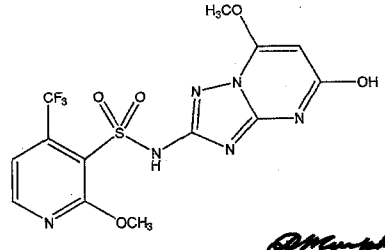
Data Evaluation Report on the Acute Toxicity of 5-OH Metabolite of Pyroxsulam (5-OH metabolite of XDE-742) to Algae, *Pseudokirchneriella subcapitata*

PMRA Submission Number 2006-4727; 1283231 EPA MRID Number 469084-47 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:	5-hydroxy-pyroxsulam or 5-hydroxy-XDE-742
Purity (%):	100%
Common name:	5-OH Metabolite of XDE-742
Chemical name:	3-pyridinesulfonamide, N-(1,5-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
IUPAC:	N-(5-hydroxy-7-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS name:	3-pyridinesulfonamide, N-(1,35-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)- (Note: the reference to "1,35-dihydro-7-methoxy" is made in the study report's Certificate of Analysis (page 57 of the study report). It is believed this should be "1,5-dihydro etc.".
CAS No.:	Not available.
Synonyms:	5-desmethyl XDE-742 metabolite, 5-OH-XDE-742

Chemical Structure:



Primary Reviewer: Daryl Murphy **Date:** 20 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland **Date:** 24 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Émilie Larivière **Date:** 31 July 2007
Environmental Assessment Directorate, PMRA

Christopher Salice **Date:** 07 December 2007
Environmental Protection Agency, OPP, EFED, ERBIV

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

CITATION: Hoberg, J R. 2006. 5-OH Metabolite of XDE-742 - Acute Toxicity Test to the Freshwater Green Alga, *Pseudokirchneriella subcapitata.* Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts, Springborn Smithers Study No. 12550.6405, Sponsor Protocol/Project No. 050107. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana. 5 April 2006. Unpublished report.

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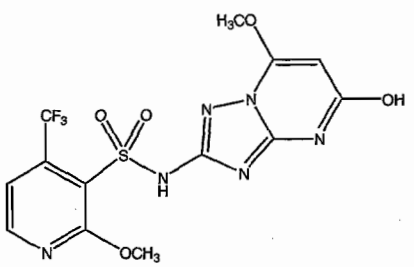
IUPAC: N-(5-hydroxy-7-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS name: 3-pyridinesulfonamide, N-(1,35-dihydro-7-methoxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
(Note: the reference to "1,35-dihydro-7-methoxy" is made in the study report's Certificate of Analysis (page 57 of the study report). It is believed this should be "1,5-dihydro etc.".
CAS No.: Not available.
Synonyms: 5-desmethyl XDE-742 metabolite, 5-OH-XDE-742

Chemical Structure:



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Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana. 5 April 2006. Unpublished report.

EXECUTIVE SUMMARY:

The purpose of this study was to determine the effect of the 5-OH metabolite of pyroxsulam (5-OH metabolite of XDE-742) on the growth of the freshwater green alga, *Pseudokirchneriella subcapitata* in a 96-hour acute toxicity study. The alga was exposed to 5-OH metabolite of pyroxsulam at nominal concentrations of 0 (control), 6.3, 13, 25, 50 and 100 mg/L (corresponding mean measured concentrations of, respectively, 0 (control), 5.2, 11, 20, 42 and 80 mg 5-OH metabolite of pyroxsulam/L). The experiment was carried out taking account of relevant OECD, European Communities and US EPA guidelines.

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 23–24°C. The light intensity range was 3900-4700 lux. The pH value in the controls was 6.9 at test initiation and 8.9 at test termination. In the solutions containing the 5-OH metabolite of pyroxsulam the pH ranged from 4.8 to 6.6 at test initiation and 6.2 to 9.3 at test termination with solution pH inversely proportional to the test concentration. The pH of 4.8 at time 0 has confounded the study's interpretation as the observed lack of algal growth seen at the mean measured concentration of 80 mg/L could be caused by the presence of the 5-OH metabolite of pyroxsulam at this concentration, the pH of the test solutions, or a combination of the two factors. As the four related hydroxyl metabolites all exhibited the same problem and had nearly identical results, inhibition was clearly caused by test acidity. This has resulted in the study being classed as supplemental by the Australian Government Department of the Environment, Water, Heritage and the Arts.

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 ~1.8 X 10^6 cells/mL is ~52% of the recommended value. Consequently, the US guideline value was not reached and the US EPA requirement has not been met. This is a second major reason for the study being classed as supplemental by the Australian Government Department of the Environment, Water, Heritage and the Arts. The OECD validity criteria for exponential growth have been met.

The pH of the control media increased by more than 1.5 pH unit (note the latest version of OECD 201 states that the control medium pH should not increase by greater than 1.5 units during the test). Other deviations or deficiencies included the light intensity exceeding OECD requirements. None of these latter deviations and deficiencies was considered to have adversely affected the study or its results.

After 72 hours, inhibition of mean specific growth rate relative to controls ranged from -2% (growth stimulation) at 11 mg/L to 35% at 80 mg/L. The inhibition of biomass relative to controls ranged from -9% at 11 mg/L to 81% at 80 mg/L. After 96 hours, inhibition of cell density relative to controls ranged from -6% at 42 mg/L to 89% at 80 mg/L.

Based on the results of this study, as shown below, the 5-OH 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 SUPPLEMENTAL by the Australian Government Department of the Environment, Water, Heritage and the Arts as it fails to fully satisfy the guideline requirements for an acute toxicity study with the unicellular green alga, *Pseudokirchneriella subcapitata* as the toxicity was the result of the acid pH of the highest test concentration.

The US EPA considered that, because of the observed issues associated with pH and the lack of reaching EPA guideline specified exponential growth, the study does not meet guideline requirements, however, it is classified as supplemental.

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The PMRA does not share the same acceptability classification as DEWHA. This study is classified by the PMRA as being of limited utility due to the pH shift, the uncertainty related to exponential growth in the controls and the effect of the acidity of the test substance. Some useful information can be obtained from this study and used in a risk assessment (EC50 expected to be >42 mg 5-OH metabolite of pyroxsulam/L). A new study would not provide additional information. The low toxicity of the test substance has been adequately demonstrated.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type: Static

Although the study is rated as supplemental by the Australian Government Department of the Environment, Water, Heritage and the Arts, the following endpoints are provided for information as they give an indication of the lack of toxicity of the 5-OH metabolite of pyroxsulam to *Pseudokirchneriella subcapitata.* Actual EC50 values are all expected to be >42 mg 5-OH metabolite of pyroxsulam/L.

Results Based on Mean Measured Concentrations, the study's reported endpoints were:

Biological Parameter	Based on Mean Measured Concentrations (mg 5-OH metabolite of pyroxsulam/L)	
	EC50 (95% confidence limits)	NOEC
96-Hour Cell Density	63 (58-64)	42
0-72 hour Total Biomass	57 (50-62)	20
0-72-Hour Average Growth Rate	>80 (not applicable)	80

Results Based on Mean Measured Concentrations which will be used for risk assessment as a result of the omission of the nominal 100 mg/L solutions having a pH of 4.8 at time 0 are:

Biological Parameter	Based on Mean Measured Concentrations (mg 5-OH metabolite of pyroxsulam/L)	
	EC50	NOEC
96-Hour Cell Density	>42	42
0-72 hour Total Biomass	>42	20
0-72-Hour Average Growth Rate	>42	42

Endpoint(s) effected: Cell count, biomass and growth rate of the *Pseudokirchneriella subcapitata.*

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 this protocol meet the requirements specified in:

- Guideline for Testing of Chemicals. Alga, Growth Inhibition Test #201. Adopted 7 June 1984. Organization for Economic Cooperation and Development. Paris, France;

- Official Journal of the European Communities. 1992. Commission Directive 92/69/EEC of 31 July 1992. Part C: Methods for the determination of Ecotoxicity. Method C.3. Algal Inhibition Test. L383A Volume 35, 29 December 1992; and

- The U.S. Environmental Protection Agency's (US EPA) *Pesticide Assessment Guidelines,* Subdivision J Hazard Evaluation: Non-target Plants, Report No. EPA 540/9-82-020, U.S. Environmental Protection Agency,, Washington, D.C. 1982.

Guidelines appear to have been generally followed with the exception of certain parameters (e.g. growth medium, pH at the highest test concentration etc.). For further details see the relevant text entries below and the deviations/deficiencies table, page 36 of this DER.

The study author reported the following deviation from the study protocol:

> The protocol states that the control mean coefficient of variation (CV) for the section-by-section growth rates should not exceed 35% and the CV for the 0 to 72-hour average growth rate should not exceed 7%. The results of this test indicated that the CV for the 0 to 24 hour growth rate of the control was 42%. All other CV values for growth rate were within the required limits. Of the six control replicates, replicate B was considered an outlier, and was dropped from the mean and statistical analysis which provided a 0 to 24 hour CV value of 33%, which is within the above criterion. Therefore, the NOEC, EC25 and EC50 calculations were repeated excluding control replicate B (N = 5). Deletion of this replicate from the data analysis provides a more conservative estimate of the NOEC and EC values than when this data point is included in the analyses.

The study author considered such deviation did not have a negative impact on the results or interpretation of the study. It is also noted that the CV requirement relates to the 2006 OECD 201 guideline, and not to the 1984 version, to which the study was conducted.

This specific issue is considered further on page 27 of this DER under "Validity of test".

As indicated above, OECD 201 was originally adopted in 1984 with a revised version adopted in March 2006. The study report has been assessed primarily against the 2006 version with its requirements largely met (the study was conducted in late February to early March 2006, at about the same time as the changes to the OECD 201 test guideline were being published) and deviations from the current OECD Guideline are generally considered minor with the exception of the pH of 4.8 in the 100 mg 5-OH metabolite of pyroxsulam/L test concentration which renders the test being classified as supplementary by the Australian Government Department of the Environment, Water, Heritage and the Arts, based on recommendations for a pH above 5.0 at the first measurement in US EPA OPPTS 850.5400.

COMPLIANCE:
The data and report for "5-OH Metabolite of XDE-742 - 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 (40 CFR, Part 160) Good Laboratory Practice regulations, namely:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris, France. 1998. 41 pp.; and

- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, 48 (230); 34052-34074, 1989.

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 Claims, Good Laboratory Practice Compliance and Quality Assurance statements were provided.

A. MATERIALS:

1. **Test Material:** 5-OH metabolite of XDE-742 (5-OH metabolite of pyroxsulam)
   Description: Solid
   Lot No./Batch No.: E2008-48
   Test substance No.: TSN105231
   Purity: 100%.
   Stability of Compound Under Test Conditions: **Stable.** Test substance concentrations were measured at 0 hour (test initiation) and 96 hours (test termination) with the reported mean measured concentrations ranging from 80 to 84% of the nominal concentrations of 6.3 to 100 mg 5-OH metabolite of pyroxsulam/L (page 17 of this DER refers). Such results indicate the test substance was stable for at least 96 hours under the test conditions.
   Storage conditions of test chemicals: The test substance (also identified as SSL No. 112-86) was stored at room temperature in the original container in a dark ventilated cabinet.
   Physicochemical properties of the 5-OH metabolite of pyroxsulam: Physicochemical data for the 5-OH metabolite of pyroxsulam were not available to the laboratory when the study report was being written (Hoberg, 2006a). The study report also stated that characterization and verification of the test substance's identity were the responsibility of the Study Sponsor. Consequently, the values for physicochemical parameters were not given in the study report

2. **Test organism:**
   Name: Freshwater green alga
   Class: Chlorophyceae
   Species: *Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum*
   Strain: 1648
   Source: In-house laboratory cultures maintained in stock culture at Springborn Smithers and originally obtained from the University of Texas.
   Age of inoculum: The inoculum used to initiate the toxicity test with 5-OH metabolite of pyroxsulam was taken from a stock culture that had been transferred to fresh medium three days before testing.

Method of cultivation: The stock cultures were maintained in AAP medium (Algal Assay Procedure (AAP) medium prepared with sterile, deionised water) 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 3900 to 4700 lux. Lighting was supplied by fluorescent bulbs. Culture flasks were agitated continuously on an orbital shaker. Temperature was controlled using an environmental chamber. Stock cultures were grown in 250-mL glass flasks each containing 100 mL of medium. The flasks were covered with stainless steel caps which permitted gas exchange.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study:
A preliminary range-finding exposure was conducted at Springborn Smithers at nominal 5-OH metabolite of pyroxsulam concentrations of 0.010, 0.10, 1.0, 10 and 100 mg/L, and a control. Two exposure vessels were established for each concentration and the control. At test termination, cells exposed to all treatment levels tested and the control were observed to be normal. Following 96 hours of exposure, cell densities in the 0.010, 0.10, 1.0, 10 and 100 mg/L treatment levels averaged 440.25, 370.75, 481.50, 469.50 and 0.50 X 10^4 cells/mL, respectively. The control averaged 366.00 X 10^4 cells/mL. Based on these results and consultation with the Study Sponsor, the study reported that nominal 5-OH metabolite of pyroxsulam concentrations of 6.3, 13, 25, 50 and 100 mg/L were selected for the definitive exposure.

b. Definitive Study
The purpose of the study was to determine the effect of 5-OH metabolite of pyroxsulam on the growth of the freshwater green alga, *Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum*. The results were based on mean measured concentrations of 5-OH metabolite of pyroxsulam and were reported as the 24, 48, 72 and 96 hour EC25 and EC50 values and the 96-hour No-Observed-Effect Concentration (NOEC) for cell density and EC50 values for 72-hour total biomass and average growth rate data, denoted as EbC50 and ErC50, respectively, and the NOEC values for total biomass and average specific growth rate.

