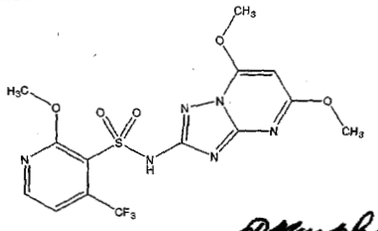
Data Evaluation Report on the Acute Toxicity of Pyroxsulam (XDE-742) Technical to Saltwater Diatom, *Skeletonema costatum*

PMRA Submission Number 2006-4727; ID 1283251 EPA MRID Number 469084-41 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE	Marine algae: 9.8.3
	EPA DP Barcode	D332116
	OECD Data Point	201
	EPA Guideline	850.5400 (123-2)
Test material:	Pyroxsulam (provisionally approved, ISO 175, Compendium of Pesticide Common Names, http://www.alanwood.net/pesticides/pyroxsulam.html)	
Purity:	98%	
Common name:	XDE-742	
Chemical name:	3-pyridinesulfonamide,N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl).	
IUPAC:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
CAS name:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
CAS No.:	422556-08-9	
Synonyms:	XR-742, X666742	

Chemical Structure:



Primary Reviewers: David McAdam and Daryl Murphy **Date:** 11 May 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Phillip Sinclair/Jack Holland **Date:** 16 May 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Émilie Lavière (#1269) **Date:** 14 June 2007
Environmental Assessment Directorate, PMRA

Christopher Salice **Date:** 20 July 2007
US Environmental Protection Agency, Environmental Fate and Effects Division

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

CITATION: Hancock G. A., Hales C. A., McClymont E. L. and Najar J. R. 2005. XDE-742: Growth Inhibition Test with the Saltwater Diatom, *Skeletonema costatum*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Study ID: 051039. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 08 June 2005. Unpublished report.

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IUPAC:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
CAS name:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
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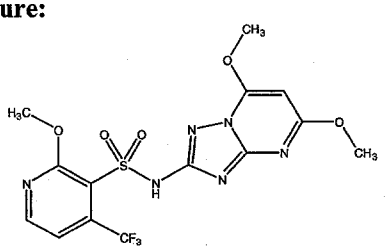

Primary Reviewers: David McAdam and Daryl Murphy **Date:** 11 May 2007
Australian Government Department of the Environment and Water Resources (DEW)

Secondary Reviewers: Phillip Sinclair/Jack Holland **Date:** 16 May 2007
Australian Government Department of the Environment and Water Resources

Émilie Larivière (#1269) **Date:** 14 June 2007
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Dow Chemical Company, Midland, Michigan. Study ID: 051039. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 08 June 2005. Unpublished report.

EXECUTIVE SUMMARY:

The purpose of this study was to assess the effects of pyroxsulam on the growth of *Skeletonema costatum*, a saltwater diatom. 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. The nominal test concentration were 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg pyroxsulam/L and mean measured concentration were 3.40, 6.80, 13.6, 26.7, 52.8, and 105 mg pyroxsulam/L.

Temperatures during the exposure period ranged from 18.7–20.8°C. The mean (± standard deviation) light intensity was 4320 ± 248 lux, with a range of 3890–4710 lux. The pH values ranged from 7.9 to 8.2 at test initiation, from 8.0 to 8.1 in pooled replicates at day 3, and from 8.2 to 8.7 in pooled replicates with the marine diatom and was 8.1 in blank replicates without diatoms at test termination.

The results were all based on mean measured pyroxsulam concentrations. The most sensitive endpoint, based on the 120-h EC50, was cell density. The 96-hour NOEC and EC50 value (95% confidence interval) for cell density were 3.4 and 17.1 (10.2-28.7) mg pyroxsulam/L respectively.

After 120 hours, inhibition relative to controls ranged from 1% at 3.4 mg pyroxsulam/L to 58% at 105 mg pyroxsulam/L for mean specific growth rate (r), from 1% at 3.40 mg pyroxsulam/L to 93% at 105 mg pyroxsulam/L for biomass (b), and from 4% at 3.40 mL to 90% at 105 mg pyroxsulam/L for cell density. The 120-hour EC50 for mean specific growth rate (r), biomass (b) and cell density was 84.3, 13.9 and 13.1 mg pyroxsulam/L, respectively. The 120-hour NOEC was 3.4 mg pyroxsulam/L for all endpoints. Hence, pyroxsulam would be classified as slightly toxic to *Skeletonema costatum* in accordance with the classification system of the Australian Government Department of the Environment and Water Resources ($10 < EC50 \leq 100$ mg/L).

The study is considered acceptable to the PMRA, the US EPA and the APVMA.

Results Synopsis and as Reported in the Study

Test Organism:	Saltwater diatom, *Skeletonema costatum,* at an initial cell density of 10,000 cells/mL.
Test Type:	Static
Endpoint(s) Effected:	Biomass (as area under the growth curve), cell count and growth rate of *Skeletonema costatum.*

Results Based on Mean Measured Concentrations:

Time (Hours)	EC Type	NOEC (mg pyroxsulam/L)	EC Value (mg pyroxsulam/L)	95% Confidence Limits (mg pyroxsulam/L)
96	**EC50**	3.40	17.1	10.2-28.7
	ErC50	3.40	59.0	31.3-111
	EbC50	3.40	14.4	9.50-21.9
120	**EC50**	3.40	13.1	8.42-20.5
	ErC50	3.40	84.3	59.8-109
	EbC50	3.40	13.9	9.49-20.3

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:
The study generally conformed to the procedures current at the time the study was performed (2005) and as described in the following:

- the Organisation for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals OECD guideline 201, "Alga, Growth Inhibition Test", adopted 7 June 1984;
- The European Economic Community (EEC) Commission Directive 92/69/EEC Annex, C.3 Algal Inhibition Test; and
- The U.S. Environmental Protection Agency's (US EPA) *Pesticide Assessment Guidelines,* Subdivision J Hazard Evaluation: Non-target Plants, Guideline 123-2, EPA 540/9-82-020, Washington, D.C. 1982, and
- The US EPA 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. 1986.

COMPLIANCE:
All phases of the study were reported as conducted in compliance with 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;
- European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004); and
- Environmental Protection Agency-FIFRA GLPs and Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule).

Signed and dated No Data Confidentiality, Compliance with Good Laboratory Practice Standards and Quality Assurance statements were provided in the study report.

A. MATERIALS:

1. **Test Material:** XDE-742, i.e. pyroxsulam
Description: Solid
Lot No./Batch No.: E0952-52-01/ TSN103826
Purity: 98%
(This material was indicated in the study report as used for both the preparation of the test solutions and as the analytical standard.)

Stability of Compound Under Test Conditions: Stable

The study report stated that the 26-day stability of pyroxsulam in acetonitrile was determined in a related study (McClymont, 2004) by analyzing a stock solution (nominal concentration 515 µg pyroxsulam/mL acetonitrile) that had been stored for 26 days at ~8°C. The data provided an analysed concentration that was 104% of the expected concentration.

Analytical verification of the test material was conducted at 0 and 96 hours. Initial mean recoveries were 105-107% of nominal and after 120 h were 104 to 112% for test solutions with the marine diatom. Such results indicate pyroxsulam was stable under the test conditions.

Storage conditions of test chemicals: Room temperature in the dark (Hancock, 2005).

Physicochemical properties of pyroxsulam.

The physicochemical properties shown in Table 1 are taken from the Study Profile Template (Hancock 2005) which noted that the UV data were unavailable at the time of publication of the Study Profile Template.

Table 1. Summary of physicochemical properties of pyroxsulam.

Parameter	Values	Comments
Water solubility at 20°C		
pH 4	0.0164 g/L	Turner (2004a)
pH 6	0.0626 g/L	Turner (2004a)
pH 7	3.2 g/L	Turner (2004a)
Vapour pressure	<1E-7	Madsen (2003)
UV absorption	Not available	
pKa	4.670	Cathie (2004)
Kow		
pH 4	12.1 (log Pow = 1.08)	Turner (2004b)
pH 7	0.097 (log Pow = -1.01)	Turner (2004b)
pH 9	0.024 (log Pow = -1.60)	Turner (2004b)

Note: The Kow values shown in the study profile template were misordered. The correct values (confirmed by examination of Turner (2004b) in Madsen (2006)) are shown above in the physicochemical properties of pyroxsulam table.

