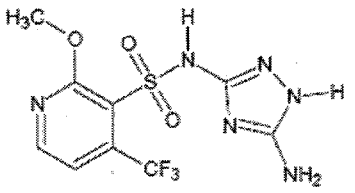
Data Evaluation Report on the Acute Toxicity of the ATSA Metabolite of Pyroxsulam (XDE-742) to Algae, *Pseudokirchneriella subcapitata*

PMRA Submission Number 2006-4727; ID 1283235 EPA MRID Number 469084-51 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:	ATSA Metabolite of XDE-742 (i.e. pyroxsulam ATSA metabolite)
Purity:	100%
Common name:	ATSA Metabolite Pyroxsulam
Chemical name:	3-pyridinesulfonamide, N-(5-amino-1H-1,2,4-triazol-3-yl)-2-methoxy-4-(trifluoromethyl)
IUPAC:	Not available (Hancock, 2006)
CAS name:	Not available (Hancock, 2006)
CAS No.:	Not listed (Hancock, 2006)
Synonyms:	X11265218

Chemical Structure:



Primary Reviewer: Daryl Murphy **Date:** 25 June 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

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

Émilie Larivière **Date:** 6 July 2007
Environmental Assessment Directorate, Pest Management Regulatory Agency, Health Canada

Brian Kiernan **Date:** 22 August 2007
US Environmental Protection Agency

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

CITATION: Hancock, G. A., Arnold; B. H., Najar, J. R. and Sushynski, J. M. (2006). ATSA metabolite of XDE-742: growth inhibition test with the freshwater green alga, *Pseudokirchneriella subcapitata*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project Study ID 061002. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences LLC, Indianapolis, Indiana 46268. 17 February 2006. Unpublished report.

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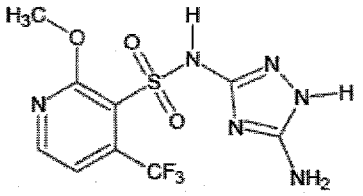

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EXECUTIVE SUMMARY:

The effect of the ATSA metabolite of pyroxsulam on the growth of the freshwater green alga, *Pseudokirchneriella subcapitata* was studied for 96 hours under static conditions and continuous illumination. The experiment was carried out taking account of relevant OECD, European Communities and US EPA guidelines. Algae were exposed to nominal concentrations of 0, (control), 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg ATSA metabolite of pyroxsulam/L. Mean measured concentrations were <0.257 (< LLQ; control), 3.06, 6.15, 12.4, 24.2, 48.0, and 99.9 g ATSA metabolite of pyroxsulam/L The growth and test medium used was algal assay medium (AAM). 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 were 24.5-24.7°C. The light intensity ranged from 4600 to 5700 lux. The pH values ranged from 6.2 to 7.5 at test initiation, and from 6.1 to 7.7 at test termination. Cell counts were conducted every 24 hours.

After 96 hours, inhibition of cell density relative to the control ranged from 4 to 98%. The 96 hour EC50 was 28.4 mg ATSA metabolite of pyroxsulam/L. Cell density was significantly less than the controls at test levels ≥12.4 mg/L; therefore, the 96-hour NOEC value for cell density was determined to be 6.15 mg/L. After 72 hours, inhibition of biomass and mean specific growth rate relative to controls ranged from 13 to 97%, and from 3 to 97%, respectively. The 72-hour EC50 for total biomass (EbC50) and for mean specific growth rate (ErC50) was 16.8 and 42.8 mg ATSA metabolite of pyroxsulam/L, respectively. Statistically significant differences in biomass and mean specific growth rate were detected between all treatment levels and controls. The 72 hour NOECs for total biomass and average growth rate were therefore set at <3.06 mg ADTP metabolite of pyroxsulam/L.

At 96 hours, microscopic evaluation of algal cells at each test concentration and the control revealed no abnormal observations at test levels ≤ 24.2 mg/L. Very few cells were observed at 48.0 and 99.9 mg/L with some noted as larger than normal with a bloated appearance (~ 10% at 48.0 mg/L and ~ 50% at 99.9 mg/L).

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

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

Results Synopsis

Test Organism Size/Age: *Pseudokirchneriella subcapitata*

Test Type: Static

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 ATSA metabolite of pyroxsulam/L),	<3.06 (Dunnett's test)	<3.06 (Dunnett's test)	6.15 (Dunnett's test)
EC50 (mg ATSA metabolite of pyroxsulam /L) (95% C.I. in brackets)	42.8 (35.8-49.8)	16.8 (15.1-18.5)	28.4 (25.8-31.0)
Reference chemical, if used	**Not applicable as no reference chemical was used.**		

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
The study was stated to have generally conformed to then current procedures described by the Organization for Economic Cooperation and Development (OECD), European Economic Community (EEC) and US Environmental Protection Agency guidelines, namely:

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

EEC Commission Directive 92/69/EEC Annex, C.3 Algal Inhibition Test;

U.S. Environmental Protection Agency (1982). Pesticide Assessment Guidelines, Subdivision J Hazard Evaluation: Non-target Plants, Guideline 123-2, EPA 540/9-82-020, Washington, D.C.; and

U.S. Environmental Protection Agency (1986). Hazard Evaluation Division: Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants Tiers 1 and 2. EPA 540/9-86-134, Washington, D.C.

COMPLIANCE:
All phases of the study were reported as the following Good Laboratory Practice Standards:

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM (98) 17.

US Environmental Protection Agency-FIFRA GLPs. Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule; and

European Community (EC). European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004).

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

A. MATERIALS:

1. **Test Material:** ATSA metabolite of XDE-742 (i.e. ATSA metabolite of pyroxsulam
   Description: Solid
   Lot No./Batch No.: 035298-95
   Purity: 100%.
   Test Substance Number: TSN105493
   Stability of Compound Under Test Conditions: **Stable.** Results from the day 0 analysis of the bulk dose solutions yielded percent of target values ranging from 96.8 to 98.8%. The pooled and blank test solution concentrations measured on day 4 had percent of target values ranging from 95.0 to 103%. From these results it was concluded that the ATSA metabolite of pyroxsulam was stable throughout the duration of the

	96-hour exposure period (consequently, all biological results were expressed as mean measured ATSA).
Storage conditions of test chemicals:	Not provided in the study report. Hancock (2006) describes the storage condition as "Ambient room temperature".
Physicochemical properties of ATSA metabolite of pyroxsulam:	Not provided.

The study report also noted that concentrations did not need to be adjusted for the purity of the test substance (i.e. it was 100%) and were presented as active ingredient (i.e. ATSA metabolite of pyroxsulam).

2. Test organism:	The freshwater green alga
Species:	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Class:	Not identified in the study report.
Strain:	UTCC 37
Source:	Axenic samples of these algae were received on 13 February 2003 from the University of Toronto Culture Collection at the University of Toronto, Toronto, Ontario, Canada. Stock cultures of this organism were maintained aseptically by periodic transfer into sterile AAM
Age of inoculum:	3 days
Method of cultivation:	The axenic stock cultures were maintained within the following conditions: a temperature of 24 ± 2°C and continuous (24 hours of light/day) illumination at 5000 ± 750 lux in Algal assay medium (AAM), as designated for the US EPA algal assay bottle test. The medium's pH was ~7.0 to 7.5. Culture vessels were 500 mL borosilicate Erlenmeyer flasks with a culture volume of 200 mL. The culture vessels were capped with Shimadzu closures. It was not stated whether there was continuous agitation of the culture vessels (Hancock (2006) indicates cultivation was done with shaking at 100 rpm).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study:
A probe study was conducted between 12 January and 16 January 2006 using four nominal ATSA metabolite of pyroxsulam concentrations of 0.0960, 0.480, 2.40, and 12.0 mg/L, plus a medium control. The percent inhibition compared to the medium control based on cell density at 96 hours was -2, -2, 1, and 16% for the 0.0960, 0.480, 2.40, and 12.0 mg ATSA metabolite of pyroxsulam/L test concentrations, respectively (negative inhibition is stimulation of growth). The percent inhibition compared to the medium control based on the growth rate parameter at 96 hours was 0, 0, 0, and 3% for the 0.0960, 0.480, 2.40, and 12.0 mg ATSA metabolite of pyroxsulam/L test concentrations, respectively. These results indicated the EC50 values for cell density and for growth rate were greater than 12.0 mg ATSA metabolite of pyroxsulam/L, the highest concentration tested. Test levels for the definitive test were correspondingly set based on the growth rate parameter. Based on this, the target concentrations for the definitive test were set at 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg ATSA metabolite of pyroxsulam/L, plus a medium control.

b. Definitive Study

The experimental phase of the 96-hour acute toxicity test was conducted from 23 to 27 January 2006 at the Dow Chemical Company, Midland, Michigan.

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

Table 1. Experimental Parameters

Parameter	**Details**	**Remarks** ***Criteria***
Acclimation period:	The inoculum used to initiate the toxicity test with the ATSA metabolite of pyroxsulam was prepared from a 3-day old stock culture of *Pseudokirchneriella subcapitata.*	Parameter considered met. OECD 201 states that an inoculum culture in the test medium is prepared 2-4 days before start of the test with the inoculum culture incubated under the same conditions as the test cultures. US EPA OPPTS 850.5400 states that the test begins when algae (inocula) from 3 to 7 day–old stock cultures are placed in the test chambers containing test solutions having the appropriate concentrations of the test substance. This guideline also states that toxicity testing should not be performed until algal cultures are shown to be actively growing (i.e. capable of logarithmic growth within the test period) in at least two subcultures lasting 7 days each prior to the start of the definitive test. *EPA recommends two week acclimation period.* This template requirement is noted but is not considered appropriate in the light of the OECD and US EPA OPPTS requirements which allow use shorter acclimatisation periods. *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*

Parameter	Details	Remarks *Criteria*
		the algal cultures contain deformed or abnormal cells, they must be discarded.
Culturing media and conditions: (same as test or not)	Culturing medium was the same as the test medium (AAM medium in both cases). Conditions of culturing and testing are considered equivalent. **Parameter** **Culture** **Temperature:** 24 ± 2°C **Light (lux):** 5000 ± 750 lux (inoculum culture) **Photoperiod:** Continuous (24 hours light/day) **Medium:** AAM **pH range:** ~7.0 to 7.5 **Culture Volume:** 200 mL **Culture Vessel:** 500-mL borosilicate Erlenmeyer flask **Culture Vessel Cap:** Shimadzu closure **Agitation** Not stated. **Growth conducted in:** Not stated	Parameter considered met. **Parameter** **Test** **Temperature:** 24 ± 2°C (measured 24.5 to 24.7°C) **Light (lux):** 5124 ± 350 lux (4600 lux – 5700 lux) **Photoperiod:** Continuous (24 hours light/day) **Medium:** AAM **pH range:** 7.5 ± 0.1 prior to test solution preparation. **Culture Volume:** 50 mL **Culture Vessel:** 250-mL borosilicate Erlenmeyer flask **Culture Vessel Cap:** Foam plugs **Agitation** Continuous (100 rpm) **Growth conducted in:** Environmental Growth Chamber

Parameter	Details	Remarks *Criteria*
Health: (any mortality observed)	Not specifically commented on but the satisfactory growth of the controls indicates the algal health was acceptable at the start of the study.	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. This was done in the study report (At 96 hours, microscopic evaluation of algal cells at each test concentration and the control revealed no abnormal observations at test levels • 24.2 mg/L (including controls).
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.