The experimental phase of the 96-hour acute toxicity test was conducted from 27 February to 3 March 2006 at Springborn Smithers Laboratories, (SSL) located in Wareham, Massachusetts. All original raw data, the protocol and the original final report produced during the study are archived by the Toxicology and Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.

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

Table 1. Experimental Parameters

Parameter	Details	Remarks/Criteria
Acclimation period:	The inoculum used to initiate the toxicity test was taken from a stock culture that had been transferred to fresh medium three days before testing.	Acclimation is considered acceptable and the requirement 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 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 current 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.*

Culturing media and conditions: (same as test or not)	The algae were maintained in stock culture in AAP medium. The AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium. Culturing and test media kept under the similar conditions: **Culture**: Environmental chamber; 24 ± 2°C; Continuous illumination; 3900 to 4700 lux; Orbital shaker; 100 ± 10 rpm **Test**: Environmental chamber; 24 ± 2°C; Continuous illumination; 3800 to 4700 lux; Orbital shaker; 100 ± 10 rpm Stock and test cultures were grown in 250-mL flasks each containing 100 mL of medium. The flasks were covered with stainless steel caps which permitted gas exchange.	Requirement considered met. Comparison of the reported typical culturing and test media conditions indicated they were equivalent.

Health: (any mortality observed)	Observations of the health of the algal cells were made at each 24-hour interval. No reference to phytotoxicity effects were identified in the study report and at test termination, cells exposed to all treatment levels tested and the control were observed to be normal. These observations are taken to indicate that the algal cultures used were healthy and growing at the test's initiation.	Requirement 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.
Test system Static/static renewal Renewal rate for static renewal	Static Not applicable for a static system in which there was no renewal of test medium.	Requirement considered met. OECD 201 does not specifically refer to static or static renewal but can be interpreted as referring to them as no mention is made of renewal of test solutions. . US EPA OPPTS 850.5400 defines a static system as one in which old nutrient medium is not renewed or replaced. It does not refer to renewal intervals.
Incubation facility	Temperature controlled environmental chamber	Incubation facility is considered acceptable. Requirements considered met. OECD 201 refers to use of a cabinet or chamber is recommended, 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	Requirement considered met. OECD 201 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 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass flasks fitted with stainless steel caps which permitted gas exchange. 250 mL 100 mL	Requirement 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		See deviations/deficiencies table, page 36 of this DER with respect to use of a sodium selenate.
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.	OECD 201 refers to use of AAP medium and provides the composition of this growth medium. Annex 3 of the OECD 201 guideline, which contains the AAP composition, does not identify sodium selenate as a constituent of the medium. However, the Annex goes on to describe the preparation of the US EPA medium and notes that sodium selenate (as the pentahydrate) is used only in the medium for stock cultures of diatom species at a final concentration in the AAP medium of 0.01 µg/L or 0.00001 mg/L. US EPA OPPTS 850.5400 states that media used for algal culture and preparation of test solutions should conform to those currently recommended by the EPA for freshwater and marine algal bioassays *EPA recommends 20X-AAP and no chelators.* This template requirement is noted but is not considered appropriate in the light of the current OECD and US EPA OPPTS requirements which allow use of chelating agents (the AAP medium used contains sodium EDTA as a chelating agent).

pH at test initiation and pH at test termination:	The AAP medium was used to prepare the exposure solutions was adjusted, if necessary, to 7.5 ± 0.1 prior to use. The pH values at 0 and 96 hours were: **Concentration* / 0 hours / 96 hours** **Control** 6.9 8.9 **6.3** 6.6 8.9 **13** 6.5 9.3 **25** 6.3 9.2 **50** 5.9 8.6 **100** 4.8 6.2 *** Nominal, mg 5-OH metabolite of pyroxsulam.** **Note: the control medium pH increased from 6.9 at time 0 to 8.9 at 96 hours, i.e. an increase of 2.0 pH units.** The study report noted that the increase in pH during the exposure is common in static algal cultures and is due to photosynthesis by the algae.	See deviations/deficiencies table, page 36 of this DER with respect to control pH change and initial and final pH values. The study report stated that the initial pH of this medium was adjusted, to 7.5 ± 0.1 prior to use. OECD 201 identifies AAP as having a pH of 7.5. OECD recommends (2006) the medium pH should not increase by greater that 1.5 pH units during the test US EPA OPPTS 850.5400 states that the pH of the nutrient medium is to be 7.5 (± 0.1) for *Selenastrum.* The US EPA guideline also states 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, and the test solution measured for pH on each day of the test. This was the situation in the study under assessment for the 100 mg/L replicates where the pH at time 0 was 4.8. However, the pH was not adjusted. *OECD recommends the medium pH after equilibration with air be ~8 with less than .001 mmol/l of chelator, if used.* This template requirement is noted but is not considered appropriate in the light of the current OECD and US EPA OPPTS requirements with respect to medium pH and specified concentrations of chelating agents.

Chelator used:	Yes, as required for AAP medium (disodium ethylenediamine tetraacetic acid dihydrate is the chelator, present at a concentration of 300 µg/L).	Requirement considered met. OECD 201 identifies the use of disodium ethylenediamine tetraacetic acid in the AAP medium. US EPA OPPTS 850.5400 states that chelating agents are included in the nutrient medium for optimum cell growth. No chelating agents are to be included in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test chemical. *EPA recommends 20X-AAP medium and no chelators.* This template requirement is noted but is not considered appropriate in the light of the current OECD and US EPA OPPTS requirements which advises on the media to use and allows use of chelating agents.
Carbon source:	Not stated in study report but identified as sodium bicarbonate in the study profile template (Hoberg, 2006a).	Requirement considered met. OECD 201 and US EPA OPPTS 850.5400 do not make specific reference to a carbon source
Salinity (for marine algae):	Not applicable as a freshwater alga was used.	Requirement not considered relevant.
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes. The medium used was standard AAP medium modified by addition of sodium selenate.	Full details of the medium's composition were provided.
Dilution water		Requirement considered met.

source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Sterile, deionised water used to prepare growth medium. Source not identified. Not reported N/A Sterilised and deionised See below Not reported See below See below Not reported. 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 have been detected at concentrations that are considered toxic in any of the water samples analysed in agreement with ASTM guidelines (2002). In addition, a representative sample of AAP medium was analysed monthly for total organic carbon (TOC) concentration. The TOC concentration of the samples collected in February and March 2006 was 0.63 and 0.41 mg/L, respectively.	No specific requirements were identified for these parameters 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. *EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *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:* *EPA: 30-35 ppt. EPA is against the use of dechlorinated water.* The template requirements are considered to be either covered by the current OECD and US EPA OPPTS guideline requirements or not identified as relevant for the present study.

Indicate how the test material is added to the medium (added directly or used stock solution).	A 100 mg/L stock solution was prepared prior to test initiation by placing 0.2004 g of 5-OH metabolite of pyroxsulam (100% purity) 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 visible undissolved test substance. Following sonication and three hours of magnetic stirring, the stock solution was observed to be clear and colourless with visible undissolved test substance. The test solution was allowed to settle and the soluble portion was removed by siphon and used to prepare test solutions by serial dilutions. All resulting test solutions were observed to be clear and colourless with no visible undissolved test substance. Additional untreated AAP medium was used for the control.	Requirement considered met with the description in the report considered satisfactory.
Aeration or agitation	Continuous agitation (approx. 100 revs./min.) by means on an orbital shaker.	Requirement 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. 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.* The use of an orbital shaker working at the rate of approximately 100 rpm is considered to have met both OECD and US EPA OPPTS requirements. *EPA recommends agitation only for Selenastrum sp. at 100 cycles per min and Skeletonema sp. at ~60 cycles per min. Aeration is not recommended.*

Initial cells density	Approximately 10,000 cells/mL (for each replicate)	Requirement 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.*
Number of replicates Control: Solvent control: Treatments:	6, inoculated with algae. N/A 3, inoculated with algae In order to estimate the impact that the presence of algal biomass had on the test substance concentration, an additional replicate flask (D) of the 25 mg/L (nominal) treatment level was prepared. This flask, which was not inoculated with algae, was analysed at 96 hours of exposure for 5-OH metabolite of pyroxsulam concentration. The results of this analysis were compared with the results for the 25 mg/L solution containing algae.	Requirement 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 in the definitive test.

		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 solubilise 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/L Ratios of nominal concentrations were 1:1.9 to 1:2.1.	Requirement considered met. OEDC 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.
Measured:	The reported concentrations were: **Nominal Conc.[a] / Measured Concentration[a] 0 hour / 96 hour / Mean[b] / % of Nominal** **Control** <0.21 <0.20 NA[c] NA **6.3** 5.2 5.1 5.2 82 **13** 11 11 11 84 **25** 20 20/20[d] 20 81 **50** 41 42 42 84 **100** 80 80 80 80 **a. mg 5-OH metabolite of pyroxsulam/L.** **b. Mean measured concentrations and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.** **c. NA = Not Applicable.** **d. Result of the additional sample without algae present to determine biological uptake/degradation** Undissolved material was noted in the 100 mg/L stock solution after 3 h stirring and the measured concentration of 80 mg/L is considered to be the limit of solubility in the test medium.	US EPA OPPTS 850.5400 states that 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 ratios of the nominal concentrations were 1:1.9 to 1:2.1 which are considered to have complied with the OECD and US EPA guidelines' requirements for this parameter. The result of the analysis of the 25 mg/L sample with and without algae present indicates that, at this concentration, the presence of the algae did not affect the concentration of the 5-OH metabolite of pyroxsulam. *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 not considered further in the light of the specific requirements in the current OECD and US EPA OPPTS guidelines.

Solvent (type, percentage, if used)	N/A; a solvent was not used	The parameter is not relevant as a solvent was not used.
Method and interval of analytical verification	Test solutions were analyzed for the presence of 5-OH-pyroxsulam at 0- and 96-hours using HPLC. All exposure solutions and QC samples were analysed for 5-OH metabolite of pyroxsulam using high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV) based on methodology validated at Springborn Smithers.	Requirement considered met. Methodology was validated (24 January 2006) to quantify the amount of 5-OH metabolite of pyroxsulam present in 20X AAP medium (a freshwater algal medium). This method validation was conducted based on the guidance document SANCO/3029/99 rev.4. Recovery samples were analysed by automated injection on a high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV). This method was validated by fortification of 20X AAP medium with 5-OH metabolite of pyroxsulam at concentrations of 0.05 and 100 mg/L. Recoveries averaged 103 ± 5.80% with a limit of quantitation (LOQ) of 0.0115 mg/L. The quality control sample range for subsequent studies was set at 80 to 120%. Conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation study. Representative chromatograms were presented as were from the analysis of a calibration standard, recovery sample and a control sample. A typical linear regression analysis for 5-OH metabolite of pyroxsulam ($r^2 = 0.99999$) was also presented in the study report.

Test conditions Temperature:	23-24°C	Temperature requirement considered met. 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. *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C* *EPA temperature: Skeletonema: 20 °C, Others: 24-25 °C;*
Photoperiod:	Continuous	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. *OECD recommended continuous uniform illumination.* *EPA photoperiod: S. costatum 14 hr light/ 10 hr dark. Others: Continuous.*

Light intensity and quality:	3900-4700 lux. The photosynthetically active radiation (PAR) of the test area measured at test initiation ranged from 62 to 75 $\mu E/m^2/s$.	See deviations/deficiencies table, page 36 of this DER with respect to light intensity. OECD 201 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 OPPTS 850.5400 states fluorescent lights providing 4300 lux are to be used for *Selenastrum.* *OECD recommended illumination provided at approximately 8000 Lux measured with a spherical collector.* *EPA light: Anabaena: 2000 lux (±15%), Others: 4000-5000 lux (±15%)* These template requirements are noted but not considered further in the light of the specific requirements in the current OECD and US EPA OPPTS guidelines.
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 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.
Other parameters, if any	Conductivity measured at test initiation and termination in the treatment and control solutions was 80 µmhos/cm.	Requirement considered met.

2. Observations:

Table 2. Observation parameters

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell densities were counted and biomass (cells/mL) and growth rate (per day) calculated. pH, conductivity, temperature, light intensity and concentrations of 5-OH metabolite of pyroxsulam in the test solutions were also determined over the course of the study.	The requirement is considered met. The parameters determined are acceptable. 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	At each 24-hour interval, a single cell count was conducted on each replicate solution of the treatment levels (A, B and C) and the six controls (A, B, C, D, E and F) using a haemocytometer and a compound microscope. Appropriate instrumental techniques were used for physico-chemical parameters listed above.	Requirement considered met. Measurement techniques used are considered acceptable. OECD 201 refers to cell counts, being made using an electronic particle counter, a microscope with counting chamber, or a flow cytometer. Other biomass surrogates can be measured using a flow cytometer, fluorimeter, spectrophotometer or colorimeter. US EPA OPPTS 850.5400 refers to the algal growth response being 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.
		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).* These template requirements are noted but not considered further in the light of the OECD and US EPA OPPTS having specific requirements.
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.

