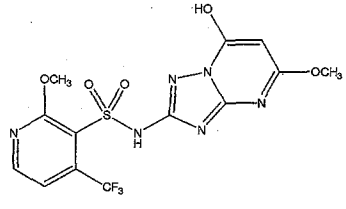
Data Evaluation Report on the Acute Toxicity of 7-OH Metabolite of Pyroxsulam (7-OH metabolite of XDE-742) to Algae, *Pseudokirchneriella subcapitata*

PMRA Submission Number 2006-4727; 1283233 EPA MRID Number 469084-50 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:	7-OH metabolite of XDE-742, i.e. 7-OH metabolite of pyroxsulam	
Purity (%):	96% (ID No. TSN 105232, used to prepare test solutions and quality control samples), and 99% (ID No. TSN 105384, used to prepare test solutions during preliminary testing and calibration standards during definitive testing).	
Common name:	7-OH metabolite of XDE-742	
Chemical name:	3-pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-	
IUPAC:	N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
CAS name:	3-Pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-	
CAS No.:	Not available	
Synonyms	7-desmethyl XDE-742 metabolite	

Chemical structure:



Primary Reviewers: Daryl Murphy **Date:** 23 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

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

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

Christopher Salice **Date:** 13 October 2007
Environmental Protection Agency, Environmental Fate and Effects Division

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

CITATION: Hoberg, J. R. 2005. 7-OH Metabolite of XDE-742: Acute Toxicity Test to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts. Springborn Smithers Study No. 12550.6408, Sponsor Protocol/Project No. 050108. The Dow

Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC Indianapolis, Indiana 46268. 16 February 2005. Unpublished report.

Note: The 2005 date is an error in the study report. Based on the dates given in the No Data Confidentiality Claims, Good Laboratory Practice Compliance and Quality Assurance statements and the date of the definitive study (26 to 30 January 2006), the correct year is 2006.

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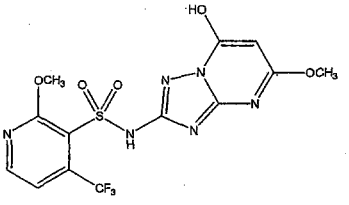
Chemical structure:



Primary Reviewers: Daryl Murphy D.Murphy 22/02/08 **Date:** 23 July 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

22/2/08

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

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

Christopher Salice **Date:** 13 October 2007
Environmental Protection Agency, Environmental Fate and Effects Division 4/04/08

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Active Code	JUA
Use Site Category:	13, 14
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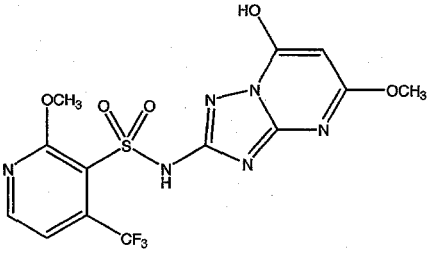

Primary Reviewers: Daryl Murphy **Date:** 23 July 2007
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EXECUTIVE SUMMARY:

The purpose of this study was to determine the effect of 7-OH metabolite of pyroxsulam (i.e. 7-OH metabolite of 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 7-OH metabolite of pyroxsulam at nominal concentrations of 0 (control), 1.0, 2.6, 6.4, 16, 40 and 100 mg/L (corresponding to mean measured concentrations of 0 (control), 0.94, 2.6, 6.2, 16, 40 and 100 mg 7-OH metabolite of pyroxsulam /L, respectively). The experiment was carried out taking account of relevant OECD, European Communities and US EPA guidelines.

Treatment groups were set in triplicate and the medium control group contained six replicates, with an initial cell density of approximately 10,000 cells/mL. Temperatures during the exposure period ranged from 23–24°C. The light intensity range was 7000-9100 lux. The pH value in the controls was 7.0 at test initiation and 9.6 at test termination. In solutions containing the 7-OH metabolite of pyroxsulam, the pH ranged from 6.9 to 4.0 at test initiation (indicative of the test solution pH being affected by the test substance with solution pH inversely proportional to the test concentration) and 9.7 to 3.9 at test termination. The pH of 4.0, which was recorded 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 7-OH metabolite of pyroxsulam at this concentration, by 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.

The pH of the control media increased by more than 1.5 pH units (note the latest version of OECD 201 states that the control medium pH should not increase by greater than 1.5 units during the test). There were other deviations or deficiencies included identified but none of these were considered to have adversely affected the study or its results.

After 72 hours, inhibition of mean specific growth rate relative to controls ranged from -8% (growth stimulation) at 2.6 mg/L to 111% at 100 mg/L. The inhibition of biomass relative to controls ranged from -39% at 2.6 mg/L to 101% at 100 mg/L. After 96 hours, inhibition of cell density relative to controls ranged from -12% at 6.2 mg/L to 100% at 100 mg/L.

Based on the results of this study, as shown below, the 7-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 fails to satisfy all the guideline requirements for an acute toxicity study with the unicellular green alga, *Pseudokirchneriella subcapitata* as the toxicity was the result of the acid pH of the highest test concentration.

The 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 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 >40 mg 7-

OH metabolite of pyroxsulam/L). A new study would not provide additional information. The low toxicity of the test substance has been adequately demonstrated.

The US EPA and APVMA agree with the PMRA's conclusions and, although there were obvious problems with pH in the test solutions that call into question the study results and a failure to satisfy guideline requirements, classify the study as Supplemental.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata,*
Test Type: Static

The following endpoints provide an indication of the lack of toxicity of the 7-OH metabolite of pyroxsulam to *Pseudokirchneriella subcapitata.* Actual EC50 values are all expected to be >40 mg 7-OH metabolite of pyroxsulam/L.

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

Biological Parameter	Based on Mean Measured Concentrations (mg 7-OH metabolite of pyroxsulam/L)	
	EC50 (95% confidence limits)	NOEC
96-Hour Cell Density	62 (58-65)	16
0-72 hour Total biomass	50 (46-53)	16
0-72-Hour Average Growth Rate	65 (61-68)	40

Based on Mean Measured Concentrations, the endpoints 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 7-OH metabolite of pyroxsulam/L)	
	EC50	NOEC
96-Hour Cell Density	>40	16
0-72 hour Total biomass	>40	16
0-72-Hour Average Growth Rate	>40	40

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The toxicity test was 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 that protocol were reported as meeting the requirements specified in:

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

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

In addition, the procedures were 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. 1982. U.S. Environmental Protection Agency, Washington, D.C.

Guidelines appear to have been generally followed with the exception of certain parameters (e.g. growth medium, pH at the highest test concentration etc.). For further details see the relevant text entries below and the deviations/deficiencies table, page 38 of this DER.

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

- The protocol stated that the photosynthetically-active radiation (PAR) within the test area will range from 90 to 140 $\mu E/m^2/s$ and the light intensity will range from 7000 to 9100 lux. At initiation of the definitive test, the PAR ranged from 108 to 145 $\mu E/m^2/s$ and the light intensity ranged from 7000 to 9100 lux. The lighting was not adjusted to reduce the PAR reading since the light intensity was within the correct range;

and

- The protocol stated that the mean coefficient of variation (CV) for the daily specific growth rates (e.g. days 0 to 1, 1 to 2 and 2 to 3) in the control should not exceed 35%, and the CV for the day 0 to 3 average growth rate should not exceed 7%. During this test, the CVs for the specific growth rates for the days 0 to 1, 1 to 2 and 2 to 3 were 22, 41 and 31%, respectively. The CV (41%) for the day 1 to 2 specific growth rate exceeded the criterion of 35%. However, the CVs for the remaining intervals were within the requested range, including the CV (4.5%) for the day 0 to 3 control average growth rate. Since the criterion was exceeded on only one day and the concentration-response was well-defined, this deviation did not impact on the results or interpretation of this study.

The PAR issue is considered further in Table 10, page 39 of this DER. The reviewer's consideration of the coefficient of variation is given on page 30 of this DER.

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 conducted in January 2006, before 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 100 mg 7-OH metabolite of pyroxsulam/L test concentration which renders the test supplementary.

COMPLIANCE:
The data and report for "7-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*" were produced and compiled in accordance with all pertinent OECD and U.S. EPA (40 CFR, Part 160) Good Laboratory Practice regulations, *viz.*

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

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

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

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

A. MATERIALS:

1. **Test Material:** 7-OH metabolite of XDE-742 (i.e. 7-OH metabolite of pyroxsulam)
   Description: Solid
   Lot No./Batch No.: E2008-46/ TSN103826
   Purity: 96%
   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 94 to 100% of the nominal concentrations of 1.00 to 100 mg 7-OH metabolite of pyroxsulam/L (page 18 of this DER refers). Such results indicate the test substance was stable for at least 96 hours under the test conditions.

   Storage conditions of test chemicals: Upon receipt at Springborn Smithers, the test substance (also identified as SSL No. 112-85) was stored at room temperature in the original container in a dark ventilated cabinet and was used to prepare test solutions and quality control samples during definitive testing. Concentrations were adjusted for the purity of the test substance and were presented as active constituent.

   Note that a second lot of test substance, 7-OH metabolite of pyroxsulam, was received on 10 November 2005 from Dow AgroSciences, Indianapolis, Indiana, for which the following information was provided:

   Name: 7-OH metabolite of XDE-742
   Synonym: 7-desmethyl XDE-742 metabolite
   ID No.: TSN 105384
   Lot No.: 35172-56
   CAS No.: Not Available
   Purity: 99%

   Upon receipt at Springborn Smithers, the test substance (identified as having the SSL No. 115-19) was stored at room temperature in the original container in a dark ventilated cabinet and was used to prepare test solutions during preliminary testing and calibration standards during definitive testing. Concentrations were adjusted for the purity of the test substance and presented as active constituent.

Physicochemical properties of 7-OH pyroxsulam:

The study report stated that characterization and verification of the test substance's identity were the responsibility of the Study Sponsor. Consequently, the values for physicochemical parameters were not given in the study report.

The study profile template (Hoberg (2006)) noted that physicochemical data for the 7-OH metabolite of pyroxsulam were not available to the laboratory when the study report was being written.

2. Test organism:

Name:	The alga used in this toxicity test was the freshwater green alga,
Species:	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Class:	Chlorophyceae
Strain:	1648
Source:	The alga was obtained from University of Texas, Austin, Texas, and was maintained in stock culture at Springborn Smithers.
Age of inoculum:	inoculum - three days since previous transfer
Method of cultivation:	The stock cultures were maintained in AAP medium (Algal Assay Procedure (AAP) medium prepared with sterile, deionised water) within the following conditions: a shaking rate of 100 ± 10 rpm, a temperature of 24 ± 2°C and continuous illumination at the surface of the medium with an intensity range of 7000 to 9100 lux (650 to 850 footcandles). Lighting was supplied by fluorescent bulbs. Culture flasks were agitated continuously on an orbital shaker. Temperature was controlled using an environmental chamber.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A preliminary range-finding exposure was conducted at Springborn Smithers at nominal 7-OH metabolite of pyroxsulam concentrations of 0.010, 0.10, 1.0, 10 and 100 mg 7-OH metabolite of pyroxsulam/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 7-OH metabolite of pyroxsulam/L treatment levels averaged 505, 482, 338, 276 and 3.75 x 10^4 cells/mL, respectively. The control averaged 347 x 10^4 cells/mL. Based on these results and consultation with the Study Sponsor, nominal 7-OH metabolite of pyroxsulam concentrations of 1.0, 2.6, 6.4, 16, 40 and 100 mg/L were selected for the definitive exposure.

b. Definitive Study
The purpose of the study was to determine the effect of 7-OH metabolite of XDE-742 on the growth of the freshwater green alga, *Pseudokirchneriella subcapitata*. The results are based on mean measured concentrations of

7-OH metabolite of pyroxsulam and are 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 0-72-hour total biomass and average growth rate data, denoted as EbC50 and ErC50, respectively, and the NOEC values for total biomass and average growth rate.