2. Test organism:

Name:	Saltwater diatom Species: *Skeletonema costatum* Class: Coscinodiscophyceae (or Bacillariophyceae);
Strain:	(Greville) Cleve
Source:	In-house culture. An axenic sample of parent was received in January 2005 from Provasoli-Guillard Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine.
Age of inoculum:	The diatom inoculum was prepared from a 5-day old stock culture of *Skeletonema costatum*.
Method of cultivation:	The culture was maintained in a temperature-controlled environmental chamber at 20 ± 2°C under 4300 ± 860 lux with a photoperiod of 14 hours light/10 hours dark per day. Stock cultures of this organism were maintained aseptically by periodic transfer into fresh f/2 medium. The culture used for this test was maintained under the same conditions as those used for testing.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study:
The exposure concentrations selected for evaluating the effects of pyroxsulam on the growth of *Skeletonema costatum* were based on the results of a preliminary test. A probe study was conducted between 07 February and 11 February 2005 using five nominal pyroxsulam concentrations of 0.0160, 0.0800, 0.400, 2.00, and 10.0 mg pyroxsulam/L, plus a medium control. The percent inhibition compared to the medium control based on cell density at 96 hours was 10, 2, -9, -3, and 50% for the 0.0160, 0.0800, 0.400, 2.00, and 10.0 mg 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 3, 0, -3, -1, and 19% for the 0.0160, 0.0800, 0.400, 2.00, and 10.0 mg pyroxsulam/L test concentrations, respectively. The empirically determined EC50 for cell density was approximately 10.0 mg pyroxsulam/L and for growth rate was greater than the highest level tested (10.0 mg pyroxsulam/L). 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 pyroxsulam/L, plus a medium control.

b. Definitive Study
The definitive test was conducted from 28 February to 05 March 2005. Four replicate test vessels were prepared per exposure concentration and seven replicate test vessels were prepared at the control level. Each replicate contained 50 mL of the appropriate test solution (culture vessels were 250 mL Erlenmeyer flasks with Shimadzu closures).

Nominal test concentrations were 0 (growth medium control), 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg pyroxsulam/L. The f/2 medium used for the culturing and testing of the saltwater diatom, *Skeletonema costatum*, is based on filtered seawater with added nutrients.

Three replicates at each exposure concentration and six replicates at the control level were inoculated with approximately 10,000 cells of *Skeletonema costatum*/mL. Uninoculated blank replicates 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 diatom biomass. The exposure phase was carried out aseptically under static conditions for approximately 120 hours. The replicate test flasks were incubated at 20 ± 2°C with photoperiod of 14 hours light/10 hours dark. Lights were set at an intensity of approximately 4300 ± 650 lux. Total cell counts/mL in the test cultures were made at 24, 48, 72, 96 and 120 hours and microscopic evaluation of cell morphology was conducted at 120 hours. Analyses of the test solutions were made at days 0 and 5.

Test endpoints were cell density (cells/mL), growth rate (day^{-1}), and biomass (area under the growth curve).

pH was determined at days 0, 3 and 5. Incubator temperature was continuously monitored with a minimum/maximum thermometer probe placed in a representative vessel. Light intensity was measured at test initiation.

In making this assessment of the *Skeletonema costatum* study report, it is observed that OECD 201 does not refer to this test organism whereas US EPA OPPTS 850.5400 does. On this basis, a priority is given to compliance with the US EPA guideline.

In the following two tables' Criteria columns (and elsewhere as relevant), entries in italics are those given in the PMRA's Draft Evaluation Report template for acute toxicity to the 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.

Note that some allowances have had to be made in this case as the test guidelines followed were the 1986 OECD and 1982 and 1986 US EPA requirements and not the current (2006) OECD 201 and (1996) US EPA OPPTS 850.5400 guidelines.

Table 2. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous. The parent culture was initiated in 2005 and maintained aseptically by periodic transfer into sterile medium. For this DER, priority has been assigned to the first US EPA guideline statement (i.e. the stock culture can be 3 to 7 days old). 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. As noted above, comparison of the reported typical culturing and test media conditions indicated they were equivalent.	Acclimation, culturing medium and conditions and algal health are acceptable and these requirements are considered met. US EPA OPPTS 850.5400 states that the test begins when algae (inocula) from 3 to 7day–old stock cultures are placed in the test chambers containing test solutions having the appropriate concentrations of the test substance. However, US EPA OPPTS 850.5400 also states that toxicity testing should not be performed until algal cultures are shown to be actively growing (i.e. capable of logarithmic growth within the test period) in at least two subcultures lasting 7 days each prior to the start of the definitive test. EPA recommends two week acclimation period. OECD recommends an amount of algae

Culturing media and conditions: (same as test or not)	Same as test (f/2 medium)	
Health: (any mortality observed)	No phytotoxicity effects noted (based on microscopic evaluation of cells at each test concentration and the control having revealed no abnormal observations at any test level after 120 hours exposure). This is indicative of the starting marine diatoms being healthy.	

Test system Static/static renewal	Static	Test system considered acceptable and the requirement is considered met. US EPA OPPTS 850.5400 indicates static tests are acceptable. OECD 201 does not specifically refer to static tests but can be interpreted as referring to them as no mention is made of renewal of test solutions.
Renewal rate for static renewal	Not applicable	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Temperature controlled environmental growth chamber/incubator.	Incubation facility is considered acceptable. Requirements 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	120-hours	Requirement considered met. US EPA OPPTS 850.5400 refers to cell counts at 24, 48, 72 and 96 hours. No reference is made to 120 hours. OECD 201 refers to the test normally being for 72 hours but with shorter or longer periods allowed provided that guideline's validity criteria are met. Because the 1986 US EPA guideline the test was conducted to a 120 hour exposure period for algal toxicity tests, the parameter is considered met.
		EPA requires: 96-120 hours *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 250 mL 50 mL	Requirements considered met. 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 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.
		OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 mL or use of a culturing apparatus.

<table>
<tr>
<td>Details of growth medium name</td>
<td>The medium's name is "f/2".

A comparison of the constituents and concentrations in the US EPA AAP medium, the OECD TG 201 (2006) medium and the f/2 medium are provided on page 44 of this DER.</td>
<td>.See deviations/deficiencies table, page 33 of this DER.

f/2 is not listed by the 1996 US EPA OPPTS 850.5400 or 2006 OECD 201 guidelines as a standard medium.
Examination of the 1986 US EPA reference and the 1984 OECD guideline did not identify use of the f/2 medium.

A detailed description of preparation of the medium was presented in the study report and a comparison of the AAP, OECD and f/2 media is presented on page 44 of this DER.</td>
</tr>
<tr>
<td>pH at test initiation and at 72 hours (day 3) and 120 hours (day 5):</td>
<td>The pH changes over the 120 hours were as follows:
<table>
<tr><th rowspan="2">mg/L*</th><th rowspan="2">Day 0**</th><th rowspan="2">Day 3#</th><th colspan="2">Day 5</th></tr>
<tr><th>With di-atoms#</th><th>With-out di-atoms</th></tr>
<tr><td>(Control) <LLQ&</td><td>8.2</td><td>8.1</td><td>8.7</td><td>8.1</td></tr>
<tr><td>3.40</td><td>8.2</td><td>8.1</td><td>8.7</td><td>8.1</td></tr>
<tr><td>6.80</td><td>8.2</td><td>8.1</td><td>8.6</td><td>8.1</td></tr>
<tr><td>13.6</td><td>8.1</td><td>8.1</td><td>8.5</td><td>8.1</td></tr>
<tr><td>26.7</td><td>8.1</td><td>8.0</td><td>8.4</td><td>8.1</td></tr>
<tr><td>52.8</td><td>8.0</td><td>8.0</td><td>8.3</td><td>8.1</td></tr>
<tr><td>105</td><td>7.9</td><td>8.0</td><td>8.2</td><td>8.1</td></tr>
</table>
* i.e. mean measured mg pyroxsulam/L.
** pH of bulk solutions.
# pH taken from pooled replicates.
& Less than the lowest level quantified, i.e. 0.163 mg pyroxsulam/L f/2 medium.</td>
<td>Requirement considered met.

US EPA OPPTS 850.5400 states that for Skeletonema, the pH of the nutrient medium is to be 8.1 ± 0.1.

OECD 201 does not refer to marine algae or media in which to grow them.

The day 0 results indicate some effect on the pH as a result of the presence of increasing concentrations of pyroxsulam.

EPA: Skeletonema costatum = ~8.0.

OECD recommends the medium pH after equilibration with air be ~8.</td>
</tr>
</table>

Chelator used:	Yes, Disodium EDTA dihydrate.	Requirement considered met. US EPA OPPTS 850.5400 states that chelating agents are permitted in nutrient media provided the chelator does not interact with the test chemical. OECD 201 notes that disodium EDTA is permitted in AAP medium and OECD TG 201 algal growth media. *EPA recommends 20X-AAP and no chelating agents.* *OECD recommends the medium pH after equilibration with air be ~8 with less than 0.001 mmol/l of chelator, if used.*
Carbon source:	Not specified in the test report. The Study Profile Template (Hancock, 2005) identified the carbon source as ambient carbon dioxide	Requirement considered met. References to carbon source were not identified in either US EPA OPPTS 850.5400 or OECD 201.
Salinity (for marine algae):	Not reported but commercial medium used was made from natural seawater.	See deviations/deficiencies table, page 33 of this DER. US EPA OPPTS 850.5400 states that saltwater for marine algal nutrient medium and test solutions should be prepared by adding a commercial synthetic sea salt formulation or a modified synthetic seawater formulation to distilled/deionised water to a concentration of 30 ppt (24 to 35 g/kg). OECD 201 does not address marine algae.

