.73
25	A	1.55	1.59
	B	1.60	1.64
	C	1.60	1.64
55	A	1.45	1.48
	B	1.57	1.61
	C	1.47	1.51
97	A	1.42	1.45
	B	1.28	1.31
	C	1.51	1.55

A t-test with the Microsoft Excel data analysis function of the reviewer calculated and study report's specific growth rates indicated that there was no statistically significant difference (results not shown) between the two sets of results:

0-72 Hour biomass (area under the curve)

The reported cell density data from the 0 to 72 hour period were used with the previously described formula for calculation of the biomass-area under the curve values to determine the 72 hour biomass-area under the growth curve values calculated by the reviewer and those reported in the study report are shown in Table 8.

Table 8. Comparison of reviewer calculated and study report 0-72 hour biomass values (as area under the growth curve).

Mean measured concentration as mg sulfinic acid metabolite of pyroxsulam/L	Replicate	Reviewer calculated 0-72 h biomass values	0-72 h biomass values as reported in the study report (rounded values)
Control	A	767500	674100
	B	872500	778800
	C	981250	880900
	D	976250	865000
	E	577500	522900
	F	625000	542100
6.4	A	753750	670600
	B	682500	613700
	C	620000	539600
15	A	1210850	1067600
	B	646250	570600
	C	1135850	996500
25	A	766250	676400
	B	1040000	935800
	C	951250	847800
55	A	582500	517300
	B	732500	635800
	C	645000	575400
97	A	513750	453400
	B	348750	310100
	C	662500	581900

The ToxCalc analysis of the study report's 0-72 hour biomass results and of the reviewer calculated 0-72 hour biomass results are shown on pages 46 and 47 of this DER. The ToxCalc analysis identified no statistically significant differences between the control mean's biomass and the means of the replicates containing the various sulfinic acid metabolite of pyroxsulam concentrations when using Bonferroni's test for comparison of the means. When William's test was used for this purpose, the 97 mg/L mean (448467 cells/mL) was identified as statistically significantly lower than the control mean (710633 cells/mL) based on the study report's specific growth rates and, for the reviewer calculated results, respectively 508333 and 800000 cells/mL respectively.

A t-test with the Microsoft Excel data analysis function (results not shown) indicated that there was a statistically significant difference between the two sets of results (t score 2.21, t critical (one tailed) 1.68, t critical (two tailed) 2.02.

Examination of the actual biomass values calculated by the reviewer and those given in the study report indicate they are similar and that, biologically, are probably not significantly different.

The endpoints reported in the study report and those calculated in the assessment of the study are similar but not identical with both sets of results shown in Table 9.

Table 9. Reported and reviewer calculated toxicity endpoints.

Toxicity endpoint	Endpoint value as mg sulfinic acid metabolite of pyroxsulam/L (95% confidence limits)	
0-72 hour mean specific growth rate	**As presented in the study report**	**As calculated by the reviewer using the ToxCalc program**
ErC50	>97 (95% Confidence intervals not calculated)	>97 (95% Confidence intervals not calculated)
NOEC	55 (William's test)	97 (Bonferroni's t-test) 55 (William's test)
0-72 hour biomass		
EbC50	>97 (95% Confidence intervals not calculated)	>97 (95% Confidence intervals not calculated)
NOEC	55 (William's test)	97 (Bonferroni's t-test) 55 (William's test)
96 hour cell density		
EC50	85 (72-96)	96.7 (95% Confidence intervals not calculated)
NOEC	55 (William's test)	55 (Bonferroni's t-test) 15 (William's test)

Note: the study report stated that when EC50 values were empirically estimated to be greater than the highest mean measured concentration tested, the 95% confidence limits could not be calculated.

The 96-hour cell density NOEC of 15 mg sulfinic acid metabolite of pyroxsulam/L, causing 8% inhibition, was not considered by the reviewer as significant, as 25% inhibition at 6.4 mg sulfinic acid metabolite of pyroxsulam/L was not significant. Inhibition at 55 mg sulfinic acid metabolite of pyroxsulam/L caused 24% inhibition. The NOEC is set at 55 mg sulfinic acid metabolite of pyroxsulam/L.

D. STUDY DEFICIENCIES:

Table 10 summarises deficiencies and deviations from the OECD 201 and US EPA OPPTS 850.5400 Guidelines.