Parameters	Details	Remarks Criteria
Other observations, if any	At test termination (96 hours), cells exposed to the treatment levels tested and the control were observed to be normal.	Requirement considered met.
Indicate whether there was an exponential growth in the control	The mean control 72 hour cell growth was 129.23 X 10^4 cells/mL (cf. 1 X 10^4 cells at test initiation). At 96 hours, the mean control cell density was ~1.84 X 10^6 cells/mL. The mean 0-72 hours calculated growth rate of the controls was 1.62 day^{-1}.	See deviations/deficiencies table, page 36 of this DER with respect to the attainment of exponential growth. The exponential growth parameter was satisfactorily demonstrated for OECD 201 which 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. At 72 hours, the mean cell density in the controls was ~129 X 10^4 cells/mL. This represents a factor of ~129 (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}. The 1.62 day^{-1} value meets this requirement. 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 ~1.8 X 10^6 cells/mL is ~52% of the recommended value of ~3.5 X 10^6/cells/mL. Consequently, the US guideline value was not reached and the US EPA requirement has not been met. *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.*
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 using

Parameters	Details	Remarks
		Criteria
		sterile, deionised water.
Were raw data included?	As tabulated results, yes, as laboratory notes, no. However, the tabulated data presented was made up of individual replicate values which could be used to verify the study report's results. The study report referred on occasion to results presented as being calculated from original raw data and not from the rounded values presented in the study report. All original raw data, the protocol and the original final report produced during the study are archived by the Toxicology and Environmental Research and Consulting archivist and stored at the Dow Chemical Company, Midland, Michigan.	Parameter considered met. OECD 201 lists the results which must be presented in the test report. These are not considered by the reviewer 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. Although 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 tabulated data are generally considered "raw" by the EPA and, because the tabulated results presented in the study report were sufficient to allow statistical analysis, the guideline would be considered met. While raw data were not submitted, the tabulated results presented were sufficient to allow statistical analysis.

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The study report reported the following in relation to inhibitory effects:

At test termination (96 hours), cells exposed to all treatment levels tested and the control were observed to be normal at the end of the exposure period.

Cell density

The effects of the 5-OH metabolite of pyroxsulam on the growth of *Pseudokirchneriella subcapitata* under the test conditions are shown in Table 3 by the mean cell density counts at 24, 48, 72 and 96 hours and the % inhibition after 96 hours.

In the table, significant cell density inhibition (with respect to the mean control cell density) was identified at 96 hours only in the algae exposed to a mean measured concentration of 80 mg 5-OH metabolite of pyroxsulam/L. Since this same phenomenon also occurs in 3 similar tests using 3 other metabolites of similar structure, this is

considered most likely the result of the acidity of the test solutions inhibiting algal growth rather than the specific toxicity of the 5-OH metabolite of pyroxsulam itself.

Table 3. Effect of 5-OH pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*).). Mean results from the study report at 24, 48, 72 and 96 hours (standard deviations shown in brackets) are presented.

Treatment (mean measured concentration (mg ac/L)	Initial cell density (cells/mL)	Mean cell density (x10^4 cells/mL) at				
		24-hours	48-hours	72-hours	96-hours	% inhibition[a, b]
Negative control[b, c]	1 x 10^4	5.80 (2.91)	29.45 (4.91)	129.23 (22.12)	183.62 (36.27)	NA[d]
5.2	1 x 10^4	5.17 (2.57)	30.25 (5.38)	137.58 (5.08)	169.94 (26.51)	7
11	1 x 10^4	5.67 (2.24)	31.08 (3.75)	142.72 (11.03)	178.28 (30.37)	3
20	1 x 10^4	6.33 (3.91)	33.67 (7.56)	134.08 (2.27)	162.83 (40.42)	11
42	1 x 10^4	5.00 (1.75)	26.33 (3.30)	88.33 (29.63)	195.00 (37.24)	-6
80	1 x 10^4	1.75 (2.05)	6.92 (1.81)	24.67 (6.48)	19.67 (2.08)[e]	89
Reference chemical (if used)	N/A					

a. Percent inhibition relative to the control.
b. Mean, standard deviation (SD) and percent inhibition were calculated in the study report from original raw data, not from the rounded values presented in the table in the study report.
c. Based on the 0 to 24 hour growth rate criterion, control replicate B was excluded by the study author from the mean and all statistical analyses as an outlier.
d. NA = not applicable.
e. Significantly reduced compared to the control, based on Williams' test.

Based on the 0 to 24 hour growth rate criterion, replicate B of the control was excluded as an outlier from the mean and all statistical analyses to lessen the variability within the data set. All statistical analyses were performed using five replicates of the control (A, C, D, E and F). The 96-hour cell density in the control averaged 183.62 x 10^4 cells/mL. Cell density in the 5.2, 11, 20, 42 and 80 mg/L treatment levels averaged 169.94, 178.28, 162.83, 195.00 and 19.67 x 10^4 cells/mL, respectively. Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set passed the requirements for normality and homogeneity of variance, therefore, Williams' Test was used to determine treatment-related effects. Statistical analysis (Williams' Test) determined a significant reduction in cell density in the 80 mg/L treatment level when compared to the control (183.62 x 10^4 cells/mL). Therefore, the 96 hour NOEC for cell density was determined to be 42 mg/L. The 96-hour EC50 was determined to be 63 mg/L, with 95% confidence intervals of 58 to 64 mg/L.

Growth rate and Biomass
The mean specific growth rates per day and the mean areas under the growth curves reported following exposure of *Pseudokirchneriella subcapitata* to the 5-OH metabolite of pyroxsulam are shown in Table 4 with respective percent inhibition results. The percentage inhibition is most marked at 80 mg 5-OH metabolite of pyroxsulam/L.

Table 4. Effect of 5-OH pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*) –study report data as given in Hoberg (2006).

Treatment measured concentrations (mg 5-OH metabolite of pyroxsulam/L)	Mean Specific Growth Rate per day		Mean Area Under the Growth Curve, X 10^4 cells/mL	
	0-72 hours	% Inhibition[1]	0-72 hours	%Inhibition[1]
Negative control	1.62	NA	100.02	NA
5.2	1.64	-1	104.54	-5
11	1.66	-2	108.53	-9
20	1.64	-1	107.35	-7
42	1.48	9	74.90*	25
80	1.06	35	19.00*	81

*** Significantly different from the pooled control (William's Test, $p \leq 0.05$). [1]Relative to control.**
Notes: Means and percent inhibition were reported as calculated from original raw data, not from the rounded values presented in the tabulated data presented in the study report. Based on the 0 to 24 hour growth rate criterion, control replicate B was excluded as an outlier from the mean and all statistical analyses presented in the table. NA = Not applicable.

The 0 to 72 hour growth rate in the control averaged 1.62 $days^{-1}$. The 0 to 72 hour growth rate in the 5.2, 11, 20, 42 and 80 mg/L treatment levels averaged 1.64, 1.66, 1.64, 1.48 and 1.06 $days^{-1}$, respectively. Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set passed the requirements for normality but not homogeneity of variance, therefore, Kruskal-Wallis' Test was used to determine treatment-related effects. Kruskal-Wallis' Test determined no significant reduction in average growth rate in any of the treatment levels tested as compared to the control data. Based on these results, the 72-hour NOEC was determined to be 80 mg/L. Since no concentration tested resulted in ≥ 50% reduction in average growth rate, the 72-hour ErC50 was empirically estimated to be > 80 mg/L, the highest mean measured concentration tested.

After 72 hours of exposure, the total biomass in the control averaged 100.02 x 10^4 cells/mL. Total biomass in the 5.2, 11, 20, 42 and 80 mg/L treatment levels averaged 104.54, 108.53, 107.35, 74.90 and 19.00 x 10^4 cells/mL, respectively (see Table 4). Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set passed the requirements for normality and homogeneity of variance, therefore, Williams' Test was used to determine treatment-related effects. Statistical analysis (Williams' Test) determined a significant reduction in total biomass in treatment levels ≥42 mg/L compared to the control data. Based on these results, the 72-hour NOEC for total biomass was determined to be 20 mg/L. The 72-hour EbC50 was determined to be 57 mg/L, with 95% confidence intervals of 50 to 62 mg/L.

The reported statistical endpoints are summarised in Table 5.

Table 5. Statistical endpoint values determined by the study author for the 5-OH metabolite of pyroxsulam with respect to toxicity to the freshwater green alga, *Pseudokirchneriella subcapitata.*

Statistical Endpoint	Cell Density (96 h). EC50-	Growth Rate (0-72 h) ErC50	Biomass (area under curve) (0-72 h) EbC50
NOEC (mg 5-OH metabolite of pyroxsulam/L)	42	80	20
EC_{50} (mg 5-OH metabolite of pyroxsulam/L) (95% C.I.)	63 (58-64)	>80 (N/A)	57 (50-62)
Reference chemical, if used	Not used		

Notes: N/A = not applicable. The ErC50 value was reported as empirically estimated and the corresponding 95% confidence limits could not be calculated.

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* and should not exceed 10% for other less commonly tested species.

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.

Study author's comments on test validity

The following acceptance criteria were required by the test protocol: the cell growth in the control must increase by more than 16 times after 72 hours of growth. During this study, the mean 72 hour cell growth in the control was 129.23 x 10^4 cells/mL, which exceeded the above criterion.

Additionally, the study report continued, the mean coefficient of variation (CV) for section-by-section specific growth rates (day 0 to 1, 1 to 2 and 2 to 3) in the control replicates should not exceed 35% while the CV for the average growth rate of the control for the entire test period (0 to 72-hour growth rate) should not exceed 7%.

The %CV values calculated in the test report were:

Observation	Growth Rate Coefficient of Variation (%)			
Interval (hours):	**0 – 24**	**24 – 48**	**48 – 72**	**0 – 72**
Control (N = 6)[a]	42	30	19	3.1
Control (N = 5)[b]	33	32	20	3.1

a Includes all six replicates of the control.
b Excludes control replicate B.

Based on the results presented in this table, the NOEC, EC25 and EC50 calculations were repeated excluding control replicate B (N = 5). Deletion of this replicate from the data analysis provides a more conservative estimate of the NOEC and EC values than when this data point is included in the analyses.

Reviewer's comments on test validity

The exponential growth requirement is considered to have been met with respect to the OECD 201 requirements (see below), but not with respect to the US EPA OPPTS 850.5400 requirement regarding reaching the logarithmic growth phase by 96 h (see Table 2, page 21 of this DER under the parameter "Indicate whether there was an exponential growth in the control".

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 growth rate formula shown under "Verification of Statistical Results" on page 30 of this DER. The values and calculated statistics, including the 0-72 h and 0-96 h mean % coefficient of variations (%CV), are as shown in Table 6:

Table 6. Reviewer calculated growth rates for the 0-24, 24-48 and 48-72 hour periods and associated means, standard deviations and percentage coefficients of variation with respect to exposure of *Pseudokirchneriella subcapitata* to the 5-OH metabolite of pyroxsulam.

	Reviewer calculated growth rates (/day) for the control replicates		
Replicate	**0-24 h**	**24-48 h**	**48-72 h**
1	0.916	2.493	1.305
2	0.693	2.264	1.822
3	2.169	1.019	1.668
4	1.447	1.840	1.823
5	1.504	1.855	1.501
6	2.197	1.420	1.096
Mean	1.49	1.82	1.54
Standard deviation	0.62	0.54	0.29
%CV	41.73	29.69	19.09
0-72 hour %CV	**3.05%** (see page 47 for ToxCalc values used to determine this result)		
0-96 hour %CV	4.7% (mean 1.28, standard deviation 0.06, calculations not provided in this DER)		

Only the %CV value for the 0-24 hour period exceeds the 35% limit set by the 2006 OECD 201 guideline. The value calculated by the reviewer, 41.7 or ~42%, was equivalent to that reported in the study report, namely 42%. The study report considered that one control replicate, B in the study report, number 2 in the above table, was an outlier based on 0 to 24 hour growth rate criterion and recalculated the %CV values with that replicate excluded.

The revised 0-24 hour %CV was 33%, which met the OECD requirement. Recalculation of the growth rate for the 0-24 h period by the reviewer with the B results excluded, gave a %CV of 32.8%, confirming the study report's value.

The overall 0-72 h %CV of 3.05% (derived from all six replicate results) satisfies the OECD 201 requirement of being less than 7%. Because the 2006 OECD guideline refers to the coefficient of variation of average specific growth rates during the whole test period, the 0-96 hours period %CV was also calculated and found to be 4.7% (mean 1.64 and standard deviation 0.21) which complies with the guideline's requirement for this parameter (i.e. <7%).

Use of the Grubb's test for outliers (Graphpad Software, Quickcalc on line calculator, © 2002-2005, GraphPad Software, Inc., http://www.graphpad.com/quickcalcs/Grubbs1.cfm) by the reviewer gave the following results which did not identify the presence of an outlier in the 0-24 hour growth rates:

Outlier Results - Data for the 0-24 hour growth rate statistics - Descriptive Statistics

Mean:	1.48767
SD:	0.62090
# of values:	6
Outlier detected?	No
Significance level:	0.05 (two-sided)
Critical value of Z:	1.89

Row	Value	Z	Significant Outlier?
1	0.916	0.92071	
2	0.693	1.27986	Furthest from the rest, but not a significant outlier (P > 0.05).
3	2.169	1.09733	
4	1.447	0.06550	
5	1.504	0.02631	
6	2.197	1.14243	

Although the study was conducted following the 1984 version of the OECD 201 guideline, the %CV requirements addressed in the study are in line with those set by the 2006 version of the OECD 201 guideline. The 0-24 hours %CV value of 42%, while a deficiency, is not considered further because of the 24-48 and 48-72 hour %CV values being <35% and the 0-96 hour %CV value of 4.7% for the growth rate complying with the 7% limit set by the 2006 edition of OECD 201.