The experimental phase of the 96-hour acute toxicity test was conducted from 26 to 30 January 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; Criteria columns (and elsewhere as relevant), entries in italics are those given in the PMRA's Draft Evaluation Report template for acute toxicity to algae. In its examination of the initial drafts of the aquatic invertebrate DERs, the PMRA advised (email of 3/07/2007) that the criteria in the templates were understood to have come from old US guidelines and that failure to comply with these template requirements would not be a deficiency. Provided relevant US EPA or OECD guidelines are complied with, this approach is agreed with.

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	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.	Requirement considered met. OECD 201 states that, in order to adapt the test alga to the test conditions and ensure that the algae are in the exponential growth phase when used to inoculate the test solutions, an inoculum culture in the test medium is prepared 2-4 days before start of the test. 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 alga was maintained in stock culture in AAP medium. The AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium. Culturing conditions same as test – see following comparison: **Culture**: Environmental chamber; 24 ± 2°C; Continuous illumination; 7000 to 9100 lux; Shaking rate of 100 rpm. **Test**: Environmental chamber; 23 to 24°C; Continuous illumination; 7000 to 9100 lux; Shaking rate of 100 rpm.	Requirement considered met. Comparison of the reported typical culturing and test media conditions indicated they were equivalent.
Health: (any mortality observed)	Observations of the health of the algal cells were made at each 24-hour interval. No reference to phytotoxicity effects were identified in the study report and at test termination, cells exposed to all treatment levels tested and the control were observed to be normal. These observations are taken to indicate that the algal cultures used were healthy and growing at the test's initiation.	Requirement considered met. OECD 201 states microscopic observation should be performed to verify a normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae (as may be caused by the exposure to the test substance) at the end of the test. US EPA OPPTS 850.5400 states that any unusual cell shapes, color differences, differences in chloroplast morphology, flocculation, adherence of algae to test containers, or aggregation of algal cells at the test end are to be noted.

Test system Static/static renewal Renewal rate for static renewal	Static Not applicable for a static system in which there was no renewal of test medium.	Requirement considered met. OECD 201 does not refer to static or static renewal but can be interpreted as referring to them as no mention is made of renewal of test solutions. US EPA OPPTS 850.5400 indicates static systems are acceptable.
Incubation facility	Temperature controlled environmental chamber	Requirement considered met. OECD 201 refers to a cabinet or chamber in which the chosen incubation temperature can be maintained at ± 2°C. US EPA OPPTS 850.5400 refers to use of a growth chamber or a 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 states that test duration is normally 72 hours. However, shorter or longer test durations may be used provided that all validity criteria can be met. US EPA OPPTS 850.5400 refers to measurement of algal cells in all containers at the end of 96 h, and at the end of 24, 48, and 72 h. *EPA requires: 96-120 hours* *OECD: 72 hours*

Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass flasks fitted with stainless steel caps which permitted gas exchange. 250 mL 100 mL	Requirement considered met. OECD 201 states that the test vessels will normally be glass flasks of dimensions that allow a sufficient volume of culture for measurements during the test and a sufficient mass transfer of CO_2 from the atmosphere. US EPA OPPTS 850.5400 states that Erlenmeyer flasks should be used for test containers. The flasks 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 sodium selenate. OECD 201 refers to use of AAP medium while 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 Annex 3 of the OECD 201 guideline, which contains the AAP composition, does not identify sodium selenate as a constituent of the medium. However, the Annex goes on to describe the preparation of the US EPA medium and notes that sodium selenate (as the pentahydrate) is used only in the medium for stock cultures of diatom species at a final concentration in the AAP medium of 0.01 µg/L or 0.00001 mg/L. Concentration of $K_2HPO_4 \cdot 3H_2O$ was 1.368 mg/L in the test medium. OECD 201 refers to a K_2HPO_4 concentration of 1.044 mg/L. The study report value calculated as K_2HPO_4 is 1.04 mg/L, i.e. the values are equivalent. *EPA recommends 20X-AAP medium and no chelators.* This template requirement is noted but is not considered appropriate in the light of the current OECD and US EPA OPPTS requirements which allow use of chelating agents (the AAP medium used contains sodium EDTA as a chelating agent).

pH at test initiation and test termination:	pH values at times 0 and 96 hours were: **Nominal Concentration (mg/L)*** / **pH**** **0 h** / **96 h** **Control** 7.0 9.6 **1.0** 6.9 9.6 **2.6** 6.9 9.6 **6.4** 6.8 9.7 **16** 6.5 9.5 **40** 6.0 8.8 **100** 4.0 3.9 *** i.e. mg 7-OH metabolite of pyroxsulam/L.** **** Control, replicate B solution pH at 72 hours was 9.7.** **Note: the control medium pH increased from 7.0 at time 0 to 9.6 at 96 hours, i.e. an increase of 2.6 pH units.** The study report noted that the increase in pH during the exposure is common in static algal cultures and is due to photosynthesis by the alga.	See deviations/deficiencies table, page 38 of this DER with respect to initial and final pH values. 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 4.0. 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 201 (2006) states that the pH of the control medium should not increase by more than 1.5 units during the test.

Chelator used:	Yes, $Na_2EDTA \cdot 2H_2O$ at 300 µg/L.	Requirement considered met. OECD 201 identifies Na_2EDTA as a constituent of AAP medium and at the concentration used in the study. US EPA OPPTS 850.5400 states that chelating agents are included in the nutrient medium for optimum cell growth. No chelating agents are to be included in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test chemical. *EPA recommends 20X-AAP medium and no chelators.* This template requirement is noted but is not considered appropriate in the light of the current OECD and US EPA OPPTS requirements which advises on the media to use and allows use of chelating agents.
Carbon source:	Not identified.	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 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:	Sterile, deionised water used to prepare growth medium. Source not identified.	Requirement considered met. 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.
pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Not reported N/A Sterilised and deionised. See below Not reported See below See below Not reported.	
	Representative samples of the dilution water source used in the preparation of the culture medium were analysed periodically for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analysed in agreement with ASTM guidelines (2002). In addition, a representative sample of AAP medium was analyzed monthly for total organic carbon (TOC) concentration. The TOC concentration of the sample collected in January 2006 was 0.58 mg/L	*EPA pH: Skeletonema costatum= ~8.0, Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.* *salinity:* *EPA: 30-35 ppt. EPA is against the use of dechlorinated water.* The template requirements are considered to be either covered by the current OECD and US EPA OPPTS guideline requirements or not identified as relevant for the present study.

Indicate how the test material is added to the medium (added directly or used stock solution)	A 100 mg 7-OH metabolite of pyroxsulam/L stock solution was prepared prior to test initiation by placing 0.2083 g of 7-OH metabolite of pyroxsulam (0.2000 g as pure test substance, i.e. adjusted for the 96% purity of the test substance) in a 2000-mL volumetric flask and bringing it to volume with AAP medium. The resulting stock solution was observed to be clear and colorless with visible undissolved test substance. Following approximately 15 minutes of sonication and approximately one hour of mixing with a magnetic stir plate and Teflon®-coated stir bar, the stock solution was observed to be clear and colorless with no visible undissolved test substance. Test solutions were prepared from dilutions of the 100 mg/L stock solution All resulting test solutions were observed to be clear and colorless with no visible undissolved test substance.	Requirement considered met with the description in the report considered satisfactory.
Aeration or agitation	Continuous agitation (approx. 100 revs./min.) by means on an orbital shaker.	Requirement considered met. OECD 201 states that during the test it is necessary to keep the algae in suspension and to facilitate transfer of CO_2. To this end constant shaking or stirring should be used and reference is made to an orbital or reciprocate shaker table being used at ~150 rpm. 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	10,000 cells/mL (for each replicate)	Requirement considered met. Initial cell density considered acceptable. OECD 201 recommends an initial cell concentration for *Pseudokirchneriella subcapitata*: of 5 x 10^3 - 10^4 cells/mL. US EPA OPPTS 850.5400 states that each test chamber in the definitive study should contain equal volumes of test solution and approximately 1 x 10^4 *Selenastrum* cells per millilitre of test solution. *EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Number of replicates		Requirement considered met. The numbers of replicates used are acceptable. OECD 201 states that the test design should include three replicates at each test concentration and that the number of control replicates must be at least three, and ideally should be twice the number of replicates used for each test concentration. US EPA 850.5400 states that a minimum of three replicates is required for each concentration of test chemical and control. A solvent control was not used in the definitive test. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp. tests should be conducted with four replicate.* *OECD prefers three replicates at each test concentration and ideally twice that number of controls. When co-solvents are used, include a solvent control in the test.*
Control: Solvent control: Treatments:	6, inoculated with algae. N/A 3, inoculated with algae. In order to estimate the impact that the presence of algal biomass had on the test substance concentration, an additional replicate flask (D) of the 6.4 mg7-OH metabolite of pyroxsulam/L (nominal) level was prepared. This flask, which was not inoculated with algae, was analyzed at 96 hours of exposure for 7-OH metabolite of pyroxsulam concentration. The results of this analysis were compared with the results for the 6.4 mg 7-OH metabolite of pyroxsulam/L solution containing algae.	
Test concentrations Nominal: Measured:	Nominal concentrations were 0 (control), 1.0, 2.6, 6.4, 16, 40 and 100 mg/L Ratios of nominal concentrations were in the range of approximately 1:2.5 or 1:2.6.	See deviations/deficiencies table, page 38 of this DER with respect to ratios of nominal test concentrations exceeding US EPA OPPTS 850.5400 concentration ratio requirements. OEDC 201 states that for the final definitive test at least five concentrations, arranged in a geometric series with a factor not exceeding 3.2, should be selected.

	Measured Concentration[a]	US EPA OPPTS 850.5400 states that algae should be exposed to five or more concentrations of the test chemical in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, and 64 mg/L). The result of the analysis of the 6.4 mg/L sample with and without algae present indicates that, at this concentration, the presence of the algae did not affect the concentration of the 7-OH metabolite of pyroxsulam. *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested having no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.* These template requirements are noted but not considered further in the light of the specific requirements in the current OECD and US EPA OPPTS guidelines.

Nominal Conc.[a]	0 hour	96 Hour	Mean[b]	% of Nominal
Control	<0.031	<0.032	NA[d]	NA
1.0	0.98	0.89	0.94	94
2.6	2.6	2.6	2.6	99
6.4	6.3	6.1/6.2[d]	6.2	96
16	16	16	16	98
40	40	39	40	99
100	99	100	100	100

a. mg 7-OH metabolite of pyroxsulam/L.
b. Mean measured concentrations and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.
c. NA = Not Applicable.
d. Result of the additional sample without algae present to determine biological uptake/degradation

Note that concentrations were adjusted for the purity of the test substance

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 7-OH metabolite of pyroxsulam at 0 and 96 hours. All exposure solutions and QC samples were analysed for 7-OH metabolite of pyroxsulam using high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV) based on methodology validated at Springborn Smithers.	Requirement considered met. Methodology was validated (23 January 2006) to quantify the amount of 7-OH metabolite of pyroxsulam present in 20X AAP medium (a freshwater algal medium). This method validation was conducted based on the guidance document SANCO/3029/99 rev.4. Recovery samples were analysed by automated injection on a high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV). This method was validated by fortification of 20X AAP medium with 7-OH metabolite of pyroxsulam at concentrations of 0.05 and 100 mg/L. Recoveries averaged 105 ± 1.95% with a limit of quantitation (LOQ) of 0.0141 mg 7-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, with the only exception being that only one lot of test substance was used in the method validation. Analytical results for the recovery of 7-OH metabolite of pyroxsulam from 20X AAP medium were presented as were representative chromatograms from the analysis of a calibration standard, recovery sample and a control sample. A typical linear regression analysis for 7-OH metabolite of pyroxsulam ($r^2 = 0.99981$) was also presented in the study report.