Parameter	Details	Remarks *Criteria*
Incubation facility	The replicate test flasks were placed in a walk-in environmental chamber thermostatically controlled at 24 ± 2°C with continuous light at approximately 5000 ± 750 lux.	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*

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Sterilised 250-mL borosilicate Erlenmeyer flasks with foam stoppers, 250 mL 50 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	The growth and test medium used was algal assay medium (AAM) designated for the EPA algal assay bottle test. Medium details provided in the study report were considered equivalent to the US EPA AAP medium composition recorded in OECD 201: A comparison of the AAM and US EPA and OECD algal growth media is shown in Table 11 (Appendix II, page 52 of this DER). The 1984 OECD 201 growth medium recipe is essentially the same as that described in the 2006 OECD 201 guideline.	Parameter considered met. OECD 201 does not refer to AAM medium. 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 AAM used contains sodium EDTA as a chelating agent).

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td>pH at test initiation and at test termination:</td><td>Initial pH values were:

<table><tr><th>ATSA metabolite of pyroxsulam mg/L Nominal</th><th>pH Values
Day 0[a]</th></tr><tr><td>Control</td><td>7.5</td></tr><tr><td>3.13</td><td>7.4</td></tr><tr><td>6.25</td><td>7.4</td></tr><tr><td>12.5</td><td>7.2</td></tr><tr><td>25.0</td><td>7.0</td></tr><tr><td>50.0</td><td>6.7</td></tr><tr><td>100</td><td>6.2</td></tr></table>a. pH of bulk solutions.

Final pH values were:

<table><tr><th>Nominal concen-trations*</th><th colspan="3">pH Values</th></tr><tr><th></th><th>Day 3 with algae[a]</th><th>Day 4 with algae[a]</th><th>Day 4 With-out algae</th></tr><tr><td>Control</td><td>8.5</td><td>7.5</td><td>7.0</td></tr><tr><td>3.13</td><td>8.3</td><td>7.4</td><td>6.9</td></tr><tr><td>6.25</td><td>8.4</td><td>7.5</td><td>6.9</td></tr><tr><td>12.5</td><td>8.3</td><td>7.6</td><td>6.9</td></tr><tr><td>25.0</td><td>7.8</td><td>7.7</td><td>6.8</td></tr><tr><td>50.0</td><td>6.8</td><td>7.2</td><td>6.5</td></tr><tr><td>100</td><td>5.9</td><td>6.1</td><td>5.9</td></tr></table>*** mg ATSA metabolite of pyroxsulam/L. a. pH taken from pooled sample of inoculated replicates.**

The control pH increased by 1 unit over 72 hours but was unchanged with respect to the initial pH value after the 96 hours of the exposure period.</td><td>Parameter considered met.

The changes in the control pH are in compliance with those recommended by the OECD 201 (2006) which states the control medium's pH should not increase by greater that 1.5 pH units during the test). US EPA 850.5400 states that the initial pH of the nutrient medium is to be 7.4 to 7.6 and notes that if the test chemical is highly acidic and reduces the pH of the test solution below 5.0 at the first measurement, appropriate adjustments to pH should be considered. This was not the situation in the study under assessment.

OECD recommends (2006) the (control) medium pH should not increase by greater that 1.5 pH units during the test.

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

Chelator used:	Yes, as required for AAM media $Na_2EDTA \cdot 2H_2O$ at 0.3 mg/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. Hancock (2006) refers to ambient CO_2 as the carbon source.	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)	Yes (AAM medium used, which is not referred to be either OECD or US EPA OPPTS guidelines). Full details of the medium's composition were supplied – see Table 11, page 52 of this DER.	Use of AAM is acceptable – full details of the AAM medium's constitution were provided (see Appendix II, page 52 of this DER).
Dilution water source/type:	The AAM medium was prepared using deionised water. The source water for the deionised water was Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant.	Dilution water parameters considered met.

pH:	The pH of the culture medium was adjusted to pH 7.5 ± 0.1 prior to test solution preparation.	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Salinity (for marine algae):	N/A for a freshwater alga.	*EPA salinity: 30-35 ppt.*
Water pretreatment (if any): Intervals of water quality measurement.	Deionised water was used to prepare the AAM growth medium. Intervals of water quality measurement not referred to.	No specific requirement identified in the guidelines.
Total Organic Carbon:	Not reported.	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:	Not reported.	No specific requirement identified in the guidelines.

Chlorine:	Not reported.	No specific requirement identified in the guidelines.
Indicate how the test material is added to the medium (added directly or used stock solution)	The high-dose test solution was prepared by direct addition of the test material to AAM medium. To prepare the 100 mg/L test solution, an aliquot of 50.85 mg (50.0 mg target with no adjustment for purity, 100%) of ATSA metabolite of pyroxsulam was weighed out and added to a 500-mL volumetric flask containing some AAM. The flask was stoppered and shaken vigorously to thoroughly mix. Due to the presence of undissolved test material this test solution was sonicated briefly (approximately two minutes) and subsequently appeared clear. The remaining test solutions (50.0, 25.0, 12.5, 6.25, and 3.13 mg ATSA metabolite of pyroxsulam/L) were prepared by serial dilution. All test solutions were noted as clear following preparation.	Parameter considered met.
Aeration or agitation	Not reported. Hancock (2006) refers to agitation at 100 revolutions per 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. Agitation at 100 rpm is considered not to be too dissimilar to this recommended rate. US EPA OPPTS 850.5400 states that test containers should be placed on a rotary shaking apparatus and oscillated at approximately 100 cycles/minute 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. Inoculations were made after all the replicate test vessels at each test concentration were poured. An additional replicate at each test concentration and control group was prepared but not inoculated with algae. These were to serve as counting blanks. These blanks were used to correct the daily counts for the interference of the test material and to monitor pH and concentration of the test material without the algal biomass.	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), 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg ATSA metabolite of pyroxsulam/L The nominal test concentrations were in the ratios of 1:2.	Nominal and measured test concentrations parameter considered met. Results from the day 0 analysis of the bulk dose solutions yielded percent of target values ranging from 96.8 to 98.8%. The pooled test solutions on day 4 had ATSA metabolite of pyroxsulam concentrations of 95 to 101% of nominal. The ATSA metabolite of pyroxsulam concentrations in the blank test solutions were 99.4 to 103% of nominal. There was no apparent 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 concentrations used were in the ratio of 1:2 and 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.*

<table>
<tr>
<td>Measured:</td>
<td>Mean measured concentrations at day 0 in the bulk dose solutions were:

Concentration of ATSA metabolite of pyroxsulam mg/L at time 0 (% of nominal)
<table>
<tr><th>Nominal:</th><th>Measured</th></tr>
<tr><td>AAM Control</td><td><LLQ[a]</td></tr>
<tr><td>3.13</td><td>3.03 (96.8%)</td></tr>
<tr><td>6.25</td><td>6.09 (97.4%)</td></tr>
<tr><td>13</td><td>12.2 (97.6%)</td></tr>
<tr><td>25</td><td>24.4 (97.6%)</td></tr>
<tr><td>50</td><td>48.5 (97.0%)</td></tr>
<tr><td>100</td><td>98.8 (98.8%)</td></tr>
</table>
a. <LLQ = Less than Lowest Level Quantified = 0.257 mg ATSA metabolite of pyroxsulam/L AAM.

Mean measured concentrations at 72 and 96 hours were as follows:

<table>
<tr><th rowspan="2">Nominal Conc.[a]</th><th colspan="2">Concentration, mg/L (Percent of Target)</th></tr>
<tr><th>Day 4 Exposure test solutions[b]</th><th>Day 4 Exposure blank solutions[c]</th></tr>
<tr><td>Control</td><td><LLQ</td><td><LLQ</td></tr>
<tr><td>3.13</td><td>3.08 (98.4%)</td><td>3.11 (99.4%)</td></tr>
<tr><td>6.25</td><td>6.20 (99.2%)</td><td>6.23 (99.7%)</td></tr>
<tr><td>13</td><td>12.6 (101%)</td><td>12.7 (102%)</td></tr>
<tr><td>25</td><td>24.0 (96.0%)</td><td>25.4 (102%)</td></tr>
<tr><td>50</td><td>47.5 (95.0%)</td><td>50.4 (101%)</td></tr>
<tr><td>100</td><td>101 (101%)</td><td>103 (103%)</td></tr>
</table>
a. as mg ATSA metabolite of pyroxsulam/L.
b. Three replicate test solutions containing algae at each dose level were pooled to provide one composite exposure test solution per dose level. c. Test solutions not containing algae.

Mean measured concentrations over 96 hours and derived from the time 0 and 96 hour test exposure solutions measured ATSA metabolite concentration of pyroxsulam were:

Concentration of ATSA metabolite of pyroxsulam mg/L
<table>
<tr><th>Nominal:</th><th>Mean measured (% of nominal)</th></tr>
<tr><td>Control</td><td><LLQ</td></tr>
<tr><td>3.13</td><td>3.06 (97.6%)</td></tr>
<tr><td>6.25</td><td>6.15 (98.3%)</td></tr>
<tr><td>13</td><td>12.4 (99.2%)</td></tr>
<tr><td>25</td><td>24.2 (96.8%)</td></tr>
<tr><td>50</td><td>48.0 (96.0%)</td></tr>
<tr><td>100</td><td>99.9 (99.9%)</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>Solvent (type, percentage, if used)</td>
<td>N/A; a solvent was not used</td>
<td>The parameter is not relevant as a solvent was not used.