The ToxCalc outputs for inclusion of the B replicate and for when it was omitted are given on page 47 and following of this DER.

B. REPORTED STATISTICS:

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 study reported stated that EC25 and EC50 values (the concentration of test substance which reduced cell density, total biomass and average specific 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 in the study report. 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:

While it could be argued that the following detailed analyses are superfluous because the main effect with respect to inhibition of the algae comes from the acidity of the nominal 100 mg/L solution, and that only a NOEC can be calculated, following exclusion of the nominal 100 mg/L results, the following statistical analyses have been conducted as they also consider the normality and homogeneity of the data sets presented and provide confirmation on the correctness of the study report's statistical analyses.

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 of the 5-OH metabolite of pyroxsulam exposed algae and the mean of the controls by Bonferroni's t test. Differences between the mean specific growth rate and biomass results of the 5-OH metabolite of pyroxsulam exposed algae and that of the controls were also tested by Bonferroni's t test. All NOEC values were determined using the ToxCalc package.

For the 0-72 hour growth rate ToxCalc analyses, the study report's results were also analysed using the ToxCalc's Non-parametric rank procedure.

Cell density

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 for each cell count. Means and standard deviations for cell density for each treatment and the control were calculated from individual replicate values.

Growth rate

Using the cell density data presented in the study report and the following formula for calculation of growth rate, *viz.*

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 0-72 hour's specific growth rate values for control and test replicates presented in the study report were recalculated and shown to be similar to those given in the study report. The reviewer-calculated and the study report's calculated growth rates of *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours of exposure to 5-OH metabolite of pyroxsulam are shown in Table 7.

The study report's calculations were verified as correct.

The percentage inhibition results (relative to the control mean of 1.62 day^{-1}) for the 0-72 hour growth rate at 5.2, 11, 20, 42 and 80 mg 5-OH metabolite of pyroxsulam/L were, respectively, -1, -2, -1, 9 and 35% (see Table 4, page 26 of this DER).

The 80 mg/L result is considered to most probably result from the low pH as the causative factor rather than any intrinsic 5-OH metabolite of pyroxsulam toxicity to the algae (also recalling that there was 89% inhibition of 96 hour cell density and 81% inhibition of 0-72 hour total biomass at this mean measured concentration).

A visual comparison of the study report and the reviewer-calculated growth rates indicates they are similar with the differences seen attributed to the reviewer's use of the rounded cell count values presented in the study report and also the assumption of an initial cell count of 10,000 cell/mL rather than an actual measured value.

The reviewer-calculated growth rate data, when analysed by the Bonferroni's t-test returned NOEC and EC50 values of 20 and >80 mg 5-OH metabolite of pyroxsulam/L when the six control replicates were used and also for when the five control replicates were used (ToxCalc results shown on page 47 of this DER).

When the study report's 0-72 hour growth rate data (using only 5 control replicates) were analysed using the ToxCalc non-parametric rank method, the normal distribution of the data was confirmed but unequal variance was identified. None of the concentrations tested were identified as statistically significantly different to the controls and the LC50, based on linear interpolation, was >80 mg 5-OH metabolite of pyroxsulam/L (ToxCalc results shown on page 49 of this DER).

Table 7. Reviewer-calculated and the study report's (italicised) calculated growth rates (Growth Rate ($days^{-1}$)) of *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours of exposure to the 5-OH metabolite of pyroxsulam. Individual replicate values shown.

Mean measured concentration, mg 5-OH metabolite of pyroxsulam/L	Growth Rate ($days^{-1}$) Observation Interval (Hours)					
	0-24 hours		0-48 hours		0-72 hours	
	Reviewer	*Study report*	Reviewer	*Study report*	Reviewer	*Study report*
Control (replicate A)	0.92	*0.96*	1.70	*1.74*	1.57	*1.57*
Replicate B	0.69	*0.72*	1.48	*1.51*	1.59	*1.60*
Replicate C	2.17	*2.26*	1.59	*1.63*	1.62	*1.62*
Replicate D	1.45	*1.51*	1.64	*1.68*	1.70	*1.71*
Replicate E	1.50	*1.57*	1.68	*1.71*	1.62	*1.62*
Replicate F	2.20	*2.29*	1.81	*1.85*	1.57	*1.57*
Mean and standard deviation using all six control replicate values:						
Mean	**1.49**	***1.55***	**1.65**	***1.69***	**1.61**	***1.62***
SD*	**0.62**	***0.65***	**0.11**	***0.11***	**0.05**	***0.05***
Mean and standard deviation using 5 control replicate values with replicate values for the B replicate excluded (study report approach):						
Mean	**1.65**	***1.72***	**1.68**	***1.72***	**1.62**	***1.62***
SD	**0.54**	***0.56***	**0.08**	***0.08***	**0.05**	***0.05***
5.2, A	1.10	*1.15*	1.71	*1.74*	1.65	*1.65*
B	1.50	*1.57*	1.78	*1.82*	1.65	*1.65*
C	2.08	*2.17*	1.60	*1.64*	1.63	*1.63*
Mean	**1.56**	***1.63***	**1.70**	***1.73***	**1.64**	***1.64***
SD	**0.49**	***0.51***	**0.09**	***0.09***	**0.01**	***0.01***
11, A	1.50	*1.57*	1.75	*1.79*	1.67	*1.67*
B	1.45	*1.51*	1.64	*1.68*	1.62	*1.63*
C	2.11	*2.20*	1.75	*1.79*	1.67	*1.67*
Mean	**1.69**	***1.76***	**1.71**	***1.75***	**1.65**	***1.66***
SD	**0.37**	***0.38***	**0.06**	***0.06***	**0.03**	***0.02***
20, A	1.01	*1.06*	1.67	*1.70*	1.64	*1.64*
B	1.75	*1.83*	1.87	*1.91*	1.63	*1.63*
C	2.35	*2.45*	1.71	*1.75*	1.64	*1.64*
Mean	**1.70**	***1.78***	**1.75**	***1.79***	**1.64**	***1.64***
SD	**0.67**	***0.70***	**0.11**	***0.11***	**0.01**	***0.01***
42, A	1.45	*1.51*	1.65	*1.68*	1.56	*1.56*
B	1.32	*1.38*	1.56	*1.59*	1.54	*1.55*
C	1.95	*2.03*	1.69	*1.72*	1.33	*1.33*
Mean	**1.57**	***1.64***	**1.63**	***1.66***	**1.48**	***1.48***
SD	**0.33**	***0.34***	**0.07**	***0.07***	**0.13**	***0.13***
80, A	0.00	*0.00***	0.90	*0.91*	0.95	*0.95*
B	0.22	*0.23*	1.10	*1.12*	1.13	*1.13*
C	1.39	*1.45*	0.87	*0.89*	1.10	*1.11*
Mean	**0.54**	***0.56***	**0.96**	***0.98***	**1.06**	***1.06***
SD	**0.75**	***0.78***	**0.13**	***0.13***	**0.10**	***0.10***

*** SD = standard deviation** Growth rate cannot be calculated when cell density is zero. A value of zero was entered for all further calculations.**

Notes to Table 7: Rounded data values, not original raw data, were presented in the study report and the reviewer calculated results were derived from the cell counts reported in the study report. The study report stated that replicate B was considered as outlier on the basis of the 0-24 hour results and was excluded from the mean and all statistical analyses. The reviewer included the B replicate result in the re-calculation of the rate results. When cell density was zero, growth rate could not be calculated and a value of zero was assigned in such situations.

Biomass

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:

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

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

Percent inhibition of the treatment data was calculated relative to the control data.

The 0-72 hours biomass (area under the growth curve) values for control and test replicates presented in the study report were recalculated and shown to be similar to those given in the study report. The reviewer-calculated and the study report's calculated biomass (area under the growth curve) values for *Pseudokirchneriella subcapitata* after 0-24, 24-48 and 48-72 hours of exposure to 5-OH metabolite of pyroxsulam are shown in Table 8.

Table 8. Reviewer-calculated and the study report's (italicised) calculated biomass (area under the growth curve) values for *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours of exposure to the 5-OH metabolite of pyroxsulam. Individual replicate values shown.

Mean measured concentration, mg 5-OH metabolite of pyroxsulam/L	Biomass (X 10^4 cells/mL) Observation Interval (Hours)						Total biomass 0-72 hours as cells X 10^4/mL	
	0-24 hours		24-48 hours		48-72 hours			
	Reviewer	Study report	Reviewer	Study report	Reviewer	Study report	Reviewer	Study report
Control. A*	0.75	*0.72*	15.38	*15.41*	69.88	*72.30*	86.00	*88.43*
B	0.50	*0.48*	9.63	*9.65*	68.13	*70.49*	78.25	*80.61*
C	3.88	*3.71*	15.50	*15.53*	75.38	*77.99*	94.75	*97.24*
D	1.63	*1.56*	14.50	*14.53*	95.21	*98.51*	111.34	*114.60*
E	1.75	*1.68*	15.63	*15.66*	77.88	*80.58*	95.25	*97.91*
F	4.00	*3.83*	22.13	*22.17*	73.38	*75.92*	99.50	*101.93*
Mean and standard deviation using all six control replicate values:								
Mean	**2.09**	***2.00***	**15.46**	***15.49***	**76.64**	***79.30***	**94.18**	***96.79***
SD*	**1.52**	***1.45***	**3.99**	***3.99***	**9.76**	***10.10***	**11.36**	***11.63***
Mean and standard deviation using 5 control replicate values (B replicate excluded, study report approach):								
Mean	2.40	*2.30*	16.63	*16.66*	78.35	*81.06*	97.37	*100.02*
SD	1.46	*1.39*	3.11	*3.11*	9.87	*10.21*	9.22	*9.52*
5.2, A	1.00	*0.96*	15.75	*15.78*	84.25	*87.18*	101.00	*103.92*
B	1.75	*1.68*	19.00	*19.04*	87.25	*90.28*	108.00	*111.00*
C	3.50	*3.35*	15.38	*15.41*	77.25	*79.93*	96.13	*98.69*
Mean	**2.08**	***2.00***	**16.71**	***16.74***	**82.92**	***85.80***	**101.71**	***104.54***
SD	**1.28**	***1.23***	**1.99**	***2.00***	**5.13**	***5.31***	**5.97**	***6.18***
11, A	1.75	*1.68*	17.88	*17.91*	90.46	*93.60*	110.09	*113.19*
B	1.63	*1.56*	14.50	*14.53*	77.38	*80.06*	93.50	*96.15*
C	3.63	*3.47*	19.75	*19.79*	89.88	*93.00*	113.25	*116.26*
Mean	**2.34**	***2.24***	**17.38**	***17.41***	**85.91**	***88.89***	**105.61**	***108.53***
SD	**1.12**	***1.07***	**2.66**	***2.67***	**7.39**	***7.65***	**10.61**	***10.83***
20, A	0.88	*0.84*	14.38	*14.40*	80.88	*83.68*	96.13	*98.93*
B	2.38	*2.28*	23.00	*23.05*	85.88	*88.86*	111.25	*114.18*
C	4.75	*4.55*	19.63	*19.67*	81.88	*84.72*	106.25	*108.94*
Mean	**2.67**	***2.56***	**19.00**	***19.04***	**82.88**	***85.75***	**104.54**	***107.35***
SD	**1.95**	***1.87***	**4.34**	***4.36***	**2.65**	***2.74***	**7.70**	***7.75***
42, A	1.63	*1.56*	14.63	*14.66*	66.5	*68.81*	82.75	*85.02*
B	1.38	*1.32*	12.25	*12.28*	61.75	*63.89*	75.38	*77.49*
C	3	*2.87*	17.13	*17.16*	40.75	*42.16*	60.88	*62.2*
Mean	**2.00**	***1.92***	**14.67**	***14.70***	**56.33**	***58.29***	**73.00**	***74.90***
SD	**0.87**	***0.83***	**2.44**	***2.44***	**13.70**	***14.18***	**11.13**	***11.63***
80, A	-0.5	*-0.48*	2	*2*	10.63	*10.99*	12.13	*12.52*
B	0.13	*0.12*	4.13	*4.13*	18.13	*18.75*	22.38	*23.01*
C	1.5	*1.44*	3.88	*3.88*	15.63	*16.17*	21	*21.49*
Mean	**0.38**	***0.36***	**3.34**	***3.34***	**14.80**	***15.30***	**18.50**	***19.01***
SD	**1.02**	***0.98***	**1.16**	***1.16***	**3.82**	***3.95***	**5.56**	***5.67***

*** Individual replicate results shown.**

Reviewer calculated results:

Comparison of reviewer-calculated toxicity endpoints and those given in the study report.

The endpoints reported in the study report and those calculated in the assessment of the study are shown in Table 9. The table also includes the EC25 cell density endpoints for 24, 48, 72 and 96 hours as well as the endpoints calculated when the nominal 100 mg/L results are excluded.

Table 9. Reported and calculated toxicity endpoints for the 5-OH metabolite of pyroxsulam with respect to the freshwater green alga, *Pseudokirchneriella subcapitata*.

