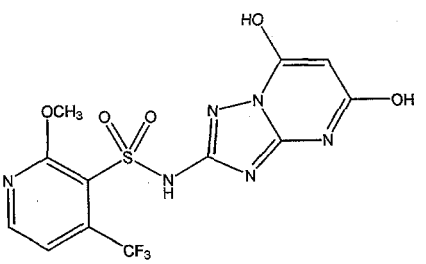
Data Evaluation Report on the Acute Toxicity of 5,7-di-OH Metabolite of Pyroxsulam (5,7-di-OH metabolite of XDE-742) to Algae, *Pseudokirchneriella subcapitata*

PMRA Submission Number 2006-4727; 1283230 EPA MRID Number 49608-xx APVMA ATS 40362

49608-49

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:	5,7-di-OH metabolite of XDE-742 i.e. 5,7-di-OH metabolite of pyroxsulam	
Purity:	98%	
Common name:	5,7-di-OH metabolite of XDE-742 5,7-di-OH-XDE-742 (i.e. 5,7-di-OH metabolite of pyroxsulam)	
Chemical name:	N-(5,7-dihydroxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridine-sulfonamide	
IUPAC:	N-(5,7-dihydroxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
CAS name:	3-Pyridinesulfonamide, N-(1,5-dihydro-7-hydroxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)	
Synonyms:	5,7-dihydroxy-XDE-742 and 5,7-di-OH-XDE-742 metabolite	

Chemical Structure:



Primary Reviewers: Daryl Murphy 22/02/08 **Date:** 26 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

22/2/08

Secondary Reviewers: Jack Holland **Date:** 26 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Émilie Larivière **Date:** 31 July 2007
Environmental Assessment Directorate, PMRA 05/03/08

Christopher Salice **Date:** 13 October 2007
Environmental Fate and Effects Division, EPA 04/08/08

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

CITATION: Hoberg, J. R. 2006. 5,7-Di-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts. Springborn Smithers Study No. 12550.6417 and Sponsor Protocol/Project No. 050109. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana 46268.5 April 2006. Unpublished report.

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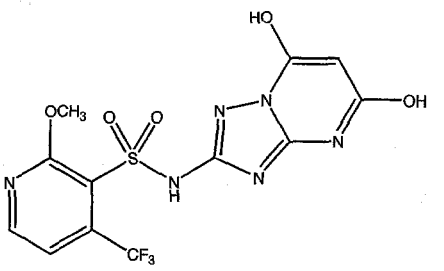
PMRA Submission Number 2006-4727; 1283230 EPA MRID Number 496084-49 APVMA ATS 40362

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:	5,7-di-OH metabolite of XDE-742 i.e. 5,7-di-OH metabolite of pyroxsulam
Purity:	98%
Common name:	5,7-di-OH metabolite of XDE-742 5,7-di-OH-XDE-742 (i.e. 5,7-di-OH metabolite of pyroxsulam)
Chemical name:	N-(5,7-dihydroxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridine-sulfonamide
IUPAC:	N-(5,7-dihydroxy-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS name:	3-Pyridinesulfonamide, N-(1,5-dihydro-7-hydroxy-5-oxo[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
Synonyms:	5,7-dihydroxy-XDE-742 and 5,7-di-OH-XDE-742 metabolite

Chemical Structure:



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

CITATION: Hoberg, J. R. 2006. 5,7-Di-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts. Springborn Smithers Study No. 12550.6417 and Sponsor Protocol/Project No. 050109. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana 46268.5 April 2006. Unpublished report.

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EXECUTIVE SUMMARY:

The purpose of this study was to determine the effect of the 5,7-di-OH metabolite of pyroxsulam (XDE-742) on the growth of the freshwater green alga, *Pseudokirchneriella subcapitata.* In a 96-hour acute toxicity study, the freshwater green alga was exposed to the 5,7-di-OH metabolite of pyroxsulam at nominal concentrations of 0 (control), 1.0, 2.6, 6.4, 16, 40 and 100 mg/L (corresponding mean measured concentrations of 0 (control), 0.5, 1.8, 4.7, 13, 36 and 92 mg/L, respectively). The experiment was carried out taking account of relevant OECD, European Community 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 22–23°C. The light intensity range was 3900-4600 lux. The pH value in the controls was 6.7 at test initiation and 8.8 at test termination. In the 5,7-di-OH metabolite of pyroxsulam containing solutions, the pH ranged from 3.8 to 6.5 at test initiation and 4.0 to 9.2 at test termination with solution pH inversely proportional to the test concentration. The pH of 3.8, for the nominal 100 mg/L concentration, at time 0 has confounded the study's interpretation as the observed lack of algal growth seen at 100 mg/L could be caused by the presence of the 5,7-di-OH metabolite of pyroxsulam at this concentration, the pH of the test solutions, or by a combination of both factors. As the four related hydroxy metabolites all exhibited the same problem and had nearly identical results, inhibition clearly was 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 and the US EPA.

The pH of the control media increased 2.1 pH units, which exceeds the 2006 OECD 201 guideline value of 1.5 units during the test. Other deviations or deficiencies included the light intensity exceeding OECD requirements. These latter deviations and deficiencies were not considered to have adversely affected the study or its results.

After 72 hours, inhibition of mean specific growth rate relative to controls ranged from -15% (growth stimulation) at 1.8 mg/L to 112% at 92 mg/L. The inhibition of biomass relative to controls ranged from -48% at 1.8 mg/L to 105% at 92 mg/L. After 96 hours, inhibition of cell density relative to controls ranged from -2% at 4.7 mg/L to 100% at 92 mg/L (mean measured concentrations).

At test termination (96 hours), cells exposed to the treatment levels tested and the control were observed to be normal.

Based on the results of this study, as shown below, the 5,7-di-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 Government Department of the Environment, Water, Heritage and the Arts and fails to 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 at the highest test concentrations.

The US EPA Reviewer classifies this study as SUPPLEMENTAL and it does not satisfy the guideline requirements for an acceptable acute toxicity study with green algae. Estimates of toxicity appear compromised due to low pH in some test concentrations.

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, 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 >36 mg 5,7-di-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 Size/Age: *Pseudokirchneriella subcapitata*
Test Type: Static

The following endpoints give an indication of the lack of toxicity of the 5,7-di-OH metabolite of pyroxsulam to *Pseudokirchneriella subcapitata*. Actual EC50 values are all expected to be >36 mg 5,7-di-OH metabolite of pyroxsulam/L.

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

Biological Parameter	Based on Mean Measured Concentrations (mg 5,7-di-OH metabolite of pyroxsulam/L)	
	EC50 (95% confidence limits)	NOEC
96-Hour Cell Density	55 (40-63)	36
0-72 hour Total Biomass	56 (38-63)	36
0-72-Hour Average Growth Rate	60 (56-62)	36

Results based on Mean Measured Concentrations which will be used for risk assessment as a result of the nominal 100 mg/L results not being considered because of the pH issue at time 0 are:

Biological Parameter	Based on Mean Measured Concentrations (mg 5,7-di-OH metabolite of pyroxsulam/L)	
	EC50	NOEC
96-Hour Cell Density	>36	36
0-72 hour Total Biomass	>36	36
0-72-Hour Average Growth Rate	>36	36

Endpoint(s) Affected: 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 the protocol were stated to meet the requirements specified by the OECD and the EC, namely:

- The OECD Guideline for Testing of Chemicals. Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Draft Revised Guideline #201. Adopted 7 June 1984; and

- The Official Journal of the European Communities. 1992. Methods for the determination of Ecotoxicity. C.3. Algal Inhibition Test. L383A Volume 35, 29 December 1992.

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

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

The study author reported the following deviation from their protocol:

- The protocol stated that the pH will be measured in one replicate of the control at 72 hours of exposure. During this study, the pH of the 72-hour control solution was inadvertently not measured. The lack of this measurement does not have a negative impact on the results or interpretation of this study.

- The protocol stated 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 48- to 72-hour growth rate of the control was 49% and the 0- to 72-hour growth rate CV for the control was 9.5%. 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 48- to 72-hour and 0- to 72-hour CV values of 36% and 6.9%, respectively, which closely approximate the above criteria. 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 reported these deviations did not affect the acceptability of the study.

  These deviations are 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. However, as the study was completed in April 2006, only shortly after the changes to the OECD 201 test guideline were published in March 2006, deviations from the current OECD Guideline are generally considered minor with the exception of the pH of 4.0 in the nominal 100 mg 5,7-di-OH metabolite of pyroxsulam/L test concentration which renders the test supplemental.

COMPLIANCE:

The data and report for "5,7-di-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 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. 41 pp. 1998; and

- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

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

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

A. MATERIALS:

1. Test Material:	5,7-di OH metabolite of XDE-742 (i.e. 5,7-di-OH metabolite of pyroxsulam)
Description:	Solid
Lot No./Batch No.:	XN8-33938-53
ID No.:	TSN 105233
Purity:	98%
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 50 to 92% 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 relatively stable for at least 96 hours under the test conditions, especially at test concentrations ≥16 mg/L (nominal). Analysis of the quality control samples ranged from 90.4 to 102% of the nominal fortified levels of 0.50, 10.0 and 100 mg/L. Overall, it is concluded that the test substance exhibited acceptable stability in the test medium over the 96 hours of the test.
Storage conditions of test chemicals:	The test substance (also identified as (SSL No. 112-84) was stored refrigerated (1 to 10°C) in the original container.
Physicochemical properties of 5,7-di-OH metabolite of pyroxsulam:	Physicochemical data for the 5,7-di-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 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
Species:	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)
Class:	Chlorophyceae
Strain:	1648
Source:	In-house laboratory cultures maintained in stock cultures at Springborn Smithers and originally obtained from the University of Texas.
Age of inoculum:	The inoculum used to initiate the toxicity test with 5,7-di-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 a culture medium of 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 3800 to 4700 lux (360 to 440 footcandles). 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 5,7-di-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. Following 96 hours of exposure, cell densities in the 0.010, 0.10, 1.0, 10 and 100 mg 5,7-di-OH metabolite of pyroxsulam/L treatment levels averaged 305, 361, 345, 310 and 0.88 x 10^4 cells/mL, respectively. The control averaged 364 x 10^4 cells/mL. Based on these results and consultation with the Study Sponsor, nominal 5,7-di-OH pyroxsulam concentrations of 1.0, 2.6, 6.4, 16, 40 and 100 mg 5,7-di-OH metabolite of pyroxsulam/L were selected for the definitive exposure.

b. Definitive Study
The purpose of this study was stated to be to determine the effect of 5,7-di-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 5,7-di-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 (area under the curve) 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 13 to 17 February 2006 at Springborn Smithers Laboratories, (SSL) located in Wareham, Massachusetts. All original raw data, the protocol and the original final report produced during this study are archived by the Toxicology and Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.

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

Table 1. Experimental Parameters

Parameter	Details	*Remarks/Criteria*
Acclimation period:	The inoculum used to initiate the toxicity test was taken from a stock culture that had been transferred to fresh medium three days before testing test.	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 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.	Requirement considered met. Comparison of the reported typical culturing and test media conditions indicated they were equivalent.

Parameter	Details	*Remarks/Criteria*
	Culturing conditions same as those specified by the test protocol – see following comparison: **Culture** / **Test** Environmental chamber / Environmental chamber 24 ± 2°C / 24 ± 2°C Continuous illumination / Continuous light 3800 to 4700 lux / 3800 to 4700 lux Orbital shaker 100 ± 10 rpm / Orbital shaker 100 ± 10 rpm Stock and test 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.	

Parameter	Details	*Remarks/Criteria*
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 (N/A) for a static system in which there was no renewal of test medium.	Requirement considered met. OECD 201 does not specifically refer to static tests 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.