Test conditions Temperature:	23-24°C	See deviations/deficiencies table, page 38 of this DER with respect to light intensity. 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 to 25°C maintained at ± 2°C* *EPA temperature: Skeletonema: 20□C, Others: 24-25□C.*
Photoperiod:	Continuous	Requirement considered met. OECD 201 refers to use of continuous light while US EPA OPPTS 850.5400 refers to test chambers containing *Selenastrum*, *Navicula*, and *Anabaena* being illuminated continuously. *OECD recommends continuous uniform illumination.* *EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous.*

Light intensity and quality	7000-9100 lux. The study report, under "Protocol Deviations" noted that the protocol states that the photosynthetically-active radiation (PAR) within the test area will range from 90 to 140 $\mu E/m^2/s$ and the light intensity will range from 7000 to 9100 lux. At initiation of the definitive test, the PAR ranged from 108 to 145 $\mu E/m^2/s$ and the light intensity ranged from 7000 to 9100 lux. The lighting was not adjusted to reduce the PAR reading since the light intensity was within the correct range.	See deviations/deficiencies table, page 38 of this DER with respect to the light intensity. OECD 201 refers to light intensity at the level of the test solutions from the range of 60-120 $\mu E \cdot m^{-2} s^{-1}$, which it states is equivalent to a range of 4440-8880 lux. US EPA light intensity requirement not met. US EPA OPPTS 850.5400 states fluorescent lights providing 4300 lux are to be used for *Selenastrum.* *EPA light: Anabaena: 2000 lux (±15%), Others: 4000-5000 lux (±15%)* *OECD: approximately 8000 Lux measured with a spherical collector.* These template requirements are noted but not considered further in the light of the specific requirements in the current OECD and US EPA OPPTS guidelines.
Reference chemical (if used) name: concentrations:	 N/A N/A	Not relevant as a reference chemical was not used. OECD 201 notes that a reference substance may be tested as a means of checking test procedures and that this should be done at least twice a year. US EPA OPPTS 850.5400 also states that positive controls using zinc chloride as a reference chemical should also be run periodically. While it is most probable that testing with a reference chemical had been conducted with satisfactory results and it is only an oversight that the relevant results were not provided, inclusion of such results would have added value to the test report.
Other parameters, if any	Conductivity measured at test initiation and termination in the treatment and control solutions ranged from 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) were determined and growth rate (per day) calculated. pH, conductivity, temperature, light intensity and concentrations of 7-OH metabolite of pyroxsulam in the test solutions were also determined over the course of the study.	The requirement is considered met. The parameters determined are acceptable. OECD 201 refers to growth and growth inhibition being quantified from measurements of the algal biomass as a function of time. US EPA OPPTS 850.5400 refers to enumeration of the algal cells to determine inhibition or stimulation of growth and the pattern of growth in test containers compared to controls. *EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks
		Criteria
Measurement technique for cell density and other end points	Single cell counts were conducted 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.

Parameters	Details	Remarks
		Criteria
Observation intervals	0, 24, 48, 72 and 96 hours	Observation intervals considered appropriate. OECD 201 refers to algal biomass in each flask being determined daily. US EPA OPPTS 850.5400 states that at the end of 96 h, and, if possible, at the end of 24, 48, and 72 h, the algal growth response (number or weight of algal cells per millilitre) in all test containers and controls is to be determined. *EPA and OECD: every 24 hours.*
Other observations, if any	Observation of cells exposed to the treatment levels and controls at test termination reported as showing the cells were normal.	Requirement considered met.

Parameters	Details	Remarks Criteria
Indicate whether there was exponential growth in the control	At 72 hours, the mean cell density in the controls was ~168 X 10^4 cells/mL. The mean control 96-hour cell growth was 277.58 x 10^4 cells/mL, i.e. ~2.8 x 10^6 cells/mL. The 0-72 hour mean specific growth rate of the controls was reported as 1.77 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 ~168 X 10^4 cells/mL. This represents a factor of ~170 (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 1.77 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 2.77 X 10^6 cells/mL is ~79% of the recommended value. Consequently, the US guideline value was not reached and the US EPA requirement has not been met. *EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Water quality was acceptable? (Yes/No)	Yes	Parameter considered met on basis of successful growth of the controls and details provided on the medium's preparation using filtered, deionised water.
Were raw data included?	As laboratory notes, no. However, the tabulated data	Parameter considered met. While raw data were not submitted, the

Parameters	Details	Remarks
		Criteria
	presented was made up of individual replicate values which could be used to verify the study report's results. The study report referred on occasion to results presented as being calculated from original raw data and not from the rounded values presented in the study report. All original raw data, the protocol and the original final report produced during the study are archived by the Toxicology and Environmental Research and Consulting archivist and stored at the Dow Chemical Company, Midland, Michigan.	tabulated results presented were sufficient to allow statistical analysis by the reviewer. Although US EPA OPPTS 850.5400 states that the sponsor must submit to the EPA all data developed by the test including those that are suggestive or predictive of acute phytotoxicity, advice from the US EPA was that tabulated data are generally considered "raw" by the EPA and, because the tabulated results presented in the study report were sufficient to allow statistical analysis, the guideline would be considered met. . . OECD 201 lists the results which must be presented in the test report. These are not considered by the reviewer to necessarily include raw, i.e. laboratory data. The tabulated data presented in the study report are considered to have complied with the OECD requirement.

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. EC50 values and corresponding 95% confidence intervals were determined by linear regression of response (percent reduction of cell density, total biomass and average growth rate as compared to the control) versus the mean measured concentration.

Cell density

The effects of the 7-OH metabolite of pyroxsulam on the growth of *Pseudokirchneriella subcapitata* under the test conditions are shown in Table 3 by the mean cell density counts at 24, 48, 72 and 96 hours and the % inhibition after 96 hours. In the table, significant cell density inhibition (with respect to the mean control cell density) was identified at 96 hours only in the algae exposed to mean measured concentration of 40 and 100 mg 7-OH metabolite of pyroxsulam/L. Since this same phenomenon also occurs in 3 similar tests using 3 other metabolites of similar structure, this is considered most likely the result of the acidity of the test solutions inhibiting algal growth rather than the specific toxicity of the 7-OH metabolite of pyroxsulam itself.

Table 3. Effect of the 7-OH metabolite of pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*). Mean study report results at 24, 48, 72 and 96 hours (standard deviations in brackets) are presented.

Treatment (mean measured concentration (mg 7-OH metabolite of pyroxsulam/L)	Initial cell density (cells/mL)	Mean cell density (x 10^4 cells/mL) at 24, 48, 72 and 96 hours. Standard deviation values shown in brackets.				
		24-hours	48-hours	72-hours	96-hours	% inhibition
Negative control	1 x 10^4	7.46 (3.29)	40.63 (14.22)	168.17 (33.75)	277.58 (12.43)	NA[c]
0.96	1 x 10^4	5.42 (1.18)	42.58 (13.36)	178.78 (33.2)	256.50 (46.00)	8
2.6	1 x 10^4	5.42 (0.88)	48.50 (13.94)	256.83 (46.05)	289.67 (33.36)	-4
6.2	1 x 10^4	4.58 (0.63)	57.50 (11.13)	225.50 (11.43)	312.00 (84.15)	-12
16	1 x 10^4	6.67 (1.76)	36.08 (11.39)	212.33 (9.46)	279.33 (47.08)	-1
40	1 x 10^4	4.33 (2.18)	19.67 (5.03)	134.31 (9.90)	224.50 (16.18)*	19
100	1 x 10^4	1.08 (0.76)	0.33 (0.14)	0.33 (0.38)	0.92 (0.76)*	100
Reference chemical (if used)	N/A					

*** Significantly different from the control at 96 hours (William's Test, $p \leq 0.05$).**
Notes: Percent inhibition is relative to the control. The mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table and NA = Not Applicable.

Based on the results of the Shapiro-Wilks' and Bartlett's test, this data set passed the requirements for normality and homogeneity of variance and William's test was used to determine treatment-related effects. Statistical analysis (William's test) determined a significant reduction in cell density in the 40 and 100 mg/L treatment levels compared to the control value (277.48 X 10^4 cells/mL). Therefore, the 96 hour NOEC for cell density was determined to be 16 mg 7-OH metabolite of pyroxsulam/L. The 96 hour EC50 was calculated to be 62 mg/L, with 95% confidence intervals of 58 to 65 mg/L.

Growth rate and biomass
The mean specific growth rates per day and the mean areas under the growth curves reported following exposure of *Pseudokirchneriella subcapitata* to the 7-OH metabolite of pyroxsulam are shown in Table 4 with respective percent inhibition results. The percentage inhibition is most marked at 100 mg 7-OH metabolite of pyroxsulam/L.

Table 4. Effect of the 7-OH metabolite of pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*). Mean results with standard deviations shown in brackets, as presented in the study report of Hoberg, are shown.

Treatment measured concentrations (mg 7-OH metabolite of pyroxsulam/L)	Mean Specific Growth Rate per day		Mean Area Under the Growth Curve, X 10^4 cells/mL	
	0-72 hours	% Inhibition	0-72 hours	% Inhibition
Negative control	1.77 (0.08)	NA	124.76 (21.75)	NA
0.96	1.80 (0.07)	-2	129.86 (14.64)	-4
2.6	1.92 (0.07)	-8	173.95 (22.03)	-39
6.2	1.88 (0.02)	-6	165.70 (4.53)	-33
16	1.86 (0.02)	-5	141.86 (9.31)	-14
40	1.70* (0.03)	4	86.14** (2.78)	31
100	-0.19 (0.25)	111	-0.85** (0.70)	101

*** For the mean specific growth rate, the Kruskal-Wallis test showed no treatment level was significantly reduced compared to the control. ** Significantly reduced from the control (William's Test). .**

The growth rate data set did not pass either Shapiro-Wilks' or Bartlett's Tests, therefore, Kruskal-Wallis' Test was used to detect treatment-related effects. Based on Kruskal-Wallis' Test, the 72-hour NOEC was empirically estimated to be 100 mg/L. However, a more reasonable 72 hour NOEC was determined to be 40 mg/L, the highest concentration tested with <10% inhibition of growth rate. The 72 hour ErC50 was calculated to be 65 mg/L, with 95% confidence intervals of 61 to 68 mg/L.

Based on the results of Shapiro-Wilks' and Bartlett's Tests, the biomass (area under the growth curve) 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 40 and 100 mg/L treatment levels as compared to the control data. Based on these results, the 72 hour NOEC for total biomass was determined to be 16 mg 7-OH metabolite of pyroxsulam/L. The 72 hour EbC50 was calculated to be 50 mg/L, with 95% confidence intervals of 46 to 53 mg/L.