If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes. Full details and a web address to the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) was provided.	Requirement considered met. The f/2 medium recipe in the test report was as reported by the CCMP web site. Both the CCMP and study report recipes refer to "dH2O" but without explanation. It is assumed distilled water is being referred to.

Dilution water source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Filtered natural seawater 8.2 (value for control at time 0 and taken from the bulk solution.) Not reported but natural seawater, Filtered Not reported but acceptable Not reported Not reported Not reported Not reported According to Hancock (2005), the study report did not give pH, salinity, Total Organic Carbon, particulate matter, metal, pesticides, and chlorine values for the dilution water. Intervals of water quality measurement were also not reported.	See deviations/deficiencies table, page 33 of this DER. US EPA OPPTS 850.5400 states that saltwater for marine algal nutrient medium and test solutions should be prepared by adding a commercial synthetic sea salt formulation or a modified synthetic seawater formulation to distilled/deionised water to a concentration of 30 ppt (24 to 35 g/kg). *EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water. OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*

Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions were prepared using test medium. The highest concentration was prepared first and then serially diluted to obtain stock solutions for all other treatment levels.	Requirement considered met. The exposure solutions were prepared as serial dilutions from the high concentration solution. To prepare the high concentration test solution (100 mg pyroxsulam/L), 102.23 mg of pyroxsulam (adjusted for a purity of 98%) was added to a 1000-mL volumetric flask containing some f/2 medium. The flask was stoppered and shaken vigorously. The flask was then brought to volume with f/2 medium, stoppered, and shaken vigorously again to thoroughly mix. Some undissolved test material was noted in the test solution after mixing. Based on this, the test solution was sonicated for approximately 10 minutes after which the solution was noted as clear. A 500-mL aliquot of this solution was then transferred directly to a second 1000-mL volumetric flask containing some f/2 medium to make the 50.0 mg pyroxsulam/L test solution. The flask was stoppered and the solution was shaken vigorously. The flask was brought to volume (1 L) with f/2 medium, stoppered, and shaken vigorously again to mix thoroughly. Subsequent bulk exposure solutions (25.0, 12.5, 6.25, and 3.13 mg pyroxsulam/L) were similarly prepared as serial dilutions of the next highest exposure solution. All test solutions were noted as clear.

Aeration or agitation	Agitation 60 rpm continuous No aeration	Requirement considered met. US EPA OPPTS 850.5400 states that test containers also should be placed on a rotary shaking apparatus and oscillated at approximately 100 cycles/min for *Selenastrum* and at approximately 60 cycles/min for *Skeletonema* during the test. OECD 201 does not refer to marine algae. Neither standard refers to aeration.
Initial cell density	10,000 cells/mL (for each replicate)	Parameter considered met. US EPA OPPTS 850.5400 states that test chambers should contain approximately 7.7 X 10^4 or 77,000 *S. costatum* cells per millilitre of test solution. OECD 201 does not refer to marine algae. The US EPA 1986 guideline, which the study was conducted to, allows for an initial *Skeletonema costatum* concentration of 10,000 cells/mL. Consequently, the parameter is considered met. *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:	7 (6 inoculated with diatoms, 1 left uninoculated as blank (i.e. without diatoms). The blank was used to correct the daily counts for interference and to monitor pH and concentrations of the test material without the diatom biomass for pH and concentration determination at the end of day 4 when the exposure period ended).	Requirement considered met. US EPA OPPTS 850.5400 states that a minimum of three replicates is required. OECD 201 refers to three replicates at each test concentration with the number of control replicates at least three, and ideally, the number of control replicates should be twice the number of replicates used for each test concentration. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp. tests should be conducted with four replicates. OECD preferably three replicates at each test concentration and ideally they should be twice the 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.*
Solvent control:	A solvent control was not used.	
Treatments:	4 per test concentration (3 inoculated with diatoms, 1 left uninoculated as blank. Blanks were used to correct the daily counts for interference and to monitor pH and concentrations of the test material without the diatom biomass)	
Test concentrations Nominal:	0 (negative control), 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg/L. These concentrations are in a geometric series the ratio of which is 2 and which complies with the relevant OECD and US EPA requirements for this parameter.	Requirement considered met. 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). 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.

<table>
<tr>
<td>Measured:</td>
<td>
<table>
<tr><th></th><th colspan="3">Analyte Measured Concentration, mg pyroxsulam/L, % of Target</th><th></th></tr>
<tr><th>Nominal mg/L</th><th>Day 0[a]</th><th>Day 5[b]</th><th>Day 5[c]</th><th>Mean[d] mg/L, % of target</th></tr>
<tr><td>0</td><td><LLQ[e]</td><td><LLQ</td><td><LLQ</td><td>Not applicable</td></tr>
<tr><td>3.13</td><td>3.34[f]
107%</td><td>3.45
110%</td><td>3.52
112%</td><td>3.40
109%</td></tr>
<tr><td>6.25</td><td>6.65
106%</td><td>6.94
111%</td><td>7.02
112%</td><td>6.80
109%</td></tr>
<tr><td>12.5</td><td>13.3
106%</td><td>13.8
110%</td><td>14.0
112%</td><td>13.6
109%</td></tr>
<tr><td>25.0</td><td>26.4
106%</td><td>26.9
108%</td><td>26.3
105%</td><td>26.7
107%</td></tr>
<tr><td>50.0</td><td>52.5
105%</td><td>53.0
106%</td><td>52.3
105%</td><td>52.8
106%</td></tr>
<tr><td>100</td><td>105[f]
105%</td><td>104
104%</td><td>109
109%</td><td>105
105%</td></tr>
</table>
a. Bulk dose solution. b. Pooled replicates with Skeletonema costatum. Three test solutions containing Skeletonema at each dose level were pooled on day 5 to provide a composite sample for analytical confirmation. c. Replicates with no Skeletonema costatum. d. Mean measured concentration determined from day 0 and day 5 results of replicates with S. costatum. e. LLQ = Lowest level quantified, 0.163 mg pyroxsulam/L f/2 media. f. This value represents the mean of 4 injections (4 samples; 1 injection/sample). Four replicate samples were collected on day 0 of the study to evaluate method precision and solution homogeneity.

In the study report's "Summary of Results from Analysis of Test Solutions for XDE-742" (Table 3 in the test report), the 0 mg/L concentration refers to "0 (f/2-Si Control)" with the footnotes to the table also including reference to "<LLQ = Less than Lowest Level Quantified = 0.163 mg XDE-742/L f/2-Si media". This is taken as an error as the medium's recipe includes sodium silicate, as required for diatoms. The CCMP website (http://ccmp.bigelow.org /future/hmenuz.php?) identifies f/2-Si as a medium prepared from distilled water and filtered seawater prepared as for the f/2 medium but with omission of sodium silicate.
</td>
<td>Results from the day 0 analyses were 105 to 107% of nominal values. The exposure test solution (containing algae) concentrations measured on day 5 had percent recovery of target dose levels of 104 to 111%. Mean analytical results (day 0 and day 5 with algae replicates) were 105 to 109% of nominal. None of the analyses of the f/2 medium controls exhibited a peak eluting at the retention time of pyroxsulam at a concentration exceeding the lowest level quantified of 0.163 mg pyroxsulam/L f/2 medium.

Typical chromatograms of a control, a standard, and a sample were presented and considered consistent with what they were stated to be.
EPA requires at least 5 test concentrations, with each at least 60% of the next higher one. OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested having no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.
</td>
</tr>
<tr>
<td>Solvent (type, percentage, if used)</td>
<td>Not applicable; a solvent was not used</td>
<td>Requirement not applicable.</td>
</tr>
</table>

Method and interval of analytical verification	The bulk dose solutions were sampled for analytical confirmation on day 0 of the study immediately following preparation. On day 5, the three test solutions containing *S. costatum* at each dose level and the 6 test solutions for the control were pooled to provide one composite *S. costatum*-containing sample per dose level for analytical confirmation; the test solutions at each dose level containing no *S. costatum* were sampled and analysed separately by high performance liquid chromatography with ultraviolet absorbance detection (HPLC/UV, 254 nm).	Requirement considered met The study report noted that, 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 analysed along with the other day 0 samples. The HPLC/UV instrumentation was reported to exhibit a linear response over a concentration range extending from approximately 0.147 to 14.7 mg pyroxsulam/L diluent, said to encompass the expected range of sample concentrations after accounting for sample dilution during sample preparation. A linear response was shown in the study report.