Parameter	Details	*Remarks/Criteria*
		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 CO2 from the atmosphere. US EPA OPPTS 850.5400 states Erlenmeyer flasks should be used for test containers and may be of any volume between 125 and 500 mL as long as the same size is used throughout a test and the test solution volume does not exceed 50 percent of the flask volume. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name	Algal Assay Procedure (AAP) medium Medium details provided in the study report were considered equivalent to the AAP medium composition recorded in OECD 201 with the following exception: The test medium contained sodium selenate at 1.88 µg/L. The study report noted this was an additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.	See deviations/deficiencies table, page 38 of this DER with respect to use of a modified AAP medium, containing sodium selenate and use of chelating agents. 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 (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 medium and no chelators*

Parameter	Details	*Remarks/Criteria*
		This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements which allow use of chelating agents (the AAP medium used contains sodium EDTA as a chelating agent).
pH at test initiation and at test termination:	The AAP medium used to prepare the exposure solutions was adjusted, if necessary, to 7.5 ± 0.1 prior to use. pH values at 0 and 96 hours were: **Nominal concentrations, mg 5,7-di-OH metabolite of pyroxsulam/L** / **Initial pH** / **pH at 96 hours** **Control** 6.7 8.8 **1.0** 6.5 9.2 **2.6** 6.4 9.1 **6.4** 6.4 9.1 **16** 6.0 8.9 **40** 5.2 8.6 **100** 3.8 4.0 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 38 of this DER with respect to initial and final pH values. OECD 201 identifies AAP as having a pH of 7.5. US EPA OPPTS 850.5400 states that the pH of the nutrient medium is to be 7.5 (± 0.1) for *Selenastrum.* US EPA 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 3.8. However, the pH was not adjusted. *OECD recommends the medium pH after equilibration with air be ~8 with less than 0.001 mmol/L chelator, if used.* This template requirement is noted but is not considered appropriate in the light of the current OECD and US EPA OPPTS requirements with respect to medium pH and specified concentrations of chelating agents. OECD recommends (2006) the medium pH should not increase by greater that 1.5 pH units during the test.
Chelator used:	Yes, as required for AAP media (disodium ethylenediaminetetreaacetic 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

Parameter	Details	*Remarks/Criteria*
		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 with respect to medium pH and specified concentrations 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 source/type:	The Algal Assay Procedure (AAP) medium prepared with sterile, deionised water. The source was not identified.	Requirements considered met for all dilution water parameters. 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. 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.
pH:	Not stated but, if necessary, the pH of the culture medium was adjusted to pH 7.5 ± 0.1.	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt.*

Parameter	Details	*Remarks/Criteria*
		OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.
Salinity (for marine algae):	Not applicable, the algae used are a freshwater species.	*salinity:* *EPA: 30-35 ppt.*
Water pretreatment (if any):	Deionisation and sterilisation	*EPA is against the use of dechlorinated water.*
Total Organic Carbon:	A representative sample of AAP medium was reported analysed monthly for total organic carbon (TOC) concentration. The TOC concentration of the sample collected in February 2006 was 0.63 mg/L.	
Particulate matter:	Not reported	
Metals: pesticides:	Not reported Not reported However, 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).	
Chlorine:	Not reported	
Indicate how the test material is added to the medium (added directly or used stock solution)	A 100 mg 5,7-di-OH metabolite of pyroxsulam/L stock solution was prepared prior to test initiation by placing 0.2040 g of 5,7-di-OH metabolite of pyroxsulam (0.1999 g as active ingredient – concentrations were adjusted for the 98% purity of the 5,7-di-OH metabolite of pyroxsulam) 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 a large amount of visible undissolved test substance. Following approximately 30 minutes of sonication and an additional 30 minutes of mixing with a magnetic stir plate and Teflon®-coated stir bar, the stock solution was observed to be clear and colourless with no visible undissolved test substance. Test solutions were prepared from serial dilutions of the 100 mg 5,7-di-OH metabolite of	Requirement considered met with the description in the report considered satisfactory.

Parameter	Details	*Remarks/Criteria*
	pyroxsulam/L stock solution 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.	
Aeration or agitation	Agitation (approx. 100 revs./min.) continuous by means of 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*

Parameter	Details	*Remarks/Criteria*
		cells/ml for S. capricornutum and S. subspicatus. When other species are used, the biomass should be comparable.
Number of replicates Control:	6, inoculated with algae.	Requirements considered met. The numbers of replicates used are acceptable. OECD 201 states that the test design should include three replicates at each test concentration and that the number of control replicates must be at least three, and ideally should be twice the number of replicates used for each test concentration. US EPA 850.5400 states that a minimum of three replicates is required for each concentration of test chemical and control.
Solvent control:	A solvent control was not used.	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Treatments:	3 per treatment level were 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 6.4 mg 5,7-di-OH metabolite of pyroxsulam/L (nominal) treatment level was prepared. This flask, which was not inoculated with algae, was analysed at 96 hours of exposure for 5,7-di-OH metabolite of pyroxsulam concentration. The results of this analysis were compared with the results for the 6.4 mg 5,7-di-OH metabolite of pyroxsulam/L solution containing algae.	
Test concentrations Nominal:	Nominal concentrations were 0 (control), 1.0, 2.6, 6.4, 16, 40 and 100 mg/L	See deviations/deficiencies table, page 38 of this DER with respect to the US EPA requirements The ratios of the nominal concentrations

Parameter	Details	*Remarks/Criteria*
Measured:	Ratios of nominal concentrations were 1:2.5 or 1:2.6 The reported measured concentrations were: **Nominal Conc.[a] / Measured Concentration[b] 0 hour / 96 Hour / Mean[c] / % of Nominal** **Control** <0.039 <0.047 NA[d] NA **1.0** 0.67 0.32 0.50 50 **2.6** 2.5 1.1 1.8 70 **6.4** 6.1 3.2/5.6[e] 4.7 73 **16** 15 11 13 82 **40** 38 34 36 90 **100** 96 88 92 92 **a, b. mg 5,7-di-OH metabolite of pyroxsulam/L.** **c. 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.** **d. NA = Not Applicable.** **e. Result of the additional sample without algae present to determine biological uptake/degradation**	were approximately 1:2.5 which exceeds the US EPA guideline's requirements for this parameter (the ratio is between 1.5 and 2.0). After 96 hours the test concentrations were below the initial values. The results of the uninoculated test at 6.4 mg/L indicate loss of test material was mainly due to uptake by the algal cells. 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. 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). *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 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 5,7-di-OH-pyroxsulam at 0- and 96-hours using HPLC. All exposure solutions and Quality Control samples were analysed for 5,7-di-OH metabolite of pyroxsulam using high performance liquid chromatographic system equipped with	Requirement considered met. Methodology was validated (24 January 2006) to quantify the amount of 5,7-di-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

Parameter	Details	*Remarks/Criteria*
	ultraviolet detection (HPLC/UV) based on methodology validated at Springborn Smithers.	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 5,7-di-OH metabolite of pyroxsulam at concentrations of 0.05 and 100 mg/L. Recoveries averaged 96.1 ± 6.13% with a limit of quantitation (LOQ) of 0.0203 mg 5,7-di-OH metabolite of pyroxsulam/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 from the analysis of a calibration standard, recovery sample and a control sample were presented. A typical linear regression analysis for 5,7-di-OH metabolite of pyroxsulam (r^2 = 0.99884) was also presented in the study report.

Parameter	Details	*Remarks/Criteria*
Test conditions Temperature:	22-23°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	Photoperiod requirement considered met. OECD 201 refers to use of continuous light while US EPA OPPTS 850.5400 refers to test chambers containing *Selenastrum*, *Navicula*, and *Anabaena*, being illuminated continuously. *OECD recommended continuous uniform illumination* *EPA photoperiod: S. costatum 14 hr light/ 10 hr dark. Others: Continuous.*
Light intensity and quality:	3900-4600 lux The photosynthetically active radiation (PAR) of the test area measured at test initiation ranged from 59 to 75 µE/m^2/s.	See deviations/deficiencies table, page 38 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 µE·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 continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector. EPA light: Anabaena:2000 lux (±15%), Others: 4000 – 5000 lux (±15%)*

Parameter	Details	*Remarks/Criteria*
		These template requirements are noted but not considered further in the light of the specific requirements in the OECD and US EPA OPPTS guidelines.
Reference chemical (if used) name: concentrations:	 N/A N/A	Parameter 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 in the treatment and control solutions was 80 (or 90 for the 100 mg/L test solution) µmhos/cm. At test termination (96 hours), the control conductivity was still 80 µmhos/cm and that of the test concentrations 80 to 100 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) were determined. pH, conductivity, temperature, light intensity and concentrations 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 current 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.*
Other observations, if	At test termination, cells exposed to all treatment levels tested and the	Requirement considered met.

Parameters	Details	Remarks/Criteria
any	control were observed to be normal.	
Indicate whether there was exponential growth in the control	The mean control 72-hour cell growth was 49.2 X 10^4 cells/mL (cf. 1 X 10^4 cells at test initiation). At 96 hours, the mean control cell density was ~179 X 10^4 or ~1.79 X 10^6 cells/mL. The mean 0-72 hours calculated growth rate of the controls was 1.3 day^{-1}.	See deviations/deficiencies table, page 38 of this DER with respect to the attainment of exponential growth. 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. At 72 hours, the mean cell density in the controls was ~49 X 10^4 cells/mL. This represents a factor of ~50 (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 1.3 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 ~51% 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 growth of the controls and details provided on the medium's preparation using 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	Parameter considered met. While raw data were not submitted, the tabulated results presented were sufficient to allow statistical analysis by the reviewer. OECD 201 lists the results which must be

Parameters	Details	Remarks/Criteria
	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.	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, because the tabulated results presented in the study report were sufficient to allow statistical analysis, the guideline would be considered met.

II. RESULTS and DISCUSSION:

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.

Cell density

The effects of the 5,7-di-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. The mean, standard deviation (SD) and percentage inhibition at 96 hours values shown in the table were calculated from original raw data, not from the rounded data presented in the study report.

Based on the 48 to 72-hour growth rate criterion (for further details, see under "Validity of the test" on page of this DER), the study report stated that replicate B of the control was excluded from the mean and all statistical analyses as an outlier 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) and the results are reported below. The 96 hour cell density in the control averaged 179.82 x 10^4 cells/mL. Cell density in the 0.50, 1.8, 4.7, 13, 36 and 92 mg/L treatment levels averaged 178.67, 181.78, 184.00, 173.83, 137.64 and 0.25 x 10^4 cells/mL, respectively. Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set was reported to have passed the requirements for normality and homogeneity of variance, therefore, Williams' Test was used to determine treatment-related effects. Statistical analysis (Williams' Test) was said to have determined a significant reduction in cell density in the 92 mg/L treatment level when compared to the control (179.82 x 10^4 cells/mL). Therefore, the NOEC for cell density was determined to be 36 mg/L. The 96-hour EC50 was calculated to be 55 mg/L, with 95% confidence intervals of 40 to 63 mg/L.

Table 3. Effect of 5,7-di-OH pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*) as

reported by Hoberg (2006). Mean counts at 24, 48, 72 and 96 hours shown with standard deviations in brackets.

Treatment (mean measured concentration (mg 5,7-di-OH metabolite of pyroxsulam/L)	Initial cell density (cells/mL)	Mean cell density ($x10^4$ x cells/mL) and standard deviation in brackets at 24, 48, 72 and 96 hours				
		24-hours	48-hours	72-hours	96-hours	Percent inhibition[1] at 96 hours
Negative control[2]	1×10^4	4.25 (2.11)	16.10 (4.07)	49.20 (10.98)	179.82 (50.98)	NA**
0.50	1×10^4	2.58 (1.13)	15.42 (6.53)	50.42 (25.48)	178.67 (11.02)	1
1.8	1×10^4	3.75 (1.00)	18.33 (8.33)	85.67 (20.53)	181.78 (43.00)	-1
4.7	1×10^4	3.75 (2.14)	15.75 (5.63)	47.00 (18.93)	184.00 (36.66)	-2
13	1×10^4	3.42 (1.77)	15.92 (4.51)	54.08 (23.78)	173.83 (40.28)	3
36	1×10^4	3.08 (1.88)	13.75 (4.63)	47.00 (16.21)	137.64 (43.33)	23
92	1×10^4	0.00 (0.00)	0.17 (0.29)	0.33 (0.29)	0.25 (0.43)*	100
Reference chemical (if used)	N/A					

*** Significantly reduced from the pooled control (William's Test, $p \leq 0.05$). ** NA = not applicable. [1]Relative to control. [2]Based on the 48 to 72 hour growth rate criterion, one control replicate (B) was excluded from the mean and all statistical analyses as an outlier and thus there were 5 replicates used for control.**

Significant cell density inhibition (with respect to the mean control cell density) was identified at 96 hours only in the algae exposed to mean measured concentration of 92 mg 5,7-di-OH metabolite of pyroxsulam/L. This is considered most likely the result of the acidity of the test solutions inhibiting algal growth rather than the specific toxicity of the 5,7-di-OH metabolite of pyroxsulam itself.