The reported statistical endpoints are summarised in Table 5.

Table 5. Statistical endpoint values for the 7-OH metabolite of pyroxsulam as determined by the study report with respect to the toxicity of the 7-OH metabolite of pyroxsulam to the freshwater green alga, *Pseudokirchneriella subcapitata*.

Statistical Endpoint	Cell Density (96 h)	Growth Rate (0-72 h)	Biomass (area under curve) (0-72 h)
NOEC (mg 7-OH metabolite of pyroxsulam/L)	16	40	16
EC_{50} (mg 7-OH metabolite of pyroxsulam/L) (95% C.I.)	62 (58-68)	**E_rC50** = 65 (61-68)	**E_bC50** = 50 (46-53)
Reference chemical, if used	N/A		

Validity of test

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

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

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

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

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. 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% while the CV for the average growth rate of the control for the entire test period (0 to 72 hour growth rate) should not exceed 7%. During the study, the mean control 72 hour cell growth (168.17 x 10^4 cells/mL) exceeded the requirement of a 16-fold increase from the initial density of 1.0 x 10^4 cells/mL. 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 were reported as 22, 41 and 31%, respectively. The CV for the average growth rate of the control for the

entire test period (0 to 72 hour growth rate) was found to be 4.5%. The study report noted that the CV (41%) for the day 1 to 2 specific growth rate exceeded the criterion of 35%, however the CVs for the remaining intervals were within the requested range, including the CV (4.5%) for the entire test period. Since the criterion was exceeded on only one day and the concentration-response was well-defined, this deviation was not considered by the study author to have impacted the results of the study.

Reviewer's comments on test validity

With respect to exponential growth, this requirement is considered to have been met (see Table 2, page 26 of this DER under the parameter "Indicate whether there was an exponential growth in the control").

The 0-24, 24-48 and 48-72 hour control replicate growth rates were calculated from the initial (10,000 cells/mL), 24, 48 and 72 hour cell density counts using the growth rate formula shown under "Verification of Statistical Results" on page 33 of this DER. The values and calculated statistics, including the overall mean % coefficient of variation (%CV), are as shown in Table 6:

Table 6. Reviewer calculated growth rates for the 0-24, 24-48 and 48-72 hour periods and associated means, standard deviations and percentage coefficients of variation.

	Reviewer calculated growth rates (/day) for the control replicates		
Replicate	**0-24 h**	**24-48 h**	**48-72 h**
1	2.42	1.45	1.16
2	2.48	0.49	2.22
3	1.83	2.19	1.24
4	1.75	1.76	1.17
5	1.61	1.89	1.79
6	1.50	2.48	1.19
Mean	1.93	1.71	1.46
Standard deviation	0.42	0.70	0.45
%CV	21.63	40.78	30.46

The %CV values for the 0-24 and 48-72 hour growth rate values do not exceed 35% while the 24-48 hours %CV does. Because the study was conducted following the 1984 version of the OECD 201 guideline, this 24-48 hours result has not been considered a deficiency of significance. Although the study was conducted following the 1984 version of the OECD 201 guideline, the %CV value of 40.78 was recognised by the study as not meeting the study's acceptance criteria. The study author's argued that, as the CVs for the remaining intervals were within the requested range, including the 0-72 hour %CV (4.5%) for the entire test period and since the criterion was exceed on only one day and the concentration-response was well-defined, this deviation did not impact the results of the study. This is accepted by the study reviewer.

The %CV for the 0-72 hours growth rate was calculated at 4.46% (mean 1.70, standard deviation 0.08, see page 50 for data used and ToxCalc values). This 0-72 hour mean %CV of 4.46% meets the OECD 201 limit of 7%. Because the 2006 OECD guideline refers to the coefficient of variation of average specific growth rates during the whole test period, the 0-96 hours period %CV was also calculated and found to be 0.81 (mean 1.40, standard deviation 0.01) which complies with the guideline's requirement for this parameter.

The coefficient of variation values found were equivalent to those reported in the study report's "Protocol Deviations".

B. REPORTED STATISTICS:

The test report stated that the EC25 and EC50 values (the concentration of test substance which reduced cell density, total biomass and average specific growth rate by 25 and 50% 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). A computer program, TOXSTAT® (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.

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

C. VERIFICATION OF STATISTICAL RESULTS:

While it could be argued that the following detailed analyses are superfluous because the main effect with respect to inhibition of the algae comes from the acidity of the nominal 100 mg/L solution, and that only a NOEC can be calculated, following exclusion of the 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 7-OH metabolite of pyroxsulam exposed algae and the mean of the controls by Bonferroni's t test. Differences between the mean specific growth rate and biomass results of the 7-OH metabolite of pyroxsulam exposed algae and that of the controls were also tested by Bonferroni's t test. All NOEC values were determined using the ToxCalc package.

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 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 7-OH metabolite of pyroxsulam are shown in Table 7.

The percentage inhibition results (relative to the control mean of 1.77 day^{-1}) for the 0-72 hour growth rate at 0.94, 2.6, 6.2, 16, 40 and 100 mg 7-OH metabolite of pyroxsulam/L were, respectively, -2, -8, -6, -5, 4 and 111% (see Table 4).

The 100 mg/L result is considered to clearly result from the low pH rather than any intrinsic 7-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 7. Reviewer calculated and the study report's (italicised) calculated growth rates (Growth Rate (days^{-1})) of *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours of exposure to 7-OH metabolite of pyroxsulam. Values for each replicate are shown with there being six control replicates and three replicates per test concentration.

Mean measured concentration, mg 7-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	2.42	*2.24*	1.93	*2.05*	1.67	*1.74*
	2.48	*2.3*	1.49	*1.57*	1.73	*1.8*
	1.83	*1.7*	2.01	*2.13*	1.75	*1.83*
	1.75	*1.62*	1.76	*1.86*	1.56	*1.63*
	1.61	*1.49*	1.75	*1.86*	1.76	*1.84*
	1.50	*1.39*	1.99	*2.11*	1.72	*1.8*
Mean (sd)**	**1.93 (0.42)**	***1.79 (0.39)***	**1.82 (0.20)**	***1.93 (0.21)***	**1.70 (0.07)**	***1.77 (0.08)***
0.94	1.61	*1.49*	1.65	*1.75*	1.75	*1.83*
	1.91	*1.77*	1.97	*2.09*	1.65	*1.72*
	1.50	*1.39*	1.94	*2.06*	1.77	*1.85*
Mean (sd)**	**1.67 (0.21)**	**1.55 (0.20)**	**1.85 (0.18)**	**1.97 (0.19)**	**1.72 (0.06)**	**1.80 (0.07)**
2.6	1.70	*1.58*	2.00	*2.12*	1.90	*1.97*
	1.83	*1.7*	1.74	*1.84*	1.87	*1.94*
	1.50	*1.39*	2.03	*2.15*	1.78	*1.85*
Mean (sd)**	**1.68 (0.17)**	**1.56 (0.16)**	**1.92 (0.16)**	**2.04 (0.17)**	**1.85 (0.06)**	**1.92 0.06)**
6.2	1.66	*1.53*	1.90	*2.01*	1.82	*1.9*
	1.50	*1.39*	2.09	*2.21*	1.80	*1.87*
	1.39	*1.28*	2.07	*2.19*	1.79	*1.87*
Mean (sd)**	**1.52 (0.14)**	**1.40 (0.13)**	**2.02 (0.10)**	**2.14 (0.11)**	**1.80 (0.02)**	**1.88 (0.02)**
16	1.87	*1.73*	1.57	*1.66*	1.79	*1.87*
	2.14	*1.98*	1.86	*1.97*	1.77	*1.84*
	1.61	*1.49*	1.89	*2*	1.80	*1.87*
Mean (sd)**	**1.87 (0.27)**	**1.73 (0.25)**	**1.77 (0.18)**	**1.88 (0.19)**	**1.79 (0.02)**	**1.86 (0.02)**
40	1.91	*1.77*	1.47	*1.56*	1.64	*1.71*
	1.32	*1.22*	1.61	*1.71*	1.60	*1.67*
	0.92	*0.85*	1.35	*1.43*	1.65	*1.72*
Mean (sd)**	**1.38 (0.50)**	**1.28 (0.46)**	**1.48 (0.13)**	**1.57 (0.14)**	**1.63 (0.03)**	**1.70 (0.03)**
100	0.22	*0.21*	-0.69	*-0.73*	0.00	*0.00**
	-1.39	*-1.28*	-0.69	*-0.73*	-0.10	*-0.1*
	0.56	*0.52*	-0.35	*-0.37*	-0.46	*-0.48*
Mean (sd)**	**-0.20 (1.04)**	**-0.18 (0.96)**	**-0.58 (0.20)**	**-0.61 (0.21)**	**-0.19 (0.24)**	**-0.19 (0.25)**

*** Growth rate cannot be calculated when cell density is zero. A value of zero was entered for all further calculations.**
**** sd = standard deviation.**
Notes to Table 7: Rounded data values, not original raw data, were presented in the study report, and the reviewer calculated results were derived from the cell counts reported in the study report. The study report stated that, based on the results of Kruskal-Wallis' Test, no treatment level was significantly reduced compared to the control and the NOEC was empirically estimated to be the highest concentration tested with < 10% inhibition.

In Table 8, 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.

Biomass

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

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

where:

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

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

The 0-72 hours biomass (area under the growth curve) values for control and test replicates presented in the study report were recalculated and shown to be similar to those given in the study report. The reviewer calculated and the study report's calculated biomass (area under the growth curve) values for *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours of exposure to 7-OH metabolite of pyroxsulam are shown in Table 8.

Table 8. Reviewer calculated and the study report's (italicised) calculated biomass (area under the growth curve) values for *Pseudokirchneriella subcapitata* after 24, 48 and 72 hours of exposure to 7-OH metabolite of pyroxsulam. Individual replicate values shown.