OECD 201 and US EPA OPPTS</td>
</tr>
</table>

		850.5400 allow, but do not require, the use of solvents.
Method and interval of analytical verification Limit of quantitation: Limit of detection:	All samples were analysed by high performance liquid chromatography with and ultra-violet absorbance detector (HPLC/UV). To assess analytical method precision and solution homogeneity, three additional samples were collected on day 0 from the 3.13 and 100 mg/L bulk dose solutions. These additional samples were collected, diluted and analyzed along with the other day 0 samples and resulted in percent relative standard deviation (%RSD) values of 0.190 and 0.409%, respectively (data not shown in the test report). The HPLC/UV instrumentation exhibited a linear response over a concentration range of 0.234 to 58.5 mg ATSA metabolite of pyroxsulam/L diluent on both days 0 and 4. The lowest level quantified was set at 0.257 mg ATSA metabolite of XDE-742/L AAM based on the concentration of analyte in the lowest standard analyzed multiplied by the lowest dilution factor. Test solutions were analyzed for the presence of pyroxsulam at 0 and 96 hours. Limit of detection not reported.	Parameter considered met. Typical chromatograms of a control, a standard and a sample were shown in the study report as was a typical linear regression analysis for ATSA metabolite of pyroxsulam.
<u>Test conditions</u>		The test conditions meet US EPA and OECD Guidelines

Temperature:	Temperature Range: 24.5–24.7°C	Parameter 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. *EPA temperature: Skeletonema: 20°C, Others: 24-25°C.* *OECD recommended the temperature be in the range of 21 to25°C maintained at ± 2°C.*
Photoperiod:	Continuous	Photoperiod requirement considered met. OECD 201 refers to use of continuous light while US EPA OPPTS 850.5400 refers to test chambers containing *Selenastrum*, *Navicula*, and *Anabaena* being illuminated continuously. *EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous. OECD recommends and continuous uniform illumination.*
Light intensity and quality:	Mean Light Intensity: 5124 lux Standard Deviation: 350 lux Minimum Light Intensity: 4600 lux Maximum Light Intensity: 5700 lux The study report noted that continuous light at approximately 5000 ± 750 lux was used. The light intensity was set lower than OECD 201 requirement for two reasons. First, the lower light intensity is more in line with the US EPA guidance of approximately 4300 lux. In addition, the April 2004 draft revised OECD 201 guideline suggests a light intensity range of 4400-8800 lux. The light intensity on the test is in line with both guidelines.	See deviations/deficiencies table, page 34 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 4300 lux 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%)*
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	At test termination, morphological observations were done on a composited sample of the three inoculated replicates at each test concentration.	Acceptable.

2. Observations:

Table 2. Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms.	Cell density and biomass (area under the growth curve), growth rate (per day). Cell appearance was observed at 24, 48, 72 and 96 hours. pH, temperature, light intensity and concentrations of the ATSA 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. Morphological observations of cells at 96 hours were by microscope using a Bright Line Hemacytometer Counting Chamber.	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 observations of the cells at each test concentration were made.	Requirement considered met. Observation made is appropriate

Parameters	Details	Remarks Criteria
Indicate whether there was an exponential growth in the control	Yes. The mean control 72-hour cell growth was 213 x 10^4 cells/mL. This represents an approximate 213-fold increase in cell numbers from the original 10,000 cells/mL. At 96 hours, the mean control cell density was ~ 549 X 10^4 cells/mL, i.e. ~5.49 X 10^6 cells/mL. The 0 to 72 hour growth rate in the control averaged 0.07444 $hour^{-1}$ or ~1.8 (0.07444 X 24) $days^{-1}$.	Parameter is considered met. OECD 201 requires, *inter alia,* that biomass in the control cultures should have increased by a factor of at least 16 within the 72 hour test period (note that cell count has been used as the measure of biomass in this situation). OECD 201 also states that the desired increase in biomass corresponds to a specific growth rate of 0.92 day^{-1}. Note that OECD 201 states that *P. subcapitata* is expected to have a growth rate of 1.5 to 1.7 in light intensity of approximately 70 $\mu E/m^2/sec$ at 21°C when grown in OECD medium. US EPA OPPTS 850.5400 states that algal growth in controls should reach the logarithmic growth phase by 96 h (at which time the number of algal cells should be approximately 3.5 X 10^6/mL for *Selenastrum*. The mean measured value of ~5.49 X 10^6 cells/mL meets this requirement *EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Water quality was acceptable? (Yes/No)	Yes	Parameter considered met on basis of successful growth of the controls and details provided on the medium's preparation from sterile, deionised water.
Were raw data included?	As laboratory data, no. Individual	Parameter considered met.

Parameters	Details	Remarks Criteria
	replicate data were presented. The data, protocol, protocol changes/revisions, and final report are archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.	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 and notes that electronic data submission (raw data) is encouraged to reduce data entry time required to conduct statistical analyses.

II. RESULTS and DISCUSSION:

INHIBITORY EFFECTS:

Mean cell densities at 96 hours were 549, 517, 526, 463, 352, 76.2, and 11.2 X 10^4 cells/mL for the control, 3.06, 6.15, 12.4, 24.2, 48.0 and 99.9 mg/L exposure concentrations, respectively. Response relative to the controls ranged from 4 to 98% inhibition of mean cell density. The 96 hour EC50 (95% confidence interval) was 28.4 (25.8-31.0) mg ATSA metabolite of pyroxsulam/L. Based on the Dunnett's test, the 96-hour cell density was significantly less than the controls at test levels ≥ 12.4 mg/L; therefore, the 96-hour NOEC value for cell density was determined to be 6.15 mg/L.

Specific growth rates at 72 hours were 0.07444, 0.07249, 0.07265, 0.06946, 0.05784, 0.02852, and 0.01827 $hour^{-1}$ for the control, 3.06, 6.15, 12.4, 24.2, 48.0, and 99.9 mg/L exposure concentrations, respectively. Response relative to the controls ranged from 2 to 75% inhibition of specific growth rate. The specific growth rate data was normally distributed (Shapiro-Wilk, $p > 0.1$). The variance was heterogeneous for both the raw and transformed values (Levene's Test, $p < 0.1$) so a nonparametric analysis was performed on the ranks of the data. The 72-hour calculated ErC50 (95% confidence interval)

value for specific growth rate was 42.8 (35.8-49.8) mg/L. Based on the Dunnett's test, the 72-hour specific growth rate was significantly less than the controls at all test levels; therefore, the 72-hour NOEC value for specific growth rate was determined to be < 3.06 mg/L, the lowest level tested. In lieu of a NOEC, the ErC10 and ErC20 values were calculated. The 72-hour calculated ErC10 (95% confidence interval) value for specific growth rate was 10.7 (5.61-15.8) mg/L. The 72-hour calculated ErC20 (95% confidence interval) value for specific growth rate was 17.9 (11.8-24.0) mg/L.

Mean biomass areas at 72 hours were 3874, 3380, 3386, 2599, 1185, 209, and 127 for the control, 3.06, 6.15, 12.4, 24.2, 48.0 and 99.9 mg/L exposure concentrations, respectively. Response relative to the controls ranged from 13 to 97% inhibition of mean biomass area. The biomass area data was normally distributed (Shapiro-Wilk, p > 0.1) and the variance was homogenous (Levene's Test, p > 0.1). The 72 hour calculated EbC50 (95% confidence interval) value for mean biomass area was 16.8 (15.1-18.5) mg/L. Based on the Dunnett's test, the 72 hour mean biomass area was significantly less than the controls at all test levels; therefore, the 72 hour NOEC value for mean biomass area was determined to be < 3.06 mg/L, the lowest level tested. In lieu of a NOEC, the EbC10 and EbC20 values were calculated. The 72 hour calculated EbC10 (95% confidence interval) value for biomass area was 6.70 (5.00-8.40) mg/L. The 72 hour calculated EbC20 (95% confidence interval) value for biomass area was 9.40 (7.63-11.2) mg/L.

At 96 hours, microscopic evaluation of algal cells at each test concentration and the control revealed no abnormal observations at test levels • 24.2 mg/L (including controls). Very few cells were observed at 48.0 and 99.9 mg/L. Some cells at both of these levels were noted as larger than normal with a bloated appearance (~ 10% at 48.0 mg/L and ~ 50% at 99.9 mg/L).

The effects of the ATSA 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. The reported mean specific growth rates and biomass (area under the growth curve) results are shown in Table 4.

Table 3. Effect of ATSA 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	Percent inhibition[1] at 72 hours	96 hours	Percent inhibition at 96 hours
Negative control	1 X 10^4	6.94 0.99)	50.5 (5.1)	213 (12)	NA[2]	549 (27)	NA
3.06	1 X 10^4	6.47 (0.47)	44.2 (4.8)	185 (17)	13	517 (10)	6
6.15	1 X 10^4	6.12 (0.16)	43.8 (5.2)	187 (17)	12	526 (9)	4
12.4	1 X 10^4	5.72 (0.29)	30.4 (3.8)	149 (18)	30	463 (75)[3]	16
24.2	1 X 10^4	4.93 (1.44)	13.4 (3.4)	67.1 (21.8)	68	352 (53)[3]	36
48.0	1 X 10^4	2.66 (0.99)	4.06 (1.99)	9.01 (6.02)	96	76.2 (57.9)[3]	86
99.9	1 X 10^4	2.67 (0.65)	3.15 (0.87)	3.91 (1.54)	98	11.2 (5.3)[3]	98
Reference chemical (if used)	Not applicable as a reference chemical was not used.						

1. Relative to control. Mean, standard deviation (SD) and percent inhibition were calculated from original raw data, not from the rounded values presented in this table. 2. Not applicable. 3. Significantly reduced compared to the control, based on Dunnett's Test.

The 96 hour exposure means for 12.4, 24.2, 48.0 and 99.9 mg ATSA metabolite of pyroxsulam/L was statistically significantly reduced from the mean control count at that time ((Dunnett's Test, p • 0.05, one-tailed).

Table 4. Effect of ATSA metabolite of pyroxsulam on algal growth (Green Alga, *Pseudokirchneriella subcapitata*) – Mean specific growth rates and biomass (area under the growth curve) and percentage inhibition after 72 hours.