Toxicity endpoints:	Mean measured 5-OH metabolite of pyroxsulam concentration, mg/L (95% confidence limits), mg 5-OH metabolite of pyroxsulam/L.		
	As presented in the study report	As calculated by the reviewer with the ToxCalc program	
		Endpoints based on use of all (5.2 to 80 mg/L, mean measured) test concentrations.	Endpoints based on use of mean measured concentrations of 5.2 to 42 mg/L with the 80 mg/L results excluded.
24 hour cell density			
EC50	67 (42-77)	67.8 (Not calculated)	>42
EC25	50 (9.8-63)	52 (Not calculated)	>42
NOEC	Not reported	80	42
48 hour cell density			
EC50	63 (59-67)	63.5 (57.0-68.7)	>42
EC25	48 (39-53)	48 (36-56)	>42
NOEC	Not reported	42	42
72 hour cell density			
EC50	54 (39-61)	54.2 (33.6-65.4)	>42
EC25	36 (28-46)	35 (24-52)	35.4 (Not calculated)
NOEC	Not reported	20	20
96 hour cell density			
EC50	63 (58-64)	63.4 (56.1-63.8)	>42
EC25	51 (44-53)	53 (41-53)	>42
NOEC	42	42 (Bonferroni t-test)	42 (Bonferroni t-test)
0-72 hour mean specific growth rate			
ErC50	>80	>80	>42
NOEC	80	20 (Bonferroni t-test)	20 (Wilcoxon Rank Sum test)
0-72 hour biomass area			
EbC50	57 (50-62)	57.5 (49.6-63.9)	>42
NOEC	20	20 (Bonferroni t-test)	20 (Bonferroni t-test)

The reviewer's and the study report's EC results for cell density, mean specific growth rate and biomass are considered equivalent as are the NOECs except for the mean specific growth rate where the reviewer's value is 20 and the study report's 80 mg 5-OH metabolite of pyroxsulam/L. It is possible that the difference results from the study report's use of the Kruskal-Wallis' Test to determine the NOEC when the data did not pass the homogeneity

and normality tests whereas the ToxCalc program did not have this test available and used the Bonferroni t Test to establish the NOEC. Recalculation of the 0-72 hour growth rate endpoints using the study report's calculated growth rates (with 5 control replicates) confirmed an EC50 of >80 mg 5-OH metabolite of pyroxsulam/L and indicated no significant differences existed between any of the test concentrations and the controls, i.e. the study report's NOEC of 80 mg/L was supported.

The above table also includes the Australian reviewer-calculated results when the 80 mg/L mean measured results are excluded from the ToxCalc calculations based on the study report's cell counts, 0-72 hours mean specific growth rates and 0-72 hour biomass results. The relevant ToxCalc outputs are given in Appendix II (page 51 and following of this DER).

NOECs determined from these calculations were the same as those determined when the 80 mg/L data were included except for the 24 hour cell density results where the NOECs were, respectively, 80 and 42 mg 5-OH metabolite of pyroxsulam/L.

The PMRA reviewer has verified the NOECs using numbers provided in the study report, and including all control replicates (Appendix III). SigmaStat Version for Windows 203 (1992-1997) was used. Assumptions of normality and homogeneity of variances were met for all endpoints, and a one-way analysis of variance (ANOVA) followed by multiple comparisons tests (Dunnett's and/or Bonferroni) were run. NOECs of 42 mg/L and 20 mg/L were obtained for cell density and biomass, respectively, confirming the conclusions of the study report and the Australian reviewer. For growth rate, a significant difference between the control and the two highest concentrations was detected when using Dunnett's method for multiple comparisons. However, using Bonferroni's multiple comparisons, a significant difference was only detected between the control and the highest concentration. The 9% inhibition of growth rate in algae at the 42 mg/L is unlikely to be biologically significant. For this reason, the PMRA reviewer would set the NOEC at 42 mg/L for this endpoint. The EC50 values would be set at >42 mg/L, due to the uncertainty associated with the effect of pH at the highest treatment level.

D. STUDY DEFICIENCIES:

Table 10 summarises deficiencies and deviations from the OECD 201 and US EPA OPPTS 850.5400 Guidelines and the template's identified OECD or US EPA requirements.

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 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. The AAP medium contains sodium EDTA as a chelator.	OECD 201 refers to use of AAP medium and provides the composition of this growth medium. Annex 3 of the guideline, which contains the AAP composition, does not identify sodium selenate as a constituent of the medium. However, the Annex goes on to describe the preparation of the US EPA medium and notes that sodium selenate is used only in the medium for stock cultures of diatom species at a final concentration in the AAP	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

		medium of 0.01 µg/L or 0.00001 mg/L.	
pH at test initiation	The pH values at test initiation were: **Nominal concentration* / 0 hours** **Control** 6.9 **6.3** 6.6 **13** 6.5 **25** 6.3 **50** 5.9 **100** 4.8 *** mg 5-OH metabolite of pyroxsulam.**	OECD 201 indicates that the AAP media has its pH adjusted to 7.5± 0.1. The study report stated that the initial pH of this medium was adjusted, to 7.5 ± 0.1 prior to use.	US EPA OPPTS 850.5400 states that the pH of the nutrient medium is to be7.5 (± 0.1) for *Selenastrum.* US EPA OPPTS 850.5400 also states 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 the situation in the study under assessment for the 100 mg/L replicates where the pH at time 0 was 4.8
pH at test termination	The pH values at test completion (96 hours) were: **Nominal concentration* / 96 hours** **Control** 8.9 **6.3** 8.9 **13** 9.3 **25** 9.2 **50** 8.6 **100** 6.2 *** mg 5-OH metabolite of pyroxsulam.**	The 2006 OECD guideline states that the pH of the control medium should not increase by more than 1.5 units during the test.	No specific comment other than as above with the need to consider pH adjustments.
Light intensity and quality:	3900-4700 lux. The PAR of the test area measured at test initiation ranged from 62 to 75 $\mu E/m^2/s$.	OECD 201 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 OPPTS 850.5400 states fluorescent lights providing 4300 lux are to be used for *Selenastrum.*
Indicate whether there was an exponential growth in the control	The mean control 72 hour cell growth was 129.23 X 10^4 cells/mL (cf. 1 X 10^4 cells at test initiation), i.e. an increase by a factor of 129. At 96 hours, the mean control cell density was ~1.84 X 10^6 cells/mL. The mean 0-72 hours calculated growth rate of the controls was 1.62 day^{-1}.	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. The guideline notes that this corresponds to a specific growth rate of 0.92 day^{-1}.	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.*

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. It is also noted that, according to OECD 201, this micronutrient should only be used in medium to be used for stock cultures of diatom species.

The 4.8 pH recorded for the nominal 100 mg/L test concentration at time 0 is a crucial issue. The observed inhibition of growth at 100 mg/L, as seen in the mean cell density count of 19.67 X 10^4 cells/mL at 96 hours (control at that time was 186.62 X 10^4 cells/mL) could be due to either the concentration of the 5-OH metabolite of pyroxsulam present or, most likely, the pH of 4.8 recorded at 0 hours. This was a situation in which the US EPA advice on appropriate adjustment of pH would have been appropriate to follow. As a result, the 100 mg/L results need to be treated with caution and, consequently, the study is considered to be significantly flawed and is rated as "supplementary".

The reason for the initial control pH being 6.9 instead of 7.5 at time zero is not known. The change in the pH of the controls from 6.9 at day 0 to 8.9 at day 4 in the pooled replicates which contained algae exceeds the OECD (2006) recommendation that the pH of the control medium should not increase by more than 1.5 units during the test. However, the guideline does not appear to make this mandatory and some increase can be expected when growing algae under static conditions.

The light used satisfied the OECD 201 PAR requirement but, as lux, was on occasion less than the lux range referred to in the OECD guideline. Although the control algae appeared to have grown successfully, the failure to demonstrate exponential growth had been achieved by 96 hours (see below) could be attributed to the use of the lower light intensity. The lux intensity is considered to have satisfied the US EPA OPPTS requirement of a 4.3 X 10^3 lux.

With respect to the reaching of exponential growth, the OECD parameters of an increase in control biomass by a factor of at least 16 within the 72 hour test period and the attainment of a specific growth rate of 0.92 day^{-1} were attained. However, the US EPA OPPTS requirement that by 96 h, the number of algal cells in the control should be approximately 3.5 X 10^6/mL for *Selenastrum* was not reached. The mean count in the controls at that time was 183.6 X 10^4 cells/mL or ~1.84 X 10^6 cells/mL (~52% of the US EPA value). As noted above, this may be attributed to the use of a lower light intensity but must still be classed as a significant deficiency with respect to compliance with the US EPA OPPTS guideline and adds support to the classification of the study as supplemental. Because a plotting cell counts against time using the Microsoft Excel Chart Wizard function and fitting the data points to an exponential curve (data and curve shown on page 61 of this DER) returned an r^2 value of 0.96, a value that indicates an exponential approximating growth occurred in the study's control algae. While the visual examination of the data points and the fitted exponential curve clearly show some deviation from each other, the r^2 value is taken to indicate this deficiency is not expected to have invalidated the study.

.Apart from the pH being 4.8 at time zero in the 100 mg 7-OH metabolite of pyroxsulam/L and the failure to reach the 96 hour cell count specified by the US EPA OPPTS guideline as required to shown exponential growth had been achieved, these deficiencies/deviations were not considered to have significantly adversely affected the study's conduct or results. The pH being 4.8 at time zero is considered, in contrast, a major deficiency. Similarly, failure to meet the US criterion for exponential growth indicates some problem existed during the study's conduct.

E. REVIEWER'S COMMENTS:

Australian 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 but with significant issues regarding the initial pH of the test solution and the demonstration that logarithmic growth had not been reached by 96 hours as required by US EPA OPPTS 850.5400.

The test solution were prepared used a stock solution where the test material had not fully dissolved despite 3 hours of stirring. This appears to have effected the subsequent test concentrations as these were 81-84% of nominal, similar to the stock solution which was used as the highest test solution and was 80% of nominal.

Although the study was completed in April 2006, after changes to OECD 201 test guideline were announced in March 2006, it is expected that the requirement of the 1984 edition of this guideline would have been paramount in the study's design and conduct. Consequently, any failure to comply with the 2006 guideline is not automatically considered a deficiency or deviation.

The study report's decision to exclude the specific growth rate data result for replicate B on the basis that the %CV for the 0-24 hour period of 42% exceeded the 2006 OECD 201 recommendation of 35% and that with its exclusion the %CV was 33% and met the OECD criterion is noted. The reviewer believes it would have been helpful to have conducted a statistical analysis of the control replicate results for the 0-24 hour period to see if the B replicate's value was statistically significantly different from the other replicate results.

The 96 hour cell density counts indicate significant inhibition (89% compared to the mean control cell counts at that time) occurred only at 80 mg 5-OH metabolite of pyroxsulam/L and the effect of the initial of pH of 4.8 in the test solutions at this concentration is expected to be the primary reason for the inhibition, rather than the inherent toxicity of the 5-OH metabolite of pyroxsulam. For the 0-72 hour specific growth rate, inhibition occurred only at the 42 and 80 mg 5-OH metabolite of pyroxsulam/L rates (respectively, 9 and 35%) but with neither result being statistically significantly different from the control values over that time period. For the biomass results, inhibition again only occurred at the 42 and 80 mg 5-OH metabolite of pyroxsulam/L concentrations (respectively 25 and 81% inhibition, the latter result supportive of the pH effect being the cause of the growth inhibition) with the mean total biomass at the 0-72 hours period for these two concentrations being statistically significant reduced compared to the mean control value.

Based on the results of this study, as shown below, the 5-OH 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 **supplemental by the Australian reviewer** because of the pH of the 100 mg/L test solution being 4.8 at time 0 and because of the failure to meet the US criterion for exponential growth after 96 hours. Consequently, the study does not satisfy all the guideline requirements for an acute toxicity study with the unicellular green alga, *Pseudokirchneriella subcapitata.*

While detailed statistical analyses of the data were conducted, they could be considered superfluous because the main effect with respect to inhibition of the algae appears to come from the acidity, and that only a NOEC can be calculated, following exclusion of the nominal 100 mg/L results. However, the statistical analyses were conducted as they also considered the normality and homogeneity of the data sets presented and provided confirmation on the correctness of the study report's statistical analyses.

However, the Australian reviewer does not recommend a repeat of the test as the low toxicity of this metabolite has been adequately demonstrated.

PMRA reviewer: The PMRA reviewer agrees with the recommendation not to request a repeat of the test, as the low toxicity of the transformation product has been adequately demonstrated. A new study would likely not produce different results.

The PMRA does not share the same acceptability classification as the Australian Government Department of the Environment, Water, Heritage and the Arts. This study is considered by the PMRA as of limited utility due to the pH shift greater than 1.5 in the control. Despite the effect of the acidity of the test substance, some useful information can be obtained from this study and used in a risk assessment (EC50 expected to be >42 mg 5-OH metabolite of pyroxsulam/L).

F. CONCLUSIONS:

While the study is in general scientifically sound, it is classified as **SUPPLEMENTAL** by the Australian Government Department of the Environment, Water, Heritage and the Arts because of the pH of 4.8 observed in the 100 mg/L test solution at time 0 and the failure to demonstrate that logarithmic growth had been achieved by 96 hours. With respect to the pH, the occurrence of similar results from other similar hydroxy metabolites points to the acidity as the dominant factor in the growth inhibition seen at higher test concentrations. The US EPA has similarly classified the study as supplemental.

The PMRA does not share the same acceptability classification as the APVMA or the US EPA. This study is of limited utility due to the pH shift greater than 1.5 units in the controls and the effect of the acidity of the test substance. Some useful information can be obtained from this study and used in a risk assessment (EC50 expected to be >42 mg 5-OH metabolite of pyroxsulam/L). A new study would likely not provide additional information. The low toxicity of the test substance has been adequately demonstrated.