Mean measured concentration, mg 7-OH metabolite of pyroxsulam/L	Biomass (X 10^4 cells/mL) Observation Interval (Hours)						Total biomass 0-72 hours	
	0-24 hours		24-48 hours		48-72 hours			
	Reviewer	Study report	Reviewer	Study report	Reviewer	Study report	Reviewer	Study report
Control	5.13	*5.54*	28.50	*23.00*	98.71	*98.02*	132.34	*126.56*
	5.50	*5.94*	14.75	*11.90*	98.92	*98.23*	119.17	*116.08*
	2.63	*2.84*	30.13	*24.31*	123.67	*122.81*	156.42	*149.95*
	2.38	*2.57*	18.63	*15.03*	69.75	*69.27*	90.75	*86.86*
	2.00	*2.16*	18.13	*14.63*	115.13	*114.33*	135.25	*131.11*
	1.75	*1.89*	28.13	*22.70*	114.21	*113.42*	144.09	*138.00*
Mean (sd*)	**3.23 (1.50)**	***3.49 (1.78)***	**23.04 (6.03)**	***18.59 (5.33)***	**103.40 (17.51)**	***102.68 (19.05)***	**129.67 (20.76)**	***124.76 (21.75)***
0.94	2.00	*2.16*	15.13	*12.21*	108.63	*107.87*	125.75	*122.24*
	2.88	*3.11*	28.25	*22.80*	95.38	*94.71*	126.50	*120.62*
	1.75	*1.89*	25.63	*20.68*	125.04	*124.17*	152.42	*146.74*
Mean (sd)	**2.21 (0.59)**	***2.39 (0.64)***	**23.00 (6.95)**	***18.56 (5.60)***	**109.68 (14.86)**	***108.92 (14.76)***	**134.89 (15.18)**	***129.86 (14.64)***
2.6	2.25	*2.43*	29.25	*23.60*	173.75	*172.54*	205.25	*198.58*
	2.63	*2.84*	18.38	*14.83*	150.50	*149.45*	171.50	*167.12*
	1.75	*1.89*	30.25	*24.41*	130.75	*129.84*	162.75	*156.14*
Mean (sd)	**2.21 (0.44)**	***2.39 (0.47)***	**25.96 (6.59)**	***20.95 (5.31)***	**151.67 (21.52)**	***150.61 (21.37)***	**179.83 (22.44)**	***173.95 (22.03)***
6.2	2.13	*2.30*	24.00	*19.37*	140.63	*139.65*	166.75	*161.31*
	1.75	*1.89*	33.88	*27.34*	142.13	*141.14*	177.75	*170.36*
	1.50	*1.62*	32.25	*26.02*	138.75	*137.79*	172.50	*165.43*
Mean (sd)	**1.79 (0.31)**	***1.94 (0.34)***	**30.04 (5.29)**	***24.24 (4.27)***	**140.50 (1.69)**	***139.52 (1.68)***	**172.33 (5.50)**	***165.70 (4.53)***
16	2.75	*2.97*	13.75	*11.10*	118.75	*117.93*	135.25	*131.99*
	3.75	*4.05*	24.00	*19.37*	120.50	*119.66*	148.25	*143.08*
	2.00	*2.16*	23.38	*18.86*	130.38	*129.47*	155.75	*150.49*
Mean (sd)	**2.83 (0.88)**	***3.06 (0.95)***	**20.38 (5.75)**	***16.44 (4.64)***	**123.21 (6.27)**	***122.35 (6.22)***	**146.42 (10.37)**	***141.86 (9.31)***
40	2.88	*3.11*	11.88	*9.58*	77.17	*76.63*	91.92	*89.32*
	1.38	*1.49*	13.38	*10.79*	73.13	*72.62*	87.88	*84.90*
	0.75	*0.81*	7.75	*6.25*	77.67	*77.13*	86.17	*84.19*
Mean (sd)	**1.67 (1.09)**	***1.80 (1.18)***	**11.00 (2.91)**	***8.88 (2.35)***	**75.99 (2.49)**	***75.46 (2.47)***	**88.65** (2.95)**	***86.14** (2.78)***
100	0.13	*0.14*	-0.25	*-0.20*	-0.88	*-0.87*	-1.00	*-0.94*
	-0.38	*-0.41*	-0.75	*-0.61*	-0.50	*-0.50*	-1.63	*-1.51*
	0.38	*0.41*	0.13	*0.10*	-0.63	*-0.62*	-0.13	*-0.11*
Mean (sd)	**0.04 (0.38)**	***0.05 (0.41)***	**-0.29 (0.44)**	***-0.24 (0.35)***	**-0.67 (0.19)**	***-0.66 (0.19)***	**-0.92** (0.75)**	***-0.85** (0.70)***

*** sd = standard deviation. ** The mean total biomass results for the 40 and 100 mg 7-OH metabolite of pyroxsulam/L exposures were significantly reduced compared to the control, based on Williams' Test.**

Reviewer calculated results:

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

Table 9. Reported and reviewer calculated toxicity endpoints.

Toxicity endpoint	Mean measured 7-OH metabolite of pyroxsulam concentration, as mg/L (95% confidence limits)		
	As presented in the study report	As calculated by the reviewer with the ToxCalc program. Endpoints based on use of all (0.94 to 100 mg/L, mean measured) test concentrations.	Endpoints based on use of all (0.94 to 40 mg/L, mean measured) with the 100 mg/L results excluded.
24 hour cell density			
EC50	51 (21-75)	51 (2.1-91)	>50
EC25	<0.94 (EC25 value empirically estimated and 95% confidence intervals could not be calculated)	0.91 (0.33-86)	0.90 (less than the lowest concentration used)*
NOEC	Not reported	40	40
48 hour cell density			
EC50	35 (21-45)	34 (18-50)	34.2*
EC25	18 (16-28)	17 (7.6-33))	16.9 (7.4-33.4)
NOEC	Not reported	40	40
72 hour cell density			
EC50	55 (50-59)	54 (44.9-59.5)	>40*
EC25	34 (31-38)	33 (28-38)	32.3 (28.0-38.4)
NOEC	Not reported	40	40
96 hour cell density			
EC50	62 (58-65)	62 (56-66)	>40*
EC25	43 (36-47)	43 (31-50)	>40*
NOEC	16	40 (Bonferroni t-test) 16 (Williams' test)	40(Bonferroni t-test) 40 (Williams' test)
72 hour mean specific growth rate			
ErC50	65 (61-68)	67 (66-68)	>40*
NOEC	40	40	40
72 hour biomass area			
EbC50	50 (46-53)	48 (43-52)	>40
NOEC	16	16	16

*** 95% confidence limits not calculated by the ToxCalc program.**

The above table also includes the reviewer calculated results when the 100 mg/L results are excluded from the ToxCalc calculations based on the study report's cell counts, 0-72 hours mean specific growth rates and 0-72 hour biomass results. The relevant ToxCalc outputs are given in Appendix II (page 52 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 10 summarises deficiencies and deviations from the OECD 201 and US EPA OPPTS 850.5400 Guidelines.

Table 10. Deviations from Guidelines and other deficiencies

Parameter	Study reported results	OECD 201 Freshwater alga and Cyanobacteria, Growth Inhibition Test	US EPA OPPTS 850.5400 Algal Toxicity, Tiers I and II
Details of growth medium name	The test medium contained sodium selenate at 1.88 µg/L. The study report noted this was an additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.	OECD 201 refers to AAP medium and provides a comparison (Annex 3) of the US EPA AAP medium and the OECD 201 medium. The guideline identifies both as suitable growth media. OECD 201 states that sodium selenate is to be used only in medium for stock cultures of diatom species. 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 0 were: **Nominal Concentration (mg/L)* / pH 0 h** **Control** 7.0 **1.0** 6.9 **2.6** 6.9 **6.4** 6.8 **16** 6.5 **40** 6.0 **100** 4.0 *** i.e. mg 7-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 be7.5 (± 0.1) for *Selenastrum.* US EPA OPPTS 850.5400 also states that if the test chemical is highly acidic and reduces the pH of the test solution below 5.0 at the first measurement, appropriate adjustments to pH should be considered. This was the situation in the study under assessment for the 100 mg/L replicates where the pH at time 0 was 4.0. This initial pH of 4.0 has invalidated the results from the 100 mg/L test solutions (see below).

pH at test termination:	pH values at time 96 hours were: **Nominal Concentration (mg/L)* — pH** 96 h** **Control** 9.6 **1.0** 9.6 **2.6** 9.6 **6.4** 9.7 **16** 9.5 **40** 8.8 **100** 3.9 *** i.e. mg 7-OH metabolite of pyroxsulam/L.**	OECD 201 states that the pH of the control medium should not increase by more than 1.5 units during the test.	No specific comment other than as above with the need to consider pH adjustments.
Test concentrations Nominal:	Ratios of nominal concentrations were in the range of approximately 1:2.5 or 1:2.6.	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).
Test conditions Light intensity and quality	7000-9100 lux. At initiation of the definitive test, the PAR ranged from 108 to 145 $\mu E/m^2/s$, whereas the protocol refers to a range of 90 to 140 $\mu E/m^2/s$ and a light intensity of 7000 to 9100 lux. The lighting was not adjusted to reduce the PAR reading since the light intensity was within the correct range.	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 light intensity requirement not met (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	At 72 hours, the mean cell density in the controls was ~168 X 10^4 cells/mL, i.e. there was an increase of a factor of 16.8. The mean control 96-hour cell growth was 277.58 x 10^4 cells/mL, i.e. ~2.8 x 10^6 cells/mL. The 0-72 hour mean specific growth rate of the controls was reported as 1.77 day^{-1}.	OECD 201 requires, *inter alia,* that biomass in the control cultures should have increased by a factor of at least 16 within the 72 hour test period. The guideline notes that this corresponds to a specific growth rate of 0.92 day^{-1}.	US EPA OPPTS 850.5400 states that algal growth in controls should reach the logarithmic growth phase by 96 h (at which time the number of algal cells should be approximately 3.5 X 10^6/mL for *Selenastrum*.

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

The 4.0 pH value recorded at time 0 in the 100 mg/L test concentrations is a crucial issue. The observed lack of growth at 100 mg/L (96 hour mean cell density was 0.92 X 10^4 cells/mL, the control at that time had a mean of 277.82 X 10^4 cells/mL) could be due to the concentration of the 7-OH metabolite of pyroxsulam present , by (most likely) the pH of 4.0 recorded at 0 hours, or by 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 and is rated as "supplemental" by the Australian Government Department of the Environment, Water, Heritage and the Arts. An acceptable alternative would have been to ignore the results of the higher concentration as the remaining treatments all had pH values above 5 at 0 hours.

The reason for the initial control pH being 7.0 instead of 7.5 at time zero is not known. The change in the pH of the controls from 7.0 at day 0 to 9.6 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 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 and did not show the increases in pH seen in the other concentrations and the control.

The ratios of the nominal concentrations were in the range of approximately 1:2.5 or 1:2.6 which exceeds the US EPA requirement that the ratio be between 1.5 and 2.0. This deviation is not considered to have adversely affected the study or its results. The 1984 validity criteria of the OECD were met.

The light used satisfied the OECD 201 requirement but exceeded that referenced in US EPA OPPTS 850.5400. There was no obvious adverse effect arising from this event, in that the control algae appeared to have grown successfully.

Apart from the pH being 4.0 throughout in the 100 mg 7-OH metabolite of pyroxsulam/L, 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 is considered, in contrast, a major deficiency. Results from this concentration should be treated with caution.

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 ~278 X 10^4 cells/mL or ~2.8 X 10^6 cells/mL, which equates to ~80% of the US EPA value. This is a deficiency with respect to compliance with the US EPA OPPTS guideline and adds support to the classification of the study as supplemental. Because a plotting cell counts against time using the Microsoft Excel Chart Wizard function and fitting the data points to an exponential curve (data and curve shown on page 58 of this DER) returned an r^2 value of 0.9619, a value that indicates an exponential approximating growth occurred in the study's control algae. While the visual examination of the data points and the fitted exponential curve clearly show some deviation from each other, the r^2 value is taken to indicate this deficiency is not expected to have invalidated the study.

E. REVIEWER'S COMMENTS:

Australian reviewer's comments:
In general, the reviewer's recalculated toxicity endpoint results were similar to the study author's. The study author's EC50 estimates for 96 h are equivalent to the reviewer's 96 h EC50 and while the reviewer's NOEC was higher based on a Bonferroni-t-Test, the William's Test gave the same result as the study author's result. For the 72-h biomass the results were similar.

As the study was completed in February 2006, before changes to OECD 201 test guideline were announced in March 2006, any failure to comply with the 2006 guideline is not automatically considered a deficiency or deviation.

The reviewer's analyses for cell density were conducted using a parametric test, Bonferroni t-Test, The data meet the conditions of the Shapiro-Wilk's test for normality. This gave 40 mg 7-OH metabolite of pyroxsulam/L as the NOEC while the study author's was 16 mg 7-OH metabolite of pyroxsulam/L using a Williams's test, which was confirmed by the reviewer.

