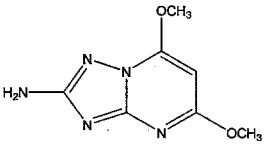
Data Evaluation Report on the Acute Toxicity of the ADTP Metabolite of Pyroxsulam (XDE-742) to Algae, *Pseudokirchneriella subcapitata*

PMRA Submission Number 2006-4727; ID 1283234 EPA MRID Number 469084-46 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:	ADTP Metabolite of Pyroxsulam	Purity: 98%
Common name:	ADTP Metabolite of Pyroxsulam (XDE-742)	
Chemical name:	[1,2,4]triazolo[1,5-a]pyrimidin-2-amine, 5,7-dimethoxy	
IUPAC:	5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-amine	
CAS name:	[1,2,4]triazolo[1,5-a]pyrimidin-2-amine, 5,7-dimethoxy	
Synonyms:	ADTP. X666738	

Chemical Structure:



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

Secondary Reviewers: Jack Holland 22/2/08 **Date:** 27 June 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Émilie Larivière **Date:** 5 July 2007 05/03/08
Environmental Assessment Directorate, Pest Management Regulatory Agency, Health Canada

Christopher Salice **Date:** 12 September 2007
Environmental Fate and Effects Division, US Environmental Protection Agency 4/03/08

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

CITATION: Hoberg, J R. (2006): ADTP 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. 12550.6414 and Sponsor Protocol/Project No. 050111. The Dow Chemical Company Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana 46268. 5 April 2006. Unpublished report

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CAS name:	[1,2,4]triazolo[1,5-a]pyrimidin-2-amine, 5,7-dimethoxy	
Synonyms:	ADTP. X666738	

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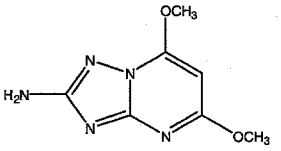

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EXECUTIVE SUMMARY: In a 96-hour acute toxicity study, the freshwater green alga (*Pseudokirchneriella subcapitata,* formerly known as *Selenastrum capricornutum*) was exposed to the ADTP metabolite of pyroxsulam at nominal concentrations of 0 (control), 6.13, 13, 25.0, 50.0, and 100 mg/L (corresponding mean measured: 0 (control), 5.7, 12, 23, 47 and 92 mg/L, respectively). The experiment was carried out in accordance with OECD Guideline for Testing of Chemicals #201, Alga, Growth Inhibition Test (OECD, 1984), EC Guideline L383A, Method C.3, Algal Inhibition Test (EC, 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 (U.S. EPA, 1982). The study was conducted in Algal Assay Procedure (AAP) medium with continuous illumination. Treatment groups were set in triplicate and the medium control group contained six replicates, with an initial cell density of approximately 10,000 cells/mL. Temperatures during the exposure period ranged from 22 to 24°C. The light intensity ranged from 3900 to 4700 lux. The pH values ranged from 6.8 to 6.9 at test initiation, and from 8.4 to 9.0 at test termination. Cell counts were conducted every 24 hours.

After 96 hours, inhibition of cell density relative to the control mean ranged from -37% (i.e. growth stimulation) at 5.7 mg ADTP metabolite of pyroxsulam/L to 18% at 92 mg/L. Inhibition of biomass relative to controls at 72 hours ranged from -6% at 47 mg ADTP metabolite of pyroxsulam/L to 28% at 23 mg/L. Inhibition of mean specific growth rate relative to controls at 72 hours ranged from -1% at 47 mg ADTP metabolite of pyroxsulam/L to 6% at 23 and 92 mg/L. Since no concentration tested resulted in ≥ 50% inhibition of cell density, 72-hour total biomass or 72-hour average growth rate, the EC50 values for each endpoint were empirically estimated to be > 92 mg ADTP metabolite of pyroxsulam/L, the highest mean measured concentration tested. The 96 hour NOEC for cell density and the 72 hour NOECs for total biomass and average growth rate were all set at 92 mg ADTP metabolite of pyroxsulam/L..

This study is classified as acceptable and satisfies the guideline requirements for an acute toxicity study with freshwater green alga.

Results Synopsis
Test Organism Size/Age: *Pseudokirchneriella subcapitata*
Test Type: Static without renewal

Results Based on Mean Measured Concentrations and expressed as mg ADTP metabolite of pyroxsulam/L:

Hours	EC Type	EC Value (mg ADTP metabolite of pyroxsulam/L)	95% Confidence Limits (mg ADTP metabolite of pyroxsulam/L)	NOEC (mg ADTP metabolite of pyroxsulam/L)
0-72	ErC50	>92	Not determined	92
	EbC50	>92	Not determined	92
96	EC50	>92	Not determined	92

I. MATERIALS AND METHODS

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

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

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

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

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

The protocol states that the control mean coefficient of variation (CV) for the section-by-section growth rates should not exceed 35% and the CV for the 0- to 72-hour average growth rate should not exceed 7%. The results of this test indicated that the CV for the 0-to 24-hour growth rate of the control was 54%. All other CV values for growth rate were within the required limits. Of the six control replicates, replicate A was considered an outlier, and was dropped from the mean and statistical analysis which then provided a 0 to 24-hour CV value of 23%, which is within the above criterion. Therefore, the NOEC, EC25 and EC50 calculations were repeated excluding control replicate A (N = 5).

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

> OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris, France. 41 pp. and
>
> Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

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

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

A. MATERIALS:

1. Test Material:	**ADTP Metabolite of XDE-742**
Description:	Solid
Lot No./Batch No.:	104425/TSN 103822
Purity:	98%
Stability of Compound Under Test Conditions:	**Stable.** Test substance concentrations were measured at 0 hour (test initiation) and 96 hours (test termination). Measured concentrations closely approximated the desired nominal concentrations, decreased slightly between sampling intervals, but maintained the expected concentration gradient. Mean measured concentrations ranged from 90 to 96% of nominal concentrations over the 96 hour period.
Storage conditions of test chemicals:	Stored at room temperature in the original container in a dark ventilated cabinet.
Physicochemical properties of ADTP metabolite of pyroxsulam:	The study report stated that determination of stability and characterization, verification of the test substance identity, maintenance of records on the test substance, and archival of a sample of the test substance are the responsibility of the Study Sponsor. Consequently, the physicochemical parameters for water solubility, vapour pressure, UV absorption, pKa and Kow were not presented in the study report.
2. Test organism:	The freshwater green alga
Species:	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Class:	Chlorophyceae
Strain:	1648
Source:	In-house stock cultures originally obtained from the University of Texas, Austin, Texas.
Age of inoculum:	7 days
Method of cultivation:	The culture was maintained in a temperature-controlled environmental chamber under continuous light. Stock cultures of this organism were maintained aseptically by periodic transfer into sterile medium. The culture used for this test was maintained under the same conditions as those used for testing.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study:
An initial preliminary range-finding exposure was conducted at nominal ADTP metabolite of pyroxsulam concentrations of 0.010, 0.10, 1.0, 10 and 100 mg/L, and a control. Two replicates were established for each concentration and the control. At test termination, cells exposed to all treatment levels tested and the control were observed to be normal. Following 96 hours of exposure, cell densities in the 0.010, 0.10, 1.0, 10 and 100 mg/L treatment levels averaged 440, 470, 431, 387 and 216 x 10^4 cells/mL, respectively. The control averaged 609 x 10^4 cells/mL.
Since a well-defined concentration response was not achieved, a second preliminary exposure was conducted at the same nominal concentrations (i.e., 0.010, 0.10, 1.0, 10 and 100 mg ADTP metabolite of pyroxsulam/L, and a control) using two replicates for each concentration and the control. At test termination, cells exposed to all treatment levels tested and the control were observed to be normal. Following 96 hours of exposure, cell densities in the 0.010, 0.10, 1.0, 10 and 100 mg/L treatment levels averaged 547, 375, 444, 306 and 149 x 10^4 cells/mL, respectively. The control averaged 606 x 10^4 cells/mL. Due to the unexpected shallow concentration-response observed again, a third range-finding test was conducted.

