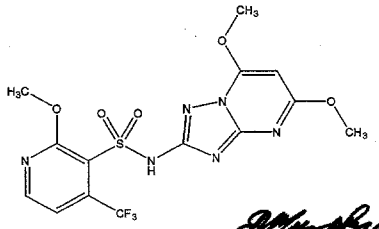
Data Evaluation Report on the Acute Toxicity of Pyroxsulam (XDE-742) Technical to Freshwater Diatom, *Navicula pelliculosa*

PMRA Submission Number 2006-4727; ID 1323248 EPA MRID Number 469084-~~xx~~ 22 APVMA ATS 40362

[A1] [A2]

Data Requirement:	PMRA DATA CODE	Fresh water algae: 9.8.2
	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) or XDE-742 Purity: 98%	
Common name:	XR-742 (i.e. XDE-742 or pyroxsulam)	
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:	X666742	

Chemical Structure:



22/02/07 22/02/07

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

22/2/08

Secondary Reviewers: Phil Sinclair/Jack Holland **Date:** 14 May 2007
Australian Government Department of the Environment, Water, ~~Heritage~~ and the Arts

Émilie Larivière (#1269) **Date:** 14 June 2007
Environmental Assessment Directorate, PMRA Émilie Larivière 05/03/08

Christopher Salice 4/09/08 **Date:** 6 July, 2007
Environmental Protection Agency, Environmental Fate and Effects Division

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

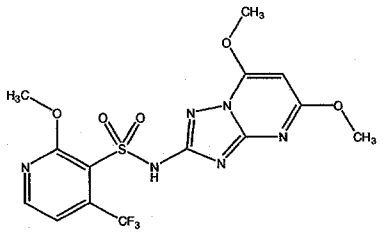
CITATION: Hoberg, J. R. 2005. XDE-742 - Growth inhibition test with freshwater diatom (*Navicula pelliculosa*). Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts. Springborn Smithers Study No. 12550.6367, Sponsor Protocol/Project No. 050283. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences Indianapolis, Indiana 46268. 14 June 2005. Unpublished report.

Data Evaluation Report on the Acute Toxicity of Pyroxsulam (XDE-742) Technical to Freshwater Diatom, *Navicula pelliculosa*

PMRA Submission Number 2006-4727; ID 1323248 EPA MRID Number 469084-32 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE	Fresh water algae: 9.8.2
	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) or XDE-742 Purity: 98%	
Common name:	XR-742 (i.e. XDE-742 or pyroxsulam)	
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:	X666742	

Chemical Structure:



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

Secondary Reviewers: Phil Sinclair/Jack Holland **Date:** 14 May 2007
Australian Government Department of the Environment and Water Resources

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

Christopher Salice **Date:** 6 July, 2007
Environmental Protection Agency, Environmental Fate and Effects Division

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

CITATION: Hoberg, J. R. 2005. XDE-742 - Growth inhibition test with freshwater diatom (*Navicula pelliculosa*). Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts. Springborn Smithers Study No. 12550.6367, Sponsor Protocol/Project No. 050283. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences Indianapolis, Indiana 46268. 14 June 2005. Unpublished report.

Data Evaluation Report on the Acute Toxicity of Pyroxsulam (XDE-742) Technical to Freshwater Diatom, *Navicula pelliculosa*
PMRA Submission Number 2006-4727; ID 1323248 EPA MRID Number 469084-32 APVMA ATS 40362

EXECUTIVE SUMMARY:

The purpose of this study was to determine the effect of pyroxsulam on the growth of the freshwater diatom, *Navicula pelliculosa*. Cultures of *Navicula pelliculosa* were exposed to nominal concentrations of 0.10, 0.26, 0.64, 1.6, 4.0, 10 mg pyroxsulam/L (0.10, 0.29, 0.67, 1.7, 4.0, 10 mg pyroxsulam/L (measured)) under static conditions.

Treatment and the medium control groups were set in triplicate, with an initial cell density of approximately 10,000 cells/mL. The reason for not using four replicates, as required by the US guideline is not known. This is both a deviation and deficiency and reduces the sensitivity and reliability of the study.The temperature was 23°C during the test period with continuous illumination at 3200 to 5400 lux. The pH of the test and control solutions ranged from 6.8 to 7.1 at test initiation. At test termination, the test solution pH values in the control, solvent control, 0.10 and 10 mg pyroxsulam/L treatment levels were unchanged. The pH in the mid-level treatment range (0.26 to 4.0 mg pyroxsulam/L) increased to a range of 7.3 to 9.2, reported as common in static algal cultures due to photosynthesis by the algae. Conductivity measured at test initiation and termination in the treatment and control solutions was 100 µmhos/cm. Modifications to the AAP medium were identified as necessary for the correct growth of the *Navicula pelliculosa*. While the modifications are clear, the concentration of sodium selenate used (1.88 µg/L) is significantly greater than that allowed according to the OECD 201 description of the AAP medium (namely ~0.007 µg/L).

At 24-hour intervals, cell counts were conducted on each replicate vessel of the treatment levels and the controls. Observations of the health of the algal cells were also made at each 24-hour interval. Due to the tendency of *Navicula pelliculosa* cells to clump, the solutions were vigorously pipetted multiple times to disperse clumped cells and achieve a homogeneous suspension prior to removing a sample for cell counts. However, the success of vigorous, multiple pipetting in breaking up the aggregates/filaments is not known and the pipetting procedure is not considered to have shown to be acceptable; indeed US EPA 850.5400 specifically states that"Sonification, ultrasonic bath, blender, syringe, or any other methods of cell separation, other than manual or rotary shaking are not allowed for *Selenastrum*, *Skeletonema*, or *Navicula*."

The OECD 201 guideline's stating that the concentration series should preferably cover the range causing 5-75% inhibition of algal growth rate was not met. There was >90% inhibition at the highest concentration (10 mg/L) with respect to cell density, specific growth rate and biomass but growth stimulation was observed at all other concentrations. Whereas sustained exponential growth was achieved in the negative control and most treatments, this was not the case in the solvent control where cell density fell between 72 and 96 hours and had barely recovered to the 72 hour level at 120 hours.

This study is classified as **INVALID** because of uncertainties relating to the successful disruption of aggregates/filaments of the *Navicula pelliculosa*, the use of a smaller number of replicates than required by the US EPA, the lack of inhibitory effects at all but the highest (10 mg/L) exposure concentration and the lack of sustained exponential growth in the solvent control. Results of this study are not to be used in a risk assessment.

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED:

The toxicity test was performed according to the protocol entitled "Growth Inhibition Test with Freshwater Diatom, *Navicula pelliculosa*", Springborn Smithers Laboratories Protocol No.: 032405/120-Hr Navicula//Dow. The methods described in this protocol were reported as meeting the requirements specified in:

- US EPA FIFRA Subdivision J Guidelines 122-2 and 123-2 as specified in the US EPA Pesticide Assessment Guidelines, Subdivision J. Hazard Evaluation: Nontarget Plants. Report No. EPA 540/9-82-020, PB83-153940. 1982. U.S. Environmental Protection Agency, Washington, D.C.;

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

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

COMPLIANCE:

The data and report for the study, "XDE-742 - Growth Inhibition Test with Freshwater Diatom *Navicula pelliculosa)"* were reported as produced and compiled in accordance with all pertinent OECD and US EPA Good Laboratory Practice regulations, namely

- OECD Good Laboratory Practice in the Testing of Chemicals. Paris. France, as revised 1997 and

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

with the following exceptions: routine dilution water contaminant screening analyses for pesticides, PCBs and toxic metals were conducted using standard U.S. EPA procedures by GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. Since the analyses were conducted following standard validated methods, these exceptions were reported as having had no impact on the study results.

A. MATERIALS:

1. Test Material:	XR-742 (i.e. pyroxsulam or XDE-742)
Description:	White powder (Mercer, 2006)
Lot No./Batch No.:	E0952-52-01/ TSN103826
Purity:	98.0%
Stability of Compound Under Test Conditions:	**Stable.** Analytical verification of the test material was conducted at 0 and 120 hours. Mean recoveries over the 120 hour period were 100% of nominal for the nominal 0.10, 1.6, 4.0 and 10 mg pyroxsulam/L test concentrations and 110% for the nominal 0.26 and 0.64 mg pyroxsulam/L test concentrations. These results indicate the pyroxsulam was stable in the test medium over the 120 hours of exposure.
Storage conditions of test chemicals:	The test substance was stored at room temperature in the original container in a dark ventilated cabinet.

Physicochemical properties of pyroxsulam.

The physicochemical properties shown in Table 1. are taken from the Study Profile Template (Mercer, 2006) 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: Freshwater diatom, *Navicula pelliculosa* (Class: Bacillariophyceae)

Strain: Not reported

Source: Originally from University of Texas, Austin and kept in culture at Springborn Smithers (Wareham).

Age of inoculum: The inoculum used to initiate the toxicity test with XDE-742 was taken from a stock culture that had been transferred to fresh medium seven days prior to test initiation.

Method of cultivation: Algal assay procedure (AAP) medium prepared with sterile deionised water. The culture was maintained in a temperature-controlled environmental chamber at 24 ± 2°C under continuous illumination (3200 to 5400 lux). 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:

A preliminary range-finding exposure was conducted at nominal pyroxsulam concentrations of 0 (control and solvent control), 0.0010, 0.010, 0.10, 1.0 and 10 mg pyroxsulam/L. Following 120 hours of exposure, cell densities in the 0.0010, 0.010, 0.10, 1.0 and 10 mg pyroxsulam/L treatment levels averaged 203, 206, 226, 171 and 3.0 x 10^4 cells/mL, respectively. The control and solvent control averaged 232 and 184 x 10^4 cells/mL,

respectively. Based on these data, nominal pyroxsulam concentrations of 0.10, 0.26, 0.64, 1.6, 4.0 and 10 mg pyroxsulam/L were selected for the definitive exposure.

b. Definitive Study

The definitive study was conducted under static exposure conditions from 22 to 27 April 2005 with replicate 250-mL flasks, three per treatment level and the controls. One hundred milliliters of the appropriate test solution prepared from (modified) Algal Assay Procedure (AAP) medium was then placed in each replicate flask. Nominal pyroxsulam concentrations tested were 0.10, 0.26, 0.64, 1.6, 4.0 and 10 mg pyroxsulam/L (Concentrations were adjusted for the purity of the test substance and are presented as active constituent). An untreated algal medium was used to prepare the control with a solvent control (dimethylformamide, DMF) also prepared with the concentration of DMF in the solvent control equal to the concentration of DMF present in each test solution (0.10 mL/L). An inoculum of *Navicula pelliculosa* cells was aseptically introduced into each flask to provide the required cell density of approximately 1.0×10^4 cells/mL. The exposure period was for 120 hours (5 days) in an incubator at 24 ± 2°C with continuous light and constant shaking. Temperature, light intensity, pH and water conductivity were determined during the course of the exposure. At 24-hour intervals, cell counts were conducted on each replicate vessel of the treatment levels and the controls with observations of the health of the algal cells also made at each 24-hour interval. Due to the tendency of *Navicula pelliculosa* cells to clump, the solutions were vigorously pipetted multiple times to disperse clumped cells and achieve a homogeneous suspension prior to removing a sample for cell counts. Analytical determinations of pyroxsulam in the test vessels were made at test initiation and test termination (120 hours),

The effect criteria considered were inhibition of 120-hour cell density, 0- to 72-hour total biomass (area under the growth curve) and 0 to 72-hour average growth rate relative to the performance of the appropriate control data.