Growth rate

As reported by the study, the mean specific growth rates per day (and the mean areas under the growth curves) reported following exposure of *Pseudokirchneriella subcapitata* to the 5,7-di-OH metabolite of pyroxsulam are shown in Table 4 with respective percent inhibition results. Means and percent inhibition were 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 in the study report as an outlier from the mean and all statistical analyses presented in the table. Significant inhibition again occurs at 92 mg 5,7-di-OH metabolite of pyroxsulam/L.

The 0 to 72-hour growth rate in the control was reported as averaging 1.30 $days^{-1}$. Similarly, it was reported that the 0 to 72-hour growth rate in the 0.50, 1.8, 4.7, 13, 36 and 92 mg/L treatment levels averaged 1.29, 1.49, 1.28, 1.32, 1.28 and –0.16 $days^{-1}$, respectively and, based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set passed the requirements for normality and homogeneity of variance. Consequently, Williams' Test was used by the study author to determine treatment-related effects. A significant reduction in average growth rate was reported detected in the 92 mg/L treatment level as compared to the control data. Based on these results, the 72-hour NOEC was determined to be 36 mg/L. The 72-hour ErC50 was calculated to be 60 mg/L, with 95% confidence intervals of 56 to 62 mg/L. Extensive inhibition was only seen at the 92 mg/L concentration, attributed by the reviewer to pH of the test solutions rather than toxicity of the 5,7-di-hydroxy metabolite itself.

Biomass

The following was reported for the study - After 72 hours of exposure, the total biomass in the control averaged 42.90 x 10^4 cells•days/mL. Total biomass in the 0.50, 1.8, 4.7, 13, 36 and 92 mg/L treatment levels averaged 41.24, 63.47, 40.93, 44.44, 38.32 and –2.13 x 10^4 cells•days/mL, respectively.

Note that the biomass has frequently been reported in the algal toxicity studies seen by the reviewer as "cells/mL". While this unit is frequently used, the present study's use of "cells•days/mL" is understood to be the more correct unit.

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. A significant reduction in total biomass was detected in the 92 mg/L treatment level as compared to the control data. Based on these results, the 72-hour NOEC for total biomass was determined to be 36 mg/L. The 72-hour EbC50 was calculated to be 56 mg/L, with 95% confidence intervals of 38 to 63 mg/L. Extensive inhibition was only seen at the 92 mg/L concentration, attributed by the reviewer to pH of the test solutions rather than toxicity of the 5,7-di-hydroxy metabolite itself.

As noted above, the mean areas under the growth curves reported following exposure of *Pseudokirchneriella subcapitata* to the 5,7-di-OH metabolite of pyroxsulam are shown in Table 4 along with respective percent inhibition results.

Table 4. Effect of 5,7-di-OH pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*) as reported by Hoberg (2006). Growth rate and mean area under the growth curve (biomass) results are shown.

Treatment measured concentrations (mg 5,7-di-OH metabolite of pyroxsulam/L)	Mean Specific Growth Rate per day (Standard deviation in brackets)		Mean Area Under the Growth Curve (Standard deviation in brackets), X 10^4 cells/mL	
	0-72 hours	Percent Inhibition[1]	0-72 hours	Percent Inhibition[1]
Negative control[2]	1.30 (0.09)	NA	42.9 (8.26)	NA**
0.50	1.29 (0.16)	1	41.24 (14.45)	4
1.8	1.49 (0.09)	-15	63.47 (13.44)	-48
4.7	1.28 (0.13)	2	40.93 (16.34)	5
13	1.32 (0.17)	-2	44.44 (13.59	-4
36	1.28 (0.12)	2	38.32 (14.68)	11
92	-0.16 (0.13)[1]	112	-2.13 (0.38)*	105

*** Significantly different from the pooled control (William's Test, p≤0.05). ** NA = not applicable. [1] Relative to control. [2]Based on the 48 to 72 hour growth rate criterion, one control replicate (B) was excluded from the mean and all statistical analyses as an outlier and thus there were 5 replicates used for control.**

The reported statistical endpoints are summarised in Table 5.

Table 5. Statistical endpoint values determined by the study report for the 5,7-di-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 5,7-di-OH metabolite of pyroxsulam/L)	36	36	36
EC50 (mg 5,7-di-OH metabolite of pyroxsulam/L) (95% C.I.)	55 (40-63)	60 (56-62)	56 (38-63)
Reference chemical, if used	Not applicable		

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

In contrast, OECD 201 (1984), the guideline version the study followed, does 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 report's comments on test validity

The study report stated that the following acceptance criteria were required by the protocol: the cell growth in the control must increase by more than 16 times after 72 hours of growth. During this study, the 72 hour cell growth in the control was 49.20 x 10^4 cells/mL, which exceeds the above criterion.

Additionally, 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%. 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 results of this test, from the study report, are presented in the following table:

Observation	Growth Rate Coefficient of Variation (%)			
Interval (hours):	**0-24**	**24-48**	**48-72**	**0-72**
Control(N=6)a	30	17	49	9.5
Control(N=5)b	34	19	36	6.9

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 reported as repeated excluding control replicate B (N = 5). Deletion of this replicate from the data analysis was said to provide 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 in Table 1, page 23 of this DER under the parameter "Indicate whether there was an exponential growth in the control".

The US EPA OPPTS 850.5400 states that algal growth in controls should reach the logarithmic growth phase by 96 h. At that 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 ~51% 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.

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 31 of this DER. The values and calculated statistics, including the 0-72 h mean % coefficient of variation (%CV), are as shown in Table 6:

Table 6. 5,7-di-OH metabolite of pyroxsulam - 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. The study report's %CV values are presented in brackets. Also shown are the mean, standard deviation and %CV for the situation in which the number 2 replicate was excluded from the data analysis. The 0-72 and 0-96 hour %CV values for growth rates are also shown.

Reviewer calculated growth rates (/day) for the control replicates			
Replicate	**0-24 h**	**24-48 h**	**48-72 h**
1	1.45	1.44	0.51
2	1.61	1.34	0.31
3	0.81	1.75	1.40
4	1.10	1.34	1.53
5	2.05	1.03	0.98
6	1.39	1.40	1.17
Mean	1.40	1.38	0.98
Standard deviation	0.43	0.23	0.49
%CV (study report %CV)	30.4 (30)	16.7 (17)	49.3 (49)
Results when the number 2 replicate was excluded from the data analysis.			
Mean	1.36	1.39	1.12
Standard deviation	0.46	0.26	0.40
%CV (study report %CV)	34 (34)	18 (19)	36 (36)
0-72 hour %CV	9.2% (see page 50 for ToxCalc values used to determine this result) based on all six control replicates. (9.5% reported by the study report).		
0-96 hour %CV	6.8% based on five control replicates (6.9% reported by the study report) 7.7% using all control replicate values and 5.3% using five replicates (calculations not shown).		

Only the %CV value for the 48-72 hour period exceeded the 35% limit set by the 2006 OECD 201 guideline. The value reported, ~49%, was the same as that reported in the study report. The study report considered that one control replicate, B in the study report, number 2 in the above table, was an outlier based on this 48-72 hour growth rate result and recalculated the %CV values with that replicate excluded. The revised 48-72 hour %CV was 36%, which, the study report noted, closely approximated the 35% OECD requirement. Recalculation of the growth rate for the 48-72 h period by the reviewer with the 2 ("B" in the study report) results excluded, gave a %CV of 36%, confirming the study report's value.

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) gave the following results (Table 7) for the control replicate growth rates over the 48-72 hour period. The results do not identify the presence of an outlier in the 48-72 hour growth rates:

Table 7. Grubb's test for outliers analysis of control replicate growth rates over the 48-72 hour period.

Outlier Results - Data for the 0-24 hour growth rate statistics - Descriptive Statistics		Mean:	0.9833
		SD:	0.4868
		# of values:	6
		Outlier detected?	No
		Significance level:	0.05 (two-sided)
		Critical value of Z:	1.89

Row	Value	Z	Significant Outlier?
1	0.51	0.9724	
2	0.31	1.3833	Furthest from the rest, but not a significant outlier (P > 0.05).
3	1.40	0.8560	
4	1.53	1.1230	
5	0.98	0.0068	
6	1.17	0.3835	

As a result, the 48-72 hour %CV value of 49% is taken as a deficiency and considered further on page 38 (Table 11, deficiencies etc.) and following of this DER.

The overall 0-72 h %CV of 9.2%, derived from use of the six replicate values, exceeds the OECD 201 requirement of being less than 7%. When the "B" replicate was excluded from the data calculations, the %CV was 6.8% (6.9% reported by the study), which meets the OECD requirement. As the 2006 version of OECD 201 refers to 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*, the %CV for this parameter over 0-96 hours was also calculated and found to be 7.7% with all replicates used and 5.3% with the B replicate omitted. The 0-96 hour %CV results are considered to meet the 2006 OECD 201 requirement for the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures.

The ToxCalc outputs for inclusion of the B replicate and for when it was omitted for the 0-72 hour period are given on page 50 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 report stated that EC25 and EC50 values (the concentration of test substance which reduced cell density, total biomass and average growth rate by 25 and 50%, respectively, relative to the control) were calculated for the 24-, 48-, 72- and 96-hour observation intervals for cell density and EC50 values for the 72-hour observation interval for total biomass, denoted as EbC50, and average growth rate, denoted as ErC50. The EC50 values and their 95% confidence intervals were determined by linear regression of response (percent reduction of cell density, total biomass and average growth rate as compared with the control) versus the mean measured concentration (Norberg-King, 1993). TOXSTAT® version 3.5 (Gulley *et al.,* 1996), was used to assist in these computations. If less than the designated percent inhibition was observed for the noted parameter, the EC value was empirically estimated to be greater than the highest concentration tested.

Of the six control replicates, the study report considered replicate B an outlier, and deleted it from the mean and statistical analysis which provided 48 to 72 hour and 0 to 72 hour CV values of 36% and 6.9%, respectively, which closely approximate the above criteria. 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.

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 stated as first checked for normality using Shapiro-Wilks' Test (Weber, et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). If the data sets passed the test for homogeneity and normality, Williams' Test (Williams, 1971, 1972) was used to determine the NOEC. If the data did not pass the tests for homogeneity and normality, then Kruskal-Wallis' Test was used to determine the NOEC. All statistical determinations were made at the 95% level of certainty, except in the case of Shapiro-Wilks' and Bartlett's Tests, where the 99% level of certainty was applied.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s):
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 at the 92 mg/L level, 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 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 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.

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 5,7-di-OH metabolite of pyroxsulam are shown in Table 8.

The study report's calculations were verified as correct.

The percentage inhibition results (relative to the control mean of 1.30 day^{-1}) for the 0-72 hour growth rate at 0.50, 1.8, 4.7, 13, 36 and 92 mg 5,7-di-OH metabolite of pyroxsulam/L were, respectively, 1, -15, 2, -2, 2 and 112% (see Table 4, page 26 of this DER).

The 92 mg/L result is considered to clearly implicate the low pH as the causative factor rather than any intrinsic 5,7-di-OH metabolite of pyroxsulam toxicity to the algae.

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.

Table 8. Reviewer calculated and the study report's (italicised) calculated specific growth rates (days-1) of Pseudokirchneriella subcapitata after 24, 48 and 72 hours of exposure to 5,7-di-OH metabolite of pyroxsulam. Individual replicate values shown.