Treatment measured concentrations (mg ATSA metabolite of pyroxsulam/L)	Mean Specific Growth Rate per hour and per day			Mean Area Under the Growth Curve (Biomass)	
	0-72 hours		Percent Inhibition[1]	0-72 hours	Percent Inhibition[1]
	$Hour^{-1}$	Day^{-1}			
Negative control	0.07444	1.787	NA	3874	NA
3.06	0.07249*	1.740	3	3380*	13
6.15	0.07265*	1.744	2	3386*	13
12.4	0.06946*	1.667	7	2599*	33
24.2	0.05784*	1.388	22	1185*	69
48.0	0.02852*	0.684	62	209*	95
99.9	0.01827*	0.438	75	127*	97

1. Relative to control at 72 hours. *. Significantly reduced compared to the control, based on Dunnett's Test. NA = not applicable.

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

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 considered to have been complied with.

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

Table 5. Study report's statistical endpoint values. EC50 and NOEC results expressed as mg ATSA metabolite of pyroxsulam/L. (95% C.I. in brackets except where this parameter was not calculated)

Statistical Endpoint	Cell Density				Growth Rate (0-72 h)	Biomass (0-72 h)
	24 h	48 h	72 h	96 h		
NOEC (mg ATSA metabolite of pyroxsulam/L)	Not reported	Not reported	Not reported	6.15	<3.06	<3.06
EC50 (mg ATSA metabolite of pyroxsulam/L)	Not reported	Not reported	Not reported	28.4 (25.8-31.0)	42.8 (35.8-49.8)	16.8 (15.1-18.5)
EC20 (mg ATSA metabolite of pyroxsulam/L)	Not reported	Not reported	Not reported	Not reported	17.9 (11.8-24.0)	9.40 (7.63-11.2)
EC10 (mg ATSA metabolite of pyroxsulam/L)	Not reported	Not reported	Not reported	Not reported	10.7 (5.61-15.8)	6.70 (5.00-8.40)
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 24 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 ~213 x 10^4 cells/mL. This represents an approximate 213-fold increase in cell numbers from the original 10,000 cells/mL. At 96 hours, the mean control cell density was ~549 X 10^4 cells/mL,

i.e. ~5.49 X 10^6 cells/mL. This meets 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.

The 0 to 72 hour growth rate in the control averaged ~1. 8 $days^{-1}$ (or 0.07444 $hour^{-1}$) 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 30 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 also 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's results	Study report's results*
1	2.183	1.753	1.520	1.818	1.818
2	1.904	2.010	1.466	1.793	1.794
3	1.929	1.804	1.545	1.759	1.759
4	1.913	2.046	1.374	1.778	1.778
5	1.816	2.096	1.449	1.787	1.786
6	1.833	2.217	1.302	1.784	1.784
Mean, standard deviation and %CV determined from all six replicate results:					
Mean	1.93	1.99	1.44	1.787	1.787
Standard deviation	0.13	0.18	0.09	0.019	0.019
%CV	6.85	8.92	6.32	1.09	1.08

* **Values shown = study report's values ($hour^{-1}$) X 24 = day^{-1}.**

None of the growth rate %CV values (0-24, 24-48 and 48-72 hours) exceeded the 35% value set by the 2006 OECD 201 guideline.

The 0-72 h %CV was calculated by the reviewer as ~1.1% (mean 1.787, standard deviation 0.019, see page 47 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 calculated as ~1.1%.

B. REPORTED STATISTICS:

The study report gave the following information with respect to the statistical analysis of the cell count data.

The results (study endpoints) of the study were evaluated based on the mean measured ATSA metabolite of pyroxsulam concentrations. The primary endpoint of this test was the response of the alga to the test material expressed as a function of specific growth rate (per day). Results were also expressed in terms of cell density (cells/mL x 10,000) and biomass (as area under the growth curve). For each endpoint, mean values were calculated for each exposure concentration level and control level. Percent inhibition values were calculated based on differences in mean exposure concentration values compared to control values.

The following formula was used to calculate growth rate:

$$\mu_{i-j} = \frac{\ln N_j - \ln N_i}{t_j - t_i}$$

Where: μ = mean specific growth rate from moment i to j ($days^{-1}$)
ln = natural logarithm
Ni = initial cell density at time i (cells/ml x 10^4)
Nj = cell density at time j
ti = the moment time for the start of the period
tj = the moment time for the end of the period

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

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

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

The data (untransformed, square root transform for cell density, and the log transform for biomass and growth rate) was tested for normality using the Shapiro-Wilk's Test ($\alpha = 0.01$) and for homogeneity of variance using the Levene's Test ($\alpha = 0.01$). If the assumptions of normality and homogeneity were met for the untransformed data or one of the transformations, no-observed-effect concentrations (NOECs) were determined using a one-tailed Dunnett's mean comparison procedure ($\alpha = 0.05$). If the assumptions normality and/or homogeneity were not met and a nonparametric analysis was required, the ranks of the values were determined and then the above analysis was performed on these ranks.

The 72 hour ErC50 value (the concentration which reduces the algal growth rate by 50% relative to the control), the 72 hour EbC50 value (the concentration which reduces biomass as area under the growth curve by 50% relative to the control), and the 96 hour EC50 value (the concentration which reduces cell density by 50% relative to the control) were determined. For endpoints where a NOEC could not be determined (*i.e.*, lower than the lowest level tested) an EC10 and EC20 value were determined and reported. Two sigmoid-shape nonlinear models were used for calculation of ECx values and 95% confidence intervals, if possible. One model used to describe the response to

increasing concentrations was the four-parameter logistic model (percent inhibition versus concentration). The second model used was the cumulative normal model (response compared to log concentration) (Bruce and Versteeg, 1992). The model that best fitted the data was reported. All statistical analyses were conducted in SAS version 6.12 (SAS Institute, Cary, North Carolina).

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s):

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

The ToxCalc results for the 24, 48, 72 and 96 hour cell counts are respectively given on pages 38, 40, 42 and 45 of this DER (Results are given for use of Bonferroni's t-test and for Dunnett's test). The 96 hour EC50 values calculated by the study report and by the reviewer were, respectively, 28.4 and 30.9 mg ATSA metabolite of pyroxsulam/L. The 96 hour cell density NOECs were 6.15 mg ATSA metabolite of pyroxsulam/L in both cases.

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 0-72 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 (as shown in Table 7 of this DER).

The ToxCalc analysis of the study report calculated 0-72 hour specific growth rates using Bonferroni's and Dunnett's tests are shown on, respectively, pages 47 and 48 of this DER.

The ToxCalc analysis identified statistically significant differences between the control mean's specific growth rate and the means of the replicates containing the various ATSA metabolite of pyroxsulam concentrations of ≥24.2 mg ATSA metabolite of pyroxsulam/L when using Bonferroni's and Dunnett's tests for comparison of the means. This result differed from the study report where all concentrations of the ATSA metabolite of pyroxsulam tested (i.e. ≥3.06 mg ATSA metabolite of pyroxsulam/L) were found to be statistically significantly less than the control mean.

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

Mean measured concentration as mg ATSA metabolite of pyroxsulam/L	Replicate	Reviewer calculated specific growth rates (day^{-1})	Specific growth rates (day^{-1}) determined from multiplication of the $hour^{-1}$ results (in column to right) X 24.	Specific growth rates reported in the study report ($hour^{-1}$) as rounded values.
Control (<LLQ)	A	1.8184	1.8182	0.07576
	B	1.7933	1.7935	0.07473
	C	1.7594	1.7594	0.07331
	D	1.7776	1.7782	0.07409
	E	1.7871	1.7863	0.07443
	F	1.7840	1.7842	0.07434
3.06	A	1.7472	1.7465	0.07277
	B	1.7060	1.7054	0.07106
	C	1.7678	1.7676	0.07365
6.15	A	1.7678	1.7681	0.07367
	B	1.7100	1.7095	0.07123
	C	1.7525	1.7530	0.07304
12.4	A	1.6199	1.6198	0.06749
	B	1.6811	1.6814	0.07006
	C	1.7000	1.7004	0.07085
24.2	A	1.4632	1.4630	0.06096
	B	1.4561	1.4558	0.06066
	C	1.2459	1.2456	0.0519
48.0	A	0.9200	0.9202	0.03834
	B	0.6414	0.6415	0.02673
	C	0.4916	0.4915	0.02048
99.9	A	0.3929	0.3929	0.01637
	B	0.5784	0.5782	0.02409
	C	0.3444	0.3444	0.01435

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

0-72 Hour biomass (area under the curve)

The reported cell density data from the 0 to 72 hour period were used in 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. The calculated and reported results are considered equivalent.

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

Mean measured concentration as mg ATSA 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)
<LLQ	A	4190	4188
	B	3907	3908
	C	3460	3462
	D	3844	3848
	E	3844	3838
	F	4000	4003
3.06	A	3545	3538
	B	3030	3025
	C	3578	3576
6.15	A	3493	3496
	B	3073	3071
	C	3587	3590
12.4	A	2247	2245
	B	2695	2696
	C	2855	2856
24.2	A	1414	1413
	B	1383	1383
	C	758	757
48	A	370	370
	B	164	164
	C	93	93
99.9	A	118	118
	B	183	183
	C	78	78

The ToxCalc analysis of the study report's 0-72 hour biomass results are shown on pages 50 and 51 of this DER for, respectively, Bonferroni's t-test and Dunnett's test analyses.

The ToxCalc analysis identified statistically significant differences between the control mean's biomass and the means of the replicates of all concentrations of ATSA metabolite of pyroxsulam when using Bonferroni's test and Dunnett's for comparison of the means. Consequently, the 0-72 hour biomass NOEC was set at <3.06 mg ATSA metabolite of pyroxsulam/L, the same value as given by 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 study report's toxicity endpoints are considered to have been validated by the results determined by the reviewer.

Table 9. Reported and reviewer calculated toxicity endpoints.