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

Table 2. Experimental Parameters

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td>Acclimation period:</td><td>The inoculum used to initiate the toxicity test with pyroxsulam was taken from a stock culture that had been transferred to fresh medium seven days prior to test initiation.</td><td>See deviations/deficiencies table, page 31 of this DER with respect to acclimatisation period.

Culturing media and conditions and algal health were considered acceptable.</td></tr>
<tr><td>Culturing media and conditions: (same as test or not)</td><td>Algal Assay Procedure (AAP) medium
Same as test

<table>
<tr><th>Parameter</th><th>Culture</th><th>Test</th></tr>
<tr><td>Temperature:</td><td>24 ± 2°C</td><td>24 ± 2°C</td></tr>
<tr><td>Light (lux):</td><td>3200 to 5400</td><td>3200 to 5400</td></tr>
<tr><td>Photoperiod:</td><td>Continuous (24 hours light/day)</td><td>Continuous</td></tr>
<tr><td>Medium:</td><td>AAP</td><td>AAP</td></tr>
<tr><td>pH range:</td><td>~7.0-7.5</td><td>Final pH adjusted to 7.5 ± 0.1</td></tr>
<tr><td>Culture Volume:</td><td>200 mL</td><td>100 mL</td></tr>
<tr><td>Culture Vessel:</td><td>500 mL Erlenmeyer flask</td><td>250 mL flasks</td></tr>
<tr><td>Culture Vessel Cap:</td><td>Shimadzu closure</td><td>Stainless steel caps which permitted gas exchange.</td></tr>
<tr><td>Agitation</td><td>Continuous at 100 ± 10 rpm</td><td>Continuous at 100 ± 10 rpm</td></tr>
</table>
Note that the AAP medium was modified by addition of both sodium selenate and sodium silicate – see below under "Details of growth medium".</td><td>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 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. This guideline also 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.

EPA recommends 3-7 day acclimation period. OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</td></tr>
</table>

Health: (any mortality observed)	Observations of the health of the algal cells were made at each 24-hour interval.	
Test system Static/static renewal Renewal rate for static renewal	Static Not applicable (N/A).	Test system is acceptable. Requirements considered met. OECD 201 does not specifically refer to static tests but can be interpreted as referring to them as no mention is made of renewal of test solutions. US EPA OPPTS 850.5400 both indicate static tests are acceptable. *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 chamber	Incubation facility is 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	See deviations/deficiencies table, page 31 of this DER. Test duration is acceptable. OECD 201 refers to the test normally being for 72 hours but with shorter or longer periods allowed provided that guideline's validity criteria are met. US EPA OPPTS 850.5400 refers to cell counts at 24, 48, 72 and 96 hours. *EPA requires: 96-120 hours* *OECD: 72 hours with the 2006 version stating shorter or longer periods allowed provided all validity criteria specified in that version are met.*

Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 250 mL 100 mL	Requirements considered met. OECD 201 states that the test vessels will normally be glass flasks of dimensions that allow a sufficient volume of culture for measurements during the test and a sufficient mass transfer of CO_2 from the atmosphere. US EPA OPPTS 850.5400 states Erlenmeyer flasks should be used for test containers and may be of any volume between 125 and 500 mL as long as the same size is used throughout a test and the test solution volume does not exceed 50 percent of the flask volume. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name	Algal Assay Procedure (AAP) medium with additions of sodium silicate and sodium selenate (Na_2SeO_4). The addition of sodium selenate was noted in the study report's description of the composition of the AAP medium as being an additional nutritional requirement (see Remarks column regarding the use of sodium selenate and disodium silicate). The concentrations of ingredients listed in the study report as being in the AAP medium corresponded to the values listed in OECD 201's AAP medium recipe, except for the silicon level. The amount of hydrated sodium silicate present was reported as 20 mg/L, calculated as equivalent to ~2 mg Si/L, cf. the 1.4 mg Si/L recommended by OECD 201.	See deviations/deficiencies table, page 31 of this DER with respect to details of the growth medium. OECD 201 refers to AAP medium and provides a comparison (Annex 3) of the US EPA AAP medium and the OECD 201 medium. The guideline identifies both as suitable growth media. The guideline also states that in tests with the diatom *Navicula pelliculosa,* both media must be supplemented with $Na_2SiO_3.9H_20$ to obtain a concentration of 1.4 mg Si/L. Although Annex 3 of the guideline, which contains the AAP composition, does not identify sodium selenate as a constituent of the medium, it goes on to describe the preparation of the US EPA medium and notes that sodium selenate is used only in the medium for stock cultures of diatom species at a final concentration in the AAP medium of ~0.007 µg/L. US EPA OPPTS 850.5400 does not specifically refer to media composition, instead referring to other sources for this information. *EPA recommends 20X-AAP medium*

<table>
<tr>
<td>pH at test initiation:</td>
<td>The control media had a pH of 6.9 at test initiation.</td>
<td>See deviations/deficiencies table, page 31 of this DER with respect to initial pH.

OECD 201 states that the pH of AAP medium is 7.5.

US EPA OPPTS 850.5400 states that the pH of the nutrient medium is to be 7.5 (± 0.1) for Navicula at the start of the test.

The study report stated that the initial pH of the AAP medium was adjusted, if necessary, to 7.5 ± 0.1 prior to use.

The reason for the control pH being 6.9 at time 0 is not immediately apparent.</td>
</tr>
<tr>
<td>pH at test termination:</td>
<td>The initial and final pH values of the control and test solutions were:

<table>
<tr><th rowspan="2">Nominal Concentrations mg pyroxsulam/L</th><th colspan="2">pH</th></tr>
<tr><th>0 h</th><th>120 h</th></tr>
<tr><td>Control</td><td>6.9</td><td>6.9</td></tr>
<tr><td>Solvent Control</td><td>7.0</td><td>7.0</td></tr>
<tr><td>0.10</td><td>7.1</td><td>7.1</td></tr>
<tr><td>0.26</td><td>7.1</td><td>7.4</td></tr>
<tr><td>0.64</td><td>7.1</td><td>9.2</td></tr>
<tr><td>1.6</td><td>7.1</td><td>8.7</td></tr>
<tr><td>4.0</td><td>7.0</td><td>7.3</td></tr>
<tr><td>10</td><td>6.8</td><td>6.8</td></tr>
</table>
</td>
<td>OECD (2006) recommends the pH of the control medium should not increase by more than 1.5 units during the test.

US EPA OPPTS 850.5400 states that the pH of the nutrient medium is to be 7.5 (± 0.1) for Navicula at the start of the test.

The US EPA guideline also states that if the test chemical is highly acidic and reduces the pH of the test solution below 5.0 at the first measurement, appropriate adjustments to pH should be considered, and the test solution measured for pH on each day of the test.

EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt.
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</td>
</tr>
</table>

Chelator used:	Yes, Na_2EDTA in the AAP medium	Requirements for chelator 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 medium and no chelators.* *OECD recommends the medium pH after equilibration with air be ~8 with less than .001 mmol/l of chelator, if used.*
Carbon source:	Not reported.	Requirements for carbon source considered met.
Salinity (for marine algae)	N/A, *Navicula pelliculosa* is a freshwater diatom.	Requirement not considered relevant.
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	A modified standard medium used (AAP medium modified by addition of sodium selenate at a concentration greater than indicated by OECD 201 for the US EPA AAP medium and by addition of sodium silicate. The use of sodium selenate and sodium silicate was identified in the test report as a required additional nutrient.	Requirement considered met with respect to a detailed description of the medium being given in the study report.

Dilution water source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Sterile deionised water with source unidentified. Not given. The pH of the medium was adjusted to 7.5. Salinity not applicable, *Navicula pelliculosa* is a freshwater species. The AAP medium was prepared with sterile, deionised water. A representative sample of AAP medium was analyzed monthly for total organic carbon (TOC) concentration. The TOC concentration was 0.53 mg/L for April 2005. Not determined. Representative samples of the dilution water source used to prepare the medium were analyzed for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed in agreement with ASTM (ASTM, 2002) standard practice.	Requirements considered met. *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)	A 100 mg pyroxsulam/mL primary stock solution was prepared prior to test initiation by placing 2.5510 g of pyroxsulam (2.5000 g as 100% pyroxsulam) in a 25-mL volumetric flask and bringing it to volume with dimethylformamide (DMF). The resulting stock solution was clear and brown in color with no visible undissolved test substance. Secondary stock solutions were prepared by dilution with DMF and then diluted with AAP medium to give the nominal test concentrations.	Description in the study report considered satisfactory. Concentrations were adjusted for the purity of the test substance and are presented as active constituent (pyroxsulam). All test solutions were clear and colorless with no visible undissolved test substance. Untreated algal medium was used to prepare the control. A solvent control was prepared by bringing 0.10 mL of DMF to a final volume of 1000 mL with AAP medium. The concentration of DMF in the solvent control was equal to the concentration of DMF present in each test solution (0.10 mL/L).

Aeration or agitation	An orbital shaker table provided a shaking table rate of 100 ± 10 rpm. No reference was made to aeration.	Requirement considered met. OECD 201 states that during the test it is necessary to keep the algae in suspension and to facilitate transfer of CO_2. To this end constant shaking or stirring should be used and reference is made to an orbital or reciprocate shaker being used at ~150 rpm. US EPA OPPTS 850.5400 states that test containers should be placed on a rotary shaking apparatus and oscillated at approximately 100 cycles/min for *Selenastrum* and at approximately 60 cycles/min for *Skeletonema* during the test. No oscillation rate is referred to for *Navicula.*
Initial cells density	10,000 cells/mL (for each replicate)	Requirement considered met. OECD 201 recommends an initial cell concentration for *Navicula pelliculosa* of 1 X 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 *Navicula* 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:	3	See deviations/deficiencies table, page 31 of this DER with respect to number of replicates used. 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. This was effectively achieved once the controls were pooled having no difference between the untreated and solvent controls. The US EPA OPPTS refers to use of four replicates for *N. pelliculosa.*
Solvent control:	3	*EPA requires a negative and/or solvent control with 3 or more replicates per dose. Navicula sp. tests should be conducted with four replicates. OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Treatments	3 inoculated with algae In order to estimate the impact that the presence of algal biomass had on the test substance concentration, an additional replicate flask of the 0.64 mg pyroxsulam/L (nominal) treatment level was prepared. This flask, which was not inoculated with algae, was analyzed at 120 hours of exposure for pyroxsulam concentration. The results of this analysis were compared with the results for the 0.64 mg pyroxsulam/L solution containing algae.	

Test concentrations

Nominal:

0.10, 0.26, 0.64, 1.6, 4.0, 10 mg/L

These nominal concentrations are in the ratio of
1 to 2.5 or 1 to 2.6.

Measured:

Nominal and measured concentrations at 0 and 120 hours were:

Nominal*	Measured Concentration*			
	0 h	120 h	Mean[a]	%**
Control	<0.013	<0.013	NA[b]	NA
Solvent Control	<0.013	<0.013	NA	NA
0.10	0.10	0.10	0.10	100
0.26	0.29	0.30	0.29	110
0.64	0.67	0.67/0.67[c]	0.67	110
1.6	1.7	1.6	1.7	100
4.0	4.0	4.0	4.0	100
10	10	10	10	100

*** mg pyroxsulam/L. ** Percentage of nominal concentration.**
a Mean measured concentrations and percent of nominal were calculated using actual analytical data and not the rounded (2 significant figures) data presented in the study report.
b NA = Not Applicable.
c Result of the additional sample without algae present to determine biological uptake/degradation.