Due to the unexpected shallow concentration-response observed in the first two preliminary tests, a third range-finding test was conducted at the same concentrations, again with two replicates per treatment level and control. At test termination, cells exposed to all treatment levels tested and the control were observed to be normal. Following 96 hours of exposure, cell densities in the 0.010, 0.10, 1.0, 10 and 100 mg/L treatment levels averaged 316, 300, 316, 266 and 122 x 10^4 cells/mL, respectively. The control averaged 243 x 10^4 cells/mL. Based on these results, and consultation with the Study Sponsor, the nominal ADTP metabolite of XDE-742 concentrations of 6.3, 13, 25, 50 and 100 mg/L were selected for the definitive exposure.

The study report stated that the shallow response curve observed in the initial two preliminary tests was believed to be due to a combination of stimulation of cell growth by the test substance at sub-toxic levels and the high light intensity required for 72-hour tests. Since this study required a 96-hour exposure, cell density was only determined at 96 hours. Consequently, it was believed that the alga exceeded the growth medium in the test solutions before the 96-hour observation and declined to below the control density, yielding the apparent shallow-response curve. The study report stated that this was supported by other concurrent testing under these conditions where cell density was monitored on a daily basis, and consequently, the light intensity was reduced during the definitive tests to the range required by the U.S. EPA for a 96-hour test.

b. Definitive Study
The definitive test was conducted from 20 to 24 February 2006.

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

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*

Acclimation period:	The inoculum used to initiate the toxicity test with ADTP metabolite of pyroxsulam was taken from a stock culture that had been transferred to fresh medium seven days before testing.	See deviations/deficiencies table, page 36 of this DER with respect to the OECD's 2-4 day requirement. OECD 201 states that an inoculum culture in the test medium is prepared 2-4 days before start of the test with the inoculum culture incubated under the same conditions as the test cultures. US EPA OPPTS 850.5400 states that the test begins when algae (inocula) from 3 to 7 day–old stock cultures are placed in the test chambers containing test solutions having the appropriate concentrations of the test substance. This guideline also states that toxicity testing should not be performed until algal cultures are shown to be actively growing (i.e. capable of logarithmic growth within the test period) in at least two subcultures lasting 7 days each prior to the start of the definitive test. *EPA recommends two week acclimation period.* *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.* These template requirements are noted but are not considered further in the light of the specific guideline requirement of the OECD and US EPA OPPTS.

<table>
<tr>
<td>Culturing media and conditions: (same as test or not)</td>
<td>Culturing medium was the same as the test medium (AAP medium in both cases)
.
Conditions same as test (see following comparisons).

<table>
<tr><th>Parameter</th><th>Culture</th><th>Test</th></tr>
<tr><td>Temperature :</td><td>24 ± 2°C</td><td>24 ± 2°C</td></tr>
<tr><td>Light (lux):</td><td>3900 to 4700 lux</td><td>3800 to 4700 lux</td></tr>
<tr><td>Photoperiod:</td><td>Continuous (24 hours light/day)</td><td>Continuous (24 hours light/day)</td></tr>
<tr><td>Medium:</td><td>AAP</td><td>AAP</td></tr>
<tr><td>pH range:</td><td>7.5 ± 0.1</td><td>7.5 ± 0.1</td></tr>
<tr><td>Culture Volume:</td><td>100 mL</td><td>100 mL</td></tr>
<tr><td>Culture Vessel:</td><td>250 mL glass flask</td><td>250-mL flask</td></tr>
<tr><td>Culture Vessel Cap:</td><td>Stainless steel caps which permitted gas exchange.</td><td>Not identified</td></tr>
<tr><td>Agitation</td><td>Continuous (100 rpm)</td><td>Continuous (100 rpm)</td></tr>
<tr><td>Growth conducted in:</td><td>Environ-mental chamber</td><td>Environ-mental chamber</td></tr>
</table>
</td>
<td>Parameter considered met.</td>
</tr>
<tr>
<td>Health: (any mortality observed)</td>
<td>No phytotoxicity effects noted.

Observations of the health of the algal cells were made at each 24-hour interval.

The satisfactory growth of the controls indicates the algal health was acceptable at the start of the study.</td>
<td>Parameter considered met.

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

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

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

Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass flasks 250 mL 100 mL	Parameter considered met. OECD 201 states that the test vessels will normally be glass flasks of dimensions that allow a sufficient volume of culture for measurements during the test and a sufficient mass transfer of CO_2 from the atmosphere. US EPA OPPTS 850.5400 states Erlenmeyer flasks should be used for test containers and may be of any volume between 125 and 500 mL as long as the same size is used throughout a test and the test solution volume does not exceed 50 percent of the flask volume. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name	Algal Assay Procedure (AAP) medium. Medium details provided in the study report were considered equivalent to the AAP medium composition recorded in OECD 201 with the following exception: The test medium contained sodium selenate at 1.88 µg/L. The study report noted this was an additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.	See deviations/deficiencies table, page 36 of this DER with respect to use of sodium selenate. OECD 201 refers to AAP medium and provides a comparison (Annex 3 of the Guidelines) of the US EPA AAP medium and the OECD 201 medium. The guideline identifies both as suitable growth media. US EPA OPPTS 850.5400 does not specifically refer to media composition, instead referring to other sources for this information. *EPA recommends 20-AAP medium and no chelators.* This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements which allow use of chelating agents (the AAP medium used contains sodium EDTA as a chelating agent).

pH at test initiation and at test termination:	**Time: 0 h 96 h** **Control** 6.8 8.4 **6.3*** 6.9 8.4 **13** 6.9 9.0 **25** 6.9 8.9 **50** 6.9 8.9 **100** 6.8 8.8 *** mg ADTP metabolite of pyroxsulam/L.** The control pH increased by 1.6 units over the 96 hours of the exposure period. pH was measured at test initiation and at the termination of the 96-hour exposure period. Measurements at test initiation were conducted on the test solution remaining after the individual test flasks had been filled. At test termination, after cell counts were completed, the three replicate solutions for each treatment and the six controls were respectively composited for pH.	See deviations/deficiencies table, page 36 of this DER with respect to the pH change observed over 96 hours and the initial control pH value. The changes in the control pH are greater than recommended by the OECD 201 (2006) which recommends that the (control) medium pH should not increase by greater that 1.5 pH units during the test. US EPA 850.5400 states that the initial pH of the nutrient medium is to be 7.4 to 7.6 and notes that if the test chemical is highly acidic and reduces the pH of the test solution below 5.0 at the first measurement, appropriate adjustments to pH should be considered, The latter was not the situation in the study under assessment. This increase in pH is considered a common occurrence, especially when there is no inhibition. *OECD recommends the medium pH after equilibration with air be ~8 with less than 0.001 mmol/L chelator, if used.* This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements with respect to medium pH and specified concentrations of chelating agents.

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

Dilution water source/type:	Source not identified in study report. The water used to make up the medium was sterile and deionised. (The study profile template, Hoberg, 2006, referred to well water being the source of the deionised water used for algal medium preparation).	Dilution water parameters considered met.
pH:	The pH of the culture medium was adjusted to pH 7.5 ± 0.1	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Salinity (for marine algae):	N/A for a freshwater alga.	*EPA salinity: 30-35 ppt.*
Water pretreatment (if any):	Sterile, deionised water was used to prepare the AAP growth medium.	No specific requirement identified in the guidelines.
Total Organic Carbon:	A representative sample of AAP medium was analysed monthly for total organic carbon (TOC) concentration. The TOC concentration of the sample collected in February 2006 was 0.63 mg/L. Note that the study profile template refers to a TOC of 0.25 mg/L, which is understood to be an error.	No specific requirements identified for these parameters (TOC, particulate matter, metal and pesticides and chlorine levels) in OECD 201 or US EPA OPPTS 850.5400 other than OECD 201 refers to use of deionised water to prepare the growth media while the US EPA guideline refers to use of water of sufficient quality (e.g. ASTM Type I water) to prepare the nutrient medium. The successful maintenance of the algae and their acceptable growth in the controls indicate the dilution water was of acceptable quality.