Toxicity endpoint	Endpoint value as mg ATSA 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	42.8 (35.8-49.8)	40.9 (35.0-55.2)
ErC20	17.9 (11.8-24.0)	22.5 (18.4-27.4)
ErC10	10.7 (5.61-15.8)	14.9 (12.3-18.4)
NOEC	<3.06 (Dunnett's test)	12.4 (Dunnett's test)
0-72 hour biomass		
EbC50	16.8 (15.1-18.5)	17.9 (14.9-21.3)
EbC20	9.40 (7.63-11.2)	8.4 (3.6-11.0)
EbC10	6.70 (5.00-8.40)	2.4 (1.3-9.6)
NOEC	<3.06 (Dunnett's test)	<3.06 (Dunnett's test)
96 hour cell density		
EC50	28.4 (25.8-31.0)	30.9 (24.1-36.1)
NOEC	6.15 (Dunnett's test)	6.15 (Bonferroni's t-test and Dunnett's test)

*** The study report stated that based on the Dunnett's test, the 72-hour specific growth rate was significantly less than the controls at all test levels; therefore, the 72-hour NOEC value for specific growth rate was determined to be < 3.06 mg/L, the lowest level tested. In lieu of a NOEC, the ErC10 and ErC20 values were calculated.**

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	AAM used.	The 2006 guideline refers to two alternative growth media, namely the OECD and the AAP medium. No reference to AAM	The guideline states formulation of nutrient medium should conform to those currently recommended by the EPA for freshwater and marine algal bioassays with reference made to relevant entries in 1991 Annual Book of ASTM Standards. No reference to AAM.
Light intensity and quality:	Mean Light Intensity: 5124 lux Minimum Light Intensity: 4600 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-	US EPA light intensity requirement not met (US EPA OPPTS 850.5400 states fluorescent lights providing 4300 lux are to be used for

Maximum Light Intensity: 5700 lux	8880 lux.	*Selenastrum.*

While neither OECD 201 nor US EPA OPPTS 850.5400 specifically refers to AAM, the provision of that medium's composition (see Appendix II, page 52 of this DER) and satisfactory algal growth in the controls indicates this is not a serious deviation from those guidelines.

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

E. REVIEWER'S COMMENTS:

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

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

Based on the results of this study, as shown below, the ATSA 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 reviewer agrees that a statistical difference may have been detected between the controls and all treatment levels for biomass and growth rate. Thus, the NOEC would be <3.06 mg ATSA metabolite of pyroxsulam/L for these two endpoints. However, the PMRA reviewer notes that although statistically significant, the inhibitions observed at the lower treatment levels are unlikely to be biologically significant (for example, inhibitions of 3 to 7 % for growth rate at 3.13 to 12.5 mg ATSA metabolite or pyroxsulam/L). The PMRA uses the EC50s for aquatic risk assessments. The NOECs of <3.06 mg ATSA metabolite of pyroxsulam/L will not used. The PMRA reviewer agrees with the other conclusions of the APVMA reviewer. This study is acceptable to the PMRA.

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 ATSA metabolite of pyroxsulam/L),	<3.06 (Dunnett's test)	<3.06 (Dunnett's test)	6.15 (Dunnett's test)
EC50 (mg ATSA metabolite of pyroxsulam/L) (95% C.I. in brackets)	42.8 (35.8-49.8)	16.8 (15.1-18.5)	28.4 (25.8-31.0)
Reference chemical, if used	**Not applicable as no reference chemical was used.**		

III. REFERENCES:

Annex to EC Commission Directive 92/69/EEC Annex, C.3 Algal Inhibition Test.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

24 hour cell density – Bonferroni's t-Test

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

Conc-mg/L	1	2	3	4	5	6
D-Control	88700	67100	68800	67700	61500	62500
3.06	70100	61300	62800			
6.15	61500	59500	62600			
12.4	54100	59900	57400			
24.2	64300	47900	35700			
48	37400	24300	18000			
99.9	28500	32100	19500			

			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	69383.3	1.0000	69383.3	61500	88700	14.283	6				69383.3	1.0000
3.06	64733.3	0.9330	64733.3	61300	70100	7.273	3	0.767	2.655	16093.7	64733.3	0.9330
6.15	61200	0.8821	61200	59500	62600	2.568	3	1.350	2.655	16093.7	61200	0.8821
12.4	57133.3	0.8234	57133.3	54100	59900	5.092	3	2.021	2.655	16093.7	57133.3	0.8234
*24.2	49300	0.7105	49300	35700	64300	29.110	3	3.313	2.655	16093.7	49300	0.7105
*48	26566.7	0.3829	26566.7	18000	37400	37.252	3	7.064	2.655	16093.7	26633.3	0.3839
*99.9	26700	0.3848	26700	19500	32100	24.307	3	7.042	2.655	16093.7	26633.3	0.3839

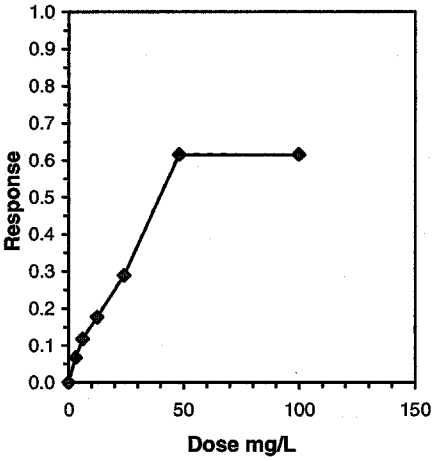
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92788	0.884	0.91833	1.42773
Bartlett's Test indicates equal variances (p = 0.18)	8.96839	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	12.4	24.2	17.3228		16093.7	0.23195	1.1E+09	7.3E+07	6.8E-06	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	2.283	1.818	0.000	9.403	0.9129
IC10	5.061	4.815	0.000	35.879	2.2417
IC15	9.568	6.537	0.000	37.351	1.1531
IC20	14.850	6.813	0.135	38.011	0.4836
IC25	20.076	6.280	4.295	39.305	0.1905
IC40	32.254	5.554	14.183	47.349	-0.2603
IC50	39.539	6.272	23.184	60.259	1.7296

* indicates IC estimate less than the lowest concentration



24 hour cell density – Dunnett's Test

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

Conc-mg/L	1	2	3	4	5	6
D-Control	88700	67100	68800	67700	61500	62500
3.06	70100	61300	62800			
6.15	61500	59500	62600			
12.4	54100	59900	57400			
24.2	64300	47900	35700			
48	37400	24300	18000			
99.9	28500	32100	19500			

			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	69383.3	1.0000	69383.3	61500	88700	14.283	6				69383.3	1.0000
3.06	64733.3	0.9330	64733.3	61300	70100	7.273	3	0.767	2.490	15093.5	64733.3	0.9330
6.15	61200	0.8821	61200	59500	62600	2.568	3	1.350	2.490	15093.5	61200	0.8821
12.4	57133.3	0.8234	57133.3	54100	59900	5.092	3	2.021	2.490	15093.5	57133.3	0.8234
*24.2	49300	0.7105	49300	35700	64300	29.110	3	3.313	2.490	15093.5	49300	0.7105
*48	26566.7	0.3829	26566.7	18000	37400	37.252	3	7.064	2.490	15093.5	26633.3	0.3839
*99.9	26700	0.3848	26700	19500	32100	24.307	3	7.042	2.490	15093.5	26633.3	0.3839

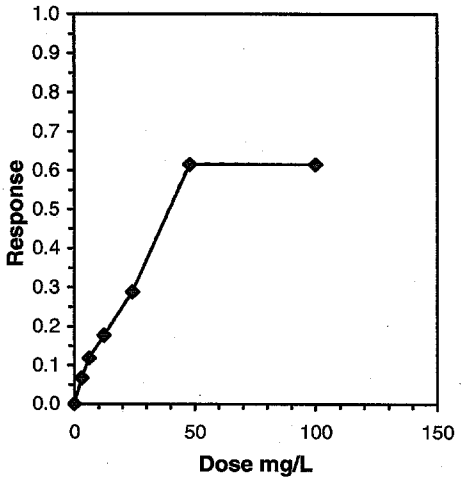
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92788	0.884	0.91833	1.42773
Bartlett's Test indicates equal variances (p = 0.18)	8.96839	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	12.4	24.2	17.3228		15093.5	0.21754	1.1E+09	7.3E+07	6.8E-06	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	2.283	1.836	0.078	9.484	0.9840
IC10	5.061	3.747	0.000	17.795	1.8666
IC15	9.568	6.011	0.000	35.650	1.0089
IC20	14.850	6.813	0.000	36.574	0.2847
IC25	20.076	6.238	4.264	37.705	0.0246
IC40	32.254	5.963	12.378	46.297	-0.4085
IC50	39.539	7.176	18.348	72.963	1.7097

* indicates IC estimate less than the lowest concentration



48 hour cell density – Bonferroni's t-Test

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
D-Control	512000	501000	418000	524000	500000	574000
3.06	487000	391000	448000			
6.15	414000	401000	497000			
12.4	262000	313000	337000			
24.2	147000	159000	95100			
48	62700	34900	24100			
99.9	29600	40900	23900			

			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	504833	1.0000	504833	418000	574000	10.015	6				504833	1.0000
3.06	442000	0.8755	442000	391000	487000	10.923	3	2.150	2.655	77608.1	442000	0.8755
6.15	437333	0.8663	437333	401000	497000	11.909	3	2.309	2.655	77608.1	437333	0.8663
*12.4	304000	0.6022	304000	262000	337000	12.599	3	6.871	2.655	77608.1	304000	0.6022
*24.2	133700	0.2648	133700	95100	159000	25.402	3	12.697	2.655	77608.1	133700	0.2648
*48	40566.7	0.0804	40566.7	24100	62700	49.090	3	15.883	2.655	77608.1	40566.7	0.0804
*99.9	31466.7	0.0623	31466.7	23900	40900	27.497	3	16.194	2.655	77608.1	31466.7	0.0623

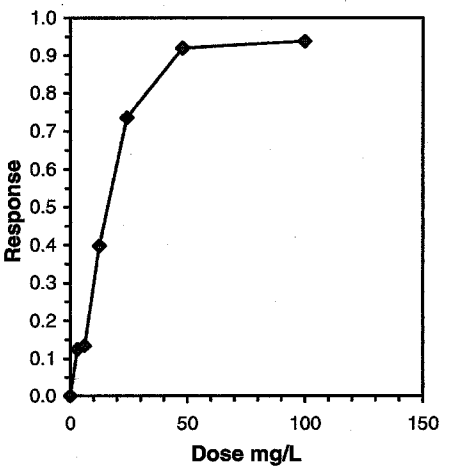
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98101	0.884	-0.2916	0.55535
Bartlett's Test indicates equal variances (p = 0.46)	5.7136	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	6.15	12.4	8.7327		77608.1	0.15373	1.5E+11	1.7E+09	1.0E-11	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.229	1.226	0.468	9.452	2.3152
IC10*	2.459	1.923	0.936	10.404	0.6757
IC15	6.536	1.941	0.000	9.525	-0.3466
IC20	7.719	1.401	0.190	10.445	-1.0062
IC25	8.902	0.991	5.459	11.618	-0.0110
IC40	12.476	1.074	9.602	15.959	0.2236
IC50	15.974	1.187	11.562	19.050	-0.4219