Mean measured concentration, mg 5,7-di-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	1.45	*1.43*	1.45	*1.49*	1.13	*1.14*
Replicate B	1.61	*1.59*	1.47	*1.52*	1.09	*1.10*
Replicate C	0.81	*0.80*	1.28	*1.32*	1.32	*1.33*
Replicate D	1.10	*1.09*	1.22	*1.26*	1.32	*1.34*
Replicate E	2.05	*2.03*	1.54	*1.59*	1.35	*1.37*
Replicate F	1.39	*1.37*	1.39	*1.44*	1.32	*1.33*
Mean and standard deviation	**1.40 (0.43)**	***1.39 (0.42)***	**1.39 (0.12)**	***1.44 (0.13)***	**1.26 (0.12)**	***1.27 (0.12)***
Mean (standard deviation) using 5 control replicate values with replicate values for the B replicate excluded (study report approach):	**1.36 (0.46)**	***1.34 (0.46)***	**1.38 (0.13)**	***1.42 (0.13)***	**1.29 (0.09)**	***1.30 (0.09)***
0.5, replicate A	0.92	*0.91*	1.29	*1.33*	1.46	*1.47*
Replicate B	1.32	*1.31*	1.56	*1.61*	1.23	*1.24*
Replicate C	0.41	*0.40*	1.16	*1.20*	1.16	*1.17*
Mean (standard deviation)	**0.88 (0.46)**	***0.88 (0.45)***	**1.34 (0.20)**	***1.38 (0.21)***	**1.28 (0.16)**	***1.29 (0.16)***
1.8, replicate A	1.01	*1.00*	1.10	*1.13*	1.50	*1.52*
Replicate B	1.56	*1.54*	1.52	*1.57*	1.38	*1.39*
Replicate C	1.32	*1.31*	1.61	*1.66*	1.54	*1.56*
Mean (standard deviation)	**1.30 (0.27)**	***1.28 (0.27)***	**1.41 (0.27)**	***1.46 (0.28)***	**1.48 (0.09)**	***1.49 (0.09)***
4.7, replicate A	0.56	*0.55*	1.39	*1.43*	1.18	*1.19*
Replicate B	1.79	*1.77*	1.53	*1.58*	1.41	*1.42*
Replicate C	1.25	*1.24*	1.15	*1.19*	1.21	*1.22*
Mean (standard deviation)	**1.20 (0.62)**	***1.19 (0.61)***	**1.36 (0.19)**	***1.40 (0.20)***	**1.27 (0.13)**	***1.28 (0.13)***
13, replicate A	1.61	*1.59*	1.39	*1.44*	1.11	*1.12*
Replicate B	1.32	*1.31*	1.50	*1.55*	1.43	*1.45*
Replicate C	0.41	*0.40*	1.21	*1.25*	1.37	*1.38*
Mean (standard deviation)	**1.11 (0.63)**	***1.10 (0.62)***	**1.37 (0.15)**	***1.41 (0.15)***	**1.30 (0.17)**	***1.32 (0.17)***
36, replicate A	1.10	*1.09*	1.32	*1.36*	1.26	*1.27*
Replicate B	1.61	*1.59*	1.45	*1.50*	1.39	*1.40*
Replicate C	0.22	*0.22*	1.10	*1.13*	1.16	*1.17*
Mean (standard deviation)	**0.98 (0.70)**	***0.97 (0.69)***	**1.29 (0.18)**	***1.33 (0.18)***	**1.27 (0.11)**	***1.28 (0.12)***
92, replicate A	0*	*0.00*	0	*0.00*	0	*0.00*
Replicate B	0	*0.00*	0	*0.00*	-0.23	*-0.23*
Replicate C	0	*0.00*	-0.35	*-0.36*	-0.23	*-0.23*
Mean (standard deviation)	**0 (0)**	***0 (0)***	**-0.12 (0.20)**	***-0.12 (0.21)***	**-0.15 (0.13)**	***-0.15 (0.13)***

Notes to table: 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 48-72 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 (see "Validity of results", page 27 of this DER). * 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 hour's biomass 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 values for *Pseudokirchneriella subcapitata* after 0-24, 24-48 and 48-72 hours of exposure to 5,7-di-OH metabolite of pyroxsulam are shown in Table 9. For Table 9, rounded data values, not original raw data, were presented in the study report, the reviewer calculated results were derived from the cell counts reported in the study report.

Table 9. 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 5,7-di-OH metabolite of pyroxsulam. Individual replicate values shown.

Mean measured concentration, mg 5. 7-di-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	1.62	*1.64*	10.12	*9.39*	23.00	*23.80*	34.75	*34.83*
	2.00	*2.02*	11.00	*10.20*	21.50	*22.25*	34.50	*34.47*
	0.62	*0.63*	6.62	*6.14*	31.75	*32.85*	39.00	*39.63*
	1.00	*1.01*	6.25	*5.79*	31.38	*32.46*	38.62	*39.27*
	3.38	*3.41*	13.75	*12.75*	38.88	*40.22*	56.00	*56.38*
	1.50	*1.52*	9.12	*8.46*	33.25	*34.40*	43.88	*44.38*
Mean and standard deviation	**1.69 (0.96)**	***1.71 (0.97)***	**9.48 (2.82)**	***8.79 (2.61)***	**30.0 (6.6)**	***31 (6.8)***	**41 (8.1)**	***41.5 (8.2)***
Mean (standard deviation) using 5 control replicate values with replicate values for the B replicate excluded (study report approach):	**1.63 (1.06)**	***1.64 (1.07)***	**9.18 (3.04)**	***8.51 (2.82)***	**31.6 (5.7)**	***32.8 (5.9)***	**42.4 (8.2)**	***42.9 (8.3)***
0.5	0.75	*0.76*	6.88	*6.37*	45.38	*46.95*	53.00	*54.08*
	1.38	*1.39*	12.25	*11.36*	30.25	*31.30*	43.88	*44.05*
	0.25	*0.25*	4.88	*4.52*	20.12	*20.82*	25.25	*25.60*
Mean (standard deviation)	**0.79 (0.56)**	***0.80 (0.57)***	**8.0 (3.8)**	***7.4 (3.5)***	**31.9 (12.7)**	***33.0 (13.5)***	**40.7 (14.1)**	***41.2 (14.4)***
1.8	0.88	*0.88*	4.88	*4.52*	49.00	*50.70*	54.75	*56.11*
	1.88	*1.89*	11.88	*11.01*	41.00	*42.42*	54.75	*55.33*
	1.38	*1.39*	13.38	*12.40*	63.00	*65.19*	77.75	*78.98*
Mean (standard deviation)	**1.38 (0.50)**	***1.39 (0.51)***	**10.0 (4.5)**	***9.3 (4.2)***	**51 (11)**	***52.8 (11.5)***	**62.4 (13.3)**	***63.5 (13.4)***
4.7	0.38	*0.38*	7.88	*7.30*	24.12	*24.96*	32.38	*32.64*
	2.50	*2.53*	12.62	*11.70*	44.00	*45.53*	59.12	*59.76*
	1.25	*1.26*	5.75	*5.33*	23.00	*23.80*	30.00	*30.39*
Mean (standard deviation)	**1.38 (1.07)**	***1.39 (1.08)***	**8.8 (3.5)**	***8.1 (3.3)***	**30.4 (11.8)**	***31.4 (12.2)***	**40.5 (16.2)**	***40.9 (16.4)***
13	2.00	*2.02*	9.62	*8.92*	21.00	*21.73*	32.62	*32.67*
	1.38	*1.39*	11.00	*10.20*	46.12	*47.73*	58.50	*59.31*
	0.25	*0.25*	5.38	*4.98*	34.88	*36.09*	40.50	*41.32*
Mean (standard deviation)	**1.21 (0.89)**	***1.22 (0.90)***	**8.67 (2.93)**	***8.03 (2.72)***	**34.0 (12.6)**	***35.2 (13.0)***	**43.9 (13.6)**	***44.4 (13.6)***
36	1.00	*1.01*	7.50	*6.95*	28.00	*28.97*	36.50	*36.94*
	2.00	*2.02*	10.62	*9.85*	40.38	*41.78*	53.00	*53.65*
	0.12	*0.13*	4.12	*3.82*	19.75	*20.44*	24.00	*24.39*
Mean (standard deviation)	**1.04 (0.94)**	***1.05 (0.95)***	**7.42 (3.25)**	***6.87 (3.02)***	**29.4 (10.4)**	***30.4 (10.7)***	**37.8 (14.6)**	***38.3 (14.7)***
92	-0.50	*-0.51*	-1.00	*-0.93*	-1.00	*-1.03*	-2.50	*-2.47*
	-0.50	*-0.51*	-1.00	*-0.93*	-0.75	*-0.78*	-2.25	*-2.21*
	-0.50	*-0.51*	-0.75	*-0.70*	-0.50	*-0.52*	-1.75	*-1.72*
Mean (standard deviation)	**-0.50 (0)**	***-0.51 (0)***	**-0.92 (0.14)**	***-0.85 (0.13)***	**-0.75 (0.25)**	***-0.78 (0.26)***	**-2.17 (0.38)**	***-2.13 (0.38)***

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 10. The table also includes the EC25 cell density endpoints for 24, 48, 72 and 96 hours as well as the endpoints when the nominal 100 mg 5,7-di-OH metabolite of pyroxsulam/L results are omitted.

Table 10. Reported and calculated toxicity endpoints.

Toxicity endpoint	Mean measured pyroxsulam concentration, mg/L (95% confidence limits), mg 5,7-di-OH metabolite of pyroxsulam/L. As calculated by the reviewer with the ToxCalc program.		
	As presented in the study report	Endpoints based on use of all (0.5 to 92 mg/L, mean measured) test concentrations.	Endpoints based on use of all (0.5 to 36 mg/L, mean measured) with the 92 mg/L results excluded.
24 hour cell density			
EC50	53 (10-64)	52.3 (0.00-71.0)	>36
EC25	28 (2.2-50)	20 (0.0-68)	20.4 (95% confidence limits not reported)
NOEC	Not reported	36	36
48 hour cell density			
EC50	59 (40-64)	58.1 (26.9-65.9)	>36
EC25	42 (4.3-50)	41 (0.0-52)	>36
NOEC	Not reported	36	36
72 hour cell density			
EC50	57 (39-64)	56.1 (27.6-68.9)	>36
EC25	39 (3.8-49)	40 (0.0-57)	>36
NOEC	Not reported	36	36
96 hour cell density			
EC50	55 (40-63)	56.0 (34.4-68.9)	>36
EC25	37 (5.6-48)	38 (7.9-57)	>36
NOEC	36	36	36
0-72 hour mean specific growth rate			
ErC50	60 (56-62)	62.4 (57.4-64.9)	>36
NOEC	36	36	36
0-72 hour biomass			
EbC50	56 (38-63)	56.4 (31.4-67.7)	>36
NOEC	36	36	36

The ToxCalc determinations of the 24, 48, 72 and 96 hour cell count results are shown on page 45, 46, 47 and 48 of this DER. The ToxCalc determinations for the 24, 48 and 72 hour cell counts indicated that the untransformed data had normal distributions (Shapiro-Wilk's test) and equal variances (Bartlett's test). In contrast, the statistical

treatment of the 96 hour cell counts determined that the untransformed data had normal distributions (Shapiro-Wilk's test) but unequal variances (Bartlett's test). A log (x + 1) transformation of the 96 hour cell count data resulted in normal distribution and equality of variances.

ToxCalc determinations of the 0-72 hour specific growth rate results are shown on page 50 of this DER while the biomass results are shown on page 52 of this DER. For the specific growth rate, the ToxCalc determinations indicated the untransformed data had normal variances (Shapiro-Wilk's test) but equality of variances (Bartlett's test) could not be confirmed. Omission of the 92 mg/L results rectified this, with a normal distributions (Shapiro-Wilk's test) and equality of variance (Bartlett's test) being confirmed. With respect to biomass, the ToxCalc determinations indicated the untransformed data had normal variances (Shapiro-Wilk's test) but equality of variances (Bartlett's test) could not be confirmed. Omission of the 92 mg/L results rectified this, with a normal distributions (Shapiro-Wilk's test) and equality of variance (Bartlett's test) being confirmed.

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 NOEC values.

With respect to the toxicity endpoints determined when the nominal 100 mg/L (mean measured 92 mg/L) results are excluded, the relevant ToxCalc outputs are given in Appendix II (page 54 and following of this DER). NOECs determined from these calculations were the same as those determined when the 100 mg/L data were included.

D. STUDY DEFICIENCIES:

Table 11 summarises deficiencies and deviations from the OECD 201 and US EPA OPPTS 850.5400 Guidelines requirements.