However, the presence of stimulatory effects in the lower concentrations tested between dose concentrations of 0.94 and 16 mg 7-OH metabolite of pyroxsulam/L is noted.

A significant deficiency with the study was the pH of 4.0 in the 100 mg/L test concentration throughout the test. The inhibition seen at this concentration is attributed to the pH.

Based on the results of this study, as shown below, the 7-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 the US EPA because of the pH of the 100 mg/L test solution being 4.0 at time 0 and does not satisfy all the guideline requirements for an acute toxicity study with the unicellular green alga, *Pseudokirchneriella subcapitata.*

While detailed statistical analyses of the data were conducted, they could be considered superfluous because the main effect with respect to inhibition of the algae 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.

PMRA reviewer comments:

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. Useful information can be obtained from this study and used in a risk assessment (EC50 expected to be >40 mg 7-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 because of the pH of 4.0 observed in the 100 mg/L test solution at time 0 and throughout the test. This in turn 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. Useful information can be obtained from this study and used in a risk assessment (EC50 expected to be >40 mg 7-OH metabolite of pyroxsulam/L).

The study's reported endpoints were:

Statistical Endpoint	**Cell Density (72 h)**	**Growth Rate (72 h)**	**Biomass (area under growth curve) (72 h)**	**Cell Density (96 h)**
NOEC (mg 7-OH metabolite of pyroxsulam/L)	16	40	16	16
EC50 (mg 7-OH metabolite of pyroxsulam/L) (95% C.I.)	53 (45-59)	ErC50 = 65 (61-68)	EbC50 = 50 (46-53)	62 (56-66)
Reference chemical, if used	N/A			

Based on Mean Measured Concentrations, the endpoints 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 7-OH metabolite of pyroxsulam/L)**	
	EC50	**NOEC**
96-Hour Cell Density	>40	16
0-72 hour Total biomass	>40	16
0-72-Hour Average Growth Rate	>40	40

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

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density at 24 hours

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

Conc-mg/L	1	2	3	4	5	6
D-Control	11.2500	12.0000	6.2500	5.7500	5.0000	4.5000
0.94	5.0000	6.7500	4.5000			
2.6	5.5000	6.2500	4.5000			
6.2	5.2500	4.5000	4.0000			
16	6.5000	8.5000	5.0000			
40	6.7500	3.7500	2.5000			
100	1.2500	0.2500	1.7500			

			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	7.4583	1.0000	7.4583	4.5000	12.0000	44.135	6				7.4583	1.0000
0.94	5.4167	0.7263	5.4167	4.5000	6.7500	21.811	3	1.364	2.655	3.9741	5.5208	0.7402
2.6	5.4167	0.7263	5.4167	4.5000	6.2500	16.209	3	1.364	2.655	3.9741	5.5208	0.7402
6.2	4.5833	0.6145	4.5833	4.0000	5.2500	13.727	3	1.921	2.655	3.9741	5.5208	0.7402
16	6.6667	0.8939	6.6667	5.0000	8.5000	26.339	3	0.529	2.655	3.9741	5.5208	0.7402
40	4.3333	0.5810	4.3333	2.5000	6.7500	50.405	3	2.088	2.655	3.9741	4.3333	0.5810
*100	1.0833	0.1453	1.0833	0.2500	1.7500	70.501	3	4.259	2.655	3.9741	1.0833	0.1453

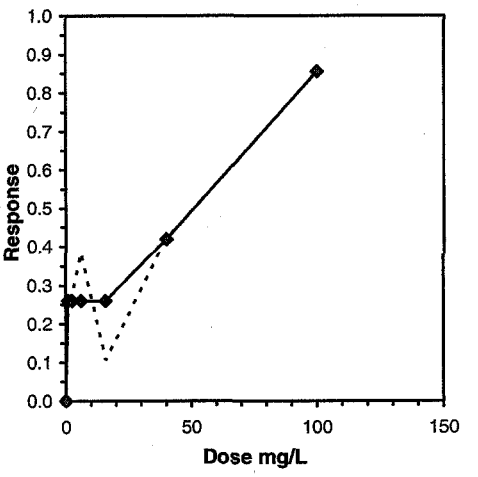
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94577	0.884	0.87449	0.81943
Bartlett's Test indicates equal variances (p = 0.15)	9.35824	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	40	100	63.2456		3.97408	0.53284	15.2201	4.481	0.02177	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	0.181	7.203	0.067	37.278	4.4417
IC10*	0.362	9.274	0.133	66.468	3.2248
IC15*	0.543	12.239	0.200	73.123	2.2227
IC20*	0.724	14.628	0.267	79.778	1.5497
IC25*	0.905	16.923	0.334	86.419	0.9539
IC40	37.137	17.712	0.000	84.850	-0.1796
IC50	51.154	14.939	2.109	90.773	-0.0067

* indicates IC estimate less than the lowest concentration



The 100 mg/L mean cell count at 24 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 48 hours

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

Conc-mg/L	1	2	3	4	5	6
D-Control	47.750	19.500	56.000	33.500	33.250	53.750
0.94	27.250	51.750	48.750			
2.6	55.000	32.500	58.000			
6.2	44.750	65.250	62.500			
16	23.000	41.500	43.750			
40	19.000	25.000	15.000			
100	0.250	0.250	0.500			

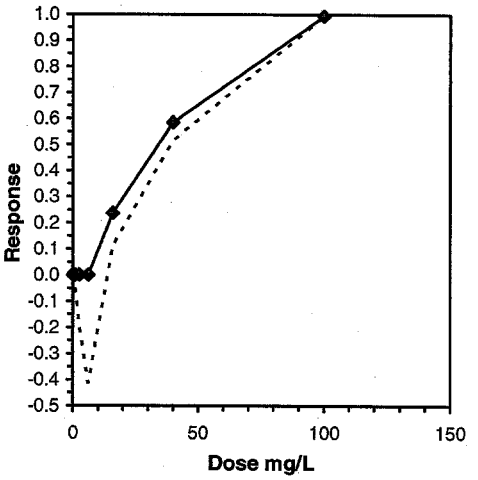
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	40.625	1.0000	40.625	19.500	56.000	35.000	6				47.302	1.0000
0.94	42.583	1.0482	42.583	27.250	51.750	31.382	3	-0.237	2.655	21.904	47.302	1.0000
2.6	48.500	1.1938	48.500	32.500	58.000	28.737	3	-0.955	2.655	21.904	47.302	1.0000
6.2	57.500	1.4154	57.500	44.750	65.250	19.351	3	-2.045	2.655	21.904	47.302	1.0000
16	36.083	0.8882	36.083	23.000	43.750	31.555	3	0.551	2.655	21.904	36.083	0.7628
40	19.667	0.4841	19.667	15.000	25.000	25.593	3	2.540	2.655	21.904	19.667	0.4158
*100	0.333	0.0082	0.333	0.250	0.500	43.301	3	4.884	2.655	21.904	0.333	0.0070

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93226	0.884	-0.5903	-0.6594
Bartlett's Test indicates equal variances (p = 0.02)	15.3153	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	40	100	63.2456		21.9038	0.53917	1121.49	136.126	2.8E-04	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	8.266	3.314	0.000	24.195	0.9822
IC10	10.332	3.808	5.880	26.511	0.6432
IC15	12.398	3.920	6.986	28.827	0.8526
IC20	14.464	4.325	7.392	31.143	0.5874
IC25	16.887	4.817	7.657	33.494	0.3352
IC40	27.260	6.059	6.762	40.614	-0.4978
IC50	34.175	5.515	17.735	50.164	-0.4676



The 100 mg/L mean cell count at 48 hours is identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using William's test, did not identify this result as statistically significantly reduced compared to the control.

APPENDIX I (Continued)

Cell density at 72 hours

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

Conc-mg/L	1	2	3	4	5	6
D-Control	151.67	180.33	193.33	108.00	199.00	176.67
0.94	192.00	141.00	203.33			
2.6	294.50	270.50	205.50			
6.2	238.50	221.00	217.00			
16	216.50	201.50	219.00			
40	137.33	123.25	142.33			
100	0.00	0.75	0.25			

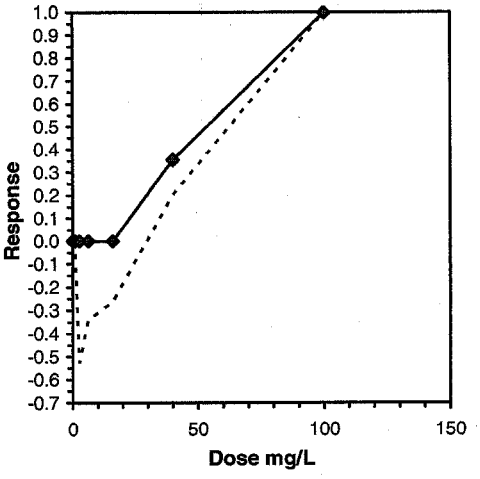
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	168.17	1.0000	168.17	108.00	199.00	20.071	6				208.32	1.0000
0.94	178.78	1.0631	178.78	141.00	203.33	18.572	3	-0.547	2.655	51.47	208.32	1.0000
2.6	256.83	1.5273	256.83	205.50	294.50	17.929	3	-4.574	2.655	51.47	208.32	1.0000
6.2	225.50	1.3409	225.50	217.00	238.50	5.071	3	-2.957	2.655	51.47	208.32	1.0000
16	212.33	1.2626	212.33	201.50	219.00	4.458	3	-2.278	2.655	51.47	208.32	1.0000
40	134.30	0.7986	134.30	123.25	142.33	7.367	3	1.747	2.655	51.47	134.30	0.6447
*100	0.33	0.0020	0.33	0.00	0.75	114.564	3	8.657	2.655	51.47	0.33	0.0016

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91379	0.884	-1.0537	1.32142
Bartlett's Test indicates unequal variances (p = 2.31E-03)	20.4388	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	40	100	63.2456		51.4712	0.30607	21263.4	751.676	6.0E-08	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	19.377	1.120	14.825	20.386	-2.8873
IC10	22.755	1.104	17.967	24.771	-1.2636
IC15	26.132	1.251	20.975	29.157	-0.4422
IC20	29.509	1.445	23.988	33.542	0.0596
IC25	32.887	1.668	27.852	37.928	0.3236
IC40	44.170	2.633	35.404	51.328	0.0675
IC50	53.500	2.274	44.920	59.483	-0.1078



The 100 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 96 hour algal cell count data in the study report gave the following results (cell count data as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	273.00	275.50	289.50	287.50	256.00	284.00
0.94	213.50	305.00	251.00			
2.6	315.50	301.50	252.00			
6.2	251.00	408.00	277.00			
16	245.00	333.00	260.00			
40	206.00	236.00	231.50			
100	1.75	0.75	0.25			