Particulate matter and metals and pesticides:	Within acceptable limits Representative samples of the dilution water source used in the preparation of the culture medium were reported analysed periodically for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were reported as having been detected at concentrations that were considered toxic in any of the water samples analysed in agreement with ASTM guidelines (2002).	
Chlorine:	Below detectable limits	*EPA is against the use of dechlorinated water.* Sterile, deionised water was used to prepare the AAP medium.
Water pretreatment (if any): Intervals of water quality measurement	Not reported. Routine dilution water contaminant screening and analyses for pesticides, PCBs and toxic metals were conducted using standard US EPA procedures by GeoLabs, Inc. Braintree, Massachusetts.	No specific requirement identified in the guidelines.

Indicate how the test material is added to the medium (added directly or used stock solution)	A 100 mg ADTP metabolite of pyroxsulam/L stock solution was prepared prior to test initiation by placing 0.2041 g of ADTP metabolite of pyroxsulam (0.2000 g as active ingredient) in a 2000-mL volumetric flask and bringing it to volume with AAP medium. The resulting stock solution was observed to be clear and colourless with a large amount of visible undissolved test substance. Following approximately ten minutes of sonication and an additional two hours of mixing with a magnetic stir plate and Teflon®-coated stir bar, the stock solution was observed to be clear and colourless with no visible undissolved test substance. Test solutions were prepared from the 100 mg/L stock solution by serial dilutions.	Parameter considered met. Note that the nominal test concentrations were adjusted for the 98% purity of the test substance.
Aeration or agitation	Continuous agitation at approximately 100 revolutions/minute (rpm).	Parameter considered met. OECD 201 states that during the test it is necessary to keep the algae in suspension and to facilitate transfer of CO_2. To this end constant shaking or stirring should be used and reference is made to an orbital or reciprocate shaker table being used at ~150 rpm. US EPA OPPTS 850.5400 states that test containers should be placed on a rotary shaking apparatus and oscillated at approximately 100 cycles/min for *Selenastrum*.

Initial cell density	Approximately 10,000 cells/mL (for each replicate).	Parameter considered met. Initial cell density considered acceptable. OECD 201 recommends an initial cell concentration for *Pseudokirchneriella subcapitata*: of 5 x 10^3 - 10^4 cells/mL. US EPA OPPTS 850.5400 states that each test chamber in the definitive study should contain equal volumes of test solution and approximately 1 x 10^4 *Selenastrum* cells per millilitre of test solution. *EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Number of replicates Control: Solvent control: Treatments:	6 replicates inoculated with algae. N/A 3 replicates/treatment level inoculated with algae, One extra replicate was prepared at 25 mg/L without algae to allow determination of the effect of the algal cells on uptake/degradation of the ADTP metabolite of pyroxsulam.	Parameter requirements considered met. The numbers of replicates used are acceptable. OECD 201 states that the test design should include three replicates at each test concentration and that the number of control replicates must be at least three, and ideally should be twice the number of replicates used for each test concentration. US EPA 850.5400 states that a minimum of three replicates is required for each concentration of test chemical and control. A solvent control was not used. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal:	Nominal concentrations were 0 (control), 6.3 13, 25.0, 50.0 and 100 mg ADTP metabolite of pyroxsulam/L. These concentrations are in the ratios of 1:92 to 1:2.06.	Nominal and measured test concentrations parameter considered met. The mean measured concentrations were 96-100% of nominal. There was no affect from algal cells on measured concentration of the test compound. OECD 201 states that, for the final definitive test, at least five concentrations, arranged in a geometric series with a factor not exceeding 3.2, should be selected.

		The OECD guideline also states that, the concentration series should preferably cover the range causing 5-75 % inhibition of algal growth rate. US EPA OPPTS 850.5400 states algae should be exposed to five or more concentrations of the test chemical in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, and 64 mg/L). The nominal concentration ratios of 1:92 to 1:2.06 are considered to meet this criterion. *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.* These template requirements are noted but are not considered further in the light of the OECD and US EPA OPPTS having equivalent requirements.

Measured:	Mean measured concentrations at 0 and 96 hours were as follows: **Nom. Conc.[a] / 0 hours / 96 hours / Mean / % of nom.** Control / <0.22 / <0.20 / NA[b] / NA 6.3 / 6.0 / 5.4 / 5.7 / 91 13 / 13 / 12 / 12 / 96 25 / 23 / 22/25[c] / 23 / 90 50 / 48 / 46 / 47 / 94 100 / 94 / 90 / 92 / 92 **a. as mg ADTP metabolite of pyroxsulam/L.** **b. NA = not applicable.** **c. Result of the additional sample without algae present to determine biological uptake or degradation.** The mean (measured concentrations) and % of nominal results are based on actual analytical (unrounded results) and not the rounded values shown in the table. In the quality control samples (3.00, 20.0 and 100 mg ADTP metabolite of pyroxsulam/L), time 0 recoveries were 93.9 to 94.1% of nominal and, at 96 hours, 83.7 (3.00 mg/L) to 99.1% of nominal.	
Solvent (type, percentage, if used)	N/A; a solvent was not used	The parameter is not relevant as a solvent was not used. OECD 201 and US EPA OPPTS 850.5400 allow, but do not require, the use of solvents.
Method and interval of analytical verification Limit of detection: Limit of quantitation:	All exposure solutions and QC samples were analysed for ADTP metabolite of pyroxsulam using high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV) based on methodology validated at Springborn Smithers. Limit of detection not reported. The limit of quantitation was 0.012 mg ADTP metabolite of pyroxsulam/L Test solutions were analyzed for the presence of pyroxsulam at 0 and 96 hours.	Parameter considered met. The method validation study established an average recovery of 100 ± 2.73% for ADTP metabolite of pyroxsulam from 20X AAP medium (a freshwater algal medium). Defined limits for acceptance of quality control sample performance in subsequent studies with ADTP metabolite of pyroxsulam were set at 80 to 120%. Conditions and procedures used

		throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation study. Representative chromatograms from the analysis of a calibration standard, recovery sample and a control sample are presented as was a typical linear regression analysis for ADTP metabolite of pyroxsulam (response versus concentration) which had an r^2 value of 1.000.

Test conditions Temperature:	 22 to 24°C	The test conditions meet US EPA and OECD Guidelines OECD 201 states the cultures should be maintained at a temperature in the range of 21 to 24°C, controlled at ± 2°C. The 1984 OECD guideline set the range as 21 to 25°C. US EPA OPPTS 850.5400 states the test temperature is to be 24°C for *Selenastrum* and that excursions from the test temperature should be no greater than ± 2°C. *EPA temperature: Skeletonema: 20°C, Others: 24-25°C.* *OECD recommended the temperature be in the range of 21 to 25°C maintained at ± 2°C.* These template requirements are noted, but not considered further in the light of the OECD and US EPA OPPTS having equivalent requirements.
Photoperiod:	Continuous	Photoperiod requirement considered met. OECD 201 refers to use of continuous light while US EPA OPPTS 850.5400 refers to test chambers containing *Selenastrum*, *Navicula*, and *Anabaena* being illuminated continuously. *EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous. OECD recommends and continuous uniform illumination.*
Light intensity and quality:	3900-4700 lux (See the section on preliminary testing, page 5 of this DER for an explanation as to why this light intensity range was used).	See deviations/deficiencies table, page 36 of this DER with respect to meeting OECD and US EPA requirements. OECD 201 (2006) refers to light intensity at the level of the test solutions from the range of 60-120

		$\mu E \cdot m^{-2} s^{-1}$, which it states is equivalent to a range of 4440-8880 lux. US EPA light intensity requirement not met (US EPA OPPTS 850.5400 states fluorescent lights providing 4.3 Klux are to be used for *Selenastrum.* *OECD states approximately 8000 Lux measured with a spherical collector.* *EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* These template requirements are noted but not considered further in the light of the OECD and US EPA OPPTS having specific requirements.
Reference chemical (if used) name: concentrations:	 N/A N/A	Not relevant as a reference chemical was not used. OECD 201 notes that a reference substance may be tested as a means of checking test procedures and that this should be done at least twice a year. US EPA OPPTS 850.5400 also states that positive controls 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 of the test solutions was determined at 0 and 96 hours. The reported results were: **Concentration*** / **Conductivity (µmhos/cm)** — **0 h** / **96 h** **Control** 90 80 **6.3** 90 90 **13** 90 90 **25** 90 90 **50** 90 90 **100** 90 90 *** mg ADTP metabolite of pyroxsulam/L.** Conductivity was measured at test initiation and at the termination of the 96-hour exposure period. Measurements at test initiation were conducted on the test solution remaining after the individual test flasks had been filled. At test termination, after cell counts were completed, the three replicate solutions for each treatment and the six controls were respectively composited for conductivity measurements. Observations of the health of the algal cells were made at each 24-hour interval.	Acceptable.