The study's reported endpoints were:

Statistical Endpoint	**Growth Rate (0-72 h)**	**Biomass (area under growth curve) (0-72 h)**	**Cell Density (96 h)**
NOEC (mg ac/L)	80	20	42
EC_{50} (mg ac/L) (95% C.I.)	>80 (95% confidence limits not calculated)	57 (50-62)	63 (56-64)
Reference chemical, if used	N/A		

The following endpoints will used for risk analysis (as a result of the omission of the nominal 100 mg/L results because of the initial pH concerns):

Statistical Endpoint	**Growth Rate (0-72 h)**	**Biomass (area under growth curve) (0-72 h)**	**Cell Density (96 h)**
NOEC (mg ac/L)	42	20	42
EC_{50} (mg ac/L) (95% C.I.)	>42 (95% confidence limits not calculated)	>42	>42
Reference chemical, if used	N/A		

Endpoint(s) Affected: Cell density, growth rate and biomass.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density at 24 hours

The ToxCalc analysis of the 24 hour algal cell count data in the study report gave the following results (cell count data as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	2.5000	2.0000	8.7500	4.2500	4.5000	9.0000
5.2	3.0000	4.5000	8.0000			
11	4.5000	4.2500	8.2500			
20	2.7500	5.7500	10.5000			
42	4.2500	3.7500	7.0000			
80	0.0000	1.2500	4.0000			

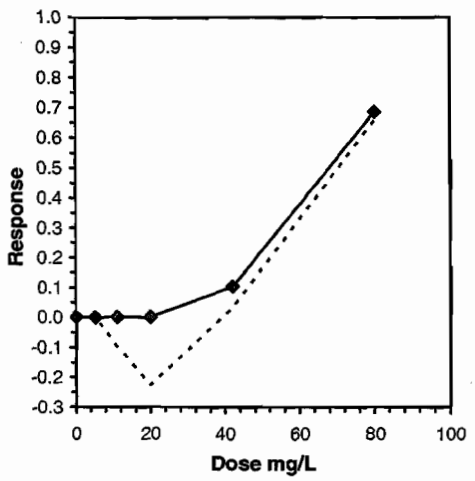
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	5.1667	1.0000	5.1667	2.0000	9.0000	58.680	6				5.5833	1.0000
5.2	5.1667	1.0000	5.1667	3.0000	8.0000	49.661	3	0.000	2.602	5.0778	5.5833	1.0000
11	5.6667	1.0968	5.6667	4.2500	8.2500	39.542	3	-0.256	2.602	5.0778	5.5833	1.0000
20	6.3333	1.2258	6.3333	2.7500	10.5000	61.702	3	-0.598	2.602	5.0778	5.5833	1.0000
42	5.0000	0.9677	5.0000	3.7500	7.0000	35.000	3	0.085	2.602	5.0778	5.0000	0.8955
80	1.7500	0.3387	1.7500	0.0000	4.0000	116.934	3	1.751	2.602	5.0778	1.7500	0.3134

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90957	0.873	0.45885	-1.0436
Bartlett's Test indicates equal variances (p = 0.91)	1.53739	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	80	>80			5.0778	0.9828	7.67262	7.61389	0.4468	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	30.529	14.636	0.000	53.625	0.0529
IC10	41.057	15.515	0.000	52.152	-0.3167
IC15	44.972	15.725	0.000	58.441	-0.5786
IC20	48.236	15.171	0.000	64.779	-0.8697
IC25	51.500				
IC40	61.292				
IC50	67.821				



APPENDIX I (Continued)
Cell density at 48 hours

The ToxCalc analysis of the 48 hour algal cell count data in the study report gave the following results (cell count data as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	30.250	19.250	24.250	26.750	28.750	37.250
5.2	30.500	35.500	24.750			
11	33.250	26.750	33.250			
20	28.000	42.250	30.750			
42	27.000	22.750	29.250			
80	6.000	9.000	5.750			

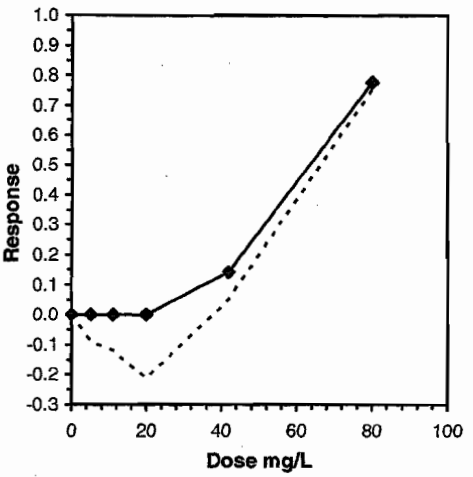
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	27.750	1.0000	27.750	19.250	37.250	21.801	6				30.688	1.0000
5.2	30.250	1.0901	30.250	24.750	35.500	17.783	3	-0.675	2.602	9.641	30.688	1.0000
11	31.083	1.1201	31.083	26.750	33.250	12.073	3	-0.900	2.602	9.641	30.688	1.0000
20	33.667	1.2132	33.667	28.000	42.250	22.454	3	-1.597	2.602	9.641	30.688	1.0000
42	26.333	0.9489	26.333	22.750	29.250	12.535	3	0.382	2.602	9.641	26.333	0.8581
*80	6.917	0.2492	6.917	5.750	9.000	26.148	3	5.624	2.602	9.641	6.917	0.2254

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97327	0.873	0.32234	0.06517
Bartlett's Test indicates equal variances (p = 0.58)	3.76323	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	42	80	57.9655		9.6405	0.34741	283.592	27.4444	1.9E-04	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	27.753	8.094	0.000	51.657	-0.9148
IC10	35.505	7.767	5.180	51.208	-1.3304
IC15	42.487	5.796	20.414	51.221	-1.8243
IC20	45.490	4.107	29.328	53.615	-1.6030
IC25	48.493	3.452	35.698	55.953	-1.4223
IC40	57.502	2.471	47.812	63.242	-0.8660
IC50	63.508	2.053	57.013	68.730	-0.6819



The 80 mg/L mean cell count at 48 hours is identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using William's test, did not identify this result as statistically significantly reduced compared to the control.

APPENDIX I (Continued)

Cell density at 72 hours

The ToxCalc analysis of the 72 hour algal cell count data in the study report gave the following results (cell count data as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	111.50	119.00	128.50	165.67	129.00	111.50
5.2	140.00	141.00	131.75			
11	149.67	130.00	148.50			
20	135.75	131.50	135.00			
42	108.00	102.75	54.25			
80	17.25	29.25	27.50			

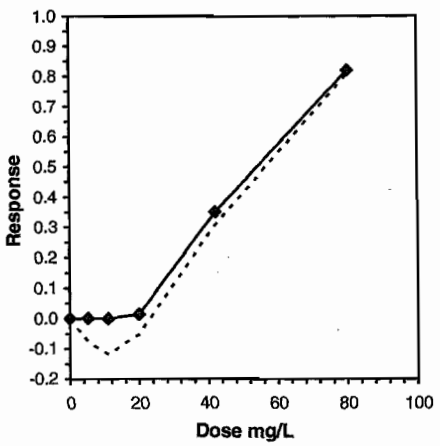
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	127.53	1.0000	127.53	111.50	165.67	15.856	6				135.95	1.0000
5.2	137.58	1.0788	137.58	131.75	141.00	3.690	3	-0.851	2.602	30.76	135.95	1.0000
11	142.72	1.1191	142.72	130.00	149.67	7.731	3	-1.286	2.602	30.76	135.95	1.0000
20	134.08	1.0514	134.08	131.50	135.75	1.692	3	-0.555	2.602	30.76	134.08	0.9863
*42	88.33	0.6927	88.33	54.25	108.00	33.547	3	3.316	2.602	30.76	88.33	0.6498
*80	24.67	0.1934	24.67	17.25	29.25	26.280	3	8.704	2.602	30.76	24.67	0.1814

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93599	0.873	0.28554	2.38582
Bartlett's Test indicates equal variances (p = 0.04)	11.9165	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	20	42	28.9828		30.7567	0.24118	6453.14	279.341	1.5E-06	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	22.373	3.074	14.390	27.877	-3.3805
IC10	25.642	2.747	20.360	35.217	0.2836
IC15	28.911	3.804	21.903	42.557	0.4469
IC20	32.179	4.933	23.228	48.938	0.3979
IC25	35.448	5.474	24.514	51.661	0.2259
IC40	46.039	6.263	30.348	60.028	-0.2245
IC50	54.153	6.457	33.602	65.432	-0.6490



The 42 and 80 mg/L mean cell count at 72 hours are identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using William's test, did not identify these results as statistically significantly reduced compared to the control.

APPENDIX I (Continued)

Cell density at 96 hours

The ToxCalc analysis of the 96 hour algal cell count data in the study report gave the following results (cell count data as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	137.25	123.75	152.33	218.00	202.50	208.00
5.2	153.00	200.50	156.33			
11	200.50	190.67	143.67			
20	140.00	209.50	139.00			
42	152.00	216.00	217.00			
80	18.00	19.00	22.00			

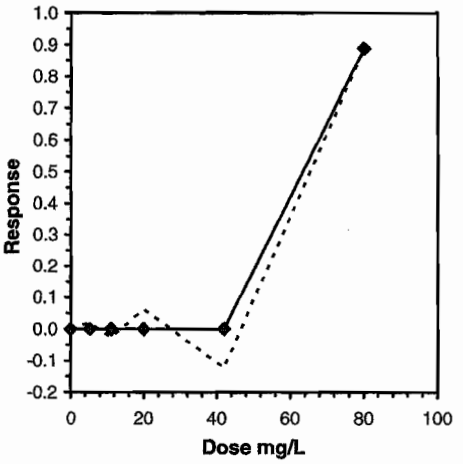
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	173.64	1.0000	173.64	123.75	218.00	23.392	6				175.94	1.0000
5.2	169.94	0.9787	169.94	153.00	200.50	15.602	3	0.153	2.602	62.95	175.94	1.0000
11	178.28	1.0267	178.28	143.67	200.50	17.037	3	-0.192	2.602	62.95	175.94	1.0000
20	162.83	0.9378	162.83	139.00	209.50	24.821	3	0.447	2.602	62.95	175.94	1.0000
42	195.00	1.1230	195.00	152.00	217.00	19.099	3	-0.883	2.602	62.95	175.94	1.0000
*80	19.67	0.1133	19.67	18.00	22.00	10.585	3	6.366	2.602	62.95	19.67	0.1118

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95437	0.873	-0.0445	-1.2289
Bartlett's Test indicates equal variances (p = 0.13)	8.47651	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	42	80	57.9655		62.9451	0.36251	12849.6	1169.98	1.4E-04	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	44.139	17.463	0.000	44.177	-0.5920
IC10	46.278	13.209	0.000	46.353	-1.7277
IC15	48.417	8.122	0.000	48.530	-3.1649
IC20	50.556	4.335	37.943	50.706	-4.9076
IC25	52.696	2.297	40.957	52.883	-1.5914
IC40	59.113	1.753	50.018	59.412	-1.3855
IC50	63.391	1.426	56.071	63.765	-1.3571



The 80 mg/L mean cell count at 96 hours is identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using William's test, similarly identified this result as statistically significantly reduced compared to the control.

APPENDIX I (Continued)

Specific growth rate (0-72 hours)

The ToxCalc analysis of the 0-72 hour reviewer calculated growth rate data (Table 7, page 32) gave the following results (growth rate data as day^{-1}).

Conc-mg/L	1	2	3	4	5	6
D-Control	1.5713	1.5930	1.6186	1.7033	1.6199	1.5713
5.2	1.6472	1.6496	1.6270			
11	1.6695	1.6225	1.6669			
20	1.6369	1.6263	1.6351			
42	1.5607	1.5441	1.3312			
80	0.9493	1.1253	1.1047			

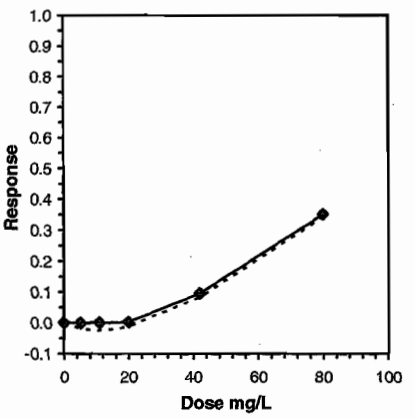
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.6129	1.0000	1.6129	1.5713	1.7033	3.051	6				1.6357	1.0000
5.2	1.6413	1.0176	1.6413	1.6270	1.6496	0.757	3	-0.608	2.602	0.1213	1.6357	1.0000
11	1.6530	1.0248	1.6530	1.6225	1.6695	1.597	3	-0.859	2.602	0.1213	1.6357	1.0000
20	1.6328	1.0123	1.6328	1.6263	1.6369	0.347	3	-0.426	2.602	0.1213	1.6328	0.9982
*42	1.4787	0.9168	1.4787	1.3312	1.5607	8.655	3	2.881	2.602	0.1213	1.4787	0.9040
*80	1.0598	0.6570	1.0598	0.9493	1.1253	9.081	3	11.870	2.602	0.1213	1.0598	0.6479

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91977	0.873	-0.8284	1.46803
Bartlett's Test indicates unequal variances (p = 6.62E-03)	16.0793	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	20	42	28.9828		0.12129	0.0752	0.16531	0.00434	5.3E-04	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.257	5.345	21.731	49.788	0.7327
IC10	42.592	4.902	24.422	53.963	-0.4433
IC15	50.011	4.606	29.545	60.627	-0.7847
IC20	57.430	4.253	36.341	67.299	-1.0416
IC25	64.849	3.746	50.387	74.096	-0.6546
IC40	>80				
IC50	>80				



The ToxCalc ErC50 value of >80 mg 5-OH metabolite of pyroxsulam/L was the same as reported in the test report for this parameter. The ToxCalc calculations and study report were both unable to set 95% confidence limits.