Table 11. 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.	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 medium of 0.01 µg/L or 0.00001 mg/L.	US EPA OPPTS 850.5400 states that formulation of nutrient medium used for algal culture and preparation of test solutions should conform to those currently recommended by the EPA for freshwater and marine algal bioassays.
pH at test initiation	pH values at time zero were **Nominal Concentration (mg/L)* — pH 0 h** **Control** 6.7 **1.0** 6.5 **2.6** 6.4 **6.4** 6.4 **16** 6.0 **40** 5.2 **100** 3.8 *** i.e. mg 5,7-di-OH metabolite of pyroxsulam/L.**	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 be 7.5 (± 0.1) for *Selenastrum.* US EPA 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 3.8. However, the pH was not adjusted. This initial pH of 3.8 has invalidated the results for the 100 mg/L test solutions (see below).
pH at test termination:	pH values at 96 hours were:	OECD recommends (2006) the medium pH should not increase by greater that 1.5 pH units during the test	No specific comment other than as above with the need to consider pH adjustments.

Nominal Concentration (mg/L)*	pH 96 h
Control	8.8
1.0	9.2
2.6	9.1
6.4	9.1
16	8.9
40	8.6
100	4.0

*** i.e. mg 5,7-di-OH metabolite of pyroxsulam**

Test concentration: Nominal	The nominal concentrations were separated by a factor of 2.5 or 2.6.	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.	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).
Light intensity and quality:	3900-4600 lux The photosynthetically active radiation (PAR) of the test area measured at test initiation ranged from 59 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 49.2 X 10^4 cells/mL (cf. 1 X 10^4 cells at test initiation). At 96 hours, the mean control cell density was ~179 X 10^4 or ~1.79 X 10^6 cells/mL. This equates to ~51% of the US EPA value of 3.5 X 10^6 cells/mL. The mean 0-72 hours calculated growth rate of the controls was 1.3 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.*
Validity of test	The %CV value for the 48-72 hour period of 49% exceeded the 35% limit set by the 2006 OECD 201 guideline and the study protocol. The 0-72 h %CV was 9.5%, which also exceeds	OECD 201 (2006) requires that, for the test to be valid, the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. The guideline also requires that the %CV of average specific growth during the	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.

the 2006 OECD requirement and the study's protocol.	whole test period not exceed 7%.

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 pH of 3.8 recorded for the 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 0.25 X 10^4 cells/mL at 96 hours (control at that time was ~179 X 10^4 cells/mL) could be due to the concentration of the 5,7-di-OH metabolite of pyroxsulam present, to the pH of 3.8 recorded at 0 hours, or to a combination of both factors. 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 deficient with respect to the results at that concentration and is rated as SUPPLEMENTAL by the Australian Government Department of the Environment, Water, Heritage and the Arts.

The reason for the initial control pH being 6.7 instead of 7.5 at time zero is not known. The change in the pH of the controls from 6.7 at day 0 to 8.8 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 in pH is expected. Additionally, it is noted that the pH increases were seen in all concentrations except at 100 mg/L where the pH remained at about 4 (3.8 at 0 hours and 4.0 at 96 hours) and did not show the increases in pH seen in the other concentrations and the control.

The nominal test concentrations did not meet the US EPA 850.5400 requirement that a geometric series be used in which the ratio is between 1.5 and 2.0 (a factor of 2.5 or 2.6 existed). This deviation is not considered a major issue.

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 (3900-4600 compared to the indicated guideline range of 4440-8880 lux). Similarly, the observed range of 3900 to 4600 lux would not always have complied with the US EPA 850.5400 OPPTS requirement of 4300 lux. There was no obvious effect arising from this event, in that the control algae appeared to have grown successfully although the failure to shown exponential growth (see following paragraph) may have been the result of the lower light intensity.

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 179 X 10^4 cells/mL or ~1.79 X 10^6 cells/mL, which equates to ~51% of the US EPA value. This is classed as a significant deficiency with respect to compliance with the US EPA OPPTS guideline and supports 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 60 of this DER) returned an r^2 value of 0.9981, a value that indicates exponential growth occurred in the study's control algae, the deficiency is not considered to be such to have invalidated the study.

The 48-72 hour %CV of 49% is a failure to meet the 2006 OECD 201 requirements and the study's own acceptance criterion value that the %CV not exceed 35%. Similarly the study's 0-72 hour average growth rate of 9.5 exceeds the 7% value set by the OECD and as one of the study's validity criteria. While these values fall within acceptable

limits if one of the control results is treated as an outlier, this action would have been more meaningful if statistical evidence that the result was an outlier had been presented. For the 0-96 hour period, the %CV value of 7.7% using all the control replicate data is taken as meeting the 2006 guideline requirement.

Apart from the pH being 4.0 throughout in the 100 mg 7-OH metabolite of pyroxsulam/L and the failure to demonstrate exponential growth was achieved according to US EPA OPPTS 850.4500, these deficiencies/deviations were not considered to have significantly adversely affected the study's conduct or results. The pH being 4.0 at time zero and throughout and the failure to demonstrate exponential growth according to US EPA OPPTS 850.4500, are considered, in contrast, sufficient reason to class the study as supplemental by the Australian Government Department of the Environment, Water, Heritage and the Arts.

E. REVIEWER'S COMMENTS:

Australia reviewer comments:
The reviewer's recalculated toxicity endpoints were similar to the study authors' and the study is considered to have been conducted in general accordance with the relevant guideline documents but with significant deficiencies with respect to the pH of the 100 mg/L (nominal) test solution over the course of the exposure period and the demonstration that logarithmic growth had not been reached by 96 hours as required by US EPA OPPTS 850.5400.

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, while the study has been assessed primarily on the 2006 OECD 201 requirements, failure to comply with the 2006 guideline is not automatically considered a deficiency or deviation.

The study author's decision to exclude results from control replicate B on the basis that the %CV for the 48-72 hour period of 49% exceeded the 2006 OECD 201 recommendation of 35% and that with its exclusion the %CV was 36% which closely approximated the OECD criterion is noted. The reviewer believes it would have been more meaningful if the study had conducted a statistical analysis of the control replicate results for the 48-72 hour period to confirm the B replicate's value was statistically significantly different from the other replicate results.

The 96 hour cell density counts indicate significant inhibition (100% compared to the mean control cell counts at that time) occurred only at 92 mg 5,7-di-OH metabolite of pyroxsulam/L and the effect of the initial pH of 3.8 in the test solutions at this concentration are expected to be the primary reason for the inhibition, rather than the inherent toxicity of the 5,7-di-OH metabolite of pyroxsulam. For the 0-72 hour specific growth rate, inhibition occurred only at the 92 mg 5,7-di-OH metabolite of pyroxsulam/L rates (112%) with the result being statistically significantly different from the mean control value over that time period. For the biomass results, inhibition only occurred only at the 92 mg 5,7-di-OH metabolite of pyroxsulam/L concentration (105% inhibition) with the mean total biomass at the 0-72 hour period for this concentration being statistically significantly reduced compared to the mean control value.

Based on the results of this study, as shown below, the 5,7-di-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 Government Department of the Environment, Water, Heritage and the Arts because of the pH of the nominal 100 mg/L test solution being 3.8 at time 0 and 4.0 after 96 hours and because of the failure to meet the US criterion for exponential growth after 96 hours. Consequently, it does not satisfy 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 comes 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 reviewer does not recommend a repeat of the test as the low toxicity of this metabolite has been adequately demonstrated.

The EPA Reviewer classified the study as SUPPLEMENTAL and not satisfying the guideline requirements for an acceptable acute toxicity study with green algae. Estimates of toxicity appear compromised due to low pH in some test concentrations.

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 or the US EPA. This study is considered acceptable to the PMRA, despite the effect of the acidity of the test substance at the highest test concentration. Exponential growth criteria have been met according to OECD 201. Useful information can be obtained from this study and used in a risk assessment (EC50 expected to be >36 mg 5,7-di-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 and the US EPA. This is because of the pH of 3.8 observed in the 100 mg/L test solution at time 0 and the pH of 4.0 at 96 hours and the failure to demonstrate that logarithmic growth had been achieved by 96 hours, according to US EPA OPPTS 850.4500, but not OECD 201. The pH issue makes it difficult to attribute the lack of growth at that concentration to either the test concentration or the test pH, although the occurrence of similar results from other similar hydroxy metabolites points to the acidity as the dominant factor in the growth inhibition.

The PMRA does not share the same acceptability classification as the Australian Government Department of the Environment, Water, Heritage and the Arts or the US EPA. This study is considered acceptable to the PMRA, despite the effect of the acidity of the test substance at the highest test concentration. Exponential growth criteria have been met according to OECD 201. Useful information can be obtained from this study and used in a risk assessment (EC50 expected to be >36 mg 5,7-di-OH metabolite of pyroxsulam/L).

The study's reported endpoints were:

Statistical Endpoint	Growth Rate (72 h)	Biomass (area under growth curve) (72 h)	Cell Density (96 h)
NOEC (mg 5,7-di-OH metabolite of pyroxsulam/L)	36	36	36
EC_{50} (mg 5,7-di-OH metabolite of pyroxsulam/L) (95% C.I.)	60 (56-62)	56 (38-63)	55 (40-63)
Reference chemical, if used	A reference chemical was not used.		

The endpoints which will be used for risk assessment, as a result of the 100 mg/L being deleted because of the pH issue at time 0, are:

Statistical Endpoint	Growth Rate (72 h)	Biomass (area under growth curve) (72 h)	Cell Density (96 h)
NOEC (mg 5,7-di-OH metabolite of pyroxsulam/L)	36	36	36
EC_{50} (mg 5,7-di-OH metabolite of pyroxsulam/L) (95% C.I.)	>36	>36	>36
Reference chemical, if used	A reference chemical was not used.		

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

III. REFERENCES:

ASTM. 2002. Conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor drive, West Conshohocken, PA 19428.

EC, 1992. Official Journal of the European Communities. 1992. Methods for the determination of Ecotoxicity. C.3. Algal Inhibition Test. L383A Volume 35, 29 December 1992.

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

Hoberg, J. R. 2006a. Study Profile Template (SPT) for: 5,7-di-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata.* Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts. Springborn Smithers Study No. 12550.6417.SPT and Dow Study No. 050109.SPT. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences, Indianapolis, Indiana 46268. April 5, 2006. Unpublished report.

(Note that this reference is designated as Hoberg, 2006a. This is to differentiate it from the study report which would be referenced as Hoberg, 2006.

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

Miller, W.E., J.C. Greene and T. Shiroyama. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test. EPA 600/9-78-018. U.S. Environmental Protection Agency, Corvallis, Oregon.

Norberg-King, Teresa J. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. National Effluent Toxicity Assessment Center, Environmental Research Laboratory – Duluth, U.S. Environmental protection Agency, Duluth, Minnesota. Technical report 03-93.

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

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

OECD, 2004. OECD Guideline for Testing of Chemicals. Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Draft Revised Guideline #201. April 2004.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

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

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27: 103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. Biometrics 28:519-531.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density at 24 hours

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

Conc-mg/L	1	2	3	4	5	6
D-Control	4.2500	5.0000	2.2500	3.0000	7.7500	4.0000
0.5	2.5000	3.7500	1.5000			
1.8	2.7500	4.7500	3.7500			
4.7	1.7500	6.0000	3.5000			
13	5.0000	3.7500	1.5000			
36	3.0000	5.0000	1.2500			
92	0.0000	0.0000	0.0000			

			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	4.3750	1.0000	4.3750	2.2500	7.7500	43.781	6				4.3750	1.0000
0.5	2.5833	0.5905	2.5833	1.5000	3.7500	43.638	3	1.457	2.602	3.1995	3.3750	0.7714
1.8	3.7500	0.8571	3.7500	2.7500	4.7500	26.667	3	0.508	2.602	3.1995	3.3750	0.7714
4.7	3.7500	0.8571	3.7500	1.7500	6.0000	56.960	3	0.508	2.602	3.1995	3.3750	0.7714
13	3.4167	0.7810	3.4167	1.5000	5.0000	51.912	3	0.780	2.602	3.1995	3.3750	0.7714
36	3.0833	0.7048	3.0833	1.2500	5.0000	60.856	3	1.051	2.602	3.1995	3.0833	0.7048
92	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000