Test conditions Temperature:	Temperature ranged from 18.7-20.8°C over the 5 days of the exposure period. Minima were 18.7 to 19.6°C and maxima, 20.2 to 20.8°C. Temperature was recorded using a Minimum/Maximum thermometer.	Requirement considered met. US EPA OPPTS 850.5400 states that the test temperature is to be 20°C for *Skeletonema.* Excursions from the test temperature should be no greater than ± 2 °C. Temperature should be recorded hourly during the test. A continuous recording device is suitable for this purpose. OECD 201 states the cultures should be maintained at a temperature in the range of 21 to 24°C, controlled at ± 2°C but this is for freshwater, not marine, algae.
		EPA temperature: Skeletonema: 20°C, Others: 24-25°C; *OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C.*
Photoperiod:	14 h light/10 h dark	Requirement considered met. US EPA OPPTS 850.5400 states that the test chambers containing *Skeletonema* are to be provided a 14 h light/10 h dark photoperiod. OECD 201 does not refer to *Skeletonema costatum* requirements. *EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous.* *OECD recommended continuous uniform illumination*

Light intensity and quality:	Range: 3890-4710 lux Mean: 4320 lux (standard deviation 248 lux). Light source not identified.	Requirement considered met. US EPA OPPTS 850.5400 states fluorescent lights providing 4300 lux (4,306 lm/m^2 or 400 ± 10% footcandles) for *Skeletonema* are to be used. OECD 201 refers to the recommended algae requiring light intensity at the level of the test solutions from the range of 60-120 $\mu E \cdot m^{-2}s^{-1}$ when measured in the photosynthetically effective wavelength range of 400-700 nm and states that 4440-8880 lux corresponds approximately to the recommended light intensity range. The guideline does not refer to recommended light intensity for marine algae. *EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%);* *OECD recommended continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector*

Reference chemical (if used) name: concentrations:	N/A	A reference chemical was not used. Parameter not relevant as a reference chemical was not used. US EPA OPPTS 850.5400 states that positive controls using zinc chloride as a reference chemical should also be run periodically. 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. The study report could profitably have presented the most recent results from reference chemicals tested against algae in their laboratory.
Other parameters, if any	None identified.	Absence of other parameters determined is noted.

2. Observations:

Table 3. Observation parameters

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell densities (cells/mL) were determined and used to calculate area under the growth curve and growth rate. Morphological observations were conducted at study termination. pH, temperature, light intensity and concentrations of pyroxsulam in the test solutions were also determined over the course of the study.	The requirement is considered met. The parameters determined are acceptable. 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. OECD 201 does not address marine algae. *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	Marine diatom cell densities of the initial inoculum and test cultures were determined by electron particle counting using a Coulter Multisizer 3. Morphological observations were made using an optical microscope with 20x or 40x objective lens; WF10x eyepiece; 1.25x Dual Observation Deck using a Bright Line Hemacytometer Counting Chamber. Appropriate instrumental techniques were used for physico-chemical parameters described above.	Requirement considered met. US EPA OPPTS 850.5400 refers to the algal growth response being determined by an indirect (spectrophotometry, electronic cell counters, dry weight, etc.) or a direct (actual microscopic cell count of at least 400 cells per flask) method. OECD 201 refers to cell counts, being made using an electronic particle counter, a microscope with counting chamber, or a flow cytometer. Other biomass surrogates can be measured using a flow cytometer, fluorimeter, spectrophotometer or colorimeter. *EPA recommends the measurement technique of cell counts or chlorophyll a. OECD recommends electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, or 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).*
Observation intervals	24, 48, 72, 96 and 120-hours	Requirement considered met. 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. OECD 201 refers to algal biomass in each flask being determined daily. *EPA and OECD: every 24 hours.*
Other observations, if any	Microscopic evaluation of cells at each test concentration and	Requirement considered met.

Parameters	Details	Remarks Criteria
	the control revealed no abnormal observations at any test level.	Observation made is appropriate.
Indicate whether there was exponential growth in the control	Yes. Cell density in the control increased by 23.5 fold at 96 hours and 52 fold by test termination (120 hours). At 72 hours, the mean cell density in the control cultures was 10.87 X 10^4 cell/mL. This represents a 10.9 fold increase. At 96 hours, the mean cell density in the control medium replicates was 23.52 X 10^4 cells/mL or 0.2352 X 10^6 cells/mL, i.e. an approximately 24-fold increase.	See deviations/deficiencies table, page 33 of this DER. 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*). Note that the starting cell density was 1 X 10^4 cells/mL as required by the 1986 US EPA guideline. As US EPA 85.0.5400 requires an initial inoculum of 7.7 X 10^4 cells of *Skeletonema*/mL and only 10,000 cells/mL were used, the 1.5 X 10^6 cells/mL value may not be expected to be reached. On a pro-rata basis, a starting cell count of 10,000 would be expected to reach a count of ~1.9 X 10^5 cells after 96 hours. As the actual cell count at that time was ~2.3 X 10^5, exponential growth is indicated to have occurred. OECD 201 requires, *inter alia,* that biomass in the control cultures should have increased by a factor of at least 16 within the 72 hour test period. The measured factor for this period was 10.9, which does not meet the 16 –fold factor. As the 1984 and 2006 versions of this guideline relate to freshwater algae, the reviewer believes it inappropriate to use the 16-fold factor in this instance. .

Parameters	Details	Remarks Criteria
		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 or for species that grow slower, test duration should be increased to obtain at least 16-fold growth.
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.
Were raw data included?	As laboratory notes, no. The study report notes that all data generated are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. While raw data were not submitted, the tabulated results presented were of the individual replicate values and were sufficient to allow statistical analysis. 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 would have complied with the OECD requirement had that guideline included marine algae.	Parameter considered met. While US EPA OPPTS 850.5400 requires that the sponsor must submit to the EPA all data developed by the test including those that are suggestive or predictive of acute phytotoxicity, advice from the US EPA was that, because the tabulated results presented in the study report were sufficient to allow statistical analysis, the guideline would be considered met.

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Results were calculated based on mean measured concentrations.

Inhibition of mean specific growth rate (r) relative to controls at 96 hours ranged from -2% at 3.40 mg pyroxsulam/L to 67% at 105 mg pyroxsulam/L. The 96-hour NOEC and ErC50 (95% confidence interval) value for mean specific growth rate were 3.40 and 59.0 (31.3-111) mg pyroxsulam/L, respectively. Inhibition of mean specific growth rate relative to controls at 120 hours ranged from 1% at 3.4 mg pyroxsulam/L to 58% at 105 mg pyroxsulam/L. The 120-hour NOEC and ErC50 (95% confidence interval) value for mean specific growth rate were 3.4 and 84.3 (59.8-109) mg pyroxsulam/L respectively.

Inhibition of biomass (b) relative to controls at 96 hours ranged from 0% at 3.40 mg/L to 95% at 105 mg pyroxsulam/L. The 96-hour NOEC and EbC50 value (95% confidence interval) for biomass were 3.4 and 14.4 (9.50-21.9) mg pyroxsulam/L respectively. Inhibition of biomass relative to controls at 120 hours ranged from 1% at 3.40 mg pyroxsulam/L to 93% at 105 mg pyroxsulam/L. The 120-hour NOEC and EbC50 value (95% confidence interval) for biomass were 3.40 and 13.9 (9.49-20.3) mg pyroxsulam/L respectively.

Inhibition of cell density relative to controls at 96 hours ranged from -5% at 3.40 mL to 88% at 105 mg pyroxsulam/L. The 96-hour NOEC and EC50 value (95% confidence interval) for cell density were 3.4 and 17.1 (10.2-28.7) mg pyroxsulam/L respectively. Inhibition of cell density relative to controls at 120 hours ranged from 4% at 3.40 mL to 90% at 105 mg pyroxsulam/L. The 120-hour NOEC and EC50 value (95% confidence interval) for cell density were 3.4 and 13.1 (8.42-20.5) mg pyroxsulam/L respectively.

pH in the control and test replicates did not show change of greater than 1 unit over the 5 day exposure period. The range of values reported is shown in the following table.

Table 4. Reported pH maxima and minima in control and pyroxsulam test media with and without the marine diatom, *Skeletonema costatum*, present.

pH values:	Minimum	Maximum
Day 0 range, bulk solution	7.9	8.2
Day 3 range	8.0	8.1
Day 5 range with the marine diatoms	8.2	8.7
Day 5 range without the marine diatoms	8.1	8.1

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

The effects of pyroxsulam on the growth of *Skeletonema costatum* under the test conditions are shown in Table 5 by the cell density counts at 24, 48, 72, 96 and 120 hours and the % inhibition after 96 and 120 hours.

Table 5. Effect of pyroxsulam on the growth of the saltwater diatom *Skeletonema costatum*

Treatment (mean measured concentration (mg pyroxsulam/L)	Initial cell density	Mean cell density ($x10^4$) at						
		24-hours	48-hours	72-hours	96-hours	% inhibition	120-hours	% inhibition
Negative control	1 X 10^4	2.19	3.80	10.9	23.52	---	52.02	---
3.4	1 X 10^4	1.81	3.80	10.5	24.80	-5	50.00	4
6.8	1 X 10^4	1.52	3.10	8.32	17.53*	25	34.15*	34
13.6	1 X 10^4	1.28	2.44	6.08	11.89*	49	23.56*	55
26.7	1 X 10^4	1.00	1.73	3.78	8.971*	62	18.17*	65
52.8	1 X 10^4	0.92	1.51	3.00	6.032*	74	13.71*	74
105	1 X 10^4	0.87	0.98	1.48	2.925*	88	5.317*	90
Reference chemical (if used)	Not applicable as no reference chemical was used.							