See deviations/deficiencies table, page 31 of this DER with respect to test concentrations geometric series ratio.

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 guideline also states that the concentration series should preferably cover the range causing 5-75 % inhibition of algal growth rate. There was >90% inhibition at the highest concentration with respect to cell density, specific growth rate and biomass but at all other concentrations, there was growth stimulation.

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).

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% relative to the control and, preferably, stop growth completely.

Solvent (type, percentage, if used)	Dimethylformamide (DMF). 0.10 mL of DMF in a final volume of 1000 mL of AAP medium. The concentration of DMF in the solvent control was equal to the concentration of DMF present in each test solution (0.10 mL/L, i.e. 100 µL/L).	Requirement considered met. OECD 201 states solvents may be used as carriers but the concentration of solvent should not exceed 100 µl/L, and the same concentration of solvent should be added to all cultures (including controls) in the test series. Also that when solvents are used to prepare the test solutions, the solvent controls rather than the controls without solvents should be used in calculation of percent inhibition. US EPA OPPTS 850.5400 states that if a carrier (or solvent) other than nutrient medium is absolutely necessary to dissolve the chemical, the volume used should not exceed the minimum volume necessary to dissolve or suspend the chemical in the test solution. The upper limit of carrier volume is 0.5 mL/L and the same amount of carrier should be added to each concentration.
Method and interval of analytical verification	Test solutions were analyzed for the presence of pyroxsulam at 0 and 120 hours using HPLC. The limit of detection was 0.0155 mg pyroxsulam/L	Requirement considered met. Methodology was validated (20 April 2005) to quantify the amount of pyroxsulam present in recovery samples prepared in AAP medium (a freshwater algal medium). Recovery samples were analyzed by automated injection on a high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV). This method was validated by fortification of AAP medium with pyroxsulam at concentrations of 0.0500, 2.00 and 35.0 mg/L. Recoveries averaged 100 ± 1.85% with a limit of quantitation of 0.0155 mg/L. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 70.0 to 120%. Analytical results for the recovery of pyroxsulam from AAP medium were presented as were representative chromatograms from the analysis of a calibration standard, recovery sample and a control sample. A typical linear regression analysis for pyroxsulam ($r^2 = 0.99998$) was also presented in the study report.

<table>
<tr>
<td>Test conditions
Temperature:</td>
<td>24 ±2°C specified, measured temperature was 23°C over the 120 hours of the exposure period.</td>
<td>Temperature requirement considered met.

OECD 201 states the cultures should be maintained at a temperature in the range of 21 to 24°C, controlled at ± 2°C. The 1984 OECD guideline set the range as 21 to 25°C.

US EPA OPPTS 850.5400 states the test temperature is to be 24°C for Navicula and that excursions from the test temperature should be no greater than ± 2°C.

EPA temperature: Skeletonema: 20 °C, Others: 24-25 °C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%) OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</td>
</tr>
<tr>
<td>Photoperiod:</td>
<td>Continuous</td>
<td>Photoperiod requirement considered met.

OECD 201 states that the cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate.

US EPA OPPTS 850.5400 states that test chambers containing Navicula must be illuminated continuously.</td>
</tr>
<tr>
<td>Light intensity and quality:</td>
<td>The measured light intensity ranges (as lux) over the 120 hours were:

<table>
<tr><th>0 h</th><th>24 h</th><th>48 h</th><th>72 h</th><th>96 h</th><th>120 h</th></tr>
<tr><td>3500 to 5400</td><td>3200 to 5400</td><td>3200 to 5400</td><td>3500 to 5400</td><td>3200 to 5400</td><td>3200 to 5400</td></tr>
</table>

The photosynthetically active radiation (PAR) measured at test initiation ranged from 63 to 87 µE/m2/s.</td>
<td>Requirement considered met.

OECD 201 refers to light intensity at the level of the test solutions from the range of 60-120 $\mu E \cdot m^{-2} s^{-1}$, which it states is equivalent to a range of 4440-8880 lux.

US EPA light intensity requires that fluorescent lights providing 4300 lux for Navicula are to be used and that these lamps should have a photosynthetically active radiation of approximately 66.5 ±10% $mEin/m^2/sec$.</td>
</tr>
</table>

Reference chemical (if used) name: concentrations:	N/A	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. The study report could profitably have presented the most recent results from reference chemicals test against algae in their laboratory.
Other parameters, if any	Conductivity was determined as 100 µmohs/cm in all vessels, test and controls, at both 0 and 120 hours.	Requirement considered met.

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. pH, temperature, light intensity and concentrations of pyroxsulam in the test solutions were also determined over the course of the study. Observations of algal health were also made at 24 hour intervals.	Requirement considered met with the parameters determined acceptable. OECD 201 refers to growth and growth inhibition being quantified from measurements of the algal biomass as a function of time. US EPA OPPTS 850.5400 refers to enumeration of the algal cells to determine inhibition or stimulation of growth and the pattern of growth in test containers compared to controls. *EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Measurement technique for cell density and other end points.	Cell density calculated using a haemocytometer and compound microscope at 24 hour intervals. Observations of the health of the algal cells were made at each 24-hour interval. Due to the tendency of *Navicula pelliculosa* cells to clump, the solutions were vigorously pipetted multiple times to disperse clumped cells and achieve a homogeneous suspension prior to removing a sample for cell counts. Appropriate instrumental techniques were used for physico-chemical parameters listed above.	See deviations/deficiencies table, page 31 of this DER with respect to formation of aggregates of *N. pelliculosa*. *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).* Measurement techniques used are considered acceptable. OECD 201 refers to cell counts, being made using an electronic particle counter, a microscope with counting chamber, or a flow cytometer. Other biomass surrogates can be measured using a flow cytometer, fluorimeter, spectrophotometer or colorimeter. US EPA OPPTS 850.5400 refers to the algal growth response being determined by an indirect (spectrophotometry, electronic cell counters, dry weight, etc.) or a direct (actual microscopic cell count of at least 400 cells per flask) method. OECD 201 also notes that *Navicula pelliculosa* may form aggregates under certain growth conditions. Due to production of lipids the algal cells sometimes tend to accumulate in the surface film. Under those circumstances special measures have to be taken when sub-samples are taken for biomass determination in order to obtain representative samples. Vigorous shaking, e.g. using a vortex mixer may be required. US EPA OPPTS 850.5400 states that the procedure used to break up the filaments should result in consistent filament lengths across treatments and replicates. Sonification, ultrasonic bath, blender, syringe, or any other methods of cell separation, other than manual or rotary shaking are **not** allowed for *Selenastrum*, *Skeletonema*, or *Navicula*.

Observation intervals	24, 48, 72, 96 and 120 hours	Requirement considered met with the 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	Abnormalities (thin cell walls) in the appearance of the algal cells were reported in the 10 mg pyroxsulam/L test concentration at 24 and 48 hours - no other cell abnormalities were reported.	Requirement considered met. Observation made is appropriate.

Indicate whether there was exponential growth in the control	Yes, based on results for the control. Mean cell density in the control increased by 24-fold and by 19.75-fold in the solvent control by test termination (120 hours). At 72 hours there was a mean of 16.92 X 10^4 cells/mL in the control and of 20.58 X 10^4 cells/mL in the solvent control, i.e. the OECD's 16-fold factor is met. At 96 hours, the mean cell count in the control was 22.67 X 10^4 cells/mL and, in the solvent control, 14.67 X 10^4 cells/mL, i.e. the OECD's 16-fold factor is met for the control but not the solvent control. The 0-72 hour mean pooled control growth rate was 0.98 day^{-1} in the AAP medium used. The 0-120 hour mean pooled control growth rate was 0.61 day^{-1}, again in the AAP medium used. A plotting of mean control and solvent control cell counts against time using the Microsoft Excel Chart Wizard function and fitting the data points to an exponential curve (data and curves shown on page 47 of this DER) returned respective R^2 value of 0.8822 and 0.8037, values taken as indicative of some deviation from exponential growth curves.	Requirement considered met with respect to 72 hour results, however, the solvent control results are indicative of some problem having 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 which corresponds to a specific growth rate of 0.92 day^{-1}. The guideline also states that for *Navicula pelliculosa,* the most frequently observed growth rate in OECD medium at light intensity approx. 70 µE m^{-2} s^{-1} and 21°C the growth rate should be 1.4 day^{-1}. No comment is made on the rate in AAP 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,* but there is no value given for *Navicula pelliculosa*). *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 stated that determination of stability and characterization, verification of the test and reference substance identity, maintenance of records on the test and reference substance, and archival of a sample of the test and reference substance are the responsibility of the Study Sponsor. 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 as individual replicate values, in the study report are considered to have complied with the OECD requirement.	Requirement considered met. While raw data were not submitted, the tabulated results presented were of the individual replicate values and were sufficient to allow statistical analysis by the reviewer. While US EPA OPPTS 850.5400 states that the sponsor must submit to the EPA all data developed by the test including those that are suggestive or predictive of acute phytotoxicity, advice from the US EPA was that, because the tabulated results presented in the study report were sufficient to allow statistical analysis, the guideline would be considered met.

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At test termination (120 hours), cells exposed to the treatment levels tested and the controls were observed to be normal, except at 10 mg pyroxsulam/L where cell were noted to be abnormal with thin cell walls at 24 and 48 h. The 120-hours cell density in the control and solvent control averaged 24.33 and 19.75 x 10^4 cells/mL, respectively. A daily increase in the control and solvent control cell density was observed over the 120-hour test period, but this slowed as the test progressed. Cell density was stimulated in most treatment solutions relative to the control data, with the peak cell density present at 0.67 mg pyroxsulam /L and 669% stimulation of growth compared to pool controls. A significant reduction in cell density was observed at 10 mg pyroxsulam/L, which was consistent with the results of the preliminary study, compared to the pooled controls. The results from the 10 mg pyroxsulam/L replicates showed clearly toxic effects (Table 4. , page Figure 1, page 23 of this DER). Whereas sustained exponential growth was achieved in the negative control and most treatments, this was not the case in the solvent control where cell density fell between 72 and 96 hours and was still below to the 72 hour level at 120 hours (mean solvent control counts at 72, 96 and 120 hours were, respectively, 20.58 X 10^4, 14.67 X 10^4 and 19.75 X 10^4 cells/mL).

The total biomass in the control and solvent control averaged 14.04 and 12.56 x 10^4 cells day/mL. The 0-72 hours growth rate in the control and solvent control averaged 0.95 and 1.01 days per day. Statistical analysis determined no significant difference between the control and solvent control growth rates. The 0-72 hour growth rate in the 10 mg pyroxsulam/L treatment level could not be calculated since the cell density was zero. Thus, significant reduction in growth rate was determined in the 10 mg pyroxsulam/L treatment level as compared with the control data.

The analytical result of the 120-hour sample from the 0.64 mg pyroxsulam/L nominal treatment level without algae present was 0.67 mg pyroxsulam/L. The equivalent test solution with algae present was 0.67 mg pyroxsulam/L, indicating that the presence of algae in the test solution had no effect on the concentrations of pyroxsulam present in solution.

The effect of pyroxsulam on the freshwater diatom, *Navicula pelliculosa*, with respect to mean cell density (0 to 24, 48, 72, 96 and 120 hours), mean specific growth and biomass (both 0-72 hours) are shown in, respectively, Table 4. and Table 5. (growth and biomass).