2. Observations:

Table 2. Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density and biomass (cells/mL), growth rate (per day) Cells exposed to all treatment levels tested were observed to be normal throughout the exposure period. pH, temperature, light intensity and concentrations of the ADTP metabolite of pyroxsulam in the test solutions were also determined over the course of the study.	The parameters determined are acceptable and their requirements are considered met. OECD 201 refers to growth and growth inhibition being quantified from measurements of the algal biomass as a function of time. US EPA OPPTS 850.5400 refers to enumeration of the algal cells to determine inhibition or stimulation of growth and the pattern of growth in test containers compared to controls. *EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks Criteria
Measurement technique for cell density and other end points	Single cell counts were conducted using a haemocytometer and a compound microscope. Appropriate instrumental techniques were used for physico-chemical parameters listed above.	Observation intervals considered appropriate and the parameters met. OECD 201 refers to algal biomass in each flask being determined daily. US EPA OPPTS 850.5400 states that at the end of 96 h, and, if possible, at the end of 24, 48, and 72 h, the algal growth response (number or weight of algal cells per millilitre) in all test containers and controls is to be determined by an indirect (spectrophotometry, electronic cell counters, dry weight, etc.) or a direct (actual microscopic cell count of at least 400 cells per flask) method. Indirect methods are to be calibrated by a direct microscopic count or data should be presented that relate electronic counts with microscopic counts. *EPA recommends the measurement technique of cell counts or chlorophyll a.* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (Note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).* These template requirements are noted but not considered further in the light of the 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	At test termination morphological observations at each test concentration	Requirement considered met. Observation made is appropriate

Parameters	Details	Remarks Criteria
Indicate whether there was an exponential growth in the control	Yes. The mean control 72-hour cell growth was 57.7 x 10^4 cells/mL. This represents an approximate 58-fold increase in cell numbers from the original 10,000 cells/mL. At 96 hours, the mean control cell density was 170 X 10^4 cells/mL, i.e. 1.70 X 10^6 cells/mL. The 0 to 72 hour growth rate in the control averaged 1.38 $days^{-1}$.	See deviations/deficiencies table, page 36 of this DER with respect to meeting the US EPA 96 hours cell density requirement. OECD 201 requires, *inter alia,* that biomass in the control cultures should have increased by a factor of at least 16 within the 72 hour test period (note that cell count has been used as the measure of biomass in this situation). OECD 201 also states that the desired increase in biomass corresponds to a specific growth rate of 0.92 day^{-1}. Note that OECD 201 states that *P. subcapitata* is expected to have a growth rate of 1.5 to 1.7 in light intensity of approximately 70 $\mu E/m^2/sec$ at 21°C when grown in OECD medium. US EPA OPPTS 850.5400 states that algal growth in controls should reach the logarithmic growth phase by 96 h (at which time the number of algal cells should be approximately 3.5 X 10^6/mL for *Selenastrum*. The mean measured value of 1.70 X 10^6 cells/mL is ~50% of the US EPA OPPTS 850.5400 value of ~3.5 X 10^6/mL and does not meet this requirement, possibly as a result of the lower light intensity used. *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

Parameters	Details	Remarks Criteria
		preparation from sterile, deionised water.
Were raw data included?	As laboratory data, no. Individual replicate data were presented. The study report notes that all data generated are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.	Parameter considered met. While raw data were not submitted, the tabulated results presented provided individual replicate data which were sufficient to allow statistical analysis by the reviewer. OECD 201 lists the results which must be presented in the test report. These are not considered to necessarily include raw, i.e. laboratory data. The tabulated data presented in the study report are considered to have complied with the OECD requirement. While US EPA OPPTS 850.5400 states that the sponsor must submit to the EPA all data developed by the test including those that are suggestive or predictive of acute phytotoxicity, advice from the US EPA was that, because the tabulated results presented in the study report were sufficient to allow statistical analysis, the guideline would be considered met.

II. RESULTS and DISCUSSION:

INHIBITORY EFFECTS:

After 96 hours, inhibition of cell density relative to the control mean ranged from -37% (i.e. growth stimulation) at 5.7 mg ADTP metabolite of pyroxsulam/L to 18% at 92 mg/L. Inhibition of biomass relative to controls at 72 hours ranged from -6% at 47 mg ADTP metabolite of pyroxsulam/L to 28% at 23 mg/L. Inhibition of mean specific growth rate relative to controls at 72 hours ranged from -1% at 47 mg ADTP metabolite of pyroxsulam/L to 6% at 23 and 92 mg/L.

The 96 hour NOEC for cell density and the 72 hour NOECs for total biomass and average growth rate were all set at 92 mg ADTP metabolite of pyroxsulam/L.

There was a major change in control pH - from 6.8 at time 0 to 8.4 after 96 hours. Increases in pH were also seen in the test solutions (pH values at time 0 were 6.8 and 6.9 and, after 96 hours, 8.4 to 9.0).

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

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

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

Treatment (mean measured concentration (mg ADTP metabolite of pyroxsulam/L)	Initial cell Density, cells/mL	Mean cell density ($x10^4$) and standard deviation in brackets at 24, 48, 72 and 96 hours				
		24 hours	48 hours	72 hours	96 hours	Percent inhibition[1] at 96 hours
Negative control	1 X 10^4	3.55 (1.05)	12.80 (1.51)	57.70 (10.77)	170.42 (65.69)	NA
5.7	1 X 10^4	4.17 (1.61)	13.33 (1.38)	56.50 (4.56)	234.00 (13.43)	-37
12	1 X 10^4	1.42 (0.52)	14.67 (6.37)	58.67 (24.68)	165.58 (54.71)	3
23	1 X 10^4	1.50 (0.25)	9.00 (1.64)	45.17 (3.83)	172.28 (45.78)	-1
47	1 X 10^4	1.33 (0.38)	15.75 (3.91)	61.25 (4.77)	187.28 (33.59)	-10
92	1 X 10^4	2.58 (0.76)	9.08 (0.76)	44.83 (6.87)	140.56 (55.57)	18
Reference chemical (if used): No reference chemical used.						

[1] **Relative to control. Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table. Based on the 0- to 24-hour growth rate criterion, control replicate A was excluded from the mean and all statistical analyses as an outlier.**

No 96 hour exposure mean was statistically significantly different from the pooled control (William's Test, $p \leq 0.05$). Note that control replicate A was excluded from the mean and all statistical analyses as an outlier.

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

Treatment measured concentrations (mg ADTP metabolite of pyroxsulam/L)	Mean Specific Growth Rate per day		Mean Area Under the Growth Curve (Biomass)	
	0-72 hours	Percent Inhibition[1]	0-72 hours	Percent Inhibition[1]
Negative control	1.38	NA	42.56	NA
5.7	1.38	0	43.07	-1
12	1.37	1	42.82	-1
23	1.30	6	30.55	28
47	1.40	-1	45.11	-6
92	1.30	6	31.50	26

[1] **Relative to control.**

No 96 hour exposure specific growth rate or area under the curve mean was statistically significantly different from the respective pooled control (William's Test, $p \leq 0.05$). Note that control replicate A was excluded from both the specific growth rate and biomass means and all statistical analyses as an outlier.