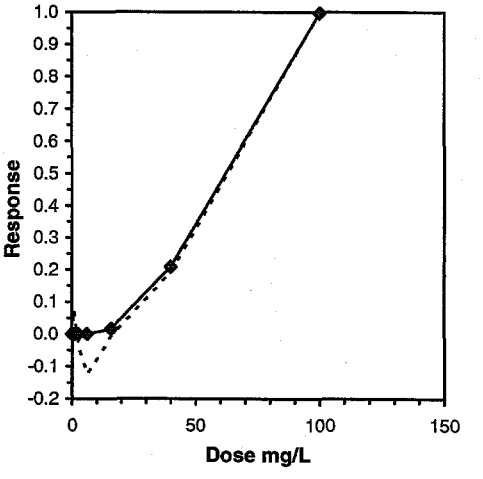
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	277.58	1.0000	277.58	256.00	289.50	4.478	6				283.94	1.0000
0.94	256.50	0.9240	256.50	213.50	305.00	17.933	3	0.757	2.655	73.91	283.94	1.0000
2.6	289.67	1.0435	289.67	252.00	315.50	11.518	3	-0.434	2.655	73.91	283.94	1.0000
6.2	312.00	1.1240	312.00	251.00	408.00	26.971	3	-1.236	2.655	73.91	283.94	1.0000
16	279.33	1.0063	279.33	245.00	333.00	16.854	3	-0.063	2.655	73.91	279.33	0.9838
40	224.50	0.8088	224.50	206.00	236.00	7.207	3	1.907	2.655	73.91	224.50	0.7907
*100	0.92	0.0033	0.92	0.25	1.75	83.320	3	9.938	2.655	73.91	0.92	0.0032

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9387	0.884	0.86836	1.81021
Bartlett's Test indicates unequal variances (p = 7.61E-04)	23.1085	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	40	100	63.2456		73.9115	0.26627	34847.9	1549.98	3.3E-04	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	20.199	6.562	0.000	24.379	-0.5878
IC10	26.412	5.919	3.015	33.062	-0.7203
IC15	32.626	5.863	4.914	44.646	-0.8575
IC20	38.840	4.356	21.065	46.570	-0.8356
IC25	43.099	2.872	30.969	49.667	-0.6212
IC40	54.528	2.121	46.607	59.767	-0.3041
IC50	62.148	1.767	55.515	66.501	-0.3020



Note that if William's test is used, the NOEC is 16 mg 7-OH metabolite of pyroxsulam/L and the 40 and 100 mg/L means are statistically significantly different from the control mean with the NOEC = 16 mg/L. The study report, using William's test, also identified the 40 and 100 mg/L means as statistically significantly reduced compared to the control mean at 96 hours and the NOEC as 16 mg/L.

APPENDIX I (Continued)

Specific growth rate (0-72 hours)

The ToxCalc analysis of the 0-72 hour reviewer calculated growth rate data (Table 7, page 34) gave the following results (growth rate data as day^{-1}).

Conc-mg/L	1	2	3	4	5	6
D-Control	1.6739	1.7316	1.7548	1.5607	1.7644	1.7248
0.94	1.7525	1.6496	1.7716			
2.6	1.8951	1.8668	1.7751			
6.2	1.8248	1.7994	1.7933			
16	1.7925	1.7686	1.7964			
40	1.6408	1.6047	1.6527			
100	0.0000	0.0000	0.0000			

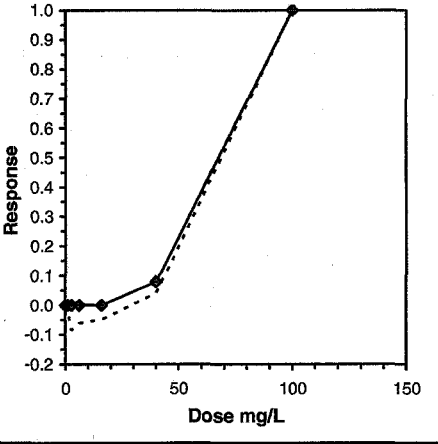
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.7017	1.0000	1.7017	1.5607	1.7644	4.462	6				1.7727	1.0000
0.94	1.7246	1.0134	1.7246	1.6496	1.7716	3.806	3	-0.611	2.655	0.0993	1.7727	1.0000
2.6	1.8457	1.0846	1.8457	1.7751	1.8951	3.397	3	-3.849	2.655	0.0993	1.7727	1.0000
6.2	1.8058	1.0612	1.8058	1.7933	1.8248	0.925	3	-2.784	2.655	0.0993	1.7727	1.0000
16	1.7858	1.0494	1.7858	1.7686	1.7964	0.842	3	-2.249	2.655	0.0993	1.7727	1.0000
40	1.6327	0.9595	1.6327	1.6047	1.6527	1.530	3	1.843	2.655	0.0993	1.6327	0.9210
*100	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3	45.496	2.655	0.0993	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.88587	0.884	-1.4712	3.02476
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 Treatments vs D-Control	40	100	63.2456		0.09931	0.05836	1.34461	0.0028	5.3E-18	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.198	1.853	25.587	38.079	0.5661
IC10	41.371	0.598	39.316	43.111	-0.3546
IC15	44.628	0.548	42.688	46.272	-0.0858
IC20	47.885	0.516	46.059	49.432	-0.0858
IC25	51.142	0.484	49.430	52.593	-0.0858
IC40	60.914	0.387	59.544	62.074	-0.0858
IC50	67.428	0.322	66.287	68.395	-0.0858



Note that the ToxCalc program has treated the calculated 0-72 hour growth rates at 100 mg/L of 0 (nominal), -0.10 and -0.46 and their calculated mean as zero values (this is considered in line with the study report's comment that growth rate cannot be calculated when cell density is zero and a value of zero was entered for all further calculations). The calculated mean value is -0.19 day^{-1}. The 100 mg/L mean result is identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using the Kruskal-Wallis' test, stated no treatment level was significantly reduced compared to the control and, therefore, the NOEC was empirically estimated to be the highest concentration tested with < 10% inhibition.

APPENDIX I (Continued)

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

The ToxCalc analysis of the 0-72 hour reviewer calculated biomass data (Table 8, page 36) gave the following results (biomass data as cell counts X 10^4 cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	132.34	119.17	156.42	90.75	135.25	144.09
0.94	125.75	126.50	152.42			
2.6	205.25	171.50	162.75			
6.2	166.75	177.75	172.50			
16	135.25	148.25	155.75			
40	91.92	87.88	86.17			
100	0.00	0.00	0.00			

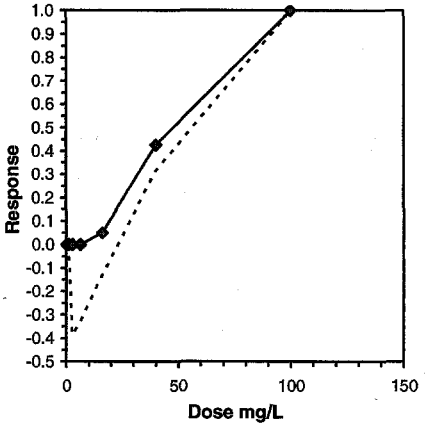
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	129.67	1.0000	129.67	90.75	156.42	17.540	6				154.18	1.0000
0.94	134.89	1.0403	134.89	125.75	152.42	11.256	3	-0.462	2.655	30.02	154.18	1.0000
2.6	179.83	1.3869	179.83	162.75	205.25	12.479	3	-4.436	2.655	30.02	154.18	1.0000
6.2	172.33	1.3290	172.33	166.75	177.75	3.193	3	-3.773	2.655	30.02	154.18	1.0000
16	146.42	1.1292	146.42	135.25	155.75	7.084	3	-1.481	2.655	30.02	146.42	0.9496
*40	88.65	0.6837	88.65	86.17	91.92	3.331	3	3.627	2.655	30.02	88.65	0.5750
*100	0.00	0.0000	0.00	0.00	0.00	0.000	3	11.466	2.655	30.02	0.00	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93992	0.884	-0.428	2.09632
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	16	40	25.2982		30.0238	0.23155	11375.2	255.761	1.8E-09	6, 17
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	15.931	2.968	6.285	20.873	-0.3891
IC10	19.180	2.286	10.259	23.755	-0.6889
IC15	22.383	2.044	14.179	26.721	-0.7003
IC20	25.586	1.804	18.494	29.649	-0.6462
IC25	28.789	1.584	22.847	32.577	-0.5643
IC40	38.398	1.202	34.845	42.602	0.0931
IC50	47.825	1.597	42.611	52.672	-0.1552



The 100 mg/L results have been allocated a zero mean value by the ToxCalc treatment of the initial data. The actual calculated values were -1.00, -1.63 and -0.13 with a calculated mean of -0.91 X 10^4 cells/mL.

The 40 and 100 mg/L mean 0- 72 hours' results are identified as statistically significantly less than the control mean at that time (Bonferrroni's t test). The study report, using William's test, also identify the 40 and 100 mg/L means as statistically significantly reduced compared to the control.

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

Cell density at 24 hours

The ToxCalc analysis of the 24 hour algal cell count data without the 100 mg/L results gave the following results using mean measured concentrations of 7-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	112500	120000	62500	57500	50000	45000
0.94	50000	67500	45000			
2.6	55000	62500	45000			
6.2	52500	45000	40000			
16	65000	85000	50000			
40	67500	37500	25000			

			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	74583.33	1.0000	74583.33	45000	120000	44.135	6				74583.3	1.0000
0.94	54166.67	0.7263	54166.67	45000	67500	21.811	3	1.291	2.602	41151.7	55208.3	0.7402
2.6	54166.67	0.7263	54166.67	45000	62500	16.209	3	1.291	2.602	41151.7	55208.3	0.7402
6.2	45833.33	0.6145	45833.33	40000	52500	13.727	3	1.818	2.602	41151.7	55208.3	0.7402
16	66666.67	0.8939	66666.67	50000	85000	26.339	3	0.501	2.602	41151.7	55208.3	0.7402
40	43333.33	0.5810	43333.33	25000	67500	50.405	3	1.976	2.602	41151.7	43333.3	0.5810

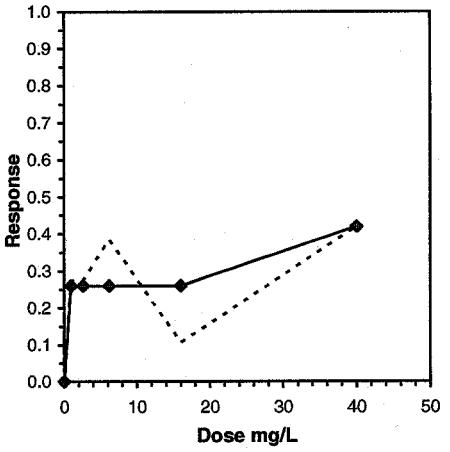
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9443	0.873	0.84766	0.45679
Bartlett's Test indicates equal variances (p = 0.20)	7.28132	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	40	>40			41151.7	0.55175	6.1E+08	5E+08	0.3508	5, 15
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05*	0.181			
IC10*	0.362			
IC15*	0.543			
IC20*	0.724			
IC25*	0.905			
IC40	37.137			
IC50	>40			

* 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 100 mg/L results gave the following results using mean measured concentrations of 7-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	477500	195000	560000	335000	332500	537500
0.94	272500	517500	487500			
2.6	550000	325000	580000			
6.2	447500	652500	625000			
16	230000	415000	437500			
40	190000	250000	150000			

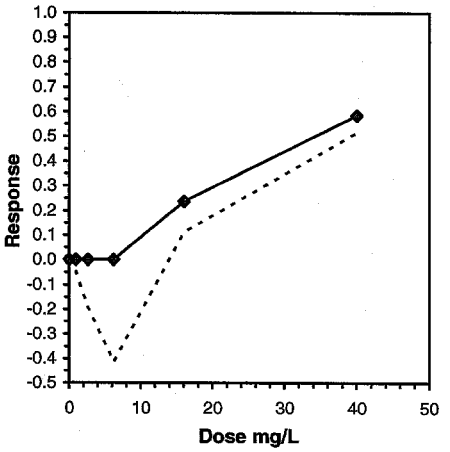
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	406250	1.0000	406250	195000	560000	35.000	6				473021	1.0000
0.94	425833.3	1.0482	425833.3	272500	517500	31.382	3	-0.223	2.602	228569	473021	1.0000
2.6	485000	1.1938	485000	325000	580000	28.737	3	-0.897	2.602	228569	473021	1.0000
6.2	575000	1.4154	575000	447500	652500	19.351	3	-1.921	2.602	228569	473021	1.0000
16	360833.3	0.8882	360833.3	230000	437500	31.555	3	0.517	2.602	228569	360833	0.7628
40	196666.7	0.4841	196666.7	150000	250000	25.593	3	2.386	2.602	228569	196667	0.4158