Table 4. Effect of pyroxsulam on the mean cell density of freshwater diatom *Navicula pelliculosa.* Standard deviations are shown in brackets.

Treatment (mean measured concentration (mg pyroxsulam/L)	Initial cell density	Mean cell density ($x10^4$) at					
		24 hours	48 hours	72-hours	96-hours	120-hours	% inhibition[1]
Negative control	1×10^4	1.00 (0.43)	6.25 (2.88)	16.92 (1.01)	22.67 (12.68)	24.33 (11.73)	N/A
Solvent control	1×10^4	0.58 (0.29)	3.25 (1.30)	20.58 (5.64)	14.67 (4.78)	19.75 (2.05)	N/A
0.10	1×10^4	1.75 (1.09)	4.17 (1.38)	27.25 (2.50)	34.5 (7.58)	44.42 (9.79)	-102[2]
0.29	1×10^4	1.92 (0.52)	7.58 (2.77)	37.33 (5.70)	63.25 (16.54)	69.58 (2.43)	-216
0.67	1×10^4	0.67 (0.38)	4.58 (2.84)	32.33 (4.47)	74.17 (7.34)	169.56 (58.46)	-669
1.7	1×10^4	0.67 (0.38)	1.58 (2.84)	27.5 (4.47)	52.92 (7.34)	84.42 (3.64)	-283
4.0	1×10^4	1.42 (0.80)	2.33 (0.38)	23.42 (7.51)	52.5 (16.04)	63.08 (11.61)	-186
10	1×10^4	0.50 (0.43)	0.50 (0.25)	0.00 (0.00)	0.08 (0.14)	0.92* (1.18)	96
Reference chemical (if used)	N/A (not applicable)						

***Significantly different from the control (William's Test, $p \leq 0.05$) [1] Percent inhibition relative to pooled controls. [2] Negative inhibition shows stimulation of cell growth.**

The algal growth curves (cell density versus time) for *N. pelliculosa* exposed to pyroxsulam are shown in Figure 1 with the drop in cell numbers in the solvent control after 72 hours most noticeable.

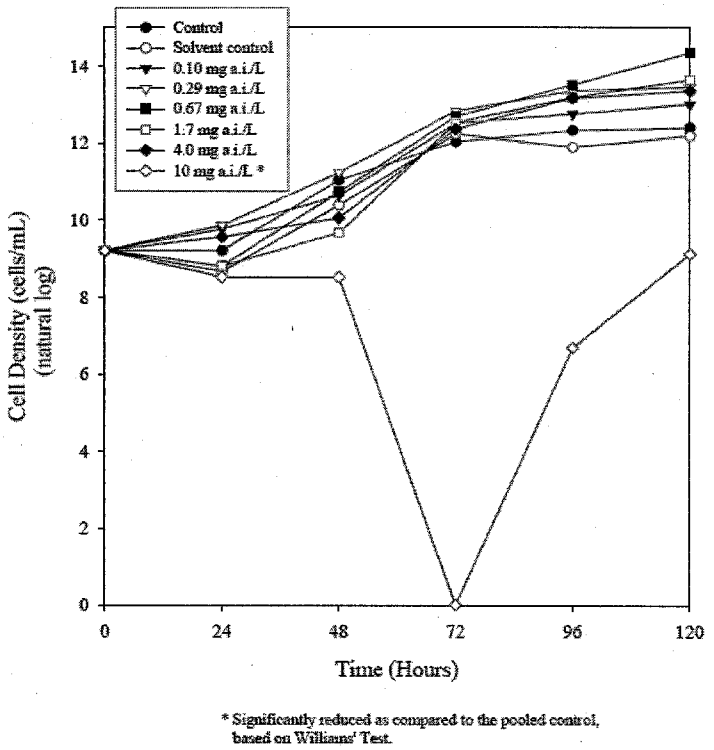


Figure 1. Algal growth curves (cell density [natural logs] vs. time) for *Navicula pelliculosa* exposed to pyroxsulam (from Hoberg, 2005).

Table 5. Effect of pyroxsulam on the growth rate and biomass of the freshwater diatom *Navicula pelliculosa*

Treatment measured concentrations (mg pyroxsulam/L)	Mean Specific Growth Rate per day, 0-72 hours		Biomass (Mean Area Under the Growth Curve), 0-72 hours	
		Percent Inhibition[1]	0-72 h	Percent Inhibition[1]
Negative control	0.95		14.04	
Solvent control	1.01		12.56	
0.10	1.11	-13[2]	18.22	-37
0.29	1.22	-24	27.37	-106
0.67	1.17	-19	20.40	-53
1.7	1.11	-13	14.69	-10
4.0	1.05	-7	13.94	-5
10	0.0*	100	-1.53*	112

*** Significantly different from the control (Dunnett's Test, p≤0.05) [1] Percent inhibition relative to pooled controls. [2] Negative inhibition shows stimulation of cell growth.**

The 0-72 hour mean specific growth rates for the 0.10 to 4.0 mg pyroxsulam/L showed stimulation relative to the controls. The same effect was observed in the 0-72 hour mean biomass results. The OECD 201 recommendation that the concentration series should preferably cover the range causing 5-75 % inhibition of algal growth rate was not met on the basis of the mean specific growth rates presented in Table 5.

Graphical representations of the 0-72 hours average growth rate and total biomass, taken from the study report, as shown in Figure 2 and Figure 3.

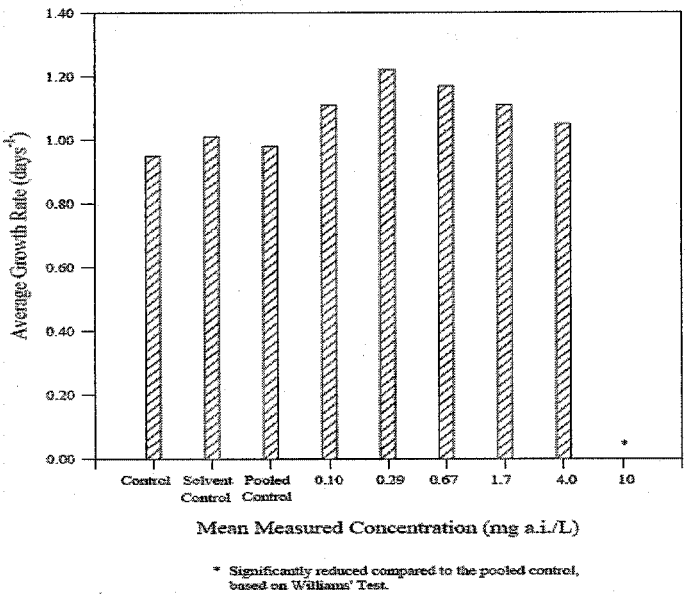


Figure 2. Average growth rate (0 to 72-hour) for *Navicula pelliculosa* exposed to pyroxsulam (from Hoberg, 2005).

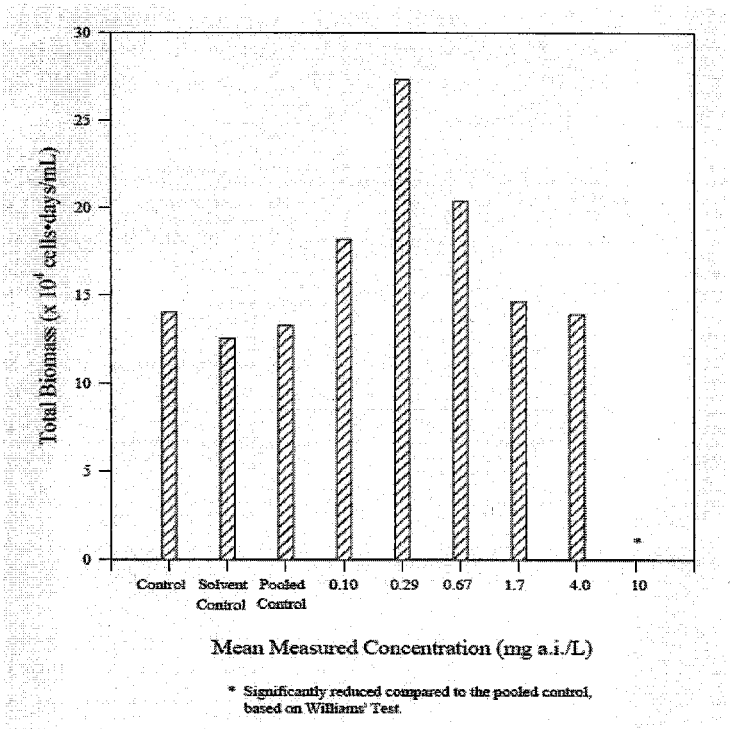


Figure 3. Total biomass (total area under the growth curve 0 to 72 hours) for *Navicula pelliculosa* exposed to pyroxsulam (from Hoberg, 2005).

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 appears to have been met for the controls but was variable for the solvent control (see page 20 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 27 of this DER. The values and calculated statistics, including the overall mean % coefficient of variation (%CV) used by the study reviewer are as shown in Table 6:

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

Reviewer calculated growth rates (/day) for the control replicates			
Replicate	**0-24 h**	**24-48 h**	**48-72 h**
1	-0.29	1.47	1.59
2	0.41	1.47	0.95
3	-0.29	2.48	0.69
Mean	-0.06	1.81	1.08
Standard deviation	0.40	0.59	0.46
%CV	-706.64	32.56	43.10

The %CV values for the 0-24 and 48-72 hour growth rate values exceed the 35% limit set by OECD 201 (2006).

The 0-72 hours mean %CV was 7.1% (mean 0.04, standard deviation 0.003, see page 43 of this DER for the data and ToxCalc results) which meets the 2006 OECD guideline requirement of not exceeding 10%. Over the entire test, as specified by the 2006 version of OECD 201, the 0-120 hour %CV was 10.2 (mean 0.61, standard deviation 0.062), considered to also meet the OECD requirement.

If the study report's pooled mean control growth rate and standard deviation values are used, the 0-72 hour %CV value is (0.07X100/0.98) or 7.1, the same as the reviewer calculated value.

Because the study was conducted following the 1984 version of the OECD 201 guideline, this has not been considered a deficiency, but the low growth rate in the initial 24 hours and the high %CV are likely to have reduced the reliability and sensitivity of the study.

The statistical endpoints reported in the study report were as shown in Table 11.

Table 7. Statistical endpoint values for the toxicity of pyroxsulam to *N. pelliculosa*.

Hour	EC Type	NOEC (mg **pyroxsulam**/L)	Value (mg **pyroxsulam**/L)	95% Confidence Limits (mg **pyroxsulam**/L)
72	ErC50	4.0	6.9	6.4-7.0
	EbC50	4.0	5.8	3.9-6.6
120	EC50	4.0	6.8	5.9-7.1
	EC25	4.0	5.1	3.9-5.5

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

B. REPORTED STATISTICS:

The cell density in each test flask was calculated for each daily interval by dividing the number of cells counted by the number of fields examined. Means and standard deviations for cell density for each treatment and the controls were calculated from individual replicate values. The study report stated that a t-test was used to compare the cell density, total biomass and average growth rate of the control to that of the solvent control. If no significant difference was determined, control and solvent control data were pooled for further statistical analysis to determine treatment level effects. If a significant difference was detected, the treatment data were compared to the solvent control data. The 120-hour cell density in the control and solvent control averaged 24.33 and 19.75 X 10^4 cells/mL, respectively (pooled control = 22.04 X 10^4 cells/mL). Based on the results of statistical analysis performed for 120

hour cell density, 0-72 hour total biomass and 0-72 hour average growth rate, the NOEC, the highest test concentration which demonstrated no statistically adverse effect ($p \leq 0.05$) when compared to the appropriate control data, was determined. The data were first checked for normality using Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test. If the data sets passed the tests for homogeneity and normality then William's Test was used to determine the NOEC. TOXSTAT was used to calculate the EC values and 95% confidence limits.