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

Table 5. Statistical endpoint values.

Statistical Endpoint	Cell Density (96 h)	Growth Rate (72 h)	Biomass (72 h)
NOEC (mg ADTP metabolite of pyroxsulam/L)	92	92	92
EC_{50} (mg ADTP metabolite of pyroxsulam/L) (95% C.I. were not calculated)	>92	>92	>92
Reference chemical, if used	No reference chemical used.		

Validity of test

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

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

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

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

With respect to exponential growth, this requirement is considered to have been met (see Table 2, page 26 of this DER under the parameter "Indicate whether there was an exponential growth in the control") because the mean control 72 hour cell growth was 57.7 x 10^4 cells/mL. This represents an approximate 58-fold increase in cell numbers from the original 10,000 cells/mL. While at 96 hours, the mean control cell density was 170 X 10^4 cells/mL, i.e. 1.70 X 10^6 cells/mL. This does not, however, meet the US EPA OPPTS 850.5400 requirement that the cell count at that time should be approximately 3.5 X 10^6 cells/mL for *Pseudokirchneriella subcapitata* at 96 hours. This is expected to be related to the use of light of a lower intensity as a result of the preliminary range finding studies' findings (page 5 of this DER refers).

The 0 to 72 hour growth rate in the control averaged 1.38 $days^{-1}$ with this value meeting the OECD 201 statement that the desired increase in biomass is shown by a specific growth rate of 0.92 day^{-1}.

The 0-24, 24-48 and 48-72 hour control replicate growth rates were calculated from the initial (10,000 cells/mL), 24, 48 and 72 hour cell density counts using the formula shown under "Reported Statistics" on page 31 of this DER. The

values and calculated statistics, including the overall mean % coefficient of variation (%CV) are as shown in Table 6.

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

Reviewer calculated growth rates (/day) for the control replicates (where the study report contained results for the same periods, they are shown in brackets in this table).

Replicate	0-24 h		24-48 h	48-72 h	0-72 hours	
	Reviewer	Study report	Reviewer	Reviewer	Reviewer	Study report
1	0.00	0.00	2.35	1.53	1.29	1.33
2	1.18	1.22	1.24	1.40	1.27	1.30
3	0.92	0.95	1.65	1.34	1.30	1.33
4	1.66	1.72	0.98	1.62	1.42	1.45
5	1.10	1.14	1.58	1.36	1.34	1.38
6	1.32	1.37	1.10	1.78	1.40	1.43
Mean, standard deviation and %CV determined from all six replicate results (results in brackets are from the study report.						
Mean	**1.03**	**1.07**	**1.48**	**1.50**	**1.34**	**1.37**
Standard deviation	**0.56**	**0.58**	**0.50**	**0.17**	**0.06**	**0.06**
%CV	**54.6**	**54**	**33.7**	**11.6**	**4.6**	**4.4**
Mean, standard deviation and %CV determined from five replicate results with replicate A's results excluded:						
Mean	**1.23**	**1.28**	**1.31**	**1.50**	**1.35**	**1.38**
Standard deviation	**0.28**	**0.29**	**0.29**	**0.19**	**0.06**	**0.06**
%CV	**22.6**	**22.6**	**22.4**	**12.9**	**4.7**	**4.6**

The %CV value for the 0-24 hour mean growth rate was calculated as 54.65% (54% reported in the study report), which exceeds the OECD 201 2006 edition requirement of the %CV not exceeding 35%. The 24-48 and 48-72 hour growth rate values do not exceed the 35% value set by the 2006 OECD 201 guideline. Because the %CV value of for the 0-24 hour period exceeded the 35% limit set by the 2006 OECD 201 guideline, it is identified as a deficiency with respect to that guideline (see the deviations table on page 36 of this DER).

The 0-72 h %CV was calculated by the reviewer as 4.6% (mean 1.34, standard deviation 0.06, see page 46 for the data and ToxCalc determinations) which meets the OECD 201 limit of 7% for *Pseudokirchneriella subcapitata*. The study report's %CV for the 0-72 hour period was 4.4% when all replicates were included and 4.6 when replicate A was excluded.

The study report stated that, of the six control replicates, replicate A was considered an outlier (because of the 0-24 hours %CV exceeding 35%), and was dropped from the mean and statistical analysis which provided a 0 to 24 hour CV value of 23%, which is within the above criterion. Therefore, the NOEC, EC25 and EC50 calculations were repeated excluding control replicate A (N = 5). Deletion of this replicate from the data analysis provides a more conservative estimate of the NOEC and EC values than when this data point is included in the analyses. The %CV results for when this omission was made are also shown in Table 6.

In both cases, the reviewer's calculated control growth rate %CV values confirmed the equivalent values given in the study report.

B. REPORTED STATISTICS:

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

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

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

where:

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

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

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

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

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

Based on the results of statistical analysis performed for 96-hour cell density and 72-hour total biomass and average growth rate data, the No-Observed-Effect Concentration (NOEC), the highest test concentration which demonstrated no statistically adverse effect ($p \leq 0.05$) for each parameter when compared to the control data, was determined. The

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s):

Cell counts
Replicate data for cell density were tested (ToxCalc™ v5.0.23j. Copyright 1994-2005 Tidepool Scientific Software, McKinleyville, CA 95519 USA) for normality and homogeneity by, respectively, the Shapiro-Wilk's and Bartlett's tests and for difference between the mean cell counts and mean specific growth rates of the pyroxsulam exposed algae and the mean of the controls by Bonferroni's t test. Differences between the mean biomass results of the ADTP metabolite of pyroxsulam exposed algae and that of the controls were tested by Dunnett's test. All NOEC values were determined using the ToxCalc package.

The ToxCalc results for the 24, 48, 72 and 96 hour cell counts are respectively given on pages 42, 43, 44 and 45 of this DER.

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

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

The ToxCalc analysis of the reviewer calculated 0-72 hour specific growth rates is shown on page 46 of this DER. The ToxCalc analysis identified no statistically significant differences between the control mean's specific growth rate and the means of the replicates containing the various ADTP metabolite of pyroxsulam concentrations.

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

Mean measured concentration as mg ADTP metabolite of pyroxsulam/L	Replicate	Reviewer calculated specific growth rates (day^{-1})	Specific growth rates reported in the study report (day^{-1}) as rounded values.
Control	A	1.29	1.33
	B	1.27	1.30
	C	1.30	1.33
	D	1.42	1.45
	E	1.34	1.38
	F	1.40	1.43
5.7	A	1.33	1.36
	B	1.37	1.41
	C	1.33	1.36
12	A	1.35	1.38
	B	1.19	1.21
	C	1.48	1.51
23	A	1.30	1.33
	B	1.25	1.28
	C	1.26	1.29
47	A	1.36	1.39
	B	1.36	1.39
	C	1.40	1.43
92	A	1.24	1.27
	B	1.32	1.35
	C	1.23	1.26

A t-test (results not shown) with the Microsoft Excel data analysis function of the reviewer calculated and study report's specific growth rates indicated that there was no statistically significant difference between the two sets of results. The t statistic calculated was -43 with the one tail and two tail critical t values being, respectively, 1.72 and 2.09:

0-72 Hour biomass (area under the curve)

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

Table 8. Comparison of reviewer calculated and study report 0-72 hour biomass values (as cells/mL).