Table 10. Deviations from Guidelines and other deficiencies

Parameter	Study reported results	OECD 201 Freshwater alga and Cyanobacteria, Growth Inhibition Test	US EPA OPPTS 850.5400 Algal Toxicity, Tiers I and II
Details of growth medium name	The AAP test medium contained sodium selenate at 1.88 µg/L. The study report noted this was an additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.	OECD 201 refers to AAP medium and provides a comparison (Annex 3) of the US EPA AAP medium and the OECD 201 medium. The guideline identifies both as suitable growth media. OECD 201 states that sodium selenate is to be used only in medium for stock cultures of diatom species.	US EPA OPPTS 850.5400 states that formulation of nutrient medium used for algal culture and preparation of test solutions should conform to those currently recommended by the EPA for freshwater and marine algal bioassays
pH at test initiation and at test termination:	Initial control pH = 6.9 (at time 0) 96 hour control pH = 10.1 Increase in pH over 96 hours 3.2 units	OECD recommends (2006) the (control) medium pH should not increase by greater that 1.5 pH units during the test	No specific requirement with respect to pH change in the controls
Temperature:	24 to 26°C	OECD 201 states the cultures should be maintained at a temperature in the range of 21 to 24°C, controlled at ± 2°C. The 1984 OECD guideline set the range as 21 to 25°C.	US EPA OPPTS 850.5400 states the test temperature is to be 24°C for *Selenastrum* and that excursions from the test temperature should be no greater than ± 2°C.
Light intensity and quality:	7000-9100 lux The photolytically active radiation (PAR) of the test area measured at test initiation ranged from 115 to 137 µE/m2/s.	OECD 201 (2006) refers to light intensity at the level of the test solutions from the range of 60-120 $\mu E \cdot m^{-2} s^{-1}$, which it states is equivalent to a range of 4440-8880 lux.	US EPA light intensity requirement not met (US EPA OPPTS 850.5400 states fluorescent lights providing 4300 lux are to be used for *Selenastrum.*
Validity of test	The %CV value for the 0-24 hour period of 44% exceeded the 35% limit set by the 2006 OECD 201 guideline.	OECD 201 (2006) requires that, for the test to be valid, the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	No %CV requirement.

Examination of the media formulation shows that it could better have been described as modified AAP medium because of the presence of the sodium selenate which OECD 201 indicates should only be used for stock cultures of diatom species.

The change in the pH of the controls from 6.9 at day 0 to 10.1 at day 4 represents an increase of 3.2 pH units which exceeds the OECD (2006) recommendation that the pH of the control medium should not increase by more than 1.5 units during the test and the 1.0 unit recommended by the 1984 version of that guideline. However, the guideline does not appear to make this mandatory. The satisfactory exponential growth of the control alga is also taken to indicate that the pH increase did not adversely affect growth. It is not immediately obvious why the control pH at time 0 was 6.9 as the medium had been adjusted to a pH of 7.5. It is possible that this initial pH was made after the addition of the algal cultures, the study report did not appear to clearly identify this point, but it was possibly after the addition of the algae which could have resulted in the pH change. The initial pH of 6.9 is not considered to have had any adverse effect on the study or its outcomes.

The 24 to 26°C temperature range is not considered to have been a significant deviation from the guideline requirements which can be read as to indicate a temperature of 26°C is acceptable.

The use of a light intensity greater than that specified by US EPA OPPTS 850.5400, while a deviation from that guideline is not considered to have had any significant adverse effect on the study's conduct or results given the satisfactory control growth. Additionally, it is noted that the study was not specifically conducted to the US EPA OPPTS 850.5400 requirements. Although exceeding the OECD 201 light intensity range to some extent, the light intensity used satisfactorily complied with OECD 201 requirements.

The 0-24 hour %CV of 44 is a failure to meet the 2006 OECD 201 requirements but, as the study was conducted to the 1984 OECD guideline, where such requirement was not specified, the deviation from the 2006 guideline is noted but is not pursued any further, especially as the 24-48 and 48-72 hour %CV values met the OECD's requirement.

E. REVIEWER'S COMMENTS:

In general, the reviewer's recalculated toxicity endpoints were similar to the study authors' and the study is considered to have been generally conducted in accordance with the relevant guideline documents.

As the study was finished in December 2005, before the changes to OECD 201 test guideline were adopted in March 2006, the study has been assessed primarily on the 1984 OECD 201 requirements and any failure to comply with the 2006 guideline is not automatically considered a deficiency or deviation.

Based on the results of this study, as shown below (under "Conclusions"), the sulfinic acid metabolite of pyroxsulam would be classified as slightly toxic to *Pseudokirchneriella subcapitata* in accordance with the classification system of the Australian Government Department of the Environment, Water, Heritage and the Arts ($10 < EC50 \leq 100$ mg/L).

This study is classified as acceptable and satisfies the guideline requirements for an acute toxicity study with the unicellular green alga, *Pseudokirchneriella subcapitata.*

F. CONCLUSIONS:

This study is scientifically sound and is classified as **ACCEPTABLE**.