The EC25 and EC50 values (concentrations of test substance which reduced cell density by 25 and 50%, respectively) and the 95% confidence limits were calculated for cell densities after 24, 48, 72, 96 and 120 hours of exposure. Additionally, EC50 values were calculated for 0-72 hour total biomass (EbC50) and average growth rate (ErC50). The EC25 and EC50 values and their 95% confidence limits were determined by linear regression of response (percent reduction of cell density, biomass and growth rate as compared with the appropriate control) versus the mean measured concentration (Norberg-King, 1993). A computer program, TOXSTAT® (Gulley et al., 1996) was used to calculate the EC values and 95% confidence limits. If less than the required response was observed (i.e. <50% response), the EC value was empirically estimated to be greater than the highest concentration tested.

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 by, respectively, the Shapiro-Wilk's and Bartlett's tests and for difference between the mean cell counts and mean specific growth rates and mean biomass results of the pyroxsulam exposed algae and the mean of the pooled controls by Bonferroni's t test. Negative and solvent controls were compared and pooled if there were no statistically significant differences. All NOEC values were determined using the ToxCalc package.

The study report's mean cell density values (and associated standard deviations) were recalculated from the summary cell count data presented in the report and found to be identical to reported means and standard deviations for cell density.

The cell density percentage inhibition results given in the study report were recalculated with the results found to be equal to those reported.

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 72 hours specific growth rate values for control and test replicates presented in the study report were recalculated and shown to be similar to those given in the study report with the small differences attributed to rounding differences between the reported replicate data and the actual raw data results.

The growth rate percentage inhibition results given in the study report were recalculated and the results found equal to those reported.

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 72 hours biomass values for control and test replicates presented in the study report were recalculated and shown to be similar to those given in the study report with the small differences presumably due to rounding differences between the reported replicate data and the actual raw data results.

.

The biomass percentage inhibition results given in the study report were recalculated with the results equal to those reported.

The reviewer calculated end points were:

Cell density 96 h:
EC50: 7.01 mg pyroxsulam/L 95% C.I.: 5.67-7.01 mg pyroxsulam/L
NOEC: 10 mg pyroxsulam/L

(Note that the 24, 48, 72 and 120 hour cell densities were also recalculated – with the values obtainable from the ToxCalc results shown in Appendix 1 on pages 38, 39, 40 and 42 respectively.)

Specific growth rate 0-72 h:
ErC50: 6.80 mg pyroxsulam/L 95% C.I.: 6.18-7.12 mg pyroxsulam/L
NOEC: 4 mg pyroxsulam/L

The ToxCalc results for the specific growth rate are shown in Appendix 1, pages 43 and 44 of this DER.

The reviewer calculated mean and standard deviation 0-72 hour specific growth rate results and the reported mean and standard deviations are shown in Table 8. . These are considered equivalent.

Table 8. Reviewer calculated and study report mean and standard deviation values for the 0-72 hour specific growth rate values for *Navicula pelliculosa* exposed to control and pyroxsulam containing solutions. Standard deviations are shown in brackets.

Mean measured concentrations mg pyroxsulam/L	Calculated (day^{-1})	Reported (day^{-1})
Control	0.94 (0.2)	0.95 (0.02)
Solvent	1.00 (0.10)	1.01 (0.10)
Pooled controls	0.97 (0.07)	0.98 (0.07)
0.1	1.10 (0.03)	1.11 (0.03)
0.29	1.20 (0.05)	1.22 (0.05)
0.67	1.16 (0.04)	1.17 (0.05)
1.7	1.10 (0.06)	1.11 (0.06)
4	1.04 (0.11)	1.05 (0.11)
10	Not calculated.	Cell density was zero, so growth could not be calculated.

Biomass 0-72 h:
EbC50: 5.71 mg pyroxsulam/L 95% C.I.: 2.20-7.08 mg pyroxsulam/L
NOEC: 4 mg pyroxsulam/L

The reviewer calculated biomass ToxCalc results are shown on pages 45 and 46 of this DER.

The reviewer calculated mean and standard deviation 0-72 hour biomass results and the reported mean and standard deviations are shown in Table 9. These are considered similar.

Table 9. Reviewer calculated and study report mean and standard deviation values for the 0-72 hour biomass for *Navicula pelliculosa* exposed to control and pyroxsulam containing solutions. Standard deviations are shown in brackets.

Mean measured concentrations mg pyroxsulam/L	Calculated biomass (X 10^4 cells/mL)	Reported biomass (X 10^4 cells/mL)
Control	13.21 (3.42)	14.04 (3.54)
Solvent	11.63 (3.97)	12.56 (4.25)
Pooled controls	12.42 (3.43)	13.3 (3.59)
0.1	17.04 (1.76)	18.22 (1.90)
0.29	25.67 (3.44)	27.37 (3.6)
0.67	18.92 (1.82)	20.4 (1.93)
1.7	13.50 (3.31)	14.69 (3.54)
4	12.96 (4.75)	13.94 (5.04)
10	-1.50 (0.50)	-1.53 (0.48)

The endpoints reported in the study report and those calculated in the assessment of the study are shown in Table 10.

Table 10. Reported and reviewer calculated toxicity endpoints.

Toxicity endpoint	Mean measured pyroxsulam concentration, mg/L (95% confidence limits)	
96 hour cell density	**As presented in the study report**	**As calculated by the ToxCalc program**
EC50	7.0 (6.3-7.0)	7.00 (5.67-7.01)
EC25	5.5 (4.4-5.5)	5.5 (3.5-5.5)
120 hour cell density		
EC50	6.8 (5.9-7.1)	6.33 (4.76-7.42)
EC25	5.1 (3.9-5.5)	4.4 (2.3-6.1)
NOEC	4.0	4.0
0-72 hour mean specific growth rate		
ErC50	6.9 (6.4-7.0)	6.80 (6.18-7.12)
NOEC	4.0	4.0
0-72 hour biomass		
EbC50	5.8 (3.9-6.6)	5.71 (2.20-7.08 with the 10 mg/L result excluded and 2.38-6.96 if included)
NOEC	4.0	4.0

The reviewer calculated endpoints are considered to be comparable to those reported in the study report.

D. STUDY DEFICIENCIES:

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

Table 11. Deviations from Guidelines and other deficiencies

Parameter	Study reported results	OECD 201 Freshwater alga and Cyanobacteria, Growth Inhibition Test	US EPA OPPTS 850.5400 Algal Toxicity, Tiers I and II
Acclimation period:	The inoculum used to initiate the toxicity test with pyroxsulam was taken from a stock culture that had been transferred to fresh medium seven days prior to test initiation.	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 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. This guideline also refers to the test beginning 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.
Duration of the test	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.	US EPA OPPTS 850.5400 refers to cell counts at 24, 48, 72 and 96 hours
Details of growth medium Name	Algal Assay Procedure (AAP) medium modified by additions of sodium silicate and sodium selenate (Na_2SeO_4) The addition of sodium selenate was noted in the study report's description of the composition of the AAP medium as being an additional nutritional requirement. The amount of hydrated sodium silicate present was reported as 20 mg/L, calculated as equivalent to ~2 mg Si/L, cf. the 1.4 mg Si/L recommended by OECD 201.	OECD 201 refers to AAP medium and provides a comparison (Annex 3) of the US EPA AAP medium and the OECD 201 medium. The guideline identifies both as suitable growth media. The guideline also states that in tests with the diatom *Navicula pelliculosa,* both media must be supplemented with $Na_2SiO_3 . 9H_20$ to obtain a concentration of 1.4 mg Si/L. The calculated concentration of silicon in the medium was ~2 mg/L, a value not considered significantly different from the 1.4 mg/L level. With respect to sodium selenate, OECD 201 states that the US EPA AAP medium can only contain sodium selenate when the medium is used to grow stock cultures of diatoms. The guideline's wording regarding use of sodium selenate can be interpreted as	US EPA OPPTS 850.5400 does not specifically refer to media composition, instead referring to other sources for this information.

	The concentration of sodium selenate in the medium was 1.88 µg/L, cf. the calculated concentration in the AAP medium of ~0.007 µg/L recommended by OECD 201.	meaning that this chemical can only be used in the stock diatom cultures and not the test cultures, which, if correct, appears unusual. This difference between the concentration of sodium selenate used and that indicated by OECD 201 appears large, but the successful growth of the control diatoms is taken to indicate no adverse effect had occurred as a result of this.	
pH at test initiation:	pH values in the control vessels at 0 hours were 6.9 (control) and 7.0 (solvent control).	OECD 201 states that the pH of AAP medium is 7.5.	US EPA OPPTS 850.5400 states that the pH of the nutrient medium is to be 7.5 (± 0.1) for *Navicula* at the start of the test
Number of replicates Control, solvent control and treatments:	3 replicates in each case.	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.	The US EPA OPPTS refers to use of four replicates for *N. pelliculosa*,
Test concentrations Nominal: (Factor used for test series)	0.10, 0.26, 0.64, 1.6, 4.0, 10 mg/L Ratio between nominal concentrations: 0.26/0.10 = 2.6 0.64/0.26 = 2.5 1.6/0.64 = 2.5 4.0/1.6 = 2.5 10/4.0 = 2.5	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 guideline also states that the concentration series should preferably cover the range causing 5-75 % inhibition of algal growth rate. There was >90% inhibition at the highest concentration with respect to cell density, specific growth rate and biomass but at all other concentrations, there was growth stimulation.	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).

Measurement technique for cell density and other end points.	Due to the tendency of *Navicula pelliculosa* cells to clump, the solutions were vigorously pipetted multiple times to disperse clumped cells and achieve a homogeneous suspension prior to removing a sample for cell counts.	OECD 201 also notes that *Navicula pelliculosa* may form aggregates under certain growth conditions. Due to production of lipids the algal cells sometimes tend to accumulate in the surface film. Under those circumstances special measures have to be taken when sub-samples are taken for biomass determination in order to obtain representative samples. Vigorous shaking, e.g. using a vortex mixer may be required.	US EPA OPPTS 850.5400 states that the procedure used to break up the filaments should result in consistent filament lengths across treatments and replicates. Sonification, ultrasonic bath, blender, syringe, or any other methods of cell separation, other than manual or rotary shaking are **not** allowed for *Navicula*.
Validity of test	The %CV values for the 0-24 and the 48-72 hour periods were reviewer calculated as -706% and 43% with such values indicative of non-compliance with the 35% limit set by the 2006 OECD 201 guideline.	OECD 201 (2006) requires that, for the test to be valid, the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	No %CV requirement.