Mean measured concentration as mg ADTP metabolite of pyroxsulam/L	Replicate	Reviewer calculated 0-72 h biomass values	0-72 h biomass values as reported in the study report (rounded values)
Control	A	332500	332200
	B	347500	346100
	C	377500	376200
	D	520000	518100
	E	431250	429700
	F	458750	457700
5.7	A	396250	395100
	B	491250	488900
	C	410000	408400
12	A	383750	383500
	B	267500	266700
	C	636250	634500
23	A	330000	329700
	B	308750	308000
	C	278750	278800
47	A	481250	479500
	B	416250	415400
	C	458750	458300
92	A	281250	280800
	B	363750	362900
	C	302500	301400

The ToxCalc analysis of the reviewer calculated 0-72 hour biomass results is shown on page 47 of this DER. The ToxCalc analysis identified no statistically significant differences between the control mean's biomass and the biomass means of the replicates containing the various ADTP metabolite of pyroxsulam concentrations.

A t-test with the Microsoft Excel data analysis function (results not shown) indicated that there was a statistically significant difference between the two sets of results. The t statistic calculated was 7.5 with the one tail and two tail critical t values being, respectively, 1.72 and 2.09.

t-Tests of the 0-24 hour, 24-48 hour and 48-72 hours reviewer calculated and study report biomass results (values not shown in this DER) showed that for the 0-24 and 24-48 hours biomass results identified statistically significant differences between the reviewer calculated and study report results (respective t values were 5.09 and 14.8, t

critical for one and two tail tests were 1.72 and 2.09 respectively), whereas there was no statistically significant difference identified for the 48-72 hour biomass results (t score was -20.9).

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

The endpoints reported in the study report and those calculated in the assessment of the study are equivalent with both sets of results shown in Table 9.

Table 9. Reported and reviewer calculated toxicity endpoints.

Toxicity endpoint	Endpoint value as mg ADTP metabolite of pyroxsulam/L (95% confidence limits)	
0-72 hour mean specific growth rate	**As presented in the study report**	**As calculated by the reviewer using the ToxCalc program**
ErC50	>92 (95% Confidence intervals not calculated)	>92 (95% Confidence intervals not calculated)
NOEC	92 (William's test)	92 (Bonferroni's t-test)
0-72 hour biomass		
EbC50	>92 (95% Confidence intervals not calculated)	>92 (95% Confidence intervals not calculated)
NOEC	92 (William's test)	92 (Bonferroni's t-test)
96 hour cell density		
EC50	>92 (95% Confidence intervals not calculated)	>92 (95% Confidence intervals not calculated)
NOEC	92 (William's test)	92 (Bonferroni's t-test)

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
Acclimation period:	The inoculum used to initiate the toxicity test with ADTP metabolite of pyroxsulam was taken from a stock culture that had been transferred to fresh medium seven days before testing.	OECD 201 states that an inoculum culture in the test medium is prepared 2-4 days before start of the test with the inoculum culture incubated under the same conditions as the test cultures.	US EPA OPPTS 850.5400 states that the test begins when algae (inocula) from 3 to 7 day–old stock cultures are placed in the test chambers containing test solutions having the appropriate concentrations of the test substance. This guideline also states that toxicity testing should not be performed until algal cultures are shown to be actively growing (i.e. capable of logarithmic growth within the test period) in at least two subcultures lasting 7 days each prior to the start of the definitive test.
Details of growth medium name	The AAP test medium contained sodium selenate at 1.88 µg/L. The study report noted this was an additional nutrient required, personal communication. Dr. R.R.L. Guillard, June 1991.	OECD 201 refers to AAP medium and provides a comparison (Annex 3) of the US EPA AAP medium and the OECD 201 medium. The guideline identifies both as suitable growth media. OECD 201 states that sodium selenate is to be used only in medium for stock cultures of diatom species.	US EPA OPPTS 850.5400 states that formulation of nutrient medium used for algal culture and preparation of test solutions should conform to those currently recommended by the EPA for freshwater and marine algal bioassays
pH at test initiation and at test termination:	Initial control pH = 6.8 (time 0) 96 hour control pH = 8.4 Increase in pH over 96 hours 1.6 units	OECD recommends (2006) the (control) medium pH should not increase by greater that 1.5 pH units during the test	No specific requirement with respect to pH change in the controls
Light intensity and quality:	3900-4700 lux	OECD 201 (2006) refers to light intensity at the level of the test solutions from the range of 60-120 $\mu E \cdot m^{-2} s^{-1}$, which it states is equivalent to a range of 4440-8880 lux.	The US EPA OPPTS 850.5400 guideline states fluorescent lights providing 4300 lux are to be used for *Selenastrum.*
Indicate whether there was an exponential	At 96 hours, the mean control cell density was 170 X 10^4 cells/mL, i.e. 1.70 X 10^6 cells/mL.	Does not specify a 96 hour cell density.	US EPA OPPTS 850.5400 states that algal growth in controls should reach the logarithmic growth phase by 96 h (at which

growth in the control	(Initial cell density was ~1 X 10^4 cells/mL.		time the number of algal cells should be approximately 3.5 X 10^6 cells/mL for *Selenastrum*.
Validity of test	The %CV value for the 0-24 hour period of 54% exceeded the 35% limit set by the 2006 OECD 201 guideline.	OECD 201 (2006) requires that, for the test to be valid, the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	No %CV requirement.

The inoculum used to initiate the toxicity test with ADTP metabolite of pyroxsulam was taken from a stock culture that had been transferred to fresh medium seven days before testing. The seven days period complies with the US EPA OPPTS 850.5400 requirement for this parameter but not that guideline's reference to growth within the test period) being demonstrated in at least two subcultures lasting 7 days each prior to the start of the definitive test. The seven day period also exceeds the 2 to 4 days period specified by OECD 201. However, OECD 201 also states, in its Annex 4, that the incubation period is normally 2 to 4 days which is taken to allow other incubation periods. The successful growth of the controls provides further evidence that the use of the 7 day old inocula was unlikely to have had any significant adverse effects on the study's conduct or outcomes.

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

The change in the pH of the controls from 6.8 at day 0 to 8.4 at day 4 represents an increase of 1.6 pH units and is marginally 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. The satisfactory exponential growth of the control alga is also taken to indicate that the pH increase did not adversely affect growth. It is not immediately obvious why the control pH at time 0 was 6.8 as the medium had been adjusted to a pH of 7.5. It is possible that this initial pH was made after the addition of the algal cultures, the study report did not appear to clearly identify this point, but it was possibly after the addition of the algae which could have resulted in the pH change. The initial pH of 6.8 is not considered to have had any adverse effect on the study or its outcomes.

As noted in the reporting of the results of the range finding studies (page 5 of this DER refers), the light intensity was reduced during the definitive tests because of a shallow response curve observed in the initial two preliminary tests. This response was believed to be due to a combination of stimulation of cell growth by the test substance at sub-toxic levels and the high light intensity required for 72-hour tests and resulted in the algal growth's exceeding the nutrients provided by the growth medium in the test solutions. This result is considered unexpected given the acceptability of the OECD 201 and US EPA OPPTS 850.5400 requirements for the satisfactory growth of *Pseudokirchneriella subcapitata* and may have been more associated with particular testing conditions rather than light intensity itself.

With use of a lower intensity of light, it might be expected that the reported mean cell count at 96 hours (1.70 X 10^6 cells/mL or ~50% of the US EPA OPPTS 850.5400 value) may not have reached the approximate 3.5 X 10^6 cells/mL value referred to in the US EPA guideline. Plotting cell counts against time using the Microsoft Excel Chart Wizard function and fitting the data points to an exponential curve (data and curve not shown) returned an r^2 value of 0.9982. This value and the visual examination of the data points and the fitted exponential curve indicates that exponential growth occurred in the study.

The use of the lower light intensity, while a deviation from the US EPA OPPTS 850.5400 guideline, is not considered to have had significant adverse effect on the study's conduct or results.

The 0-24 hour %CV of 54 is a failure to meet the 2006 OECD 201 requirements but, as the study was conducted to the 1984 OECD guideline, where such requirement was not specified, the deviation from the 2006 guideline is noted but is not pursued any further because the 24-48 and 48-72 hour %CV values met the OECD requirement.