* indicates IC estimate less than the lowest concentration



48 hour cell density – Dunnett's Test

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
D-Control	512000	501000	418000	524000	500000	574000
3.06	487000	391000	448000			
6.15	414000	401000	497000			
12.4	262000	313000	337000			
24.2	147000	159000	95100			
48	62700	34900	24100			
99.9	29600	40900	23900			

			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	504833	1.0000	504833	418000	574000	10.015	6				504833	1.0000
3.06	442000	0.8755	442000	391000	487000	10.923	3	2.150	2.490	72785.1	442000	0.8755
6.15	437333	0.8663	437333	401000	497000	11.909	3	2.309	2.490	72785.1	437333	0.8663
*12.4	304000	0.6022	304000	262000	337000	12.599	3	6.871	2.490	72785.1	304000	0.6022
*24.2	133700	0.2648	133700	95100	159000	25.402	3	12.697	2.490	72785.1	133700	0.2648
*48	40566.7	0.0804	40566.7	24100	62700	49.090	3	15.883	2.490	72785.1	40566.7	0.0804
*99.9	31466.7	0.0623	31466.7	23900	40900	27.497	3	16.194	2.490	72785.1	31466.7	0.0623

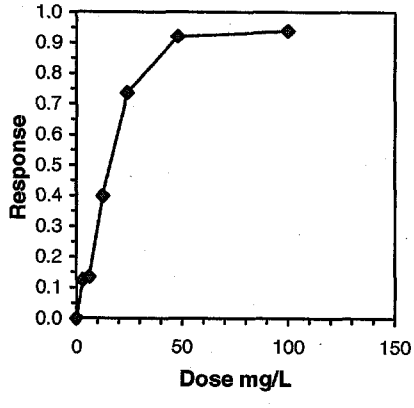
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98101	0.884	-0.2916	0.55535
Bartlett's Test indicates equal variances (p = 0.46)	5.7136	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	6.15	12.4	8.7327		72785.1	0.14418	1.5E+11	1.7E+09	1.0E-11	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.229	1.162	0.475	8.323	2.2030
IC10*	2.459	1.761	0.950	9.890	0.8906
IC15	6.536	1.864	0.000	9.203	-0.4302
IC20	7.719	1.398	0.219	10.235	-1.1541
IC25	8.902	0.961	5.808	11.386	-0.0848
IC40	12.476	1.084	9.979	16.396	0.3332
IC50	15.974	1.132	12.311	19.154	-0.4301

* indicates IC estimate less than the lowest concentration



72 hour cell density – Bonferroni's t-Test

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
D-Control	2340000	2170000	1960000	2070000	2130000	2110000
3.06	1890000	1670000	2010000			
6.15	2010000	1690000	1920000			
12.4	1290000	1550000	1640000			
24.2	806000	789000	420000			
48	158000	68500	43700			
99.9	32500	56700	28100			

			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	2130000	1.0000	2130000	1960000	2340000	5.886	6				2130000	1.0000
3.06	1856667	0.8717	1856667	1670000	2010000	9.287	3	2.651	2.655	273748	1865000	0.8756
6.15	1873333	0.8795	1873333	1690000	2010000	8.809	3	2.489	2.655	273748	1865000	0.8756
*12.4	1493333	0.7011	1493333	1290000	1640000	12.171	3	6.175	2.655	273748	1493333	0.7011
*24.2	671667	0.3153	671667	420000	806000	32.474	3	14.144	2.655	273748	671667	0.3153
*48	90066.7	0.0423	90066.7	43700	158000	66.756	3	19.785	2.655	273748	90066.7	0.0423
*99.9	39100	0.0184	39100	28100	56700	39.386	3	20.279	2.655	273748	39100	0.0184

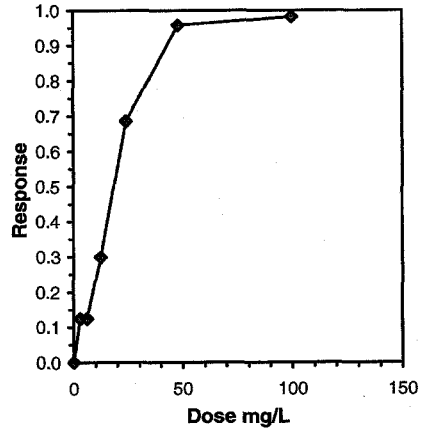
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9463	0.884	-0.4561	-0.5126
Bartlett's Test indicates equal variances (p = 0.18)	8.84435	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	6.15	12.4	8.7327		273748	0.12852	2.8E+12	2.1E+10	3.1E-13	6, 17
Treatments vs D-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.230	0.835	0.633	4.432	3.4704
IC10*	2.460	1.754	1.266	10.410	1.1041
IC15	7.066	1.831	0.000	10.041	-0.8717
IC20	8.857	1.386	3.641	13.225	-0.1949
IC25	10.648	1.358	7.297	14.990	0.0694
IC40	15.492	1.142	11.611	18.886	-0.3307
IC50	18.551	1.038	15.505	21.871	-0.1041

* indicates IC estimate less than the lowest concentration



72 hour cell density – Dunnett's-Test (1) – Interrupted dose response, LOEC = first significant treatment
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
D-Control	2340000	2170000	1960000	2070000	2130000	2110000
3.06	1890000	1670000	2010000			
6.15	2010000	1690000	1920000			
12.4	1290000	1550000	1640000			
24.2	806000	789000	420000			
48	158000	68500	43700			
99.9	32500	56700	28100			

			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	2130000	1.0000	2130000	1960000	2340000	5.886	6				2130000	1.0000
*3.06	1856667	0.8717	1856667	1670000	2010000	9.287	3	2.651	2.490	256735	1865000	0.8756
6.15	1873333	0.8795	1873333	1690000	2010000	8.809	3	2.489	2.490	256735	1865000	0.8756
*12.4	1493333	0.7011	1493333	1290000	1640000	12.171	3	6.175	2.490	256735	1493333	0.7011
*24.2	671667	0.3153	671667	420000	806000	32.474	3	14.144	2.490	256735	671667	0.3153
*48	90066.7	0.0423	90066.7	43700	158000	66.756	3	19.785	2.490	256735	90066.7	0.0423
*99.9	39100	0.0184	39100	28100	56700	39.386	3	20.279	2.490	256735	39100	0.0184

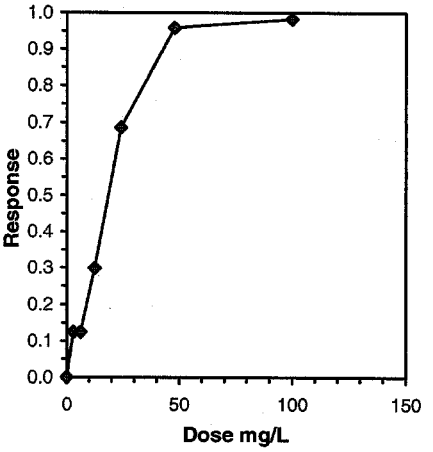
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9463	0.884	-0.4561	-0.5126
Bartlett's Test indicates equal variances (p = 0.18)	8.84435	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	<3.06	3.06			256735	0.12053	2.8E+12	2.1E+10	3.1E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.230	0.641	0.681	4.249	3.6122
IC10*	2.460	1.747	1.361	9.876	1.1055
IC15	7.066	1.932	0.016	10.125	-0.7354
IC20	8.857	1.317	4.456	12.990	0.0729
IC25	10.648	1.303	7.006	14.830	0.2438
IC40	15.492	1.076	12.132	18.722	-0.1678
IC50	18.551	0.992	15.874	21.904	0.2068

* indicates IC estimate less than the lowest concentration



Note that the ToxCalc program identified an interrupted dose response in the 72 hour cell count data. The results above have identified the first significant treatment as the LOEC.

72 hour cell density – Dunnett's-Test (2) – Interrupted dose response, LOEC not placed equal to the first significant treatment

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
D-Control	2340000	2170000	1960000	2070000	2130000	2110000
3.06	1890000	1670000	2010000			
6.15	2010000	1690000	1920000			
12.4	1290000	1550000	1640000			
24.2	806000	789000	420000			
48	158000	68500	43700			
99.9	32500	56700	28100			

			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	2130000	1.0000	2130000	1960000	2340000	5.886	6				2130000	1.0000
*3.06	1856667	0.8717	1856667	1670000	2010000	9.287	3	2.651	2.490	256735	1865000	0.8756
6.15	1873333	0.8795	1873333	1690000	2010000	8.809	3	2.489	2.490	256735	1865000	0.8756
*12.4	1493333	0.7011	1493333	1290000	1640000	12.171	3	6.175	2.490	256735	1493333	0.7011
*24.2	671667	0.3153	671667	420000	806000	32.474	3	14.144	2.490	256735	671667	0.3153
*48	90066.7	0.0423	90066.7	43700	158000	66.756	3	19.785	2.490	256735	90066.7	0.0423
*99.9	39100	0.0184	39100	28100	56700	39.386	3	20.279	2.490	256735	39100	0.0184

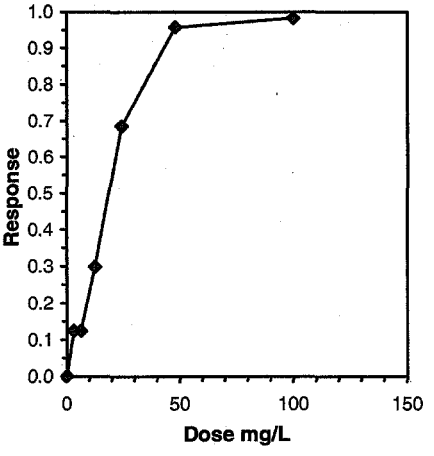
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9463	0.884	-0.4561	-0.5126
Bartlett's Test indicates equal variances (p = 0.18)	8.84435	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	6.15	12.4	8.7327		256735	0.12053	2.8E+12	2.1E+10	3.1E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.230	0.731	0.636	5.074	2.9941
IC10*	2.460	1.728	1.272	9.890	0.9298
IC15	7.066	1.781	0.000	9.343	-0.9735
IC20	8.857	1.209	4.982	11.842	-0.8531
IC25	10.648	1.226	7.350	14.234	0.1311
IC40	15.492	1.157	11.320	18.412	-0.4645
IC50	18.551	1.086	15.358	21.699	-0.1510