Statistical Endpoint from the study report	Growth Rate (0-72 h)	Biomass (area under growth curve) (0-72 h)	Cell Density (96 h)
NOEC (mg sulfinic acid metabolite of pyroxsulam/L),	55 (William's test)	55 (William's test)	55 (William's test)
EC50 (mg sulfinic acid metabolite of pyroxsulam/L) (95% C.I. in brackets)	>97 (95% Confidence intervals not calculated)	>97 (95% Confidence intervals not calculated)	85 (72-96)
Reference chemical, if used	**Not applicable as no reference chemical was used.**		

The reviewer calculated endpoints were similar to those reported although a 96 hour cell density NOEC of 15 mg sulfinic acid metabolite of pyroxsulam/L was determined using William's test and the study report's 96 hour cell density counts.
This NOEC of 15 mg sulfinic acid metabolite of pyroxsulam/L, causing 8% inhibition, was not considered by the reviewer as significant, as 25% inhibition at 6.4 mg sulfinic acid metabolite of pyroxsulam/L was not significant. Inhibition at 55 mg sulfinic acid metabolite of pyroxsulam/L caused 24% inhibition. The NOEC is set at 55 mg sulfinic acid metabolite of pyroxsulam/L.

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(Note the above study is designated as Hoberg, 2005a to distinguish it from the study report reference which would be referenced as Hoberg, 2005).

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

24 hour cell density

The ToxCalc analysis of the 24 hour algal cell count data in the study report gave the following results. Cell counts equal the number shown as cells/mL. While the ToxCalc program reported an interrupted dose response, the option to set the LOEC at the lowest response concentration (25 mg sulfinic acid metabolite of pyroxsulam/L) was not used.

Conc-mg/L	1	2	3	4	5	6
S-Control	40000	65000	35000	52500	37500	12500
6.4	27500	12500	27500			
15	20000	30000	17500			
25	22500	15000	12500			
55	30000	17500	22500			
97	12500	20000	5000			

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	40416.67	1.0000	40416.67	12500	65000	43.811	6				40416.7	1.0000
6.4	22500	0.5567	22500	12500	27500	38.490	3	2.167	2.602	21518.6	22500	0.5567
15	22500	0.5567	22500	17500	30000	29.397	3	2.167	2.602	21518.6	22500	0.5567
*25	16666.67	0.4124	16666.67	12500	22500	31.225	3	2.872	2.602	21518.6	20000	0.4948
55	23333.33	0.5773	23333.33	17500	30000	26.964	3	2.066	2.602	21518.6	20000	0.4948
*97	12500	0.3093	12500	5000	20000	60.000	3	3.376	2.602	21518.6	12500	0.3093

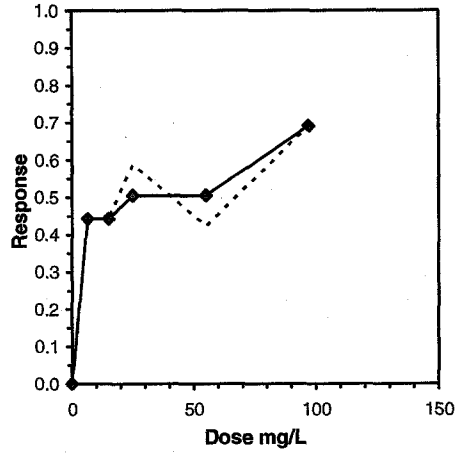
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92333	0.873	-0.2964	3.04757
Bartlett's Test indicates equal variances (p = 0.33)	5.80569	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	55	97	73.0411		21518.6	0.53242	4.3E+08	1.4E+08	0.03905	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	0.722	0.696	0.400	3.159	6.8222
IC10*	1.444	1.042	0.799	6.317	4.5361
IC15*	2.166	1.400	1.199	9.673	3.6563
IC20*	2.887	2.617	1.599	15.502	4.2026
IC25*	3.609	7.041	1.998	24.534	6.4556
IC40*	5.775				
IC50	24.167				

* indicates IC estimate less than the lowest concentration



The calculated 24 hour EC50 for cell density value is ~24 mg sulfinic acid metabolite of pyroxsulam/L which is different from the study report's 24 hour EC50 of 44 mg sulfinic acid metabolite of pyroxsulam/L with 95% confidence limits of 8.8 to 49 mg sulfinic acid metabolite of pyroxsulam/L (determined by linear regression of the response versus mean measured concentration using the ToxStat® program).

The 25 and 97 mg/L mean cell counts at 24 hours are identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report did not identify these results as statistically significantly reduced compared to the control when tested according to Williams' test.

48 hour cell density

The ToxCalc analysis of the 48 hour algal cell count data gave the following results. Cell counts equal the number shown as cells/mL.