E. REVIEWER'S COMMENTS:

The reviewer's results were equivalent to the study authors'. In the study author's statistical analysis, one of the control replicate was not used as it was considered to be an outlier based on the 0-24 h result only. The reviewer's analysis of the data did not identify an outlier in the controls and all control values were used in the reviewer's statistical analysis.

As the study was conducted in April 2006, just after the March 2006 changes to OECD 201 test guideline were adopted, deviations from the current OECD Guideline are acceptable provided they are consistent with the requirements of the 1984 OECD 201 guideline.

The observation that the measured %CV value for the 0-24 hour mean growth rate was reported as 54% and exceeded the 2006 version of the OECD guideline has not been considered a significant finding because of the absence of this criterion in the 1984 version of the standard to which the study was conducted and because the 24-48 and 48-72 hour growth rate values do not exceed the 35% value set by the 2006 OECD 201 guideline. The 0-72 hour %CV value was calculated as 4.5%, which meets the 2006 OECD 201 requirement that the coefficient of variation of average specific growth rate during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*.

The deficiencies and deviations identified were not considered to have adversely affected the study's conduct or results.

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

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

The PMRA and US EPA agree with the conclusions of the APVMA reviewer.

F. CONCLUSIONS:

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

Statistical Endpoint from the study report	Growth Rate (0-72 h)	Biomass (area under growth curve) (0-72 h)	Cell Density (96 h)
NOEC (mg ADTP metabolite of pyroxsulam/L)	92	92	92
EC_{50} (mg ADTP metabolite of pyroxsulam /L) (95% C.I. were not determined)	>92	>92	>92
Reference chemical, if used	Not applicable as no reference chemical was used.		

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

24 hour cell density

The ToxCalc analysis of the 24 hour algal cell count data gave the following results. Cell counts equal the number shown as cells/mL.

Conc-mg/L	1	2	3	4	5	6
S-Control	10000	32500	25000	52500	30000	37500
5.7	30000	60000	35000			
12	10000	12500	20000			
23	15000	17500	12500			
47	10000	17500	12500			
92	17500	32500	27500			

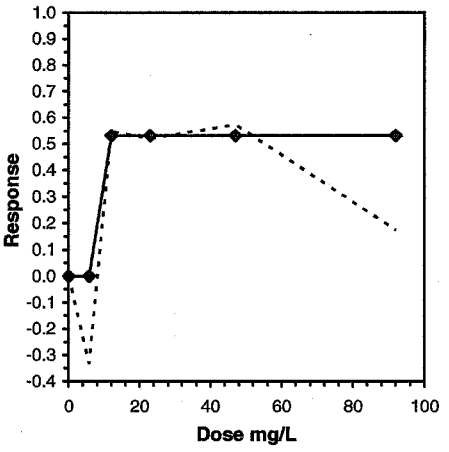
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	31250	1.0000	31250	10000	52500	44.900	6				36458.3	1.0000
5.7	41666.67	1.3333	41666.67	30000	60000	38.575	3	-1.378	2.602	19668.6	36458.3	1.0000
12	14166.67	0.4533	14166.67	10000	20000	36.735	3	2.260	2.602	19668.6	17083.3	0.4686
23	15000	0.4800	15000	12500	17500	16.667	3	2.150	2.602	19668.6	17083.3	0.4686
47	13333.33	0.4267	13333.33	10000	17500	28.641	3	2.371	2.602	19668.6	17083.3	0.4686
92	25833.33	0.8267	25833.33	17500	32500	29.565	3	0.717	2.602	19668.6	17083.3	0.4686

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94849	0.873	0.28963	1.58076
Bartlett's Test indicates equal variances (p = 0.14)	8.33441	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	92	>92			19668.6	0.6294	4.3E+08	1.1E+08	0.02162	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	6.293	0.982	0.313	6.702	-3.5788
IC10	6.885	0.662	4.046	7.704	-4.0944
IC15	7.478	0.583	5.527	8.706	-2.4535
IC20	8.071	0.595	6.336	9.708	-0.4108
IC25	8.664				
IC40	10.442				
IC50	11.627				



48 hour cell density

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

Conc-mg/L	1	2	3	4	5	6
S-Control	105000	112500	130000	140000	145000	112500
5.7	120000	147500	132500			
12	115000	105000	220000			
23	92500	105000	72500			
47	202500	132500	137500			
92	82500	92500	97500			

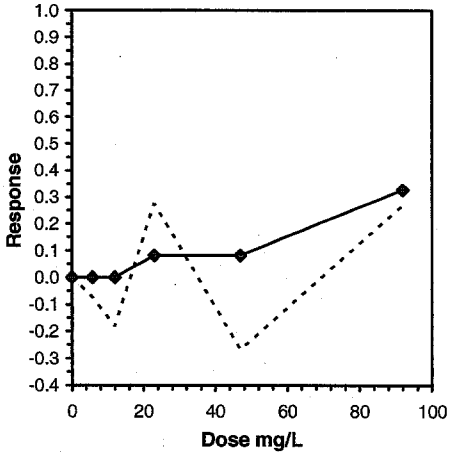
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	124166.7	1.0000	124166.7	105000	145000	13.274	6				134722	1.0000
5.7	133333.3	1.0738	133333.3	120000	147500	10.327	3	-0.431	2.602	55326.1	134722	1.0000
12	146666.7	1.1812	146666.7	105000	220000	43.435	3	-1.058	2.602	55326.1	134722	1.0000
23	90000	0.7248	90000	72500	105000	18.215	3	1.607	2.602	55326.1	123750	0.9186
47	157500	1.2685	157500	132500	202500	24.794	3	-1.568	2.602	55326.1	123750	0.9186
92	90833.33	0.7315	90833.33	82500	97500	8.408	3	1.568	2.602	55326.1	90833.3	0.6742

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9283	0.873	1.12399	2.11126
Bartlett's Test indicates equal variances (p = 0.05)	11.0515	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	92	>92			55326.1	0.44558	2.4E+09	9E+08	0.06624	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	18.753	16.186	0.728	76.550	1.1273
IC10	50.418	19.679	0.000	70.154	0.2032
IC15	59.627	19.936	0.000	77.728	-0.5229
IC20	68.835	17.392	0.000	84.977	-1.3287
IC25	78.044				
IC40	>92				
IC50	>92				



72 hour cell density

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

Conc-mg/L	1	2	3	4	5	6
S-Control	485000	455000	495000	705000	562500	667500
5.7	542500	617500	535000			
12	567500	350000	842500			
23	495000	422500	437500			
47	587500	582500	667500			
92	412500	527500	405000			

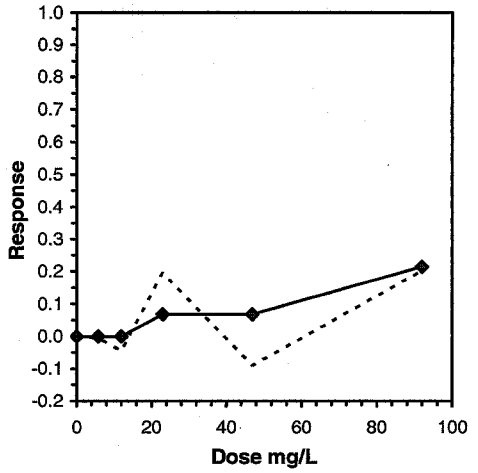
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	561666.7	1.0000	561666.7	455000	705000	18.407	6				571111	1.0000
5.7	565000	1.0059	565000	535000	617500	8.074	3	-0.041	2.602	210541	571111	1.0000
12	586666.7	1.0445	586666.7	350000	842500	42.070	3	-0.309	2.602	210541	571111	1.0000
23	451666.7	0.8042	451666.7	422500	495000	8.473	3	1.360	2.602	210541	532083	0.9317
47	612500	1.0905	612500	582500	667500	7.787	3	-0.628	2.602	210541	532083	0.9317
92	448333.3	0.7982	448333.3	405000	527500	15.315	3	1.401	2.602	210541	448333	0.7850

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92908	0.873	0.35813	2.25207
Bartlett's Test indicates equal variances (p = 0.08)	9.81489	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	92	>92			210541	0.37485	1.5E+10	1.3E+10	0.37546	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	20.048			
IC10	56.716			
IC15	72.060			
IC20	87.403			
IC25	>92			
IC40	>92			
IC50	>92			



96 hour cell density

The ToxCalc analysis of the 96 hour algal cell count data gave the following results. Cell counts equal the value shown as cells/mL.