***Significantly different from the control (Dunnett's Test, $p \leq 0.05$)**

The mean specific growth rates per day and the mean areas under the growth curves reported by the study following exposure of *Skeletonema costatum* to pyroxsulam are shown in Table 6 with respective percent inhibition results.

Table 6. Effect of pyroxsulam on the saltwater diatom *Skeletonema costatum* growth rate.

Treatment measured concentrations (mg pyroxsulam/L)	Mean Specific Growth Rate per day				Mean Area Under the Growth Curve			
	0-96 h	0-120 h	Percent Inhibition		0-96 h	0-120 h	Percent Inhibition	
			0-96 h	0-120 h			0-96 h	0-120 h
Negative control	0.788	0.790	---	---	603	1485	---	---
3.4	0.803	0.782	-2	1	601	1474	0	1
6.8	0.715*	0.706*	9	11	437*	1033*	28	30
13.6	0.619*	0.632*	21	20	294*	695*	51	53
26.7	0.547*	0.580*	31	27	180*	482*	70	68
52.8	0.449*	0.523*	43	34	119*	331*	80	78
105	0.263*	0.331*	67	58	31*	106*	95	93

***Significantly different from the control (Dunnett's Test, $p \leq 0.05$)**

The reported statistical endpoints are summarised in Table 7.

Table 7. Statistical endpoint values.

Hour	EC Type	NOEC	EC Value	95% Confidence Limits

		(mg **pyroxsulam**/L)	(mg **pyroxsulam**/L)	(mg **pyroxsulam**/L)
96	ErC50	3.40	59.0	31.3-111
	EbC50	3.40	14.4	9.50-21.9
	EC50	3.40	17.1	10.2-28.7
120	ErC50	3.40	84.3	59.8-109
	EbC50	3.40	13.9	9.49-20.3
	EC50	3.40	13.1	8.42-20.5

Note: ErC50 for growth rate, EbC50 for biomass (area under growth curve) and EC50 is for cell density

Validity of test

While noting that OECD 201 (2006) does not apply to marine algae, it is noted that that guideline requires that, for the test to be valid, the following performance criteria should be met:

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

OECD 201 (1984), the guideline version the study followed, requires that the cell concentration in the control cultures should have increased by a factor of at least 16 within three days. This was not met. However, this guideline indicates such a factor relates to freshwater green algae and makes no comment on what the factor should be for marine species such as *Skeletonema costatum*.

In contrast, the US EPA OPPTS 850.5400 specifically refers to *Skeletonema* species and 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.

Although not required by the US EPA guideline, the OECD's exponential growth requirement is considered to have been met (see Table 3 and its associated comments on page 25 of this DER under the parameter "Indicate whether there was an exponential growth in the control").

The 0-24, 24-48 and 48-72 hour control replicate growth rates were calculated from the initial (10,000 cells/mL), 24, 48 and 72 hour cell density counts using the growth rate formula shown under "Verification of Statistical Results" on page 31 of this DER. The values and calculated statistics, including the overall mean % coefficient of variation (%CV) are as shown in Table 8:

Plotting cell counts against time using the Microsoft Excel Chart Wizard function and fitting the data points to an exponential curve (data and curve not shown) returned an r^2 value of 0.9955. This value and the visual examination of the data points and the fitted exponential curve indicate that exponential growth occurred in the study.

Table 8. Reviewer calculated growth rates for the 0-24, 24-48 and 48-72 hour periods in the control replicates and associated means, standard deviations and percentage coefficients of variation.

Reviewer calculated growth rates (/day) for the control replicates			
Replicate	**0-24 h**	**24-48 h**	**48-72 h**
1	0.819	0.582	1.165
2	0.725	0.700	1.081
3	0.711	0.563	0.926
4	0.673	0.638	1.051
5	0.755	0.604	0.854
6	0.983	0.236	1.193
Mean	0.78	0.55	1.04
Standard deviation	0.11	0.16	0.13
%CV	14.38	29.41	12.71

The %CV values for the 0-24, 24-48 and 48-72 hour growth rate values do not exceed the 35% limit set by OECD 201 (2006).

The 0-72 hours %CV of was calculated as 6.29 (mean 0.79, standard deviation 0.05) which does not exceed the OECD 201 limit of 10%. Page 39 of this DER provides the data and Toxcalc calculations which give this result.

B. REPORTED STATISTICS:

The results (study endpoints) of the study were evaluated based on the mean measured pyroxsulam concentrations. The endpoints analysed were growth rate (day^{-1}), cell density (cells x 10000/mL), and biomass (area under the growth curve).

The EC50 value for cell density (the concentration that limited cell density to 50% relative to the control) was determined by a least squares linear regression of cell density against the concentration and the common log of the concentration at 96 and 120 hours. The line with the highest R^2 was then reported if the estimates were consistent with the observed data.

The ErC50 value (the concentration that inhibited the growth rate to 50% relative to the control population) was calculated by two methods. First, by regressing the percent reduction in mean specific growth rate for each exposure group compared to the control group against the natural logarithm of the concentrations for the 0- to 96-hour and 0- to 120-hour exposure periods. Second, by regressing the growth rate against concentrations. The line with the highest R^2 was then reported if the estimates were consistent with the observed data. The standard OECD formula was used to calculate growth rate (see under "Verification of Statistical Results").

The EbC50 value (the concentration that inhibited biomass to 50% relative to the control) was calculated by two methods. First, by regression of the differences in the area under the growth curves for each exposure group compared to the control against the log of the concentrations for 96 and 120 hours. Second, by regression of the area under the growth curve against the (log of) concentrations. The line with the highest R^2 was then reported if the estimates were consistent with the observed data. The standard OECD formula was used to calculate area under the growth curve (see under "Verification of Statistical Results").

The data were tested for normality using the Shapiro-Wilk's Test and for homogeneity of variance using the Bartlett's Test. The 96- and 120-hour growth rate and 96-hour biomass raw data met the assumptions of

normality and homogeneity of variance. The 120-hour biomass square root transformed data and 96- and 120-hour cell density log transformed data were homogenous and normal. Based on this, these data were analysed using the analysis of variance and Dunnett's test ($\alpha = 0.05$) to determine NOEC values.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): 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 and for difference between the mean cell counts and mean specific growth rates of the pyroxsulam exposed diatoms and the mean of the controls by Bonferroni's t test. Differences between the mean biomass results of the pyroxsulam exposed diatoms and that of the controls were tested by Dunnett's or Bonferroni's tests. All NOEC values were determined using the Toxcalc package.

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

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

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 96 and 120 hours specific growth rate values for control and test replicates presented in the study report were recalculated and shown to be identical to those given in the study report.

Similarly, the cell density data were used in the following formula for calculation of the biomass-area under the curve values, *viz.*

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

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 96 and 120 hour biomass-area under the growth curve values calculated were the same as those reported in the study report.

The ToxCalc data and statistical outputs are provided on pages 37 and 38 for cell density, pages 40 and 41 for specific growth rate and pages 42 and 43 for biomass.

Most sensitive endpoint
At 96 hours: Cell biomass (0-96 hours).

At 120 hours: Cell density.

The calculated toxicity endpoints are considered similar to those given in the study report except for the 96 hour ErC50 results where the calculated value was 8.1 mg/L higher (see Table 9 below.)

The endpoints reported in the study report and those calculated in the assessment of the study are shown in Table 9.

Table 9. Reported and reviewer calculated toxicity endpoints.

Toxicity endpoint	Mean measured pyroxsulam concentration, mg/L (95% confidence limits)	
	As presented in the study report	As calculated using the ToxCalc program
96 hour mean specific growth rate		
ErC50	59.0 (31.3-111)	67.1 (58.9-79.8)
NOEC	3.4	3.4
96 hour cell density		
EC50	17.1 (10.2-28.7)	13.4 (12.2-17.5)
NOEC	3.4	3.4
96 hour biomass		
EbC50	14.4 (9.50-21.9)	13.2 (11.4-15.8)
NOEC	3.4	3.4
120 hour mean specific growth rate		
ErC50	84.3 (59.8-109)	87.7 (79.2-104)
NOEC	3.4	
120 hour cell density		
EC50	13.1 (8.42-20.5)	12.0 (9.9-13.5)
NOEC	3.4	3.4
120 hour biomass		
EbC50	13.9 (9.49-20.3)	12.6 (10.9-13.8)
NOEC	3.4	3.4

The reviewer calculated results are considered equivalent or similar to those given in the study report.

D. STUDY DEFICIENCIES:

Table 10 summarises deficiencies and deviations from the US EPA OPPTS 850.5400 Guideline. While OECD 201 (2006) requirements are specifically for freshwater algae, that guideline's requirements for the parameters concerned have also been included, if appropriate, for comparison and/or information. Note that the study generally conformed to the procedures described in the 1984 edition of OECD 201 and 1982 and 1986 US EPA requirements and, consequently, some allowance has been made for this (page 6 of this DER also refers).