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90766	0.873	-0.5577	-0.9981
Bartlett's Test indicates equal variances (p = 0.86)	1.95273	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	40	>40			228569	0.56263	4.9E+10	1.5E+10	0.03844	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	8.266	2.879	0.000	23.153	0.9564
IC10	10.332	3.513	4.690	25.742	0.6519
IC15	12.398	3.980	6.265	28.020	0.4858
IC20	14.464	4.409	6.985	30.498	0.4078
IC25	16.887	4.919	7.377	33.382	0.2384
IC40	27.260	5.707	6.175	40.259	-0.5044
IC50	34.175				



APPENDIX II (Continued)

Cell density at 72 hours

The ToxCalc analysis of the 72 hour algal cell count data without the 100 mg/L values gave the following results using mean measured concentrations of 7-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	1516700	1803300	1933300	1080000	1990000	1766700
0.94	1920000	1410000	2033300			
2.6	2945000	2705000	2055000			
6.2	2385000	2210000	2170000			
16	2165000	2015000	2190000			
40	1373300	1232500	1423300			

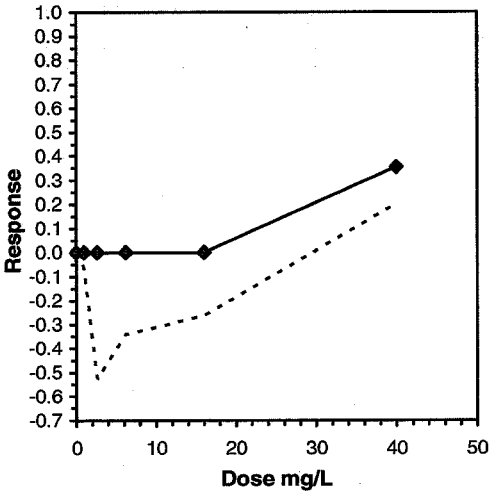
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1681667	1.0000	1681667	1080000	1990000	20.071	6				2083220	1.0000
0.94	1787767	1.0631	1787767	1410000	2033300	18.572	3	-0.514	2.602	537108	2083220	1.0000
2.6	2568333	1.5273	2568333	2055000	2945000	17.929	3	-4.296	2.602	537108	2083220	1.0000
6.2	2255000	1.3409	2255000	2170000	2385000	5.071	3	-2.778	2.602	537108	2083220	1.0000
16	2123333	1.2626	2123333	2015000	2190000	4.458	3	-2.140	2.602	537108	2083220	1.0000
40	1343033	0.7986	1343033	1232500	1423300	7.367	3	1.641	2.602	537108	1343033	0.6447

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91712	0.873	-0.9955	0.79367
Bartlett's Test indicates equal variances (p = 0.19)	7.47513	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	40	>40			537108	0.31939	6.2E+11	8.5E+10	0.00119	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	19.377	1.146	14.672	20.478	-3.4576
IC10	22.755	1.099	17.681	24.955	-1.6332
IC15	26.132	1.252	20.952	29.433	-0.6624
IC20	29.509	1.448	24.731	33.911	-0.0874
IC25	32.887	1.674	27.984	38.388	0.2188
IC40	>40				
IC50	>40				



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 (also 100 mg/L mean measured) values gave the following results using mean measured concentrations of 7-OH metabolite of pyroxsulam:

Conc-mg/L	1	2	3	4	5	6
D-Control	2730000	2755000	2895000	2875000	2560000	2840000
0.94	2135000	3050000	2510000			
2.6	3155000	3015000	2520000			
6.2	2510000	4080000	2770000			
16	2450000	3330000	2600000			
40	2060000	2360000	2315000			

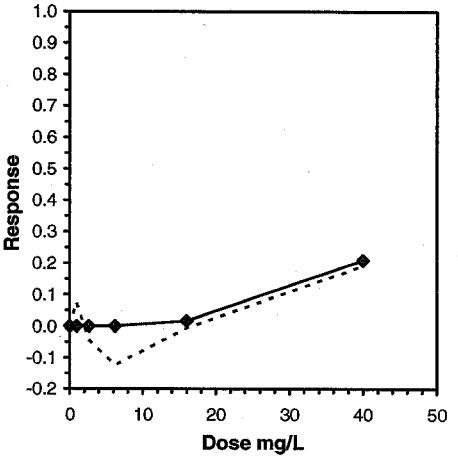
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	2775833	1.0000	2775833	2560000	2895000	4.478	6				2839375	1.0000
0.94	2565000	0.9240	2565000	2135000	3050000	17.933	3	0.711	2.602	771268	2839375	1.0000
2.6	2896667	1.0435	2896667	2520000	3155000	11.518	3	-0.408	2.602	771268	2839375	1.0000
6.2	3120000	1.1240	3120000	2510000	4080000	26.971	3	-1.161	2.602	771268	2839375	1.0000
16	2793333	1.0063	2793333	2450000	3330000	16.854	3	-0.059	2.602	771268	2793333	0.9838
40	2245000	0.8088	2245000	2060000	2360000	7.207	3	1.791	2.602	771268	2245000	0.7907

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9486	0.873	0.82046	1.236
Bartlett's Test indicates equal variances (p = 0.05)	11.2696	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	40	>40			771268	0.27785	2.7E+11	1.8E+11	0.23734	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	20.199	6.725	0.000	25.227	-0.7889
IC10	26.412	5.888	3.129	33.270	-0.6318
IC15	32.626				
IC20	38.840				
IC25	>40				
IC40	>40				
IC50	>40				



Note: When the hypothesis test was done using the Williams' test, the results were the same as those calculated from use of the Bonferroni t-test, with the NOEC being 40 mg 7-OH metabolite of pyroxsulam/L.

APPENDIX II (Continued)

Specific growth rate (0-72 hours)

The ToxCalc analysis of the 0-72 hour study report's growth rate data (Table 7, page 34) with the 100 mg/L (nominal and mean measured) results omitted gave the following results (initial growth rate data as day^{-1}).

Conc-mg/L	1	2	3	4	5	6
D-Control	1.7400	1.8000	1.8300	1.6300	1.8400	1.8000
0.94	1.8300	1.7200	1.8500			
2.6	1.9700	1.9400	1.8500			
6.2	1.9000	1.8700	1.8700			
16	1.8700	1.8400	1.8700			
40	1.7100	1.6700	1.7200			

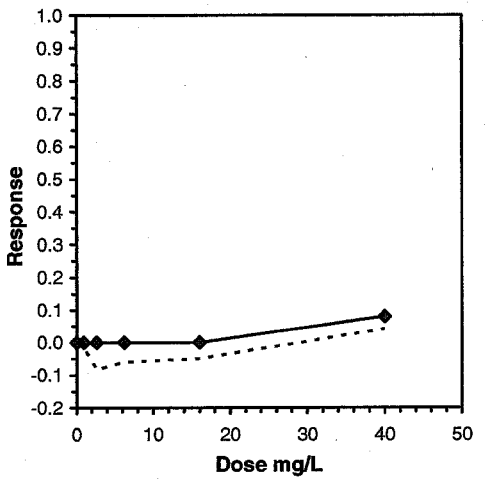
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1.7733	1.0000	1.7733	1.6300	1.8400	4.421	6				1.8467	1.0000
0.94	1.8000	1.0150	1.8000	1.7200	1.8500	3.889	3	-0.647	2.602	0.1072	1.8467	1.0000
2.6	1.9200	1.0827	1.9200	1.8500	1.9700	3.253	3	-3.560	2.602	0.1072	1.8467	1.0000
6.2	1.8800	1.0602	1.8800	1.8700	1.9000	0.921	3	-2.589	2.602	0.1072	1.8467	1.0000
16	1.8600	1.0489	1.8600	1.8400	1.8700	0.931	3	-2.103	2.602	0.1072	1.8467	1.0000
40	1.7000	0.9586	1.7000	1.6700	1.7200	1.556	3	1.780	2.602	0.1072	1.7000	0.9206

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.89728	0.873	-1.3131	2.05433
Bartlett's Test indicates equal variances (p = 0.18)	7.55922	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	40	>40			0.10723	0.06047	0.02052	0.0034	0.00295	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	31.109	1.844	25.986	37.967	0.4839
IC10	>40				
IC15	>40				
IC20	>40				
IC25	>40				
IC40	>40				
IC50	>40				



APPENDIX II (Continued)

Biomass (0-72 hours)

The ToxCalc analysis of the 0-72 hour study report's biomass data (Table 8, page 36) with the 100 mg/L results (nominal and mean measured) omitted gave the following results (biomass data as cells/mL).

Conc-mg/L	1	2	3	4	5	6
D-Control	1265600	1160800	1499500	868600	1311100	1380000
0.94	1222400	1206200	1467400			
2.6	1985800	1671200	1561400			
6.2	1613100	1703600	1654300			
16	1319900	1430800	1504900			
40	893200	849000	841900			

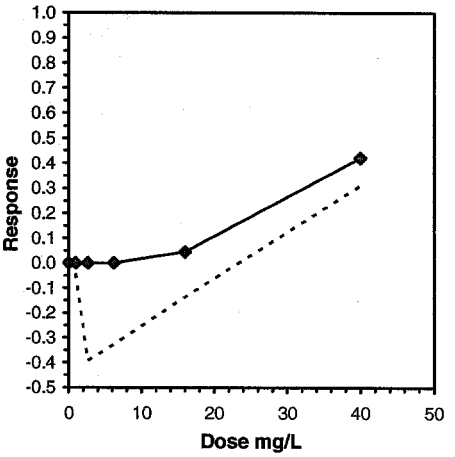
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1247600	1.0000	1247600	868600	1499500	17.433	6				1485683	1.0000
0.94	1298667	1.0409	1298667	1206200	1467400	11.269	3	-0.443	2.602	300279	1485683	1.0000
2.6	1739467	1.3943	1739467	1561400	1985800	12.664	3	-4.263	2.602	300279	1485683	1.0000
6.2	1657000	1.3282	1657000	1613100	1703600	2.734	3	-3.548	2.602	300279	1485683	1.0000
16	1418533	1.1370	1418533	1319900	1504900	6.564	3	-1.481	2.602	300279	1418533	0.9548
*40	861366.7	0.6904	861366.7	841900	893200	3.227	3	3.347	2.602	300279	861367	0.5798

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94872	0.873	-0.4749	1.71243
Bartlett's Test indicates equal variances (p = 0.10)	9.19613	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	16	40	25.2982		300279	0.24069	3.1E+11	2.7E+10	1.0E-04	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	16.307	2.841	6.538	20.734	-0.5927
IC10	19.507	2.155	11.020	23.757	-0.7569
IC15	22.707	1.939	15.321	26.830	-0.7279
IC20	25.907	1.738	19.476	29.942	-0.6620
IC25	29.106	1.555	23.663	33.055	-0.5584
IC40	38.706				
IC50	>40				



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	74600
48	406300
72	1681700
96	2775800