Conc-mg/L	1	2	3	4	5	6
S-Control	202500	270000	375000	290000	232500	157500
6.4	260000	287500	147500			
15	382500	195000	335000			
25	242500	435000	357500			
55	190000	180000	235000			
97	172500	122500	215000			

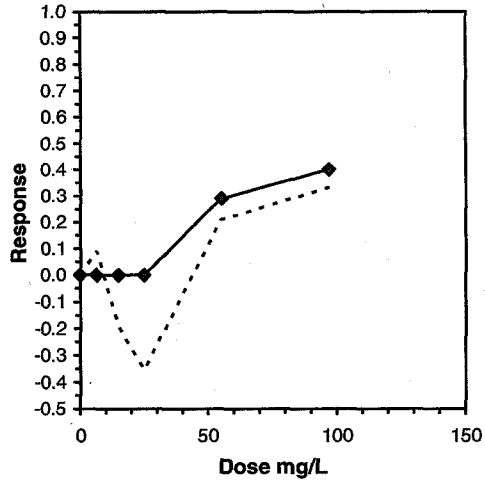
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	254583.3	1.0000	254583.3	157500	375000	29.717	6				283854	1.0000
6.4	231666.7	0.9100	231666.7	147500	287500	32.018	3	0.435	2.602	137212	283854	1.0000
15	304166.7	1.1948	304166.7	195000	382500	32.048	3	-0.940	2.602	137212	283854	1.0000
25	345000	1.3552	345000	242500	435000	28.074	3	-1.715	2.602	137212	283854	1.0000
55	201666.7	0.7921	201666.7	180000	235000	14.528	3	1.004	2.602	137212	201667	0.7105
97	170000	0.6678	170000	122500	215000	27.236	3	1.604	2.602	137212	170000	0.5989

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96705	0.873	-0.1642	-0.649
Bartlett's Test indicates equal variances (p = 0.72)	2.89177	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	97	>97			137212	0.53897	1.3E+10	5.6E+09	0.10009	5, 15
Treatments vs S-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	30.181	8.562	0.000	36.027	-2.1922
IC10	35.361	7.776	0.000	47.053	-2.6646
IC15	40.542	7.431	11.991	58.080	-1.8047
IC20	45.722				
IC25	50.903				
IC40	96.586				
IC50	>97				



The calculated 48 hour EC50 and study report's 48 hour EC50 values are equivalent, namely >97 mg sulfinic acid metabolite of pyroxsulam/L with 95% confidence limits not calculable. The ToxCalc and study report analyses of the 48 hour cell counts found no statistically significant differences between any of the sulfinic acid metabolite of pyroxsulam mean counts and the control counts at that time.

72 hour cell density

The ToxCalc analysis of the 72 hour algal cell count data gave the following results. Cell counts equal the value shown as cells/mL.

Conc-mg/L	1	2	3	4	5	6
S-Control	1100000	1125000	1192500	1317500	665000	960000
6.4	982500	815000	940000			
15	1666700	892500	1616700			
25	1052500	1230000	1212500			
55	775000	1120000	825000			
97	707500	462500	935000			

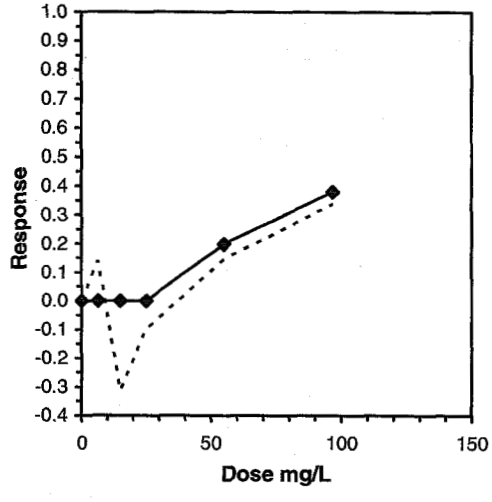
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	1060000	1.0000	1060000	665000	1317500	21.335	6				1132367	1.0000
6.4	912500	0.8608	912500	815000	982500	9.542	3	0.878	2.602	437201	1132367	1.0000
15	1391967	1.3132	1391967	892500	1666700	31.127	3	-1.976	2.602	437201	1132367	1.0000
25	1165000	1.0991	1165000	1052500	1230000	8.397	3	-0.625	2.602	437201	1132367	1.0000
55	906666.7	0.8553	906666.7	775000	1120000	20.563	3	0.913	2.602	437201	906667	0.8007
97	701666.7	0.6619	701666.7	462500	935000	33.678	3	2.133	2.602	437201	701667	0.6196

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93021	0.873	-0.8318	0.58442
Bartlett's Test indicates equal variances (p = 0.34)	5.65308	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	97	>97			437201	0.41245	1.7E+11	5.6E+10	0.04229	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	32.526	12.771	0.000	73.382	-0.1984
IC10	40.051				
IC15	47.577				
IC20	55.158				
IC25	66.758				
IC40	>97				
IC50	>97				



The calculated 72 hour EC50 and study report's 48 hour EC50 values are equivalent, namely >97 mg sulfinic acid metabolite of pyroxsulam/L with 95% confidence limits not calculable. The ToxCalc and study report analyses of the 72 hour cell counts found no statistically significant differences between any of the sulfinic acid metabolite of pyroxsulam mean counts and the control counts at that time.