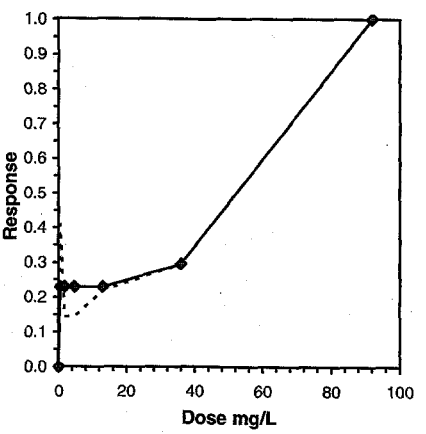
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96093	0.873	0.45283	-0.285
Bartlett's Test indicates equal variances (p = 0.91)	1.50519	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	36	92	57.55		3.19953	0.73132	1.54673	3.02292	0.76335	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	0.109	8.971	0.029	62.014	3.2044
IC10*	0.219	11.199	0.059	66.429	2.3310
IC15*	0.328	13.494	0.088	70.843	1.7176
IC20*	0.438	15.870	0.118	75.258	1.1104
IC25	20.393	17.843	0.000	67.764	0.6244
IC40	44.324	16.954	0.000	66.845	-0.5919
IC50	52.270	13.582	0.000	71.038	-0.9277

* indicates IC estimate less than the lowest concentration



APPENDIX I (Continued)
Cell density at 48 hours

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

Conc-mg/L	1	2	3	4	5	6
D-Control	18.000	19.000	13.000	11.500	21.750	16.250
0.5	13.250	22.750	10.250			
1.8	9.000	21.000	25.000			
4.7	16.000	21.250	10.000			
13	16.250	20.250	11.250			
36	14.000	18.250	9.000			
92	0.000	0.000	0.500			

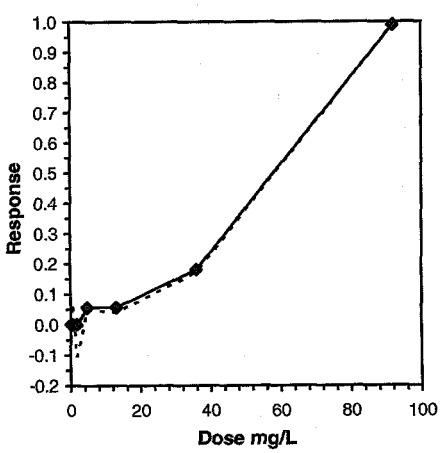
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	16.583	1.0000	16.583	11.500	21.750	23.094	6				16.778	1.0000
0.5	15.417	0.9296	15.417	10.250	22.750	42.328	3	0.323	2.655	9.597	16.778	1.0000
1.8	18.333	1.1055	18.333	9.000	25.000	45.418	3	-0.484	2.655	9.597	16.778	1.0000
4.7	15.750	0.9497	15.750	10.000	21.250	35.741	3	0.231	2.655	9.597	15.833	0.9437
13	15.917	0.9598	15.917	11.250	20.250	28.330	3	0.184	2.655	9.597	15.833	0.9437
36	13.750	0.8291	13.750	9.000	18.250	33.673	3	0.784	2.655	9.597	13.750	0.8195
*92	0.167	0.0101	0.167	0.000	0.500	173.205	3	4.542	2.655	9.597	0.167	0.0099

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96471	0.884	-0.1866	-0.6232
Bartlett's Test indicates equal variances (p = 0.12)	10.1902	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	36	92	57.55		9.59706	0.57872	116.13	26.1324	0.007	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	4.376	8.670	0.000	55.350	2.0298
IC10	21.096	11.912	0.000	50.070	1.1451
IC15	30.357	14.330	0.000	49.266	0.4019
IC20	37.351	14.491	0.000	49.822	-0.3727
IC25	40.810	12.942	0.000	52.499	-0.9099
IC40	51.185	7.464	14.122	60.531	-0.9034
IC50	58.102	6.374	26.898	65.886	-1.0006



The 92 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 gave the following results (as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	30.000	26.000	52.500	53.250	58.000	52.250
0.5	79.500	39.750	32.000			
1.8	91.000	63.000	103.000			
4.7	34.250	68.750	38.000			
13	27.750	74.000	60.500			
36	44.000	64.500	32.500			
92	0.000	0.500	0.500			

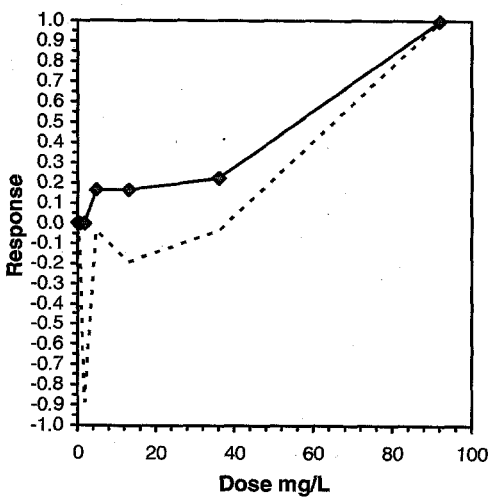
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	45.333	1.0000	45.333	26.000	58.000	30.104	6				60.472	1.0000
0.5	50.417	1.1121	50.417	32.000	79.500	50.545	3	-0.402	2.655	33.603	60.472	1.0000
1.8	85.667	1.8897	85.667	63.000	103.000	23.961	3	-3.187	2.655	33.603	60.472	1.0000
4.7	47.000	1.0368	47.000	34.250	68.750	40.275	3	-0.132	2.655	33.603	50.542	0.8358
13	54.083	1.1930	54.083	27.750	74.000	43.975	3	-0.691	2.655	33.603	50.542	0.8358
36	47.000	1.0368	47.000	32.500	64.500	34.488	3	-0.132	2.655	33.603	47.000	0.7772
*92	0.333	0.0074	0.333	0.000	0.500	86.603	3	3.556	2.655	33.603	0.333	0.0055

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96923	0.884	0.03807	-0.9549
Bartlett's Test indicates equal variances (p = 0.03)	13.8743	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	36	92	57.55		33.6026	0.74123	1870.1	320.368	0.00186	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	2.683	9.150	1.849	60.484	2.1324
IC10	3.566	12.129	1.898	64.448	1.2377
IC15	4.449	14.403	1.947	68.412	0.6928
IC20	27.053	15.623	0.000	59.343	0.1461
IC25	37.975	15.644	0.000	57.284	-0.4061
IC40	48.860	10.432	0.000	64.234	-1.7933
IC50	56.117	7.169	27.632	68.867	-1.2550



The 92 mg/L mean cell count at 72 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 96 hours

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

Conc-mg/L	1	2	3	4	5	6
D-Control	155.33	83.00	129.25	152.00	207.00	255.50
0.5	188.33	181.00	166.67			
1.8	182.33	138.50	224.50			
4.7	192.00	216.00	144.00			
13	158.67	143.33	219.50			
36	113.50	187.67	111.75			
92	0.75	0.00	0.00			

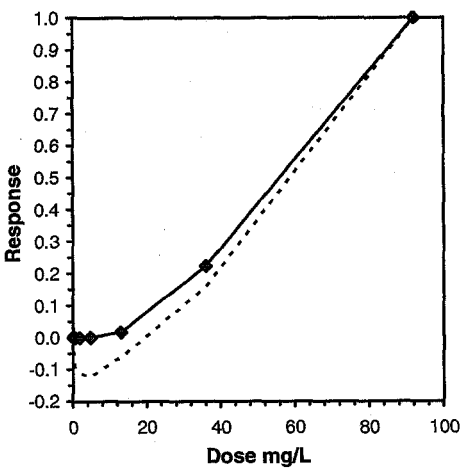
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	163.68	1.0000	163.68	83.00	255.50	36.868	6				177.03	1.0000
0.5	178.67	1.0916	178.67	166.67	188.33	6.166	3	-0.490	2.655	81.25	177.03	1.0000
1.8	181.78	1.1106	181.78	138.50	224.50	23.657	3	-0.591	2.655	81.25	177.03	1.0000
4.7	184.00	1.1241	184.00	144.00	216.00	19.924	3	-0.664	2.655	81.25	177.03	1.0000
13	173.83	1.0620	173.83	143.33	219.50	23.175	3	-0.332	2.655	81.25	173.83	0.9819
36	137.64	0.8409	137.64	111.75	187.67	31.485	3	0.851	2.655	81.25	137.64	0.7775
*92	0.25	0.0015	0.25	0.00	0.75	173.205	3	5.341	2.655	81.25	0.25	0.0014

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96744	0.884	0.3774	0.72542
Bartlett's Test indicates unequal variances (p = 5.44E-03)	18.3369	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	36	92	57.55		81.2474	0.49638	13236.9	1872.93	6.7E-04	6, 17
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	16.593	10.024	0.000	52.124	1.0473
IC10	22.218	11.260	0.000	53.229	0.4654
IC15	27.843	11.045	0.000	54.334	0.0989
IC20	33.468	10.063	0.000	55.439	-0.2222
IC25	37.984	9.100	7.919	57.210	-0.4483
IC40	48.807	7.294	24.656	64.156	-0.4614
IC50	56.023	6.072	34.411	68.786	-0.4071



The 92 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, also identified this result as statistically significantly reduced compared to the control.

APPENDIX I (Continued)
Cell density at 96 hours (continued)

The ToxCalc analysis of the log(x +1) transformed 96 hour algal cell count data gave the following results (as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	155.33	83.00	129.25	152.00	207.00	255.50
0.5	188.33	181.00	166.67			
1.8	182.33	138.50	224.50			
4.7	192.00	216.00	144.00			
13	158.67	143.33	219.50			
36	113.50	187.67	111.75			
92	0.75	0.00	0.00			

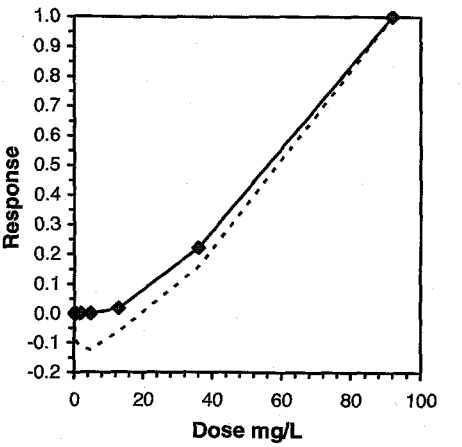
			Transform: Log (X + 1)						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	163.68	1.0000	2.1908	1.9243	2.4091	7.657	6				177.03	1.0000
0.5	178.67	1.0916	2.2539	2.2245	2.2772	1.194	3	-0.707	2.655	0.2368	177.03	1.0000
1.8	181.78	1.1106	2.2537	2.1446	2.3531	4.642	3	-0.704	2.655	0.2368	177.03	1.0000
4.7	184.00	1.1241	2.2611	2.1614	2.3365	3.983	3	-0.788	2.655	0.2368	177.03	1.0000
13	173.83	1.0620	2.2353	2.1594	2.3434	4.301	3	-0.499	2.655	0.2368	173.83	0.9819
36	137.64	0.8409	2.1289	2.0521	2.2757	5.975	3	0.695	2.655	0.2368	137.64	0.7775
*92	0.25	0.0015	0.0810	0.0000	0.2430	173.205	3	23.654	2.655	0.2368	0.25	0.0014

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96068	0.884	-0.0537	0.42483
Bartlett's Test indicates equal variances (p = 0.52)	5.15142	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	36	92	57.55		65.223	0.42032	2.00194	0.01591	3.9E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	16.593	10.269	0.000	51.949	0.8939
IC10	22.218	11.076	0.000	53.070	0.4239
IC15	27.843	10.946	0.000	54.191	-0.0275
IC20	33.468	10.053	0.000	55.311	-0.3116
IC25	37.984	9.232	7.611	57.097	-0.6716
IC40	48.807	7.308	26.205	64.090	-0.7569
IC50	56.023	5.812	36.243	68.795	-0.3351



APPENDIX I (Continued)
Specific growth rate (0-72 hours)

The ToxCalc analysis of the 0-72 hour study report calculated growth rate data (see page 32 of this DER) gave the following results (as day^{-1}). Data from all concentrations tested included in the analyses.