* indicates IC estimate less than the lowest concentration



96 hour cell density – Bonferroni's t-Test

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

Conc-mg/L	1	2	3	4	5	6
D-Control	5950000	5450000	5140000	5340000	5530000	5540000
3.06	5150000	5080000	5270000			
6.15	5170000	5320000	5310000			
12.4	4570000	3900000	5410000			
24.2	3830000	3820000	2910000			
48	1430000	485000	373000			
99.9	108000	168000	61900			

			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	5491667	1.0000	5491667	5140000	5950000	4.902	6				5491667	1.0000
3.06	5166667	0.9408	5166667	5080000	5270000	1.860	3	1.137	2.655	758885	5216667	0.9499
6.15	5266667	0.9590	5266667	5170000	5320000	1.592	3	0.787	2.655	758885	5216667	0.9499
*12.4	4626667	0.8425	4626667	3900000	5410000	16.353	3	3.026	2.655	758885	4626667	0.8425
*24.2	3520000	0.6410	3520000	2910000	3830000	15.008	3	6.898	2.655	758885	3520000	0.6410
*48	762667	0.1389	762667	373000	1430000	76.132	3	16.545	2.655	758885	762667	0.1389
*99.9	112633	0.0205	112633	61900	168000	47.234	3	18.819	2.655	758885	112633	0.0205

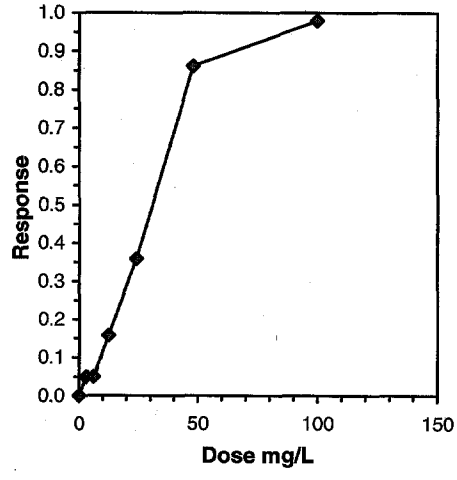
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94977	0.884	0.21743	0.81816
Bartlett's Test indicates equal variances (p = 0.01)	16.3378	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	6.15	12.4	8.7327		758885	0.13819	1.7E+13	1.6E+11	2.2E-12	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	3.055	3.129	1.027	19.751	0.8295
IC10	9.054	2.515	5.577	19.073	0.6100
IC15	11.963	2.628	6.431	20.729	0.3118
IC20	14.888	2.880	6.769	22.356	-0.0035
IC25	17.816	3.018	7.204	24.922	-0.4885
IC40	26.142	1.978	19.706	31.058	-0.4686
IC50	30.882	1.708	25.644	35.667	-0.2791

* indicates IC estimate less than the lowest concentration



The calculated 96 hour EC50 for cell density value is 30.9 mg ATSA metabolite of pyroxsulam/L which is similar to the study report's 96 hour EC50 of 28.4 mg ATSA metabolite of pyroxsulam/L with 95% confidence limits of 25.8 to 31.0 mg ATSA metabolite of pyroxsulam/L. The study report's 96 hour cell density NOEC was 6.15 mg ATSA metabolite of pyroxsulam, the same as calculated by the reviewer.

96 hour cell density – Dunnett's Test

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

Conc-mg/L	1	2	3	4	5	6
D-Control	5950000	5450000	5140000	5340000	5530000	5540000
3.06	5150000	5080000	5270000			
6.15	5170000	5320000	5310000			
12.4	4570000	3900000	5410000			
24.2	3830000	3820000	2910000			
48	1430000	485000	373000			
99.9	108000	168000	61900			

			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	5491667	1.0000	5491667	5140000	5950000	4.902	6				5491667	1.0000
3.06	5166667	0.9408	5166667	5080000	5270000	1.860	3	1.137	2.490	711724	5216667	0.9499
6.15	5266667	0.9590	5266667	5170000	5320000	1.592	3	0.787	2.490	711724	5216667	0.9499
*12.4	4626667	0.8425	4626667	3900000	5410000	16.353	3	3.026	2.490	711724	4626667	0.8425
*24.2	3520000	0.6410	3520000	2910000	3830000	15.008	3	6.898	2.490	711724	3520000	0.6410
*48	762667	0.1389	762667	373000	1430000	76.132	3	16.545	2.490	711724	762667	0.1389
*99.9	112633	0.0205	112633	61900	168000	47.234	3	18.819	2.490	711724	112633	0.0205

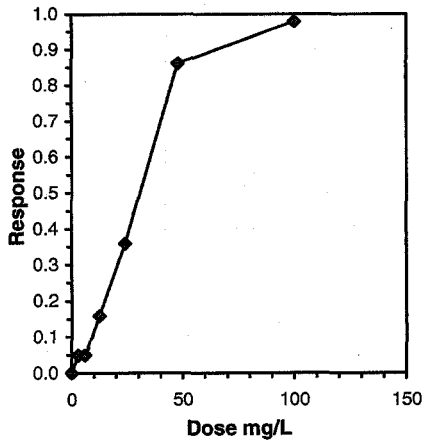
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94977	0.884	0.21743	0.81816
Bartlett's Test indicates equal variances (p = 0.01)	16.3378	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	6.15	12.4	8.7327		711724	0.1296	1.7E+13	1.6E+11	2.2E-12	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	3.055	2.850	1.074	18.289	0.7233
IC10	9.054	2.237	5.358	17.771	0.8322
IC15	11.963	2.386	6.658	19.258	0.2630
IC20	14.888	2.612	7.378	21.426	-0.1147
IC25	17.816	2.758	7.980	24.028	-0.5035
IC40	26.142	2.079	19.392	31.377	-0.4043
IC50	30.882	1.837	24.110	36.096	-0.3549

* indicates IC estimate less than the lowest concentration



As for the Bonferroni's t-test, the calculated 96 hour EC50 for cell density value is 30.9 mg ATSA metabolite of pyroxsulam/L which is similar to the study report's 96 hour EC50 of 28.4 mg ATSA metabolite of pyroxsulam/L with 95% confidence limits of 25.8 to 31.0 mg ATSA metabolite of pyroxsulam/L. The study report's 96 hour cell density NOEC was 6.15 mg ATSA metabolite of pyroxsulam, the same as calculated by the reviewer.

0-72 hour mean specific growth rate – determined from study report's results – Bonferroni's t-Test

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

Conc-mg/L	1	2	3	4	5	6
D-Control	0.0758	0.0747	0.0733	0.0741	0.0744	0.0743
3.06	0.0728	0.0711	0.0737			
6.15	0.0737	0.0712	0.0730			
12.4	0.0675	0.0701	0.0709			
24.2	0.0610	0.0607	0.0519			
48	0.0383	0.0267	0.0205			
99.9	0.0164	0.0241	0.0144			

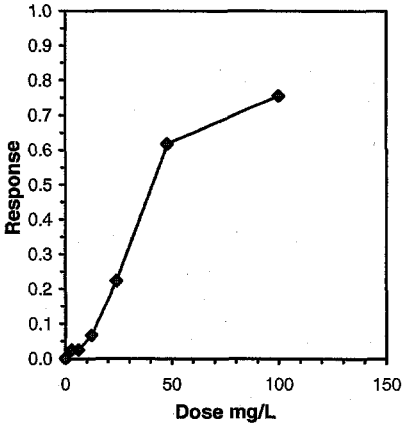
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.0744	1.0000	0.0744	0.0733	0.0758	1.081	6				0.0744	1.0000
3.06	0.0725	0.9738	0.0725	0.0711	0.0737	1.817	3	0.672	2.655	0.0077	0.0726	0.9748
6.15	0.0726	0.9759	0.0726	0.0712	0.0737	1.744	3	0.619	2.655	0.0077	0.0726	0.9748
12.4	0.0695	0.9331	0.0695	0.0675	0.0709	2.529	3	1.715	2.655	0.0077	0.0695	0.9331
*24.2	0.0578	0.7770	0.0578	0.0519	0.0610	8.898	3	5.723	2.655	0.0077	0.0578	0.7770
*48	0.0285	0.3831	0.0285	0.0205	0.0383	31.782	3	15.830	2.655	0.0077	0.0285	0.3831
*99.9	0.0183	0.2454	0.0183	0.0144	0.0241	28.136	3	19.362	2.655	0.0077	0.0183	0.2454

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92961	0.884	0.42065	2.5199
Bartlett's Test indicates unequal variances (p = 2.35E-03)	20.3951	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	12.4	24.2	17.3228		0.0077	0.10347	0.00177	1.7E-05	1.7E-12	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	9.873	1.428	6.541	14.552	0.1283
IC10	14.904	0.958	12.272	18.302	0.1609
IC15	18.682	1.396	15.528	23.155	0.2662
IC20	22.460	1.776	18.265	27.180	0.0682
IC25	25.829	1.712	21.327	30.422	-0.2655
IC40	34.893	1.943	30.608	41.916	0.7065
IC50	40.935	2.876	35.368	57.134	1.4179



The study report noted that specific growth rate data was normally distributed (Shapiro-Wilk, p > 0.1), which was confirmed in the ToxCalc analysis. The variance was heterogeneous for both the raw and transformed values (Levene's Test, p < 0.1), again confirmed by the ToxCalc analysis, so a nonparametric analysis was performed on the ranks of the data. The 0-72 hour calculated ErC50 (95% confidence interval) value for specific growth rate was 42.8 (35.8-49.8) mg/L (40.94 (25.4-57.1) mg/L as determined by the ToxCalc program using linear interpolation). Based on the Dunnett's test, the 72-hour specific growth rate was significantly less than the controls at all test levels; therefore, the 72 hour NOEC value for specific growth rate was determined to be < 3.06 mg/L, the lowest level tested. The ToxCalc program did not identify such a low NOEC.