96 hour cell density (1)

The ToxCalc analysis of the 96 hour algal cell count data gave the following results when hypothesis testing was conducted with the Bonferroni t-test. Cell counts equal the value shown as cells/mL.

Conc-mg/L	1	2	3	4	5	6
S-Control	4310000	2675000	2815000	3730000	2245000	3160000
6.4	2130000	2010000	2965000			
15	3475000	2550000	2730000			
25	2420000	1893300	2730000			
55	2050000	2595000	2575000			
97	1035000	1386700	2295000			

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	3155833	1.0000	3155833	2245000	4310000	23.876	6				3155833	1.0000
6.4	2368333	0.7505	2368333	2010000	2965000	21.965	3	1.882	2.602	1089246	2643333	0.8376
15	2918333	0.9247	2918333	2550000	3475000	16.805	3	0.567	2.602	1089246	2643333	0.8376
25	2347767	0.7439	2347767	1893300	2730000	18.017	3	1.931	2.602	1089246	2377217	0.7533
55	2406667	0.7626	2406667	2050000	2595000	12.841	3	1.790	2.602	1089246	2377217	0.7533
*97	1572233	0.4982	1572233	1035000	2295000	41.353	3	3.784	2.602	1089246	1572233	0.4982

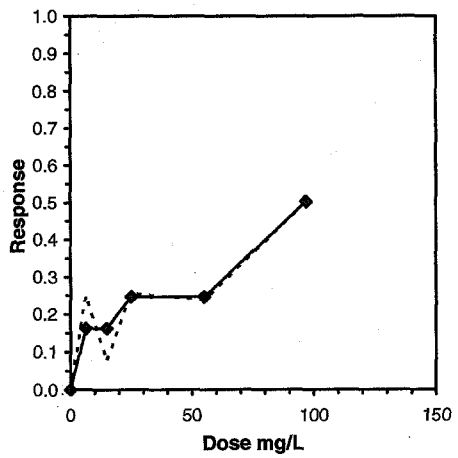
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95647	0.873	0.50613	-0.2412
Bartlett's Test indicates equal variances (p = 0.84)	2.0612	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	55	97	73.0411		1089246	0.34515	1.2E+12	3.5E+11	0.03343	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.970	6.770	0.425	33.029	2.0364
IC10*	3.941	12.446	0.851	87.467	2.4110
IC15*	5.911	17.909	1.276	95.044	1.4904
IC20	19.459				
IC25	55.540				
IC40	80.238				
IC50	96.703				

* indicates IC estimate less than the lowest concentration



The calculated 96 hour EC50 for cell density value is 96.7 mg sulfinic acid metabolite of pyroxsulam/L which is different from the study report's 96 hour EC50 of 85 mg sulfinic acid metabolite of pyroxsulam/L with 95% confidence limits of 72 to 96 mg sulfinic acid metabolite of pyroxsulam/L (determined by linear regression of the response versus mean measured concentration using the ToxStat® program).

The ToxCalc and study report analyses of the 96 hour cell counts both found a statistically significant difference between the 97 mg sulfinic acid metabolite of pyroxsulam/L mean counts and the control counts at that time.

96 hour cell density (2)

The ToxCalc analysis of the 96 hour algal cell count data gave the following results when William's test was used for hypothesis testing. Cell counts equal the value shown as cells/mL.

Conc-mg/L	1	2	3	4	5	6
D-Control	4310000	2675000	2815000	3730000	2245000	3160000
6.4	2130000	2010000	2965000			
15	3475000	2550000	2730000			
25	2420000	1893300	2730000			
55	2050000	2595000	2575000			
97	1035000	1386700	2295000			

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	3155833	1.0000	3155833	2245000	4310000	23.876	6				3155833	1.0000
6.4	2368333	0.7505	2368333	2010000	2965000	21.965	3	1.224	1.750	732448	2643333	0.8376
15	2918333	0.9247	2918333	2550000	3475000	16.805	3	1.224	1.825	763839	2643333	0.8376
*25	2347767	0.7439	2347767	1893300	2730000	18.017	3	1.860	1.850	774302	2377217	0.7533
*55	2406667	0.7626	2406667	2050000	2595000	12.841	3	1.860	1.855	776395	2377217	0.7533
*97	1572233	0.4982	1572233	1035000	2295000	41.353	3	3.784	1.865	780580	1572233	0.4982