Conc-mg/L	1	2	3	4	5	6
D-Control	1.1400	1.1000	1.3300	1.3400	1.3700	1.3300
0.5	1.4700	1.2400	1.1700			
1.8	1.5200	1.3900	1.5600			
4.7	1.1900	1.4200	1.2200			
13	1.1200	1.4500	1.3800			
36	1.2700	1.4000	1.1700			
92	0.0000	0.0000	0.0000			

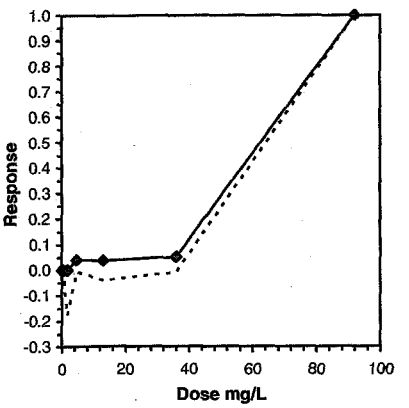
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.2683	1.0000	1.2683	1.1000	1.3700	9.187	6				1.3506	1.0000
0.5	1.2933	1.0197	1.2933	1.1700	1.4700	12.135	3	-0.291	2.655	0.2282	1.3506	1.0000
1.8	1.4900	1.1748	1.4900	1.3900	1.5600	5.965	3	-2.578	2.655	0.2282	1.3506	1.0000
4.7	1.2767	1.0066	1.2767	1.1900	1.4200	9.794	3	-0.097	2.655	0.2282	1.2967	0.9601
13	1.3167	1.0381	1.3167	1.1200	1.4500	13.206	3	-0.562	2.655	0.2282	1.2967	0.9601
36	1.2800	1.0092	1.2800	1.1700	1.4000	9.010	3	-0.136	2.655	0.2282	1.2800	0.9478
*92	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3	14.753	2.655	0.2282	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96567	0.884	-0.1916	-0.9537
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	36	92	57.55		0.22825	0.17996	0.77368	0.01478	5.0E-10	6, 17
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.822	14.395	0.000	42.987	-0.1491
IC10	38.822	9.843	0.000	43.267	-2.1399
IC15	41.776	2.868	33.168	45.974	-4.8256
IC20	44.731	1.956	36.629	48.682	-0.4086
IC25	47.685	1.834	40.090	51.389	-0.4086
IC40	56.548	1.467	50.472	59.511	-0.4086
IC50	62.457	1.223	57.393	64.926	-0.4086



Note: The ToxCalc program gave a choice of including the 92 mg/L results in the analysis or excluding them. The above results are when the 92 mg/L results are included in the analysis. The results from excluding the data are shown on the following page.

The 92 mg/L mean 0-72 hours' specific growth rate is 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 this result as statistically significantly reduced compared to the control.

APPENDIX I (Continued)
Specific growth rate (0-72 hours), continued.

The ToxCalc analysis of the 0-72 hour study report calculated growth rate data (see page 32 of this DER) gave the following results (as day^{-1}). Data from the 92 mg 5,7-di-OH metabolite of pyroxsulam are excluded from the analyses.

Conc-mg/L	1	2	3	4	5	6
D-Control	1.1400	1.1000	1.3300	1.3400	1.3700	1.3300
0.5	1.4700	1.2400	1.1700			
1.8	1.5200	1.3900	1.5600			
4.7	1.1900	1.4200	1.2200			
13	1.1200	1.4500	1.3800			
36	1.2700	1.4000	1.1700			
92	0.0000	0.0000	0.0000			

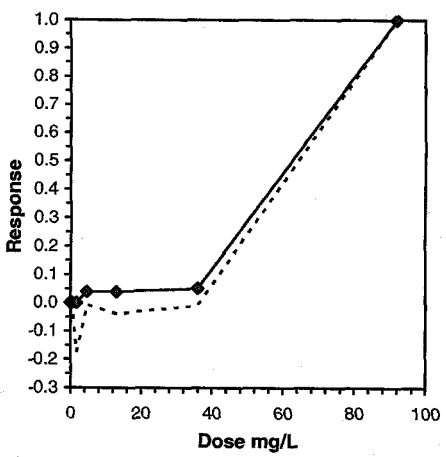
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.2683	1.0000	1.2683	1.1000	1.3700	9.187	6				1.3506	1.0000
0.5	1.2933	1.0197	1.2933	1.1700	1.4700	12.135	3	-0.273	2.602	0.2382	1.3506	1.0000
1.8	1.4900	1.1748	1.4900	1.3900	1.5600	5.965	3	-2.422	2.602	0.2382	1.3506	1.0000
4.7	1.2767	1.0066	1.2767	1.1900	1.4200	9.794	3	-0.091	2.602	0.2382	1.2967	0.9601
13	1.3167	1.0381	1.3167	1.1200	1.4500	13.206	3	-0.528	2.602	0.2382	1.2967	0.9601
36	1.2800	1.0092	1.2800	1.1700	1.4000	9.010	3	-0.127	2.602	0.2382	1.2800	0.9478
92	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9443	0.873	-0.181	-1.2644
Bartlett's Test indicates equal variances (p = 0.96)	1.02253	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	36	92	57.55		0.23818	0.18779	0.02288	0.01675	0.29168	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.822	14.389	0.000	42.611	0.1553
IC10	38.822	8.982	0.000	42.911	-2.1415
IC15	41.776	2.159	33.589	45.638	-0.3960
IC20	44.731	1.967	37.025	48.365	-0.1433
IC25	47.685	1.844	40.461	51.093	-0.1433
IC40	56.548	1.475	50.768	59.274	-0.1433
IC50	62.457	1.230	57.640	64.728	-0.1433



Note: In this case, exclusion of the 92 mg/L results in no change in the ErC50 value (62.4 mg 5,7-di-OH metabolite of pyroxsulam/L) compared to the mean when the results are included. However, the data are now found to be normally distributed and having equal variances.

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

The ToxCalc analysis of the 0-72 hour study report calculated biomass data (Table 9, page 35) gave the following results (cell counts X 10^4 cells/mL). Data from all concentrations tested included in the analyses.

Conc-mg/L	1	2	3	4	5	6
D-Control	34.830	34.470	39.630	39.270	56.380	44.380
0.5	54.080	44.050	25.600			
1.8	56.110	55.330	78.980			
4.7	32.640	59.760	30.390			
13	32.670	59.310	41.320			
36	36.940	53.650	24.390			
92	0.000	0.000	0.000			

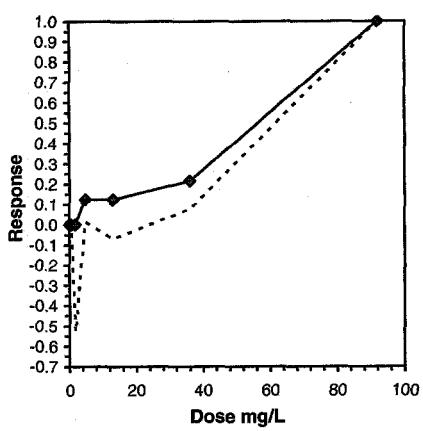
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	41.493	1.0000	41.493	34.470	56.380	19.641	6				48.737	1.0000
0.5	41.243	0.9940	41.243	25.600	54.080	35.026	3	0.029	2.655	22.515	48.737	1.0000
1.8	63.473	1.5297	63.473	55.330	78.980	21.166	3	-2.592	2.655	22.515	48.737	1.0000
4.7	40.930	0.9864	40.930	30.390	59.760	39.937	3	0.066	2.655	22.515	42.682	0.8758
13	44.433	1.0709	44.433	32.670	59.310	30.585	3	-0.347	2.655	22.515	42.682	0.8758
36	38.327	0.9237	38.327	24.390	53.650	38.300	3	0.373	2.655	22.515	38.327	0.7864
*92	0.000	0.0000	0.000	0.000	0.000	0.000	3	4.893	2.655	22.515	0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91693	0.884	0.48552	-0.8884
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	36	92	57.55		22.5154	0.54263	1085.53	143.834	4.6E-04	6, 17
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	2.967	9.577	1.904	58.599	2.0605
IC10	4.134	12.550	2.033	62.468	1.0590
IC15	19.631	14.366	0.000	57.740	0.3931
IC20	32.500	14.653	0.000	54.587	-0.1173
IC25	38.592	13.218	0.000	55.502	-0.7617
IC40	49.274	7.451	15.246	62.801	-0.7040
IC50	56.395	6.349	31.362	67.668	-0.8041



Note: The ToxCalc program gave a choice of including the 92 mg/L results in the analysis or excluding them. The above results are when the 92 mg/L results are included in the analysis. The results from excluding the data are shown on the following page.

The 92 mg/L mean 0-72 hours' biomass is 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 this result as statistically significantly reduced compared to the control.

APPENDIX I (Continued)
Biomass (area under the growth curve), 0-72 hours, continued

The ToxCalc analysis of the 0-72 hour study report calculated growth rate data (page 32 of this DER) gave the following results (cell counts X 10^4 cells/mL). Data from the 92 mg 5,7-di-OH metabolite of pyroxsulam are excluded from the analyses.

Conc-mg/L	1	2	3	4	5	6
D-Control	34.830	34.470	39.630	39.270	56.380	44.380
0.5	54.080	44.050	25.600			
1.8	56.110	55.330	78.980			
4.7	32.640	59.760	30.390			
13	32.670	59.310	41.320			
36	36.940	53.650	24.390			
92	0.000	0.000	0.000			

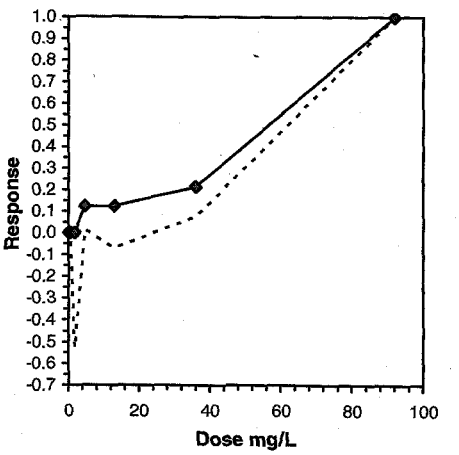
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	41.493	1.0000	41.493	34.470	56.380	19.641	6				48.737	1.0000
0.5	41.243	0.9940	41.243	25.600	54.080	35.026	3	0.028	2.602	23.495	48.737	1.0000
1.8	63.473	1.5297	63.473	55.330	78.980	21.166	3	-2.435	2.602	23.495	48.737	1.0000
4.7	40.930	0.9864	40.930	30.390	59.760	39.937	3	0.062	2.602	23.495	42.682	0.8758
13	44.433	1.0709	44.433	32.670	59.310	30.585	3	-0.326	2.602	23.495	42.682	0.8758
36	38.327	0.9237	38.327	24.390	53.650	38.300	3	0.351	2.602	23.495	38.327	0.7864
92	0.000	0.0000	0.000	0.000	0.000	0.000	3				0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90086	0.873	0.45871	-1.2053
Bartlett's Test indicates equal variances (p = 0.89)	1.69742	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	36	92	57.55		23.4953	0.56624	263.719	163.012	0.21545	5, 15
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	2.967	10.413	1.847	60.300	1.8730
IC10	4.134	13.232	1.909	64.079	1.0141
IC15	19.631	14.614	0.000	59.262	0.2744
IC20	32.500	14.466	0.000	56.020	-0.2670
IC25	38.592	13.553	0.000	56.845	-0.7170
IC40	49.274	8.348	16.419	63.876	-0.8527
IC50	56.395	7.315	22.171	68.563	-1.0058



Note: In this case, exclusion of the 92 mg/L result results in no change in the EbC50 value (56.4 mg 5,7-di-OH metabolite of pyroxsulam/L) compared to the mean when the results are included. However, the data are now found to be normally distributed and having equal variances.