Table 10. Deviations from Guidelines and other deficiencies

Parameter	Study reported results	OECD 201 Freshwater alga and Cyanobacteria, Growth Inhibition Test	US EPA OPPTS 850.5400 Algal Toxicity, Tiers I and II
Details of growth medium name	The medium's name is "f/2". A detailed description of preparation of the medium was presented in the study report.	f/2 is not listed by the 2006 OECD 201 guideline as a standard medium. The 1984 OECD guideline did not identify use of the f/2 medium which is specifically for saltwater algal species.	f/2 is not listed by the 1996 US EPA OPPTS 850.5400 guideline as a standard medium. The1986 US EPA reference did not identify use of the f/2 medium.
Salinity (for marine algae)	Not reported but filtered sea water was used.	OECD 201 does not address marine algae or media for their growth.	US EPA OPPTS 850.5400 states that saltwater for marine algal nutrient medium and test solutions should be prepared by adding a commercial synthetic sea salt formulation or a modified synthetic seawater formulation to distilled/deionised water to a concentration of 30 ppt (24 to 35 g/kg)
Dilution water source/type:	Filtered natural seawater. The f/2 medium used was commercially purchased.	OECD 201 does not refer to marine algae not to media for their growth.	US EPA OPPTS 850.5400 states that saltwater for marine algal nutrient medium and test solutions should be prepared by adding a commercial synthetic sea salt formulation or a modified synthetic seawater formulation to distilled/deionised water

Parameter	Study reported results	OECD 201 Freshwater alga and Cyanobacteria, Growth Inhibition Test	US EPA OPPTS 850.5400 Algal Toxicity, Tiers I and II
Indicate whether there was exponential growth in the control	Initial cell count: ~10^4 cells/mL Mean control cell count at 96 h: 23.52 X 10^4 cells/mL or 0.2352 X 10^6 cells/mL. Cell count had increased by a factor of 23.5 at 96 hours. (At 120 hours, the mean cell density was 0.52 X 10^6 cells/mL).	OECD 201 (1984 and 2006 editions) 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. However, this requirement is for freshwater algae. An acceptable value for marine algae is not provided by the guideline.	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* but note that this is based on an initial cell density of 7.7 X 10^4 cells/mL, and not the 1 X 10^4 cells/mL used in the study. The guideline also states that if logarithmic growth cannot be demonstrated, the test is to be repeated.

The growth and test medium was f/2 medium, a commercial product whose description was provided. The study report noted that the axenic sample of *Skeletonema costatum* used was maintained aseptically by periodic transfer into fresh f/2 medium. US EPA OPPTS recommends that nutrient medium for algal culture and preparation of test solutions should conform to those currently recommended by the EPA for freshwater and marine algal bioassays in . ASTM E1218–20 (ASTM, 1991). Use of the commercially available f/2 medium is considered to have been acceptable. An internet search provided evidence that *Skeletonema* is routinely cultured in the f/2 medium.

The lack of measured salinity of the f/2 medium and source of the dilution water results reported by the study are not considered of major concern as the reason for extending the study to 120 hours was provided (The exposure phase was extended from approximately 96 hours to approximately 120 hours due to a slight lag in growth during the first portion of the test) and the f/2 medium is a commercial product which can be taken as having acceptable salinity and based on sea water as the diluent.

The US EPA OPPTS 850.5400 requirement 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*) was not met (mean cell count at 96 hours in the control replicates was 23.52 X 10^4 or ~0.24 X 10^6 cells/mL. As such, the guideline requirement is not met. This can be seen as a result of using 10,000 cells/mL initially rather than the recommended 77,000 cells/mL. On a *pro rata* basis, the use of the recommended cell count would have given a 96 hour cell count of ~1.9 X 10^6 cells/mL, which would meet the guideline requirement.

E. REVIEWER'S COMMENTS: In general, the reviewer's recalculation of the toxicity results were considered similar to the study authors' with the differences found possibly related to the recalculated results being based on the

reported cell counts rather than the raw data used by the study authors. Pyroxsulam is considered as slightly toxic to the marine diatom, *Skeletonema costatum* (see "Conclusions" below).

The study is classified as acceptable (see "Conclusions" in the following section) because of the use of the lower starting cell density was shown to have given satisfactory exponential growth over 96 hours, despite the US EPA recommendation that a higher initial cell density be used.

F. CONCLUSIONS:

This study is scientifically sound and is classified as **acceptable** to the PMRA, US EPA and APVMA. .

The most sensitive endpoint at 96 hours was cell biomass (13.2 mg pyroxsulam/L), and at 120 hours, cell density (12.0 mg pyroxsulam/L).

The NOEC was 3.4 mg pyroxsulam/L (at both 96 and 120 hours) and for all growth parameters.

Based on the results of this study, pyroxsulam would be classified as slightly toxic to *Skeletonema costatum* in accordance with the classification system of the Australian Government Department of the Environment and Water Resources ($10 < EC50 \leq 100$ mg/L).

The study's reported endpoints were:

Hour	EC Type	NOEC (mg pyroxsulam/L)	EC Value (mg pyroxsulam/L)	95% Confidence Limits (mg pyroxsulam/L)
96	ErC50	3.40	59.0	31.3-111
	EbC50	3.40	14.4	9.50-21.9
	EC50	3.40	17.1	10.2-28.7
120	ErC50	3.40	84.3	59.8-109
	EbC50	3.40	13.9	9.49-20.3
	EC50	3.40	13.1	8.42-20.5

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APPENDIX. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

96 hour cell density

The ToxCalc analysis of the 96 hour marine diatom cell count data (log transformed) gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	247900	254300	189700	246900	235700	236500
3.4	237500	257000	249500			
6.8	171600	194000	160400			
13.6	114900	124700	117100			
26.7	81680	101300	86160			
52.8	55840	58160	66960			
105	32160	34240	21360			

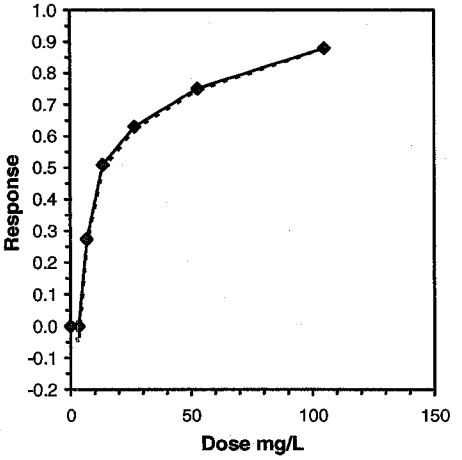
			Transform: Log						1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	235167	1.0000	5.3694	5.2781	5.4053	0.866	6				241583	1.0000
3.4	248000	1.0546	5.3942	5.3757	5.4099	0.321	3	-0.657	2.490	0.0941	241583	1.0000
*6.8	175333	0.7456	5.2425	5.2052	5.2878	0.799	3	3.358	2.490	0.0941	175333	0.7258
*13.6	118900	0.5056	5.0749	5.0603	5.0959	0.367	3	7.793	2.490	0.0941	118900	0.4922
*26.7	89713.3	0.3815	4.9510	4.9121	5.0056	0.983	3	11.072	2.490	0.0941	89713.3	0.3714
*52.8	60320	0.2565	4.7791	4.7469	4.8258	0.866	3	15.620	2.490	0.0941	60320	0.2497
*105	29253.3	0.1244	4.4572	4.3296	4.5345	2.497	3	24.140	2.490	0.0941	29253.3	0.1211

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93822	0.884	-0.9603	1.6642
Bartlett's Test indicates equal variances (p = 0.23)	8.13606	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	3.4	6.8	4.80833		45602.7	0.1948	0.39389	0.00286	1.8E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	4.020	0.118	3.758	4.480	1.2533
IC10	4.640	0.219	4.235	5.560	1.7711
IC15	5.260	0.323	4.678	6.640	1.7873
IC20	5.880	0.431	5.119	7.795	1.7296
IC25	6.500	0.596	5.549	9.231	1.5590
IC40	10.461	0.654	8.387	12.597	-0.2016
IC50	13.372	0.916	12.165	17.474	1.5016



120 hour cell density

The ToxCalc analysis of the 120 hour marine diatom cell count data (log transformed) gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	544000	581700	447300	519400	519300	509400
3.4	477800	516800	505400			
6.8	316200	368800	339400			
13.6	236800	233400	236600			
26.7	175000	189200	180900			
52.8	134800	128700	147700			
105	48800	65760	44960			