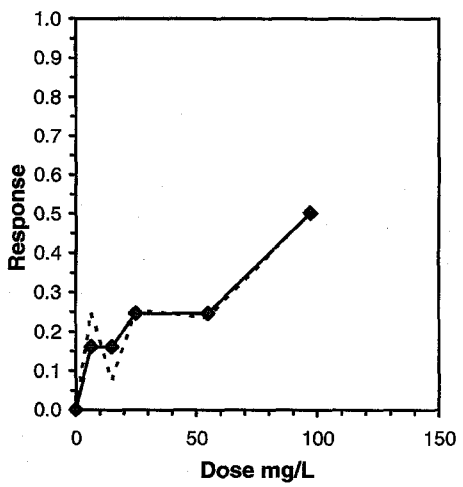
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95647	0.873	0.50613	-0.2412
Bartlett's Test indicates equal variances (p = 0.84)	2.0612	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	15	25	19.3649		780580	0.24735	1.2E+12	3.5E+11	0.03343	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.970	5.466	0.466	30.159	2.2006
IC10*	3.941	10.501	0.931	57.229	2.3839
IC15*	5.911	17.533	1.397	93.741	1.4900
IC20	19.459	22.607	0.000	99.052	0.7316
IC25	55.540				
IC40	80.238				
IC50	96.703				

* indicates IC estimate less than the lowest concentration



The ToxCalc analysis of the 96 hour cell density data, using Williams' test, showed that the 25, 55 and 96 mg/L mean cell counts were statistically significantly less than the control mean's value. In contrast, the study report identified only the 97 mg/L mean result as statistically significantly reduced, according to Williams' test.

The NOEC of 15 mg sulfinic acid metabolite of pyroxsulam/L, causing 8% inhibition, was not considered by the reviewer, as 25% inhibition at 6.4 mg sulfinic acid metabolite of pyroxsulam/L was not significant. Inhibition at 55 mg sulfinic acid metabolite of pyroxsulam/L caused 24% inhibition. The NOEC is set at 55 mg sulfinic acid metabolite of pyroxsulam/L.

0-72 hour mean specific growth rate – determined from study report's results

The ToxCalc analysis of the 0-72 hour mean specific growth rate data presented in the study report gave the following results. Growth rate data are expressed as day^{-1}.

Conc-mg/L	1	2	3	4	5	6
S-Control	1.6000	1.6100	1.6300	1.6600	1.4300	1.5600
6.4	1.5600	1.5000	1.5500			
15	1.7400	1.5300	1.7300			
25	1.5900	1.6400	1.6400			
55	1.4800	1.6100	1.5100			
97	1.4500	1.3100	1.5500			

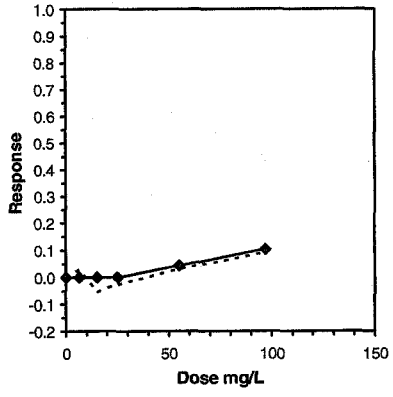
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	1.5817	1.0000	1.5817	1.4300	1.6600	5.143	6				1.6021	1.0000
6.4	1.5367	0.9715	1.5367	1.5000	1.5600	2.092	3	0.767	2.602	0.1527	1.6021	1.0000
15	1.6667	1.0537	1.6667	1.5300	1.7400	7.108	3	-1.449	2.602	0.1527	1.6021	1.0000
25	1.6233	1.0263	1.6233	1.5900	1.6400	1.778	3	-0.710	2.602	0.1527	1.6021	1.0000
55	1.5333	0.9694	1.5333	1.4800	1.6100	4.439	3	0.824	2.602	0.1527	1.5333	0.9571
97	1.4367	0.9083	1.4367	1.3100	1.5500	8.391	3	2.472	2.602	0.1527	1.4367	0.8967

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9261	0.873	-0.7648	0.13285
Bartlett's Test indicates equal variances (p = 0.40)	5.17244	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	97	>97			0.15265	0.09651	0.01954	0.00688	0.05341	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	59.933			
IC10	94.737			
IC15	>97			
IC20	>97			
IC25	>97			
IC40	>97			
IC50	>97			



If the hypothesis test is conducted with William's test, the following results are obtained, with the 97 mg/L mean identified as statistically significantly lower than the control mean:

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9261	0.873	-0.7648	0.13285
Bartlett's Test indicates equal variances (p = 0.40)	5.17244	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	55	97	73.0411		0.10939	0.06916	0.01954	0.00688	0.05341	5, 15

The ToxCalc analyses of the 0-72 hour specific growth rate means for all concentrations tested found no statistically significant differences between any of the sulfinic acid metabolite of pyroxsulam mean counts and the control counts at that time when tested by Bonferroni's t test but use of the Williams' test identified the 97 mg/L mean specific growth rate as statistically significantly less than the control mean. This latter result was also reported by the study report.