The ToxCalc determination identified the NOEC as 20 mg 5-OH metabolite of pyroxsulam/L. The study report's NOEC was 80 mg 5-OH metabolite of pyroxsulam/L.

The ToxCalc calculations with control replicate B (1.5930) excluded still reported the NOEC as 20 mg 5-OH metabolite of pyroxsulam/L. The statistical output is shown on the following page.

Specific growth rate (0-72 hours) (continued)

The ToxCalc analysis of the 0-72 hour reviewer calculated growth rate data (Table 7, page 32) but with the control replicate B value of 1.5930 excluded gave the following results (growth rate data as day^{-1}).

Conc-mg/L	1	2	3	4	5
D-Control	1.5713	1.6186	1.7033	1.6199	1.5713
5.2	1.6472	1.6496	1.6270		
11	1.6695	1.6225	1.6669		
20	1.6369	1.6263	1.6351		
42	1.5607	1.5441	1.3312		
80	0.9493	1.1253	1.1047		

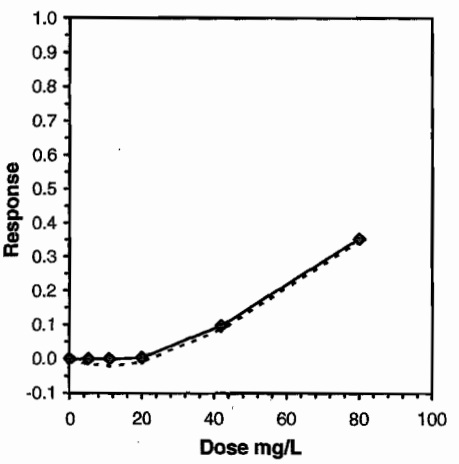
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.6169	1.0000	1.6169	1.5713	1.7033	3.335	5				1.6370	1.0000
5.2	1.6413	1.0151	1.6413	1.6270	1.6496	0.757	3	-0.490	2.624	0.1303	1.6370	1.0000
11	1.6530	1.0223	1.6530	1.6225	1.6695	1.597	3	-0.726	2.624	0.1303	1.6370	1.0000
20	1.6328	1.0098	1.6328	1.6263	1.6369	0.347	3	-0.320	2.624	0.1303	1.6328	0.9974
*42	1.4787	0.9145	1.4787	1.3312	1.5607	8.655	3	2.785	2.624	0.1303	1.4787	0.9033
*80	1.0598	0.6554	1.0598	0.9493	1.1253	9.081	3	11.224	2.624	0.1303	1.0598	0.6474

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91825	0.868	-0.8593	1.28231
Bartlett's Test indicates unequal variances (p = 7.91E-03)	15.6535	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	20	42	28.9828		0.13028	0.08057	0.1645	0.00462	1.8E-07	5, 14

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.077	5.540	19.926	54.980	0.6740
IC10	42.484	5.065	20.158	56.454	-0.2959
IC15	49.909	4.593	27.923	63.101	-0.5054
IC20	57.334	3.952	40.031	69.782	-0.6220
IC25	64.759	3.355	49.836	76.471	-0.2113
IC40	>80				
IC50	>80				



The 42 and 80 mg/L mean results at 96 hours are identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using William's test, did not identify any results as statistically significantly reduced compared to the control.

APPENDIX I (Continued)

Specific growth rate (0-72 hours) (continued) – Non-parametric rank analysis

The ToxCalc analysis of the 0-72 hour reviewer calculated growth rate data (Table 7, page 32) but with the control replicate B value of 1.5930 excluded gave the following results (growth rate data as day^{-1}) and using a non-parametric rank analysis gave the following results.

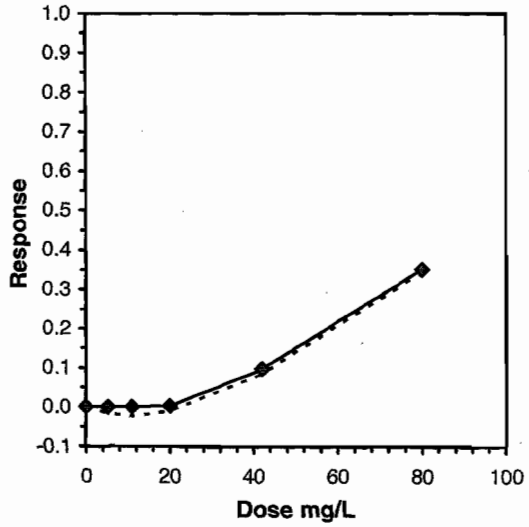
Conc-mg/L	1	2	3	4	5
D-Control	1.5700	1.6200	1.7100	1.6200	1.5700
5.2	1.6500	1.6500	1.6300		
11	1.6700	1.6300	1.6700		
20	1.6400	1.6300	1.6400		
42	1.5600	1.5500	1.3300		
80	0.9500	1.1300	1.1100		

			Transform: Untransformed					Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	1.6180	1.0000	1.6180	1.5700	1.7100	3.534	5	1.6393	1.0000
5.2	1.6433	1.0157	1.6433	1.6300	1.6500	0.703	3	1.6393	1.0000
11	1.6567	1.0239	1.6567	1.6300	1.6700	1.394	3	1.6393	1.0000
20	1.6367	1.0115	1.6367	1.6300	1.6400	0.353	3	1.6367	0.9984
42	1.4800	0.9147	1.4800	1.3300	1.5600	8.784	3	1.4800	0.9028
80	1.0633	0.6572	1.0633	0.9500	1.1300	9.278	3	1.0633	0.6486

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91735	0.868	-0.834	1.25393
Bartlett's Test indicates unequal variances (p = 6.02E-03)	16.3067	15.0863		

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.136	5.271	18.999	54.955	0.7116
IC10	42.420	4.950	20.782	56.906	-0.3437
IC15	49.895	4.620	29.264	63.600	-0.6504
IC20	57.370	4.094	39.919	70.294	-0.8976
IC25	64.846	3.660	47.273	77.055	-0.7394
IC40	>80				
IC50	>80				



APPENDIX I (Continued)

Biomass (area under the growth curve) 0-72 hours

The ToxCalc analysis of the 0-72 hour reviewer calculated biomass data (Table 8, page 34) gave the following results (biomass data as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	86.00	78.25	94.75	111.34	95.25	99.50
5.2	101.00	108.00	96.13			
11	110.09	93.50	113.25			
20	96.13	111.25	106.25			
42	82.75	75.38	60.88			
80	12.13	22.38	21.00			

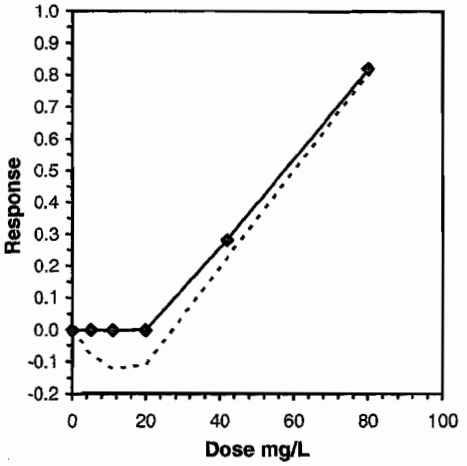
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	94.18	1.0000	94.181	78.250	111.335	12.057	6				101.51	1.0000
5.2	101.71	1.0799	101.708	96.125	108.000	5.869	3	-1.114	2.602	17.583	101.51	1.0000
11	105.61	1.1214	105.612	93.500	113.250	10.044	3	-1.692	2.602	17.583	101.51	1.0000
20	104.54	1.1100	104.542	96.125	111.250	7.371	3	-1.534	2.602	17.583	101.51	1.0000
*42	73.00	0.7751	73.000	60.875	82.750	15.245	3	3.135	2.602	17.583	73.00	0.7191
*80	18.50	0.1964	18.500	12.125	22.375	30.073	3	11.201	2.602	17.583	18.50	0.1822

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96629	0.873	-0.1828	-0.2846
Bartlett's Test indicates equal variances (p = 0.87)	1.82945	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	20	42	28.9828		17.5832	0.1867	3491.51	91.2958	5.1E-08	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	23.916	1.982	18.448	26.920	-5.4330
IC10	27.833	1.791	23.516	33.839	0.3049
IC15	31.749	2.456	26.141	40.759	0.7590
IC20	35.666	3.072	29.302	46.824	0.5538
IC25	39.582	3.242	31.651	49.590	0.0960
IC40	50.432	2.986	40.647	58.197	-0.5557
IC50	57.510	2.403	49.654	63.922	-0.5655



The 42 and 80 mg/L mean biomass values are identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using William's test, also identified these results as statistically significantly reduced compared to the control.

APPENDIX II. STATISTICAL VERIFICATION THE STUDY REPORT'S RESULTS WITH OMISSIONOF THE 100 mg/L DATA POINTS:

Cell density at 24 hours

The ToxCalc analysis of the 24 hour algal cell count data, without the nominal 100 mg/L results, gave the following results, using mean measured concentrations of the 5-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	25000	20000	87500	42500	45000	90000
5.2	30000	45000	80000			
11	45000	42500	82500			
20	27500	57500	105000			
42	42500	37500	70000			

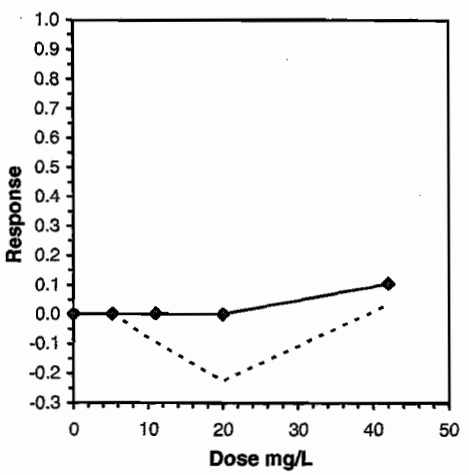
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	51666.67	1.0000	51666.67	20000	90000	58.680	6				55833.3	1.0000
5.2	51666.67	1.0000	51666.67	30000	80000	49.661	3	0.000	2.533	51097.3	55833.3	1.0000
11	56666.67	1.0968	56666.67	42500	82500	39.542	3	-0.248	2.533	51097.3	55833.3	1.0000
20	63333.33	1.2258	63333.33	27500	105000	61.702	3	-0.578	2.533	51097.3	55833.3	1.0000
42	50000	0.9677	50000	37500	70000	35.000	3	0.083	2.533	51097.3	50000	0.8955

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.89994	0.858	0.45754	-1.1143
Bartlett's Test indicates equal variances (p = 0.87)	1.25904	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	42	>42			51097.3	0.98898	9.5E+07	8.1E+08	0.97433	4, 13

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	30.529			
IC10	41.057			
IC15	>42			
IC20	>42			
IC25	>42			
IC40	>42			
IC50	>42			



APPENDIX II (Continued)

Cell density at 48 hours

The ToxCalc analysis of the 48 hour algal cell count data, without the nominal 100 mg/L results, gave the following results 100 mg/L results gave the following results, using mean measured concentrations of the 5-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	302500	192500	242500	267500	287500	372500
5.2	305000	355000	247500			
11	332500	267500	332500			
20	280000	422500	307500			
42	270000	227500	292500			

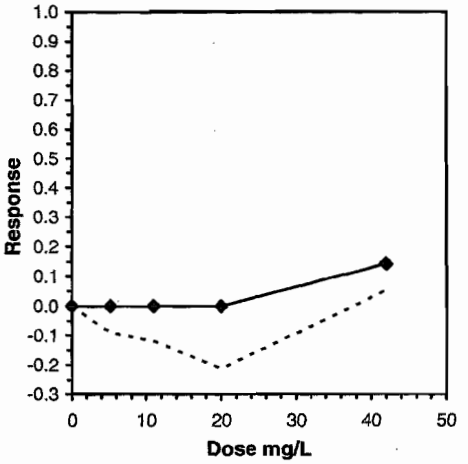
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	277500	1.0000	277500	192500	372500	21.801	6				306875	1.0000
5.2	302500	1.0901	302500	247500	355000	17.783	3	-0.633	2.533	99972.6	306875	1.0000
11	310833.3	1.1201	310833.3	267500	332500	12.073	3	-0.844	2.533	99972.6	306875	1.0000
20	336666.7	1.2132	336666.7	280000	422500	22.454	3	-1.499	2.533	99972.6	306875	1.0000
42	263333.3	0.9489	263333.3	227500	292500	12.535	3	0.359	2.533	99972.6	263333	0.8581

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96961	0.858	0.30604	-0.3068
Bartlett's Test indicates equal variances (p = 0.82)	1.54787	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	42	>42			99972.6	0.36026	2.7E+09	3.1E+09	0.50223	4, 13

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	27.753			
IC10	35.505			
IC15	>42			
IC20	>42			
IC25	>42			
IC40	>42			
IC50	>42			



APPENDIX II (Continued)

Cell density at 72 hours

The ToxCalc analysis of the 72 hour algal cell count data, without the nominal 100 mg/L results, gave the following results 100 mg/L results gave the following results, using mean measured concentrations of the 5-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	1115000	1190000	1285000	1656700	1290000	1115000
5.2	1400000	1410000	1317500			
11	1496700	1300000	1485000			
20	1357500	1315000	1350000			
42	1080000	1027500	542500			