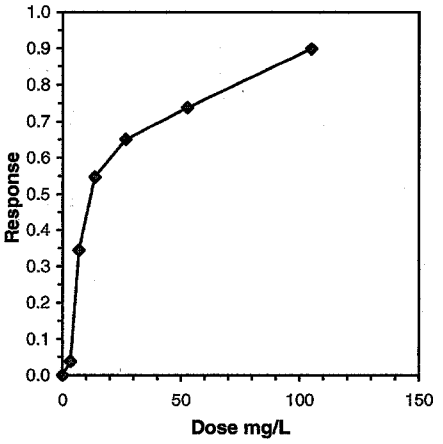
			Transform: Log					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	520183	1.0000	5.7148	5.6506	5.7647	0.660	6				520183	1.0000
3.4	500000	0.9612	5.6987	5.6792	5.7133	0.308	3	0.565	2.490	0.0708	500000	0.9612
*6.8	341467	0.6564	5.5325	5.5000	5.5668	0.605	3	6.408	2.490	0.0708	341467	0.6564
*13.6	235600	0.4529	5.3722	5.3681	5.3744	0.066	3	12.044	2.490	0.0708	235600	0.4529
*26.7	181700	0.3493	5.2591	5.2430	5.2769	0.323	3	16.017	2.490	0.0708	181700	0.3493
*52.8	137067	0.2635	5.1362	5.1096	5.1694	0.592	3	20.337	2.490	0.0708	137067	0.2635
*105	53173.3	0.1022	4.7197	4.6528	4.8180	1.841	3	34.976	2.490	0.0708	53173.3	0.1022

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93978	0.884	0.57963	2.16745
Bartlett's Test indicates equal variances (p = 0.03)	13.5616	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	3.4	6.8	4.80833		78050.9	0.15051	0.42882	0.00162	7.9E-16	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.525	0.728	0.546	4.232	-0.8272
IC10	4.083	0.349	2.872	4.805	-0.9454
IC15	4.641	0.314	3.591	5.389	-0.3088
IC20	5.198	0.313	4.174	6.003	-0.0853
IC25	5.756	0.325	4.691	6.644	0.1154
IC40	8.686	0.911	5.551	10.770	-0.2587
IC50	12.027	0.572	9.913	13.539	-0.2639



0-72 hour mean specific growth rate

The ToxCalc analysis of the 0-72 hour mean specific growth rate data (untransformed) gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	0.8552	0.8352	0.7333	0.7873	0.7377	0.8041
3.4	0.7741	0.7651	0.8118			
6.8	0.6955	0.7094	0.7132			
13.6	0.6146	0.5511	0.6330			
26.7	0.4526	0.4519	0.4255			
52.8	0.3715	0.3885	0.3365			
105	0.0281	0.2230	0.7462			

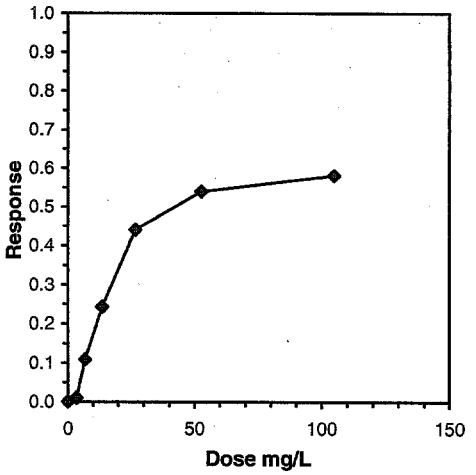
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7921	1.0000	0.7921	0.7333	0.8552	6.294	6				0.7921	1.0000
3.4	0.7837	0.9893	0.7837	0.7651	0.8118	3.159	3	0.091	2.655	0.2474	0.7837	0.9893
6.8	0.7060	0.8913	0.7060	0.6955	0.7132	1.322	3	0.924	2.655	0.2474	0.7060	0.8913
13.6	0.5996	0.7569	0.5996	0.5511	0.6330	7.170	3	2.067	2.655	0.2474	0.5996	0.7569
*26.7	0.4434	0.5597	0.4434	0.4255	0.4526	3.490	3	3.743	2.655	0.2474	0.4434	0.5597
*52.8	0.3655	0.4614	0.3655	0.3365	0.3885	7.264	3	4.579	2.655	0.2474	0.3655	0.4614
*105	0.3324	0.4196	0.3324	0.0281	0.7462	111.708	3	4.934	2.655	0.2474	0.3324	0.4196

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.69437	0.884	1.34523	9.61699
Bartlett's Test indicates unequal variances (p = 2.73E-06)	36.0227	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	13.6	26.7	19.0557		0.24738	0.3123	0.13496	0.01736	3.9E-04	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	4.764	0.674	1.985	6.046	-0.6928
IC10	6.498	0.719	4.332	8.778	0.2543
IC15	8.890	0.986	5.970	11.861	0.1209
IC20	11.419	1.193	8.393	15.246	0.3664
IC25	14.059	1.501	10.380	18.466	0.2224
IC40	24.023				
IC50	42.555				



0-96 hour mean specific growth rate

The ToxCalc analysis of the 0-96 hour mean specific growth rate data (untransformed) gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	0.8026	0.8090	0.7357	0.8016	0.7900	0.7908
3.4	0.7919	0.8116	0.8042			
6.8	0.7106	0.7413	0.6938			
13.6	0.6104	0.6308	0.6151			
26.7	0.5251	0.5789	0.5384			
52.8	0.4300	0.4402	0.4754			
105	0.2920	0.3077	0.1897			

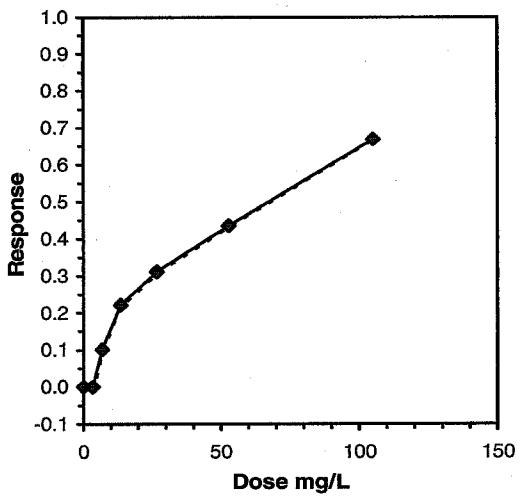
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7883	1.0000	0.7883	0.7357	0.8090	3.396	6				0.7954	1.0000
3.4	0.8026	1.0181	0.8026	0.7919	0.8116	1.242	3	-0.657	2.490	0.0542	0.7954	1.0000
*6.8	0.7152	0.9073	0.7152	0.6938	0.7413	3.370	3	3.358	2.490	0.0542	0.7152	0.8992
*13.6	0.6188	0.7850	0.6188	0.6104	0.6308	1.731	3	7.793	2.490	0.0542	0.6188	0.7779
*26.7	0.5474	0.6945	0.5474	0.5251	0.5789	5.119	3	11.072	2.490	0.0542	0.5474	0.6882
*52.8	0.4485	0.5690	0.4485	0.4300	0.4754	5.312	3	15.620	2.490	0.0542	0.4485	0.5638
*105	0.2632	0.3338	0.2632	0.1897	0.3077	24.346	3	24.140	2.490	0.0542	0.2632	0.3308

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93822	0.884	-0.9603	1.6642
Bartlett's Test indicates equal variances (p = 0.23)	8.13606	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	3.4	6.8	4.80833		0.05417	0.06871	0.13052	0.00095	1.8E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	5.086	0.311	4.478	6.470	1.0005
IC10	6.773	0.629	5.557	9.296	0.5120
IC15	9.558	0.655	7.355	11.327	-0.2744
IC20	12.361	0.516	10.941	14.068	0.5346
IC25	17.676	1.477	13.802	24.575	0.9242
IC40	45.214	2.818	38.388	55.483	0.6160
IC50	67.103	3.363	58.898	79.830	0.5151



0-120 hour mean specific growth rate

The ToxCalc analysis of the 0-120 hour mean specific growth rate data (untransformed) gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	0.7993	0.8127	0.7601	0.7900	0.7900	0.7861
3.4	0.7733	0.7890	0.7846			
6.8	0.6908	0.7215	0.7049			
13.6	0.6329	0.6300	0.6328			
26.7	0.5724	0.5880	0.5791			
52.8	0.5202	0.5110	0.5385			
105	0.3170	0.3767	0.3006			

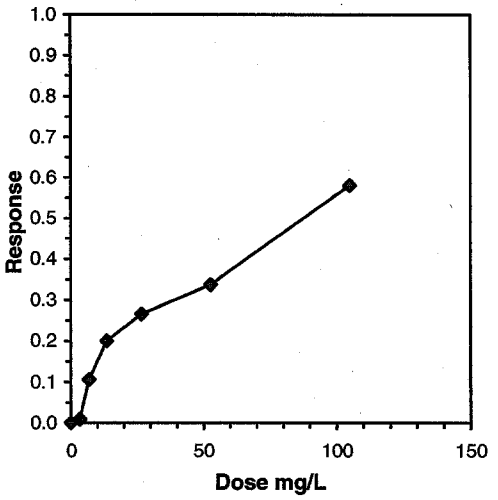
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.7897	1.0000	0.7897	0.7601	0.8127	2.198	6				0.7897	1.0000
3.4	0.7823	0.9906	0.7823	0.7733	0.7890	1.034	3	0.565	2.490	0.0326	0.7823	0.9906
*6.8	0.7057	0.8937	0.7057	0.6908	0.7215	2.183	3	6.408	2.490	0.0326	0.7057	0.8937
*13.6	0.6319	0.8002	0.6319	0.6300	0.6329	0.257	3	12.044	2.490	0.0326	0.6319	0.8002
*26.7	0.5799	0.7343	0.5799	0.5724	0.5880	1.351	3	16.017	2.490	0.0326	0.5799	0.7343
*52.8	0.5232	0.6626	0.5232	0.5110	0.5385	2.678	3	20.337	2.490	0.0326	0.5232	0.6626
*105	0.3315	0.4197	0.3315	0.3006	0.3767	12.075	3	34.976	2.490	0.0326	0.3315	0.4197