Conc-mg/L	1	2	3	4	5	6
S-Control	1107500	967500	1385000	2675000	1486700	2006700
5.7	2265000	2260000	2495000			
12	2220000	1620000	1127500			
23	1743300	1255000	2170000			
47	1880000	1533300	2205000			
92	1107500	2046700	1062500			

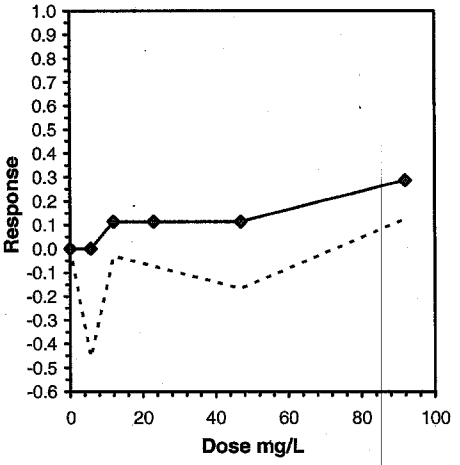
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	1604733	1.0000	1604733	967500	2675000	39.634	6				1972367	1.0000
5.7	2340000	1.4582	2340000	2260000	2495000	5.737	3	-2.034	2.602	940720	1972367	1.0000
12	1655833	1.0318	1655833	1127500	2220000	33.043	3	-0.141	2.602	940720	1750456	0.8875
23	1722767	1.0736	1722767	1255000	2170000	26.576	3	-0.327	2.602	940720	1750456	0.8875
47	1872767	1.1670	1872767	1533300	2205000	17.936	3	-0.742	2.602	940720	1750456	0.8875
92	1405567	0.8759	1405567	1062500	2046700	39.535	3	0.551	2.602	940720	1405567	0.7126

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95292	0.873	0.70897	0.0971
Bartlett's Test indicates equal variances (p = 0.55)	3.97964	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	92	>92			940720	0.58622	3.2E+11	2.6E+11	0.34547	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	8.500			
IC10	11.299			
IC15	56.648			
IC20	69.515			
IC25	82.383			
IC40	>92			
IC50	>92			



0-72 hour mean specific growth rate

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

Conc-mg/L	1	2	3	4	5	6
S-Control	1.2939	1.2726	1.3007	1.4185	1.3433	1.4003
5.7	1.3312	1.3744	1.3266			
12	1.3462	1.1851	1.4779			
23	1.3007	1.2479	1.2595			
47	1.3578	1.3549	1.4003			
92	1.2399	1.3219	1.2338			

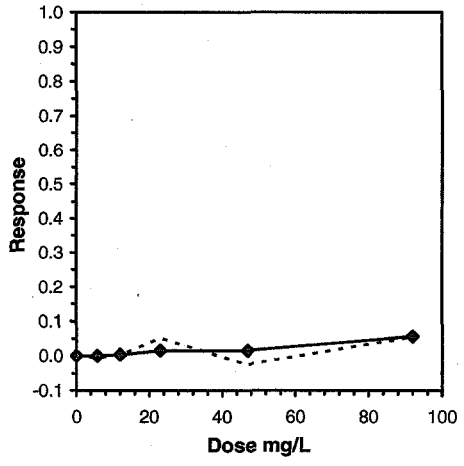
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	1.3382	1.0000	1.3382	1.2726	1.4185	4.486	6				1.3411	1.0000
5.7	1.3440	1.0044	1.3440	1.3266	1.3744	1.961	3	-0.121	2.602	0.1258	1.3411	1.0000
12	1.3364	0.9987	1.3364	1.1851	1.4779	10.974	3	0.037	2.602	0.1258	1.3364	0.9965
23	1.2693	0.9485	1.2693	1.2479	1.3007	2.185	3	1.425	2.602	0.1258	1.3202	0.9844
47	1.3710	1.0245	1.3710	1.3549	1.4003	1.855	3	-0.679	2.602	0.1258	1.3202	0.9844
92	1.2652	0.9454	1.2652	1.2338	1.3219	3.888	3	1.511	2.602	0.1258	1.2652	0.9434

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94574	0.873	-0.0266	2.10391
Bartlett's Test indicates equal variances (p = 0.10)	9.30339	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	92	>92			0.1258	0.09401	0.00577	0.00467	0.34152	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	84.721			
IC10	>92			
IC15	>92			
IC20	>92			
IC25	>92			
IC40	>92			
IC50	>92			



Note: the study report stated that the control, 5.7, 12, 23, 47 and 92 mg/L calculated 0-72 hour growth rate means were, respectively, 1.38, 1.38, 1.37, 1.30, 1.40 and 1.30 for replicates B, C, D, E and F. The ToxCalc equivalents based on the reviewer calculated 0-72 hour growth rates were similar, being 1.34, 1.34, 1.34, 1.27, 1.37 and 1.26.

0-72 hour biomass

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

Conc-mg/L	1	2	3	4	5	6
S-Control	332500	347500	377500	520000	431250	458750
5.7	396250	491250	410000			
12	383750	267500	636250			
23	330000	308750	278750			
47	481250	416250	458750			
92	281250	363750	302500			

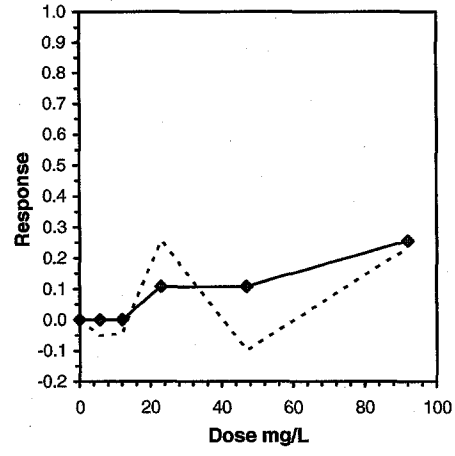
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
S-Control	411250	1.0000	411250	332500	520000	17.497	6				424306	1.0000
5.7	432500	1.0517	432500	396250	491250	11.871	3	-0.352	2.602	157170	424306	1.0000
12	429166.7	1.0436	429166.7	267500	636250	43.928	3	-0.297	2.602	157170	424306	1.0000
23	305833.3	0.7437	305833.3	278750	330000	8.419	3	1.746	2.602	157170	378958	0.8931
47	452083.3	1.0993	452083.3	416250	481250	7.301	3	-0.676	2.602	157170	378958	0.8931
92	315833.3	0.7680	315833.3	281250	363750	13.563	3	1.580	2.602	157170	315833	0.7444

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9307	0.873	0.73205	2.70088
Bartlett's Test indicates equal variances (p = 0.07)	10.1582	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs S-Control	92	>92			157170	0.38218	1.2E+10	7.3E+09	0.19716	5, 15

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	17.146	9.094	0.000	67.518	2.7823
IC10	22.292	16.871	0.000	86.373	1.4692
IC15	60.045				
IC20	75.168				
IC25	90.292				
IC40	>92				
IC50	>92				



The study report stated that, after 72 hours of exposure, the total biomass in the control averaged 42.56 x 10^4 cells•days/mL. Total biomass in the 5.7, 12, 23, 47 and 92 mg/L treatment levels averaged 43.07, 42.82, 30.55, 45.11 and 31.50 x 10^4 cells/mL, respectively (with replicate A from the controls omitted). The ToxCalc results (with replicate A's control results included) were, respectively, 41.12 (control), 43.25, 42.92, 30.58, 45.21 and 31.58 x 10^4 cells/mL, considered equivalent to those given in the study report.