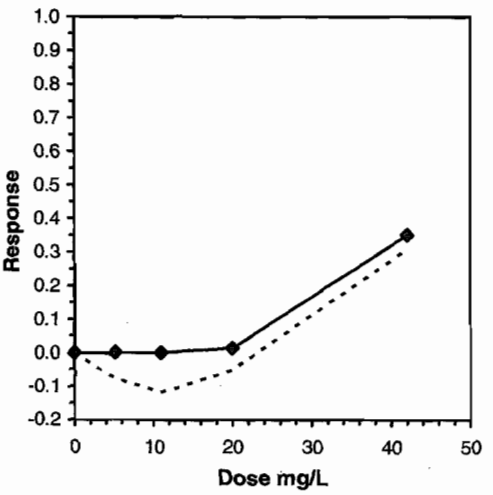
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1275283	1.0000	1275283	1115000	1656700	15.856	6				1359450	1.0000
5.2	1375833	1.0788	1375833	1317500	1410000	3.690	3	-0.800	2.533	318273	1359450	1.0000
11	1427233	1.1191	1427233	1300000	1496700	7.731	3	-1.209	2.533	318273	1359450	1.0000
20	1340833	1.0514	1340833	1315000	1357500	1.692	3	-0.522	2.533	318273	1340833	0.9863
*42	883333.3	0.6927	883333.3	542500	1080000	33.547	3	3.119	2.533	318273	883333	0.6498

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95139	0.858	0.28132	1.88632
Bartlett's Test indicates equal variances (p = 0.04)	10.0405	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	20	42	28.9828		318273	0.24957	1.4E+11	3.2E+10	0.0166	4, 13

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	22.373	2.714	14.055	27.088	-3.8419
IC10	25.642	2.749	20.103	33.640	-1.9147
IC15	28.911	3.309	22.479	40.423	0.5538
IC20	32.179				
IC25	35.448				
IC40	>42				
IC50	>42				



APPENDIX II (Continued)

Cell density at 96 hours

The ToxCalc analysis of the 96 hour algal cell count data, without the nominal 100 mg/L results, gave the following results 100 mg/L results gave the following results, using mean measured concentrations of the 5-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	1372500	1237500	1523300	2180000	2025000	2080000
5.2	1530000	2005000	1563300			
11	2005000	1906700	1436700			
20	1400000	2095000	1390000			
42	1520000	2160000	2170000			

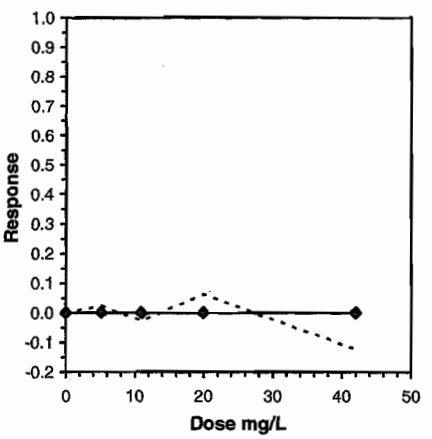
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1736383	1.0000	1736383	1237500	2180000	23.392	6				1759390	1.0000
5.2	1699433	0.9787	1699433	1530000	2005000	15.602	3	0.142	2.533	657831	1759390	1.0000
11	1782800	1.0267	1782800	1436700	2005000	17.037	3	-0.179	2.533	657831	1759390	1.0000
20	1628333	0.9378	1628333	1390000	2095000	24.821	3	0.416	2.533	657831	1759390	1.0000
42	1950000	1.1230	1950000	1520000	2170000	19.099	3	-0.822	2.533	657831	1759390	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91305	0.858	-0.0418	-1.5662
Bartlett's Test indicates equal variances (p = 0.97)	0.52823	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	42	>42			657831	0.37885	4.4E+10	1.3E+11	0.85567	4, 13

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>42			
IC10	>42			
IC15	>42			
IC20	>42			
IC25	>42			
IC40	>42			
IC50	>42			



APPENDIX II (Continued)

Specific growth rate (0-72 hours)

The ToxCalc analysis of the 0-72 hour study report's growth rate data (Table 7, page 32) with the 100 mg/L results omitted gave the following results (initial growth rate data as day^{-1}).

Conc-mg/L	1	2	3	4	5	6
D-Control	1.5700	1.6000	1.6200	1.7100	1.6200	1.5700
5.2	1.6500	1.6500	1.6300			
11	1.6700	1.6300	1.6700			
20	1.6400	1.6300	1.6400			
42	1.5600	1.5500	1.3300			

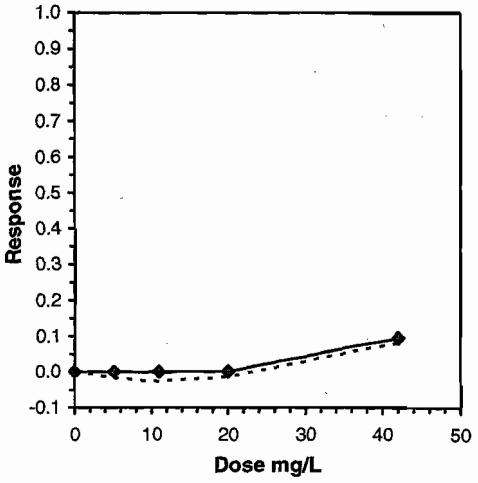
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
D-Control	1.6150	1.0000	1.6150	1.5700	1.7100	3.200	6			1.6383	1.0000
5.2	1.6433	1.0175	1.6433	1.6300	1.6500	0.703	3	21.00	6.00	1.6383	1.0000
11	1.6567	1.0258	1.6567	1.6300	1.6700	1.394	3	21.00	6.00	1.6383	1.0000
20	1.6367	1.0134	1.6367	1.6300	1.6400	0.353	3	21.00	6.00	1.6367	0.9990
*42	1.4800	0.9164	1.4800	1.3300	1.5600	8.784	3	6.00	6.00	1.4800	0.9034

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.87367	0.858	-0.8083	3.18634
Bartlett's Test indicates unequal variances (p = 3.23E-03)	15.8496	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test Treatments vs D-Control	20	42	28.9828	

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	31.269			
IC10	>42			
IC15	>42			
IC20	>42			
IC25	>42			
IC40	>42			
IC50	>42			



APPENDIX II (Continued)

Biomass (0-72 hours)

The ToxCalc analysis of the 0-72 hour study report's biomass data (Table 8, page 34) with the 100 mg/L results omitted gave the following results (biomass data as cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	88.43	80.61	97.24	114.60	97.91	101.93
5.2	103.92	111.00	98.69			
11	113.19	96.15	116.26			
20	98.93	114.18	108.94			
42	85.02	77.49	62.20			

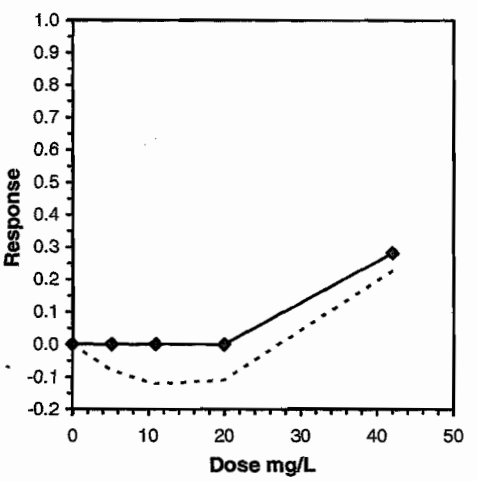
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	96.79	1.0000	96.787	80.610	114.600	12.020	6				104.30	1.0000
5.2	104.54	1.0801	104.537	98.690	111.000	5.910	3	-1.064	2.533	18.440	104.30	1.0000
11	108.53	1.1214	108.533	96.150	116.260	9.982	3	-1.613	2.533	18.440	104.30	1.0000
20	107.35	1.1091	107.350	98.930	114.180	7.218	3	-1.451	2.533	18.440	104.30	1.0000
*42	74.90	0.7739	74.903	62.200	85.020	15.524	3	3.006	2.533	18.440	74.90	0.7181

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96543	0.858	-0.1419	-0.4376
Bartlett's Test indicates equal variances (p = 0.90)	1.07919	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	20	42	28.9828		18.44	0.19052	583.026	106.024	0.00814	4, 13

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	23.903	1.857	17.748	26.744	-5.8375
IC10	27.805	1.728	23.340	33.487	0.3344
IC15	31.708	2.407	26.300	40.231	0.6457
IC20	35.611				
IC25	39.513				
IC40	>42				
IC50	>42				



APPENDIX III Statistical verification output of the PMRA reviewer

Cell Density

One Way Analysis of Variance Tuesday, July 31, 2007, 13:14:51

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.709)

Group Name	N	Missing	Mean	Std Dev	SEM
control	6	0	173.638	40.617	16.582
5.2 mg/L	3	0	169.777	26.227	15.142
11 mg/L	3	0	178.280	30.373	17.536
20 mg/L	3	0	162.833	40.418	23.335
42 mg/L	3	0	195.000	37.242	21.502
80 mg/L	3	0	19.667	2.082	1.202

Source of Variation	DF	SS	MS	F	P
Between Groups	5	64231.226	12846.245	10.999	<0.001
Residual	15	17519.324	1167.955		
Total	20	81750.550			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.999

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: treatment

Comparison	Diff of Means	q'	P	P<0.050
control vs. 80 mg/L	153.972	6.372	--	Yes
control vs. 42 mg/L	21.362	0.884	--	No
control vs. 20 mg/L	10.805	0.447	--	Do Not Test
control vs. 11 mg/L	4.642	0.192	--	Do Not Test
control vs. 5.2 mg/L	3.862	0.160	--	Do Not Test

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Biomass

One Way Analysis of Variance Tuesday, July 31, 2007, 13:16:21

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.939)

Group Name	N	Missing	Mean	Std Dev	SEM
control	6	0	96.787	11.634	4.750
5.2 mg/L	3	0	104.537	6.178	3.567
11 mg/L	3	0	108.533	10.834	6.255
20 mg/L	3	0	107.350	7.748	4.474
42 mg/L	3	0	74.903	11.628	6.713
80 mg/L	3	0	19.007	5.669	3.273

Source of Variation	DF	SS	MS	F	P
Between Groups	5	18438.451	3687.690	38.345	<0.001
Residual	15	1442.582	96.172		
Total	20	19881.033			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: treatment

Comparison	Diff of Means	t	P	P<0.050
control vs. 80 mg/L	77.780	11.217	<0.001	Yes
control vs. 42 mg/L	21.883	3.156	0.033	Yes
control vs. 11 mg/L	11.747	1.694	0.555	No
control vs. 20 mg/L	10.563	1.523	0.742	Do Not Test
control vs. 5.2 mg/L	7.750	1.118	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Growth rate

One Way Analysis of Variance Tuesday, July 31, 2007, 13:18:00

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.063)

Equal Variance Test: Passed (P = 0.395)

Group Name	N	Missing	Mean	Std Dev	SEM
control	6	0	1.615	0.0517	0.0211
5.2 mg/L	3	0	1.643	0.0115	0.00667
11 mg/L	3	0	1.657	0.0231	0.0133
20 mg/L	3	0	1.637	0.00577	0.00333
42 mg/L	3	0	1.480	0.130	0.0751
80 mg/L	3	0	1.063	0.0987	0.0570

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.825	0.165	36.387	<0.001
Residual	15	0.0680	0.00453		
Total	20	0.893			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: treatment

Comparison	Diff of Means	q'	P	P<0.050
control vs. 80 mg/L	0.552	11.586	--	Yes
control vs. 42 mg/L	0.135	2.835	--	Yes
control vs. 11 mg/L	0.0417	0.875	--	No
control vs. 5.2 mg/L	0.0283	0.595	--	Do Not Test
control vs. 20 mg/L	0.0217	0.455	--	Do Not Test

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Growth rate

One Way Analysis of Variance Tuesday, July 31, 2007, 13:18:25

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.063)

Equal Variance Test: Passed (P = 0.395)

Group Name	N	Missing	Mean	Std Dev	SEM
control	6	0	1.615	0.0517	0.0211
5.2 mg/L	3	0	1.643	0.0115	0.00667
11 mg/L	3	0	1.657	0.0231	0.0133
20 mg/L	3	0	1.637	0.00577	0.00333
42 mg/L	3	0	1.480	0.130	0.0751
80 mg/L	3	0	1.063	0.0987	0.0570

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.825	0.165	36.387	<0.001
Residual	15	0.0680	0.00453		
Total	20	0.893			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: treatment

Comparison	Diff of Means	t	P	P<0.050
control vs. 80 mg/L	0.552	11.586	<0.001	Yes
control vs. 42 mg/L	0.135	2.835	0.063	No
control vs. 11 mg/L	0.0417	0.875	1.000	Do Not Test
control vs. 5.2 mg/L	0.0283	0.595	1.000	Do Not Test
control vs. 20 mg/L	0.0217	0.455	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Exponential growth
(page 38 of this DER refers)

To examine the goodness of fit of the cell count data with an exponential growth curve, the mean cell counts at 0 to 96 hours were plotted against time using the Microsoft Excel Chart Wizard function and the data points fitted to an exponential curve. The data used and the fitted curve obtained are shown below.

Time (hours)	Mean cell count, cells/mL
0	10000
24	10000
48	58000
72	294500
96	1292300