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93978	0.884	0.57963	2.16745
Bartlett's Test indicates equal variances (p = 0.03)	13.5616	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	3.4	6.8	4.80833		0.03262	0.04131	0.09094	0.00034	7.9E-16	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	4.825	0.296	3.869	5.560	-0.0476
IC10	6.578	0.496	5.445	8.341	0.7360
IC15	9.977	0.649	7.659	11.461	-0.2983
IC20	13.637	0.849	11.999	17.256	0.9853
IC25	23.574	1.410	19.270	27.915	0.2046
IC40	66.252	2.222	60.001	73.052	0.2877
IC50	87.745	4.001	79.171	104.125	0.7092



0-96 hour biomass

ToxCalc analysis of the reported biomass (untransformed) gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	677.59	664.44	494.90	602.95	562.78	612.98
3.4	576.36	595.82	629.83			
6.8	434.88	460.70	414.62			
13.6	301.66	275.21	304.49			
26.7	166.56	197.11	176.93			
52.8	112.32	119.62	123.94			
105	23.42	52.13	17.09			

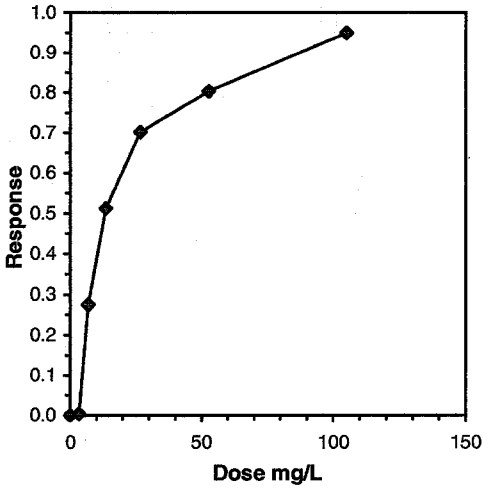
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	602.61	1.0000	602.61	494.90	677.59	11.191	6				602.61	1.0000
3.4	600.67	0.9968	600.67	576.36	629.83	4.506	3	0.069	2.490	70.22	600.67	0.9968
*6.8	436.74	0.7247	436.74	414.62	460.70	5.288	3	5.881	2.490	70.22	436.74	0.7247
*13.6	293.78	0.4875	293.78	275.21	304.49	5.497	3	10.950	2.490	70.22	293.78	0.4875
*26.7	180.20	0.2990	180.20	166.56	197.11	8.622	3	14.978	2.490	70.22	180.20	0.2990
*52.8	118.62	0.1969	118.62	112.32	123.94	4.949	3	17.161	2.490	70.22	118.62	0.1969
*105	30.88	0.0512	30.88	17.09	52.13	60.466	3	20.272	2.490	70.22	30.88	0.0512

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.8948	0.884	-0.7484	4.14016
Bartlett's Test indicates equal variances (p = 0.03)	14.1088	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	3.4	6.8	4.80833		70.2238	0.11653	192400	1590.74	5.5E-13	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.985	0.534	0.984	4.242	-2.0262
IC10	4.610	0.331	3.069	5.061	-0.9316
IC15	5.235	0.336	3.715	5.888	-0.5030
IC20	5.859	0.354	4.582	6.711	-0.0970
IC25	6.484	0.433	5.285	7.884	0.6861
IC40	10.376	0.646	8.043	12.199	-0.2631
IC50	13.242	0.696	11.412	15.823	0.7000



0-120 hour biomass

ToxCalc analysis of the reported biomass (untransformed) gave the following results.

Conc-mg/L	1	2	3	4	5	6
D-Control	1603.87	1643.64	1235.3	1498.51	1444.78	1484.06
3.4	1410.72	1500.38	1511.71			
6.8	996.24	1112.06	990.384			
13.6	699.696	680.928	704.928			
26.7	450.576	521.712	473.4			
52.8	317.088	319.848	357.528			
105	96.576	148.128	72.672			

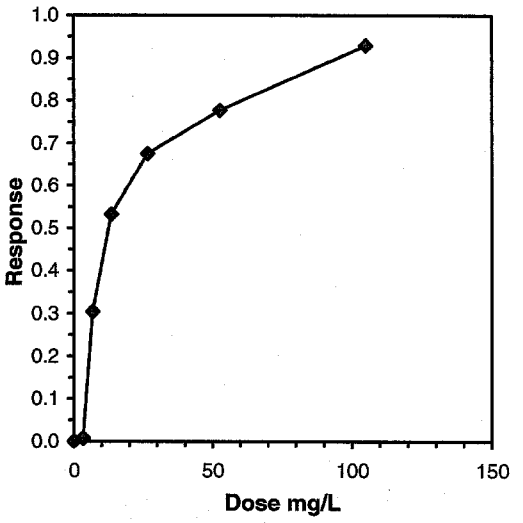
			Transform: Untransformed						1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	1485.03	1.0000	1485.03	1235.3	1643.64	9.686	6				1485.03	1.0000
3.4	1474.27	0.9928	1474.27	1410.72	1511.71	3.753	3	0.177	2.490	151.545	1474.27	0.9928
*6.8	1032.9	0.6955	1032.9	990.384	1112.06	6.644	3	7.429	2.490	151.545	1032.9	0.6955
*13.6	695.184	0.4681	695.184	680.928	704.928	1.815	3	12.978	2.490	151.545	695.184	0.4681
*26.7	481.896	0.3245	481.896	450.576	521.712	7.537	3	16.482	2.490	151.545	481.896	0.3245
*52.8	331.488	0.2232	331.488	317.088	357.528	6.816	3	18.954	2.490	151.545	331.488	0.2232
*105	105.792	0.0712	105.792	72.672	148.128	36.452	3	22.662	2.490	151.545	105.792	0.0712

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.85543	0.884	-1.1357	5.59642
Bartlett's Test indicates equal variances (p = 0.02)	14.6706	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	3.4	6.8	4.80833		151.545	0.10205	1100614	7408.22	9.9E-14	6, 17

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.889	0.481	1.228	4.153	-2.1695
IC10	4.461	0.286	3.251	4.907	-0.9812
IC15	5.033	0.288	3.836	5.636	-0.4699
IC20	5.605	0.307	4.516	6.380	-0.0457
IC25	6.177	0.345	5.105	7.153	0.4515
IC40	9.657	0.677	7.096	11.253	-0.3455
IC50	12.647	0.460	10.910	13.801	-0.2825



Comparison of AAP, OECD and f/2 media
(Page 11 of this DER refers)

	AAP-medium (US EPA) mM	OECD TG 201 (2006) mM	f/2 mM
$NaHCO_3$	0.179	0.595	
$NaNO_3$	0.300		0.883
NH_4Cl		0.280	
$MgCl_2 \cdot 6H_2O$	0.0598	0.0590	
$CaCl_2 \cdot 2H_2O$	0.0300	0.122	
$MgSO_4.7H_2O$	0.0592	0.0609	
K_2HPO_4	0.00599		
KH_2PO_4		0.00919	
$FeCl_3 \cdot 6H_2O$	0.000591	0.000237	0.01
$Na_2EDTA \cdot 2H_2O$	0.000806	0.000269	0.01
H_3BO_3	0.00300	0.00299	
$MnCl_2 \cdot 4H_2O$	0.00201	0.00210	0.0009
$ZnCl_2$	0.000024	0.0000220	
$CoCl_2 \cdot 6H_2O$	0.000006	0.00000630	0.00005
$Na_2MoO_4 \cdot 2H_2O$	0.000030	0.0000289	0.00003
$CuCl_2.2H_2O$	0.00000007	0.00000006	
$NaH_2PO_4.H_2O$			0.363
$Na_2SiO_3.9H_2O$			0.107
$CuSO_4.5H_2O$			0.00004
$ZnSO_4.7H_2O$			0.00008
Vitamin B12			0.0000001
Biotin			0.000002
Thiamine.HCl			0.0003
pH	7.5	8.1	Not referred to. Reported pH in the bulk control solution was 8.2 at time 0.

AAP and OECD TG201 values taken from OECD TG 201 (2006).
The f/2 medium is made up in filtered seawater while the OECD TG 201 medium is made up with sterilised water.
US EPA OPPTS 850.5400 refers to nutrient medium being freshly prepared using a clean water source.

The composition and preparation instructions for the f/2 media are obtainable at http://ccmp.bigelow.org/SD/display.php?strain=CCMP1318.

US EPA OPPTS 850.5400 notes that the pH of the nutrient medium for Skeletonema is to be 8.1 ±0.1.