Comments on these deficiencies/deviations

The use of a 7, rather than 2-4, day old culture to obtain the *N. pelliculosa* cells is required by the US EPA OPPTS 850.5400 guideline and, while exceeding the OECD 201 2 to 4 day requirement for the culture age, is not considered to be a significant deviation from the latter guideline. The successful growth of the control cultures adds support to this belief although it is noted that the counts in the control and solvent controls averaged, respectively, 1×10^4 and 0.58×10^4 cells/mL after 24 hours, indicative of a lag phase. The reason for the study being taken out to 120 hours was not identified in the study report, but in itself the use of a 120 hour period is not considered to be a significant deviation or deficiency. Furthermore, the data presented allowed calculation of endpoints at 24, 48, etc. hours.

The modifications to the AAP medium were identified as necessary for the correct growth of the *Navicula pelliculosa*. While the modifications are clear with a close reading of the study report, it is considered that reference to "modified AAP" throughout the study report would have been a better description because the concentration of sodium selenate used (1.88 µg/L) is significantly greater than that allowed according to the OECD 201 description of the AAP medium (namely ~0.007 µg/L).

The reason for not using four replicates, as required by the US guideline is not known. This is both a deviation and deficiency and reduces the sensitivity and reliability of the study.

While the ratio of the nominal concentrations exceeds the US EPA guideline requirement of being between 1.5 and 2.0 (the values were 2.5 or 2.6), this deviation from the US EPA guideline is not considered a serious deficiency. However, the OECD 201 guideline's stating that the concentration series should preferably cover the range causing 5-75 % inhibition of algal growth rate was not met. There was >90% inhibition at the highest concentration (10 mg/L) with respect to cell density, specific growth rate and biomass but at all other concentrations, there was growth stimulation.

While the need to break up the *Navicula pelliculosa* aggregates/filaments is identified by both guidelines and recognised in the study report, the success of vigorous, multiple pipetting in breaking up the aggregates/filaments is not known and the pipetting procedure is not considered to have shown been to be acceptable, especially as the US EPA 850.5400 specifically states, "Sonification, ultrasonic bath, blender, syringe, or any other methods of cell separation, other than manual or rotary shaking are not allowed for *Selenastrum*, *Skeletonema*, or *Navicula*." The "Validity of test" deficiencies regarding the %CV values for the 0-24 and 48-72 hour periods is considered most likely also related to the failure to properly break up aggregates/filaments.

While not included in the table of deviations, the observation the marked decline in the mean numbers of cells in the solvent control at 72 hours is also indicative of an unexplained event having occurred in the control solvent samples.

At 96 hours, the mean cell count in the control was 22.67 X 10^4 cells/mL and, in the solvent control, 14.67 X 10^4 cells/mL, i.e. the OECD's 16-fold factor is met for the control but not the solvent control.

E. REVIEWER'S COMMENTS:

While the results from the study are considered to show that pyroxsulam is moderately toxic to the freshwater diatom, *Navicula pelliculosa*, the issues of the modification of the AAP medium, the use of three rather than four replicates as required by the US EPA, the uncertainty as to whether the vigorous pipetting sufficiently disrupted the aggregates/filaments, and the absence of sustained exponential growth in the solvent control have resulted in classifying the study as INVALID. Additionally, only one concentration tested resulted in inhibition of algal growth, all others indicated varying degrees of growth stimulation. Such a result is not considered to be routinely expected.

It was also noted that there was considerable variation between replicates in several treatment levels, especially at 120 h samples. This may be due to the diatoms clumping, as indicated in the report, and not being sufficiently agitated (shaken) to break up the clumps. Whereas sustained exponential growth was achieved in the negative control and most treatments, this was not the case in the solvent control where cell density fell between 72 and 96 hours and had barely recovered to the 72 hour level at 120 hours.

This variation between replicates and the strong stimulation, which may be due to sampling problems, provide further reason for classifying the study as INVALID.

Results are therefore not reported in the Executive Summary or the Conclusions Sections of this DER, and should not be used in a risk assessment.

F. CONCLUSIONS: This study is classified as **INVALID** because of uncertainties relating to the successful disruption of aggregates/filaments of the *Navicula pelliculosa,* the use of a smaller number of replicates than required by the US EPA, the lack of inhibitory effects at all but the highest (10 mg/L) exposure concentration and the lack of sustained exponential growth in the solvent control. Results should not be used in a risk assessment. This study is of limited utility due to the issues of the modification of the AAP medium, the use of three rather than four replicates as required by the US EPA, the uncertainty as to whether the vigorous pipetting sufficiently disrupted the aggregates/filaments, and the absence of sustained exponential growth in the solvent control. These have resulted in classifying the study as invalid.

The study's 0-72 hour ErC50 of 6.9 mg pyroxsulam/L is an order of magnitude greater than the 0-72 hour ErC50 value of 0.695 mg pyroxsulam/L determined in the DER for the effect of pyroxsulam on the freshwater green alga, *Pseudokirchneriella subcapitata.* Because of the uncertainty associated with this study and its calculated endpoints, the reviewer is not confident that pyroxsulam's toxicity to *Navicula pelliculosa* has conclusively been demonstrated as likely to be less than to *Pseudokirchnerialla subcapitata.*

DEW recommends that the *Navicula* study be repeated, based upon current OECD and US EPA guideline requirements.

III. REFERENCES:

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

Cathie, C. (2004). Determination of Dissociation Constant of XR-742 using UV-Visible Spectrophotometry. 30 August 2004. Unpublished report of Dow AgroSciences LLC, Indianapolis, Indiana.

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

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

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

Madsen, S. (2003). Determination of the Surface Tension, Density, and Vapour Pressure of the Pure Active Ingredient XDE-742. 09 October 2003. Unpublished report of Dow AgroSciences LLC, Indianapolis, Indiana.

Madsen, S. (2006). Group B: Physical and Chemical Properties of XDE-742. Dow AgroSciences LLC Supply R&D Laboratory, Analytical/Product Chemistry Center of Expertise, 9330 Zionsville Road Indianapolis, Indiana 46268, Huntingdon Life Sciences Ltd., Woolley Road, Alconbury Huntingdon Cambridgeshire, PE28 4HS, England, Dow AgroSciences LLC, 89 Paritutu Road, New Plymouth, New Zealand, and BASF Aktiengesellschaft, BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, 57114 Limburgerhof, Germany. LABORATORY STUDY ID NAFST-06-086. 15-June-2006. Unpublished report.

Mercer, J. 2006. Study Profile Template (SPT) for XDE-742 Growth inhibition test with the freshwater diatom (*Navicula pelliculosa*). Springborn Smithers Laboratories, 790 Main Street, Wareham, MA. Springborn Smithers study number: 12550.6367.SPT, DAS study number: 050283.SPT. The Dow Chemical Company Midland, MI 48674 USA for Dow AgroSciences LLC, Indianapolis, IN 46268 USA. 8 February 2006. Unpublished report.

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

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

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

OECD. 1997. Good Laboratory Practice in the Testing of Chemicals. Paris. France, as revised 1997.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry* 2nd Edition. W.H. Freeman and Co., New York, NY. 859 pp.

Turner, B. J. (2004a). Determination of Water Solubility for XDE-742. 22 December 2004. Unpublished report of Dow AgroSciences LLC, Indianapolis, Indiana.

Turner, B. J. (2004b). Determination of Octanol/Water Partition Coefficient for XDE-742. 22 December 2004. Unpublished report of Dow AgroSciences LLC, Indianapolis, Indiana.

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

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

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

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

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

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell density - 24 hours

The ToxCalc analysis of the 24 hour algal cell count data (untransformed) gave the following results. Cell count data is given a cells/mL.

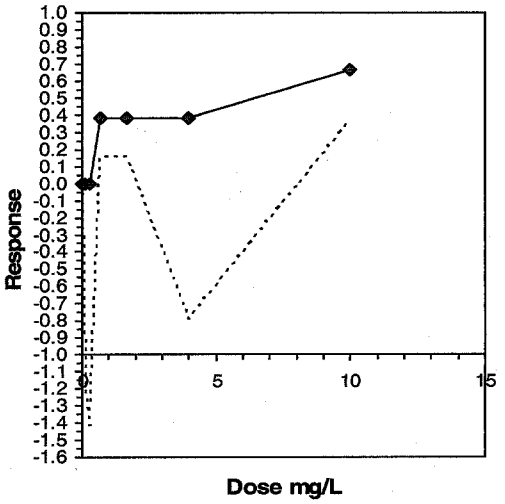
Conc-mg/L	1	2	3
D-Control	7500	15000	7500
S-Control	7500	7500	2500
0.1	12500	30000	10000
0.29	15000	17500	25000
0.67	10000	2500	7500
1.7	2500	10000	7500
4	5000	17500	20000
10	7500	7500	0

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	7916.667	1.0000	7916.667	2500	15000	50.592	6				14861.1	1.0000
0.1	17500	2.2105	17500	10000	30000	62.270	3	-2.287	2.655	11124.1	14861.1	1.0000
0.29	19166.67	2.4211	19166.67	15000	25000	27.152	3	-2.685	2.655	11124.1	14861.1	1.0000
0.67	6666.667	0.8421	6666.667	2500	10000	57.282	3	0.298	2.655	11124.1	9166.67	0.6168
1.7	6666.667	0.8421	6666.667	2500	10000	57.282	3	0.298	2.655	11124.1	9166.67	0.6168
4	14166.67	1.7895	14166.67	5000	20000	56.727	3	-1.492	2.655	11124.1	9166.67	0.6168
10	5000	0.6316	5000	0	7500	86.603	3	0.696	2.655	11124.1	5000	0.3364

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9742	0.884	0.36812	0.16555
Bartlett's Test indicates equal variances (p = 0.58)	4.72432	16.8119		
The control means are not significantly different (p = 0.24)	1.38675	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	10	>10			11124.1	1.40515	1.1E+08	3.5E+07	0.0348	6, 17
Treatments vs Pooled Controls										

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.3396	0.4164	0.3032	0.5162	9.9602
IC10	0.3892	0.6126	0.3270	0.7425	7.1060
IC15	0.4388	1.0076	0.3492	6.7401	3.9510
IC20	0.4883	1.4480	0.3690	8.1883	2.5540
IC25	0.5379	1.9426	0.3887	9.5860	1.5984
IC40	4.3600				
IC50	6.5000				



Cell density - 48 hours

The ToxCalc analysis of the 48 hour algal cell count data (untransformed) gave the following results. Cell count data is given a cells/mL.