0-72 hour mean specific growth rate – determined from reviewer's calculated results

The ToxCalc analysis of the 0-72 hour mean specific growth rate data calculated by the reviewer gave the following results. Growth rate data are expressed as day^{-1}.

Conc-mg/L	1	2	3	4	5	6
S-Control	1.5668	1.5743	1.5937	1.6270	1.3991	1.5214
6.4	1.5292	1.4669	1.5144			
15	1.7053	1.4971	1.6952			
25	1.5521	1.6041	1.5993			
55	1.4501	1.5728	1.4709			
97	1.4197	1.2780	1.5127			

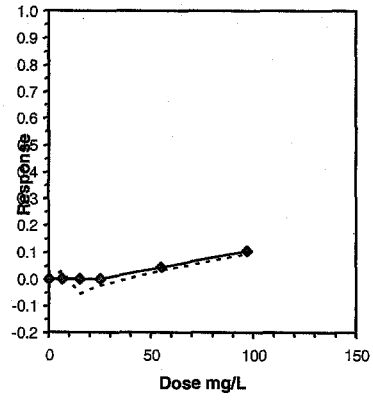
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	1.5471	1.0000	1.5471	1.3991	1.6270	5.192	6				1.5671	1.0000
6.4	1.5035	0.9718	1.5035	1.4669	1.5292	2.166	3	0.754	2.602	0.1504	1.5671	1.0000
15	1.6326	1.0553	1.6326	1.4971	1.7053	7.190	3	-1.480	2.602	0.1504	1.5671	1.0000
25	1.5852	1.0246	1.5852	1.5521	1.6041	1.811	3	-0.659	2.602	0.1504	1.5671	1.0000
55	1.4980	0.9683	1.4980	1.4501	1.5728	4.385	3	0.850	2.602	0.1504	1.4980	0.9559
97	1.4035	0.9072	1.4035	1.2780	1.5127	8.419	3	2.486	2.602	0.1504	1.4035	0.8956

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92431	0.873	-0.7651	0.09316
Bartlett's Test indicates equal variances (p = 0.41)	5.08856	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	97	>97			0.15035	0.09719	0.01913	0.00668	0.05197	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	59.107			
IC10	93.935			
IC15	>97			
IC20	>97			
IC25	>97			
IC40	>97			
IC50	>97			



If the hypothesis test is conducted with William's test, the following results are obtained, with the 97 mg/L mean identified as statistically significantly lower than the control mean:

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92431	0.873	-0.7651	0.09316
Bartlett's Test indicates equal variances (p = 0.41)	5.08856	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	55	97	73.0411		0.10775	0.06965	0.01913	0.00668	0.05197	5, 15

The ToxCalc analyses of the 0-72 hour specific growth rate means for all concentrations tested found no statistically significant differences between any of the sulfinic acid metabolite of pyroxsulam mean counts and the control counts at that time when tested by Bonferroni's t test. Williams' test identified the 97 mg/L mean specific growth rate as statistically significantly less than the control mean. This last result was also determined by the study report.

0-72 hour biomass calculated from the 0-72 hour total biomass results given in the study report.

ToxCalc analysis of the reported 0-72 hour biomass gave the following results. Biomass is expressed as area under the growth curve.

Algal Reproduction Test-Reproduction

Start Date: Test ID: 6767 Sample ID:
End Date: Lab ID: Sample Type:
Sample Date: Protocol: EPAM 94-EPA/600/4-91/003 Test Species: CP-Champia parvula
Comments:

Conc-mg/L	1	2	3	4	5	6
S-Control	674100	778800	880900	865000	522900	542100
6.4	670600	613700	539600			
15	1067600	570600	996500			
25	676400	935800	847800			
55	517300	635800	575400			
97	453400	310100	581900			