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

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

Conc-mg/L	1	2	3	4	5	6
D-Control	0.0758	0.0747	0.0733	0.0741	0.0744	0.0743
3.06	0.0728	0.0711	0.0737			
6.15	0.0737	0.0712	0.0730			
12.4	0.0675	0.0701	0.0709			
24.2	0.0610	0.0607	0.0519			
48	0.0383	0.0267	0.0205			
99.9	0.0164	0.0241	0.0144			

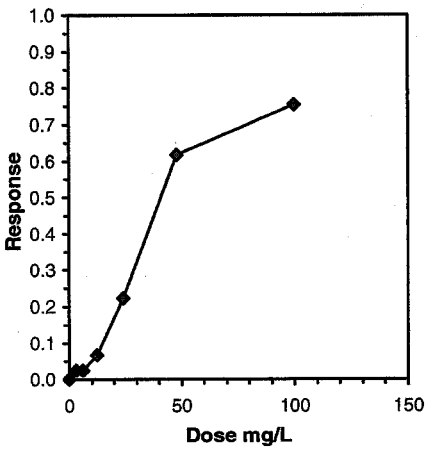
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.0744	1.0000	0.0744	0.0733	0.0758	1.081	6				0.0744	1.0000
3.06	0.0725	0.9738	0.0725	0.0711	0.0737	1.817	3	0.672	2.490	0.0072	0.0726	0.9748
6.15	0.0726	0.9759	0.0726	0.0712	0.0737	1.744	3	0.619	2.490	0.0072	0.0726	0.9748
12.4	0.0695	0.9331	0.0695	0.0675	0.0709	2.529	3	1.715	2.490	0.0072	0.0695	0.9331
*24.2	0.0578	0.7770	0.0578	0.0519	0.0610	8.898	3	5.723	2.490	0.0072	0.0578	0.7770
*48	0.0285	0.3831	0.0285	0.0205	0.0383	31.782	3	15.830	2.490	0.0072	0.0285	0.3831
*99.9	0.0183	0.2454	0.0183	0.0144	0.0241	28.136	3	19.362	2.490	0.0072	0.0183	0.2454

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.92961	0.884	0.42065	2.5199
Bartlett's Test indicates unequal variances (p = 2.35E-03)	20.3951	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	12.4	24.2	17.3228		0.00722	0.09704	0.00177	1.7E-05	1.7E-12	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	9.873	1.480	5.687	14.140	0.2363
IC10	14.904	0.953	12.298	18.408	0.2411
IC15	18.682	1.395	15.667	23.211	0.2907
IC20	22.460	1.764	18.440	27.400	0.0417
IC25	25.829	1.691	21.606	30.525	-0.3192
IC40	34.893	1.898	29.856	41.568	0.5672
IC50	40.935	2.781	35.005	55.226	1.1741



As for the previous results (Bonferroni's test), the ToxCalc concentrations with Dunnett's test gave the 0-72 hour NOEC as 12.4 mg ATSA metabolite of pyroxsulam/L, and not the study report value of < 3.06 mg/L.

0-72 hour biomass calculated from the 0-72 hour total biomass results given in the study report –Bonferroni's t-test.

The ToxCalc analysis of the 0-72 hour biomass (area under growth curve) data presented in the study report gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	4188	3908	3462	3848	3838	4003
3.06	3538	3025	3576			
6.15	3496	3071	3590			
12.4	2245	2696	2856			
24.2	1413	1383	757			
48	370	164	93			
99.9	118	183	78			

			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	3874.5	1.0000	3874.5	3462	4188	6.196	6				3874.5	1.0000
*3.06	3379.67	0.8723	3379.67	3025	3576	9.106	3	2.688	2.655	488.84	3382.67	0.8731
6.15	3385.67	0.8738	3385.67	3071	3590	8.168	3	2.655	2.655	488.84	3382.67	0.8731
*12.4	2599	0.6708	2599	2245	2856	12.191	3	6.928	2.655	488.84	2599	0.6708
*24.2	1184.33	0.3057	1184.33	757	1413	31.274	3	14.611	2.655	488.84	1184.33	0.3057
*48	209	0.0539	209	93	370	68.841	3	19.908	2.655	488.84	209	0.0539
*99.9	126.333	0.0326	126.333	78	183	41.948	3	20.357	2.655	488.84	126.333	0.0326

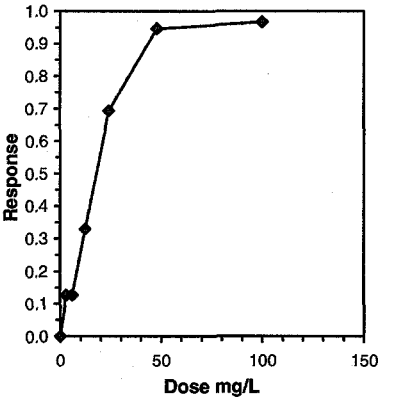
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90077	0.884	-0.7347	-0.5754
Bartlett's Test indicates equal variances (p = 0.48)	5.4923	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs D-Control	<3.06	3.06			488.84	0.12617	8863444	67801	2.9E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.205	0.650	0.672	3.904	3.8992
IC10*	2.411	1.644	1.343	9.682	1.1974
IC15	6.863	1.756	0.067	9.358	-0.8880
IC20	8.408	1.266	3.911	11.595	-0.8985
IC25	9.953	1.159	6.864	13.892	0.3025
IC40	14.688	1.209	10.767	18.338	-0.2588
IC50	17.920	1.053	14.840	21.449	-0.0971

* indicates IC estimate less than the lowest concentration



Note that the ToxCalc program identified an interrupted dose response in the 0-72 hour biomass data. The results above have identified the first significant treatment as the LOEC.

These results are considered equivalent to the study report's 0-72 hour biomass findings that all the ATSA metabolite of pyroxsulam concentrations tested were statistically significantly less than the control biomass mean value and that the NOEC was <3.06 mg ATSA metabolite of pyroxsulam/L.

0-72 hour biomass calculated from the 0-72 hour total biomass results given in the study report –Dunnett's test.

The ToxCalc analysis of the 0-72 hour biomass (area under growth curve) data presented in the study report gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	4188	3908	3462	3848	3838	4003
3.06	3538	3025	3576			
6.15	3496	3071	3590			
12.4	2245	2696	2856			
24.2	1413	1383	757			
48	370	164	93			
99.9	118	183	78			

			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	3874.5	1.0000	3874.5	3462	4188	6.196	6				3874.5	1.0000
*3.06	3379.67	0.8723	3379.67	3025	3576	9.106	3	2.688	2.490	458.461	3382.67	0.8731
*6.15	3385.67	0.8738	3385.67	3071	3590	8.168	3	2.655	2.490	458.461	3382.67	0.8731
*12.4	2599	0.6708	2599	2245	2856	12.191	3	6.928	2.490	458.461	2599	0.6708
*24.2	1184.33	0.3057	1184.33	757	1413	31.274	3	14.611	2.490	458.461	1184.33	0.3057
*48	209	0.0539	209	93	370	68.841	3	19.908	2.490	458.461	209	0.0539
*99.9	126.333	0.0326	126.333	78	183	41.948	3	20.357	2.490	458.461	126.333	0.0326

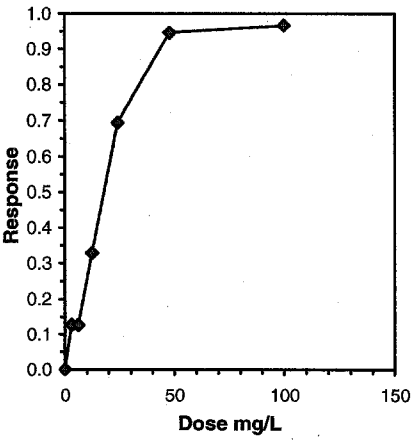
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90077	0.884	-0.7347	-0.5754
Bartlett's Test indicates equal variances (p = 0.48)	5.4923	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs D-Control	<3.06	3.06			458.461	0.11833	8863444	67801	2.9E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	1.205	0.502	0.654	3.517	1.3932
IC10*	2.411	1.557	1.307	9.620	1.2786
IC15	6.863	1.762	0.013	9.144	-0.6941
IC20	8.408	1.079	3.641	10.985	-0.4007
IC25	9.953	1.104	6.419	13.361	0.2000
IC40	14.688	1.171	10.774	18.112	-0.2486
IC50	17.920	1.042	14.892	21.340	-0.0731

* indicates IC estimate less than the lowest concentration



Note that the ToxCalc program did not identify an interrupted dose response in the 0-72 hour biomass data in this case (cf. the Bonferroni's t-test results). The ToxCalc analysis identified that all ATSA metabolite of pyroxsulam concentrations tested had mean 0-72 hour biomass values statistically significantly less than the 0-72 hour mean biomass result. This finding is the same as that reported in the study report for the 0-72 hour biomass analysis. The NOEC of <3.06 mg ATSA metabolite of pyroxsulam/L was reported by the ToxCalc and study report's analyses.

APPENDIX II COMPARISON OF AAM, US EPA AAP and OECD AAP ALGAL GROWTH MEDIA

A comparison of the AAM and US EPA and OEC D algal growth media is shown in Table 11. The 1984 OECD 201 growth medium recipe is essentially the same as that described in the 2006 OECD 201 guideline.

Table 11. Composition and pH of AAM and US and OECD AAP algal growth media.

Constituents	AAM medium mg/L	US EPA AAP medium mg/L	OECD 201 (2006) AAP medium mg/L
$NaNO_3$	25.5	25.5	0
$MgCl_2.6H_2O$	12.2	12.16	12.0
$CaCl_2.2H_2O$	4.4	4.41	18.0
$MgSO_4.7H_2O$	14.7	14.6	15.0
$NaHCO_3$	15	15.0	50.0
K_2HPO_4	1.044	1.044	0
H_3BO_3	0.186	0.186	0.185
$MnCl_2.4H_2O$	0.417	0.415	0.415
$ZnCl_2$	0.00327	0.00327	0.003
$NaMoO_4.2H_2O$	0.00726	0.00726	0.007
$CoCl_2.6H_2O$	0.00143	0.00143	0.0015
$CuCl_2.2H_2O$	0.000011	0.000012	0.00001
$Na_2EDTA.2H_2O$	0.3	0.30	0.10
$FeCl_3.6H_2O$	0.16	0.16	0.064
KH_2PO_4	0	0	1.6
NH_4Cl	0	0	15.0
pH	7.5 ± 0.1	7.5	8.1