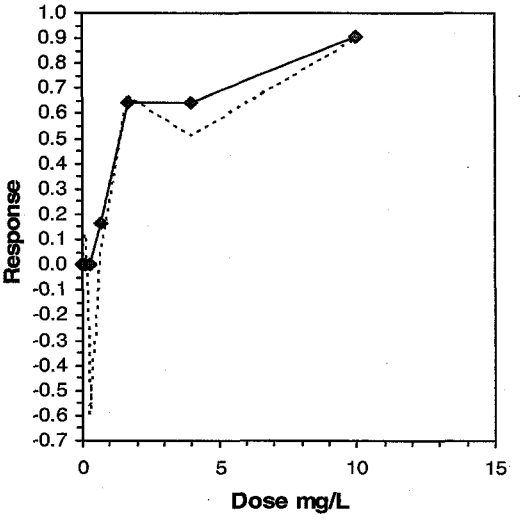
Conc-mg/L	1	2	3
D-Control	32500	65000	90000
S-Control	40000	17500	40000
0.1	42500	27500	55000
0.29	50000	105000	72500
0.67	22500	77500	37500
1.7	17500	22500	7500
4	22500	20000	27500
10	7500	2500	5000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	47500	1.0000	47500	17500	90000	54.493	6				55000	1.0000
0.1	41666.67	0.8772	41666.67	27500	55000	33.045	3	0.406	2.655	38186.1	55000	1.0000
0.29	75833.33	1.5965	75833.33	50000	105000	36.463	3	-1.970	2.655	38186.1	55000	1.0000
0.67	45833.33	0.9649	45833.33	22500	77500	62.032	3	0.116	2.655	38186.1	45833.3	0.8333
1.7	15833.33	0.3333	15833.33	7500	22500	48.238	3	2.202	2.655	38186.1	19583.3	0.3561
4	23333.33	0.4912	23333.33	20000	27500	16.366	3	1.680	2.655	38186.1	19583.3	0.3561
*10	5000	0.1053	5000	2500	7500	50.000	3	2.955	2.655	38186.1	5000	0.0909

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94689	0.884	0.67586	0.65797
Bartlett's Test indicates equal variances (p = 0.04)	13.2621	16.8119		
The control means are not significantly different (p = 0.18)	1.64317	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	4	10	6.32456		38186.1	0.80392	1.7E+09	4.1E+08	0.00894	6, 17
Treatments vs Pooled Controls										

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.4040	0.2278	0.0000	0.9746	0.0199
IC10	0.5180	0.2370	0.0000	1.0513	-0.1607
IC15	0.6320	0.2389	0.0000	1.1279	-0.2522
IC20	0.7419	0.2440	0.0626	1.2096	-0.2535
IC25	0.8498	0.2577	0.1596	1.2963	-0.2829
IC40	1.1736	0.2956	0.1413	1.5650	-0.5802
IC50	1.3894	0.3754	0.1108	1.8102	1.7700



Cell density - 72 hours

The ToxCalc analysis of the 72 hour algal cell count data (untransformed) gave the following results. Cell count data is given a cells/mL.

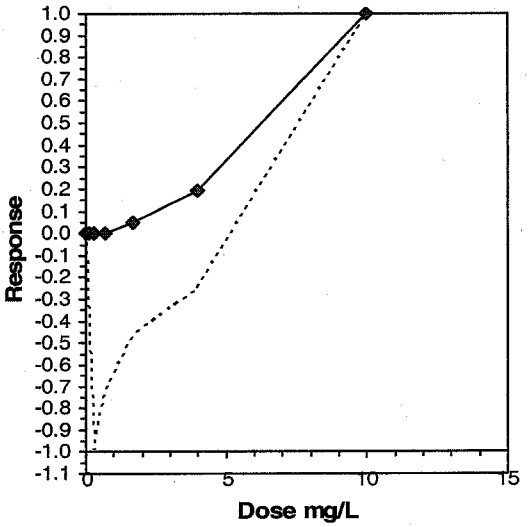
Conc-mg/L	1	2	3
D-Control	160000	167500	180000
S-Control	260000	147500	210000
0.1	247500	272500	297500
0.29	362500	322500	435000
0.67	375000	297500	297500
1.7	277500	322500	225000
4	157500	237500	307500
10	0	0	0

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	187500	1.0000	187500	147500	260000	22.087	6				289167	1.0000
0.1	272500	1.4533	272500	247500	297500	9.174	3	-2.599	2.655	86826.3	289167	1.0000
0.29	373333.3	1.9911	373333.3	322500	435000	15.275	3	-5.682	2.655	86826.3	289167	1.0000
0.67	323333.3	1.7244	323333.3	297500	375000	13.839	3	-4.154	2.655	86826.3	289167	1.0000
1.7	275000	1.4667	275000	225000	322500	17.745	3	-2.676	2.655	86826.3	275000	0.9510
4	234166.7	1.2489	234166.7	157500	307500	32.052	3	-1.427	2.655	86826.3	234167	0.8098
*10	0	0.0000	0	0	0	0.000	3	5.733	2.655	86826.3	0	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95562	0.884	0.32485	-0.3641
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.33)	1.10905	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	4	10	6.32456		86826.3	0.46307	4.5E+10	2.1E+09	5.6E-07	6, 17
Treatments vs Pooled Controls										

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	1.7164	0.9326	0.5368	5.8501	1.0062
IC10	2.5308	1.0208	0.5795	5.8415	0.4560
IC15	3.3452	0.9587	0.6039	5.8329	-0.0532
IC20	4.0726	0.8670	1.2244	5.8764	-0.3184
IC25	4.4431	0.7758	1.8761	6.1342	-0.3094
IC40	5.5544	0.6659	2.7277	6.9073	-0.6387
IC50	6.2954	0.5771	3.8151	7.4228	-0.8468



Cell density - 96 hours

The ToxCalc analysis of the 96 hour algal cell count data (untransformed) gave the following results. Cell count data is given a cells/mL.

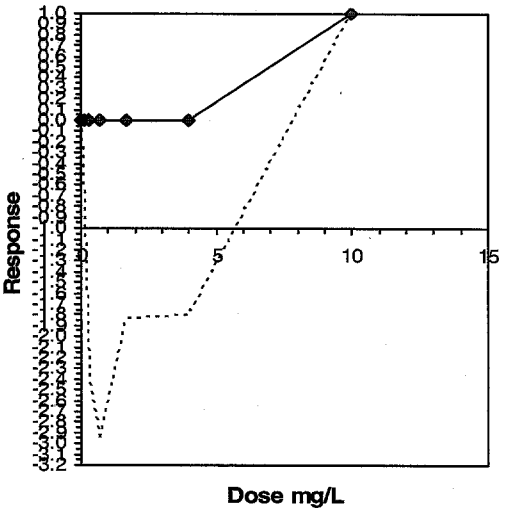
Conc-mg/L	1	2	3
D-Control	360000	212500	107500
S-Control	132500	200000	107500
0.1	302500	432500	300000
0.29	820000	507500	570000
0.67	755000	662500	807500
1.7	595000	447500	545000
4	350000	560000	665000
10	2500	0	0

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	186666.7	1.0000	186666.7	107500	360000	51.584	6				493333	1.0000
0.1	345000	1.8482	345000	300000	432500	21.967	3	-2.141	2.655	196384	493333	1.0000
0.29	632500	3.3884	632500	507500	820000	26.144	3	-6.027	2.655	196384	493333	1.0000
0.67	741666.7	3.9732	741666.7	662500	807500	9.898	3	-7.503	2.655	196384	493333	1.0000
1.7	529166.7	2.8348	529166.7	447500	595000	14.176	3	-4.630	2.655	196384	493333	1.0000
4	525000	2.8125	525000	350000	665000	30.551	3	-4.574	2.655	196384	493333	1.0000
10	833.3333	0.0045	833.3333	0	2500	173.205	3	2.512	2.655	196384	833.333	0.0017

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9666	0.884	0.3871	0.01485
Bartlett's Test indicates equal variances (p = 0.02)	15.7133	16.8119		
The control means are not significantly different (p = 0.36)	1.02209	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	10	>10			196384	1.05205	2.3E+11	1.1E+10	5.8E-07	6, 17
Treatments vs Pooled Controls										

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	4.3005	0.7671	0.2584	4.3014	-2.0795
IC10	4.6010	0.5681	1.2950	4.6028	-2.9720
IC15	4.9015	0.4121	2.3266	4.9042	-3.1830
IC20	5.2020	0.3633	3.0001	5.2056	-2.9594
IC25	5.5025	0.3309	3.5114	5.5070	-2.8959
IC40	6.4041	0.2655	4.8080	6.4112	-2.9225
IC50	7.0051	0.2212	5.6725	7.0140	-2.9250



Cell density – 120 hours

The ToxCalc analysis of the 120 hour algal cell count data (untransformed) gave the following results. Cell count data is given a cells/mL.

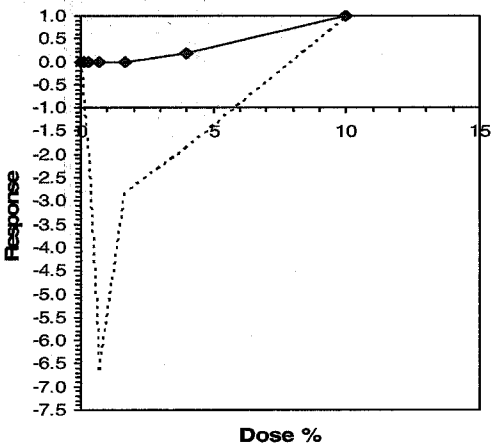
Conc- mg/L	1	2	3
B-Control	375000	205000	150000
S-Control	180000	220000	192500
0.1	337500	530000	465000
0.29	690000	722500	675000
0.67	1260000	1466700	2360000
1.7	882500	840000	810000
4	525000	755000	612500
10	25000	0	5000

			Transform: Log (X + 1)						1-Tailed		Isotonic	
Conc- mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	220417	1.0000	5.3240	5.1761	5.5740	2.536	6				780030	1.0000
0.1	444167	2.0151	5.6400	5.5283	5.7243	1.788	3	-0.547	2.655	1.5331	780030	1.0000
0.29	695833	3.1569	5.8423	5.8293	5.8588	0.258	3	-0.898	2.655	1.5331	780030	1.0000
0.67	1695567	7.6926	6.2132	6.1004	6.3729	2.288	3	-1.540	2.655	1.5331	780030	1.0000
1.7	844167	3.8299	5.9262	5.9085	5.9457	0.315	3	-1.043	2.655	1.5331	780030	1.0000
4	630833	2.8620	5.7951	5.7202	5.8779	1.367	3	-0.816	2.655	1.5331	630833	0.8087
*10	10000	0.0454	2.6990	0.0000	4.3980	87.565	3	4.546	2.655	1.5331	10000	0.0128

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.57542	0.884	-1.8837	11.068
Bartlett's Test indicates unequal variances (p = 6.58E-11)	59.1877	16.8119		
The control means are not significantly different (p = 0.64)	0.50109	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	4	10	6.32456	25	204685	0.9707	4.31532	0.66687	0.00108	6, 17

			Linear Interpolation (200 Resamples)		
Point	%	SD	95% CL(Exp)		Skew
IC05	2.3012	0.6730	0.5258	5.4398	1.2716
IC10	2.9025	0.7228	0.9938	5.5681	0.5189
IC15	3.5037	0.7105	1.4126	5.6964	0.1431
IC20	4.0658	0.6624	1.9128	5.8482	-0.0289
IC25	4.4427	0.6177	2.3132	6.1110	-0.1107
IC40	5.5735	0.5125	3.6692	6.8997	-0.3077
IC50	6.3274	0.4258	4.7639	7.4235	-0.3074



Specific growth rate (0-72 hours) – statistical analysis for means and difference from the means based on exclusion of the 10 mg/L result (no cell growth).

The ToxCalc analysis of the 0-72 hour mean specific growth rate data (untransformed, as day^{-1}) gave the following results. Growth rate data in units of day^{-1}. Data points were calculated by the reviewer from the cell density data.