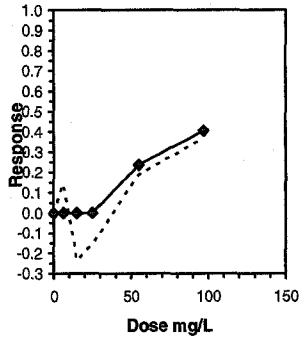
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	710633.3	1.0000	710633.3	522900	880900	22.021	6				754208	1.0000
6.4	607966.7	0.8555	607966.7	539600	670600	10.805	3	0.945	2.602	282847	754208	1.0000
15	878233.3	1.2358	878233.3	570600	1067600	30.605	3	-1.542	2.602	282847	754208	1.0000
25	820000	1.1539	820000	676400	935800	16.087	3	-1.006	2.602	282847	754208	1.0000
55	576166.7	0.8108	576166.7	517300	635800	10.284	3	1.237	2.602	282847	576167	0.7639
97	448466.7	0.6311	448466.7	310100	581900	30.318	3	2.412	2.602	282847	448467	0.5946

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95814	0.873	-0.5838	-0.2235
Bartlett's Test indicates equal variances (p = 0.41)	5.01849	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	97	>97			282847	0.39802	7.8E+10	2.4E+10	0.03265	5, 15
Treatments vs S-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.354	10.717	0.000	37.487	-1.4977
IC10	37.708	9.995	0.000	49.974	-1.9143
IC15	44.063	8.448	15.846	62.399	-0.2135
IC20	50.417				
IC25	58.457				
IC40	95.665				
IC50	>97				



If the hypothesis test is conducted with William's test, the following results are obtained, with the 97 mg/L mean identified as statistically significantly lower than the control mean:

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95814	0.873	-0.5838	-0.2235
Bartlett's Test indicates equal variances (p = 0.41)	5.01849	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test	55	97	73.0411		202695	0.28523	7.8E+10	2.4E+10	0.03265	5, 15
Treatments vs D-Control										

The ToxCalc analyses of the 0-72 hour biomass means for all concentrations tested found no statistically significant differences between any of the sulfinic acid metabolite of pyroxsulam mean counts and the control counts at that time when tested by Bonferroni's t test. Williams' test identified the 97 mg/L mean biomass result as statistically significantly less than the control mean. This last result was also determined by the study report.

0-72 hour biomass calculated from the 0-72 hour total biomass results determined by the reviewer.

ToxCalc analysis of the reviewer calculated 0-72 hour biomass gave the following results. Biomass is expressed as area under the growth curve.

Conc-mg/L	1	2	3	4	5	6
S-Control	767500	872500	981250	976250	577500	625000
6.4	753750	682500	620000			
15	1210850	646250	1135850			
25	766250	1040000	951250			
55	582500	732500	645000			
97	513750	348750	662500			

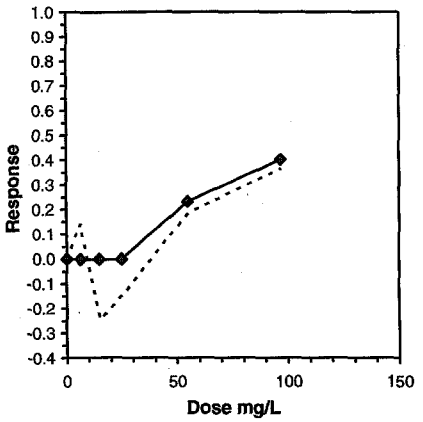
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	800000	1.0000	800000	577500	981250	21.677	6				850558	1.0000
6.4	685416.7	0.8568	685416.7	620000	753750	9.764	3	0.939	2.602	317667	850558	1.0000
15	997650	1.2471	997650	646250	1210850	30.735	3	-1.619	2.602	317667	850558	1.0000
25	919166.7	1.1490	919166.7	766250	1040000	15.195	3	-0.976	2.602	317667	850558	1.0000
55	653333.3	0.8167	653333.3	582500	732500	11.533	3	1.202	2.602	317667	653333	0.7681
97	508333.3	0.6354	508333.3	348750	662500	30.874	3	2.389	2.602	317667	508333	0.5976

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95789	0.873	-0.6229	-0.0984
Bartlett's Test indicates equal variances (p = 0.40)	5.09634	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	97	>97			317667	0.39708	9.9E+10	3E+10	0.03224	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.469	10.774	0.000	38.994	-1.4654
IC10	37.938	10.235	0.000	53.077	-1.7592
IC15	44.407	8.695	16.911	65.816	0.5430
IC20	50.876				
IC25	59.465				
IC40	96.420				
IC50	>97				



If the hypothesis test is conducted with William's test, the following results are obtained, with the 97 mg/L mean identified as statistically significantly lower than the control mean:

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95789	0.873	-0.6229	-0.0984
Bartlett's Test indicates equal variances (p = 0.40)	5.09634	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	55	97	73.0411		227648	0.28456	9.9E+10	3E+10	0.03224	5, 15