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 5,7-di-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	42500	50000	22500	30000	77500	40000
0.5	25000	37500	15000			
1.8	27500	47500	37500			
4.7	17500	60000	35000			
13	50000	37500	15000			
36	30000	50000	12500			

			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	43750	1.0000	43750	22500	77500	43.781	6				43750	1.0000
0.5	25833.33	0.5905	25833.33	15000	37500	43.638	3	1.457	2.602	31995.3	33750	0.7714
1.8	37500	0.8571	37500	27500	47500	26.667	3	0.508	2.602	31995.3	33750	0.7714
4.7	37500	0.8571	37500	17500	60000	56.960	3	0.508	2.602	31995.3	33750	0.7714
13	34166.67	0.7810	34166.67	15000	50000	51.912	3	0.780	2.602	31995.3	33750	0.7714
36	30833.33	0.7048	30833.33	12500	50000	60.856	3	1.051	2.602	31995.3	30833.3	0.7048

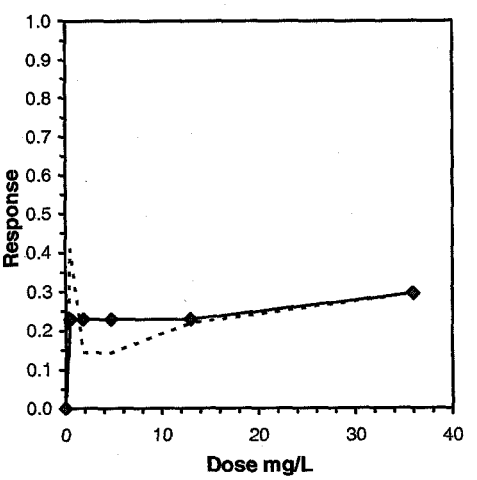
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96093	0.873	0.45283	-0.285
Bartlett's Test indicates equal variances (p = 0.91)	1.50519	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	36	>36			31995.3	0.73132	1.5E+08	3E+08	0.76335	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05*	0.109			
IC10*	0.219			
IC15*	0.328			
IC20*	0.438			
IC25	20.393			
IC40	>36			
IC50	>36			

* indicates IC estimate less than the lowest concentration



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 using mean measured concentrations of 5,7-di-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	180000	190000	130000	115000	217500	162500
0.5	132500	227500	102500			
1.8	90000	210000	250000			
4.7	160000	212500	100000			
13	162500	202500	112500			
36	140000	182500	90000			

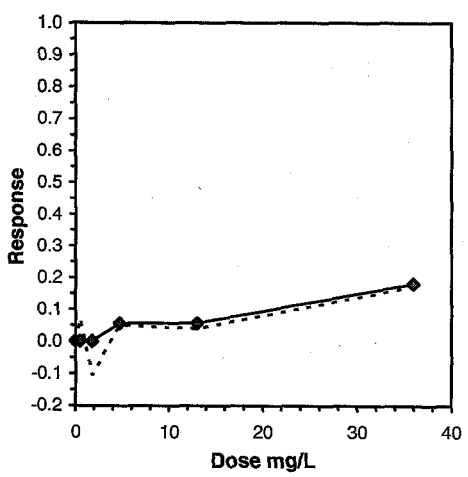
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	165833.3	1.0000	165833.3	115000	217500	23.094	6				167778	1.0000
0.5	154166.7	0.9296	154166.7	102500	227500	42.328	3	0.303	2.602	100129	167778	1.0000
1.8	183333.3	1.1055	183333.3	90000	250000	45.418	3	-0.455	2.602	100129	167778	1.0000
4.7	157500	0.9497	157500	100000	212500	35.741	3	0.217	2.602	100129	158333	0.9437
13	159166.7	0.9598	159166.7	112500	202500	28.330	3	0.173	2.602	100129	158333	0.9437
36	137500	0.8291	137500	90000	182500	33.673	3	0.736	2.602	100129	137500	0.8195

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95797	0.873	-0.1764	-0.9635
Bartlett's Test indicates equal variances (p = 0.85)	1.99405	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	36	>36			100129	0.60379	6.9E+08	3E+09	0.94116	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	4.376			
IC10	21.096			
IC15	30.357			
IC20	>36			
IC25	>36			
IC40	>36			
IC50	>36			



APPENDIX II (Continued)

Cell density at 72 hours

The ToxCalc analysis of the 72 hour algal cell count data without the nominal100 mg/L values gave the following results using mean measured concentrations of 5,7-di-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	300000	260000	525000	532500	580000	522500
0.5	795000	397500	320000			
1.8	910000	630000	1030000			
4.7	342500	687500	380000			
13	277500	740000	605000			
36	440000	645000	325000			

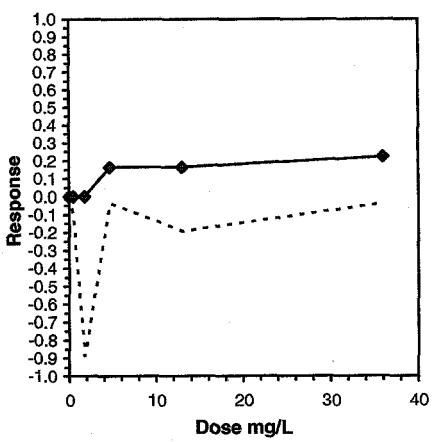
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	453333.3	1.0000	453333.3	260000	580000	30.104	6				604722	1.0000
0.5	504166.7	1.1121	504166.7	320000	795000	50.545	3	-0.377	2.602	350646	604722	1.0000
1.8	856666.7	1.8897	856666.7	630000	1030000	23.961	3	-2.994	2.602	350646	604722	1.0000
4.7	470000	1.0368	470000	342500	687500	40.275	3	-0.124	2.602	350646	505417	0.8358
13	540833.3	1.1930	540833.3	277500	740000	43.975	3	-0.649	2.602	350646	505417	0.8358
36	470000	1.0368	470000	325000	645000	34.488	3	-0.124	2.602	350646	470000	0.7772

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94816	0.873	0.03597	-1.2654
Bartlett's Test indicates equal variances (p = 0.92)	1.41526	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	36	>36			350646	0.77348	7.6E+10	3.6E+10	0.12395	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	2.683			
IC10	3.566			
IC15	4.449			
IC20	27.053			
IC25	>36			
IC40	>36			
IC50	>36			



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 values gave the following results using mean measured concentrations of 5,7-di-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	1553300	830000	1292500	1520000	2070000	2555000
0.5	1883300	1810000	1666700			
1.8	1823300	1385000	2245000			
4.7	1920000	2160000	1440000			
13	1586700	1433300	2195000			
36	1135000	1876700	1117500			

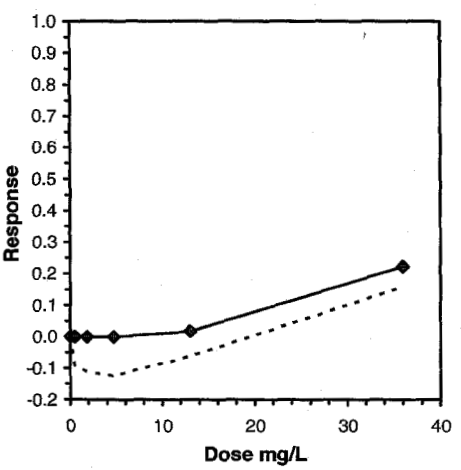
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1636800	1.0000	1636800	830000	2555000	36.868	6				1770308	1.0000
0.5	1786667	1.0916	1786667	1666700	1883300	6.166	3	-0.460	2.602	847831	1770308	1.0000
1.8	1817767	1.1106	1817767	1385000	2245000	23.657	3	-0.555	2.602	847831	1770308	1.0000
4.7	1840000	1.1241	1840000	1440000	2160000	19.924	3	-0.624	2.602	847831	1770308	1.0000
13	1738333	1.0620	1738333	1433300	2195000	23.175	3	-0.312	2.602	847831	1738333	0.9819
36	1376400	0.8409	1376400	1117500	1876700	31.485	3	0.799	2.602	847831	1376400	0.7775

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97085	0.873	0.35657	0.25448
Bartlett's Test indicates equal variances (p = 0.51)	4.24964	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	36	>36			847831	0.51798	9.3E+10	2.1E+11	0.81605	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	16.593			
IC10	22.218			
IC15	27.843			
IC20	33.468			
IC25	>36			
IC40	>36			
IC50	>36			



APPENDIX II (Continued)

Specific growth rate (0-72 hours)

The ToxCalc analysis of the 0-72 hour study report's growth rate data (Table 8, page 32) with the nominal 100 mg/L results omitted and using mean measured concentrations of 5,7-di-OH metabolite of pyroxsulam gave the following results (initial growth rate data as day^{-1}).

Conc-mg/L	1	2	3	4	5	6
D-Control	1.1400	1.1000	1.3300	1.3400	1.3700	1.3300
0.5	1.4700	1.2400	1.1700			
1.8	1.5200	1.3900	1.5600			
4.7	1.1900	1.4200	1.2200			
13	1.1200	1.4500	1.3800			
36	1.2700	1.4000	1.1700			

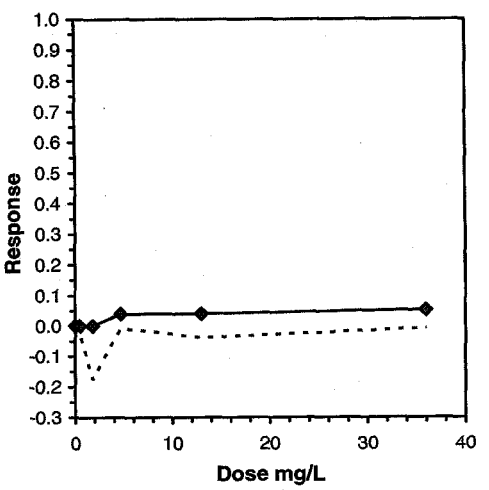
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.2683	1.0000	1.2683	1.1000	1.3700	9.187	6				1.3506	1.0000
0.5	1.2933	1.0197	1.2933	1.1700	1.4700	12.135	3	-0.273	2.602	0.2382	1.3506	1.0000
1.8	1.4900	1.1748	1.4900	1.3900	1.5600	5.965	3	-2.422	2.602	0.2382	1.3506	1.0000
4.7	1.2767	1.0066	1.2767	1.1900	1.4200	9.794	3	-0.091	2.602	0.2382	1.2967	0.9601
13	1.3167	1.0381	1.3167	1.1200	1.4500	13.206	3	-0.528	2.602	0.2382	1.2967	0.9601
36	1.2800	1.0092	1.2800	1.1700	1.4000	9.010	3	-0.127	2.602	0.2382	1.2800	0.9478

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9443	0.873	-0.181	-1.2644
Bartlett's Test indicates equal variances (p = 0.96)	1.02253	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	36	>36			0.23818	0.18779	0.02288	0.01675	0.29168	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	31.822			
IC10	>36			
IC15	>36			
IC20	>36			
IC25	>36			
IC40	>36			
IC50	>36			



APPENDIX II (Continued)

Biomass (0-72 hours)

The ToxCalc analysis of the 0-72 hour study report's biomass data (Table 9, page 35) with the nominal 100 mg/L results omitted and using mean measured concentrations of 5,7-di-OH metabolite of pyroxsulam gave the following results (biomass data as cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	34.830	34.470	39.630	39.270	56.380	44.380
0.5	54.080	44.050	25.600			
1.8	56.110	55.330	78.980			
4.7	32.640	59.760	30.390			
13	32.670	59.310	41.320			
36	36.940	53.650	24.390			

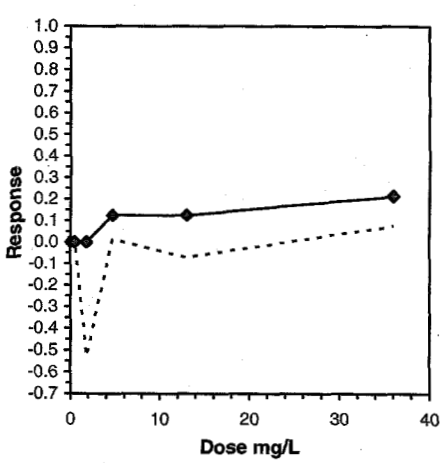
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	41.493	1.0000	41.493	34.470	56.380	19.641	6				48.737	1.0000
0.5	41.243	0.9940	41.243	25.600	54.080	35.026	3	0.028	2.602	23.495	48.737	1.0000
1.8	63.473	1.5297	63.473	55.330	78.980	21.166	3	-2.435	2.602	23.495	48.737	1.0000
4.7	40.930	0.9864	40.930	30.390	59.760	39.937	3	0.062	2.602	23.495	42.682	0.8758
13	44.433	1.0709	44.433	32.670	59.310	30.585	3	-0.326	2.602	23.495	42.682	0.8758
36	38.327	0.9237	38.327	24.390	53.650	38.300	3	0.351	2.602	23.495	38.327	0.7864

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90086	0.873	0.45871	-1.2053
Bartlett's Test indicates equal variances (p = 0.89)	1.69742	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	36	>36			23.4953	0.56624	263.719	163.012	0.21545	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	2.967			
IC10	4.134			
IC15	19.631			
IC20	32.500			
IC25	>36			
IC40	>36			
IC50	>36			



Exponential growth
(page 40 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	42500
48	161000
72	492000
96	1798200