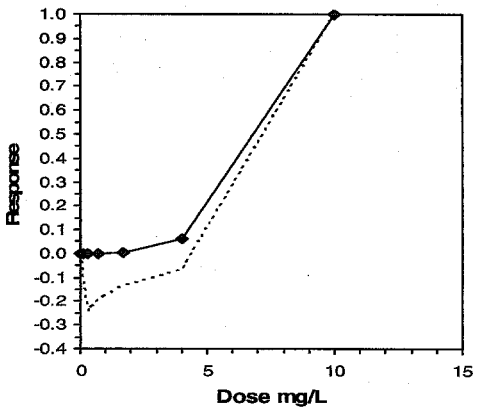
Conc-mg/L	1	2	3
B-Control	0.0385	0.0391	0.0401
S-Control	0.0453	0.0374	0.0423
0.1	0.0446	0.0459	0.0471
0.29	0.0499	0.0482	0.0524
0.67	0.0503	0.0471	0.0471
1.7	0.0462	0.0482	0.0432
4	0.0383	0.0440	0.0476
10	0.0000	0.0000	0.0000

	Transform: Untransformed								1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	0.0405	1.0000	0.0405	0.0374	0.0453	7.115	6				0.0462	1.0000
0.1	0.0459	1.1338	0.0459	0.0446	0.0471	2.787	3	-2.742	2.602	0.0051	0.0462	1.0000
0.29	0.0502	1.2402	0.0502	0.0482	0.0524	4.175	3	-4.923	2.602	0.0051	0.0462	1.0000
0.67	0.0482	1.1914	0.0482	0.0471	0.0503	3.852	3	-3.922	2.602	0.0051	0.0462	1.0000
1.7	0.0459	1.1342	0.0459	0.0432	0.0482	5.474	3	-2.750	2.602	0.0051	0.0459	0.9937
4	0.0433	1.0701	0.0433	0.0383	0.0476	10.826	3	-1.437	2.602	0.0051	0.0433	0.9376
10	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98266	0.873	0.15091	-0.0288
Bartlett's Test indicates equal variances (p = 0.64)	3.36042	15.0863		
The control means are not significantly different (p = 0.37)	1.01204	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs Pooled Controls	4	10	6.32456	25	0.00514	0.12699	5E-05	7.8E-06	0.00232	5, 15

	Linear Interpolation (200 Resamples)				
Point	%	SD	95% CL(Exp)		Skew
IC05	3.4915	0.8159	0.4775	4.7851	-0.4616
IC10	4.2405	0.4007	2.3534	4.8157	-1.2514
IC15	4.5605	0.2729	3.3674	5.1037	-0.8458
IC20	4.8805	0.2457	3.8953	5.3917	-0.5957
IC25	5.2004	0.2304	4.2768	5.6797	-0.5957
IC40	6.1603	0.1843	5.4214	6.5438	-0.5957
IC50	6.8003	0.1536	6.1845	7.1198	-0.5957



Note that these results come from excluding the 10 mg/L results from the initial analysis. If that result is included, the following results are obtained:

Specific growth rate (0-72 hours) – data from the 10 mg/L results included in the statistical analysis. Growth rate data in units of day^{-1}.

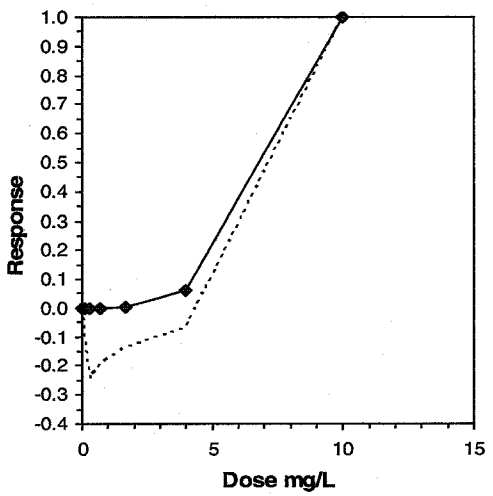
Conc-mg/L	1	2	3
D-Control	0.0385	0.0391	0.0401
S-Control	0.0453	0.0374	0.0423
0.1	0.0446	0.0459	0.0471
0.29	0.0499	0.0482	0.0524
0.67	0.0503	0.0471	0.0471
1.7	0.0462	0.0482	0.0432
4	0.0383	0.0440	0.0476
10	0.0000	0.0000	0.0000

			Transform: Log (X + 1)						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	0.0405	1.0000	0.0172	0.0159	0.0192	6.970	6				0.0462	1.0000
0.1	0.0459	1.1338	0.0195	0.0189	0.0200	2.726	3	-2.922	2.655	0.0020	0.0462	1.0000
0.29	0.0502	1.2402	0.0213	0.0205	0.0222	4.074	3	-5.234	2.655	0.0020	0.0462	1.0000
0.67	0.0482	1.1914	0.0204	0.0200	0.0213	3.761	3	-4.174	2.655	0.0020	0.0462	1.0000
1.7	0.0459	1.1342	0.0195	0.0184	0.0205	5.354	3	-2.930	2.655	0.0020	0.0459	0.9937
4	0.0433	1.0701	0.0184	0.0163	0.0202	10.606	3	-1.530	2.655	0.0020	0.0433	0.9376
*10	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3	22.317	2.655	0.0020	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9784	0.884	0.15757	0.42429
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.37)	1.01059	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	4	10	6.32456		0.0049	0.00471	0.00017	1.2E-06	1.7E-13	6, 17
Treatments vs Pooled Controls										

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.4915	0.8111	0.5507	4.7851	-0.1571
IC10	4.2405	0.4409	2.2838	4.8157	-1.0769
IC15	4.5605	0.2866	3.3114	5.1037	-0.8292
IC20	4.8805	0.2521	3.8814	5.3917	-0.4440
IC25	5.2004	0.2363	4.2638	5.6797	-0.4440
IC40	6.1603	0.1890	5.4110	6.5438	-0.4440
IC50	6.8003	0.1575	6.1759	7.1198	-0.4440



Biomass (area under the curve) for 0-72 hours – data from the 10 mg/L results excluded from the statistical analysis.

ToxCalc analysis of the reported biomass results (untransformed) gave the following results. Biomass data were calculated by the reviewer from the reported cell counts and are expressed as cell count X 10,000 cells/mL.

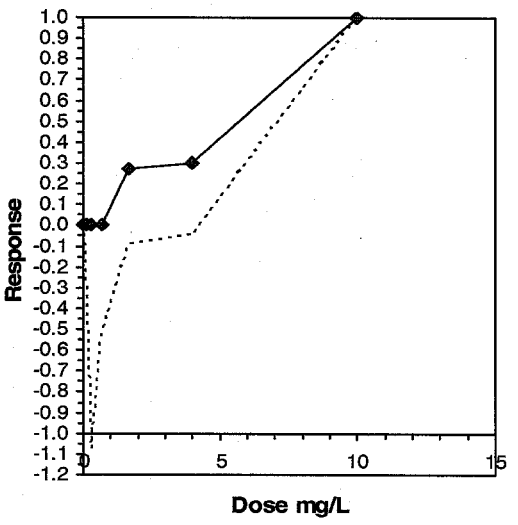
Conc-mg/L	1	2	3
D-Control	9.500	13.875	16.250
S-Control	15.250	7.375	12.250
0.1	15.375	16.875	18.875
0.29	22.125	25.875	29.000
0.67	19.500	20.375	16.875
1.7	13.375	16.875	10.250
4	8.125	13.125	17.625
10	0.000	0.000	0.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	12.417	1.0000	12.417	7.375	16.250	27.619	6				18.510	1.0000
0.1	17.042	1.3725	17.042	15.375	18.875	10.304	3	-1.988	2.602	6.056	18.510	1.0000
0.29	25.667	2.0671	25.667	22.125	29.000	13.411	3	-5.694	2.602	6.056	18.510	1.0000
0.67	18.917	1.5235	18.917	16.875	20.375	9.629	3	-2.793	2.602	6.056	18.510	1.0000
1.7	13.500	1.0872	13.500	10.250	16.875	24.550	3	-0.466	2.602	6.056	13.500	0.7293
4	12.958	1.0436	12.958	8.125	17.625	36.673	3	-0.233	2.602	6.056	12.958	0.7001
10	0.000	0.0000	0.000	0.000	0.000	0.000	3				0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96295	0.873	-0.2125	-0.8603
Bartlett's Test indicates equal variances (p = 0.80)	2.37124	15.0863		
The control means are not significantly different (p = 0.63)	0.52276	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test Treatments vs Pooled Controls	4	10	6.32456		6.05578	0.48771	86.4592	10.8292	7.6E-04	5, 15

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.8603	0.1477	0.2897	1.4114	0.1929
IC10	1.0505	0.3876	0.5680	2.1739	5.2454
IC15	1.2408	0.6113	0.8484	5.9273	3.6304
IC20	1.4310	0.9060	0.9744	6.3619	1.9868
IC25	1.6213	1.2359	1.1117	6.7964	0.6283
IC40	4.8576	0.9465	0.9021	6.5008	-1.0586
IC50	5.7146	0.7554	2.2046	7.0840	-1.0793



Note that these results come from excluding the 10 mg/L results from the initial ToxCalc analysis. If that result is included, the following results are obtained:

Biomass (area under the curve) for 0-72 hours – data from the 10 mg/L results included in the statistical analysis. Biomass data were calculated by the reviewer from the reported cell counts and are expressed as cell count X 10,000 cells/mL.

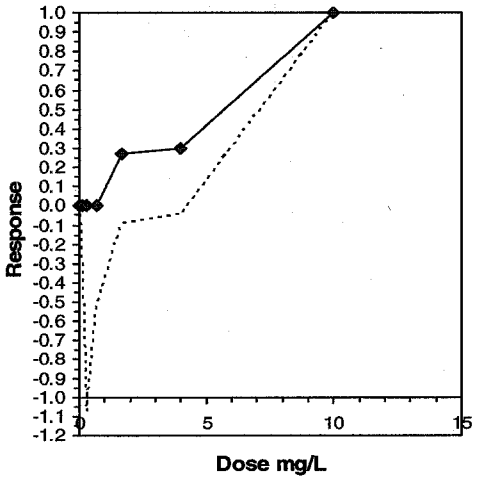
Conc-mg/L	1	2	3
D-Control	9.500	13.875	16.250
S-Control	15.250	7.375	12.250
0.1	15.375	16.875	18.875
0.29	22.125	25.875	29.000
0.67	19.500	20.375	16.875
1.7	13.375	16.875	10.250
4	8.125	13.125	17.625
10	0.000	0.000	0.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Pooled	12.417	1.0000	12.417	7.375	16.250	27.619	6				18.510	1.0000
0.1	17.042	1.3725	17.042	15.375	18.875	10.304	3	-2.116	2.655	5.803	18.510	1.0000
0.29	25.667	2.0671	25.667	22.125	29.000	13.411	3	-6.062	2.655	5.803	18.510	1.0000
0.67	18.917	1.5235	18.917	16.875	20.375	9.629	3	-2.974	2.655	5.803	18.510	1.0000
1.7	13.500	1.0872	13.500	10.250	16.875	24.550	3	-0.496	2.655	5.803	13.500	0.7293
4	12.958	1.0436	12.958	8.125	17.625	36.673	3	-0.248	2.655	5.803	12.958	0.7001
*10	0.000	0.0000	0.000	0.000	0.000	0.000	3	5.681	2.655	5.803	0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95916	0.884	-0.225	-0.507
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.63)	0.52276	2.77645		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	4	10	6.32456		5.8032	0.46737	185.89	9.55515	9.5E-07	6, 17
Treatments vs Pooled Controls										

			Linear Interpolation (200 Resamples)		
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	0.8603	0.1465	0.2863	1.2522	-0.0992
IC10	1.0505	0.3066	0.5405	1.8496	5.7523
IC15	1.2408	0.6140	0.7938	5.7221	3.4076
IC20	1.4310	0.8492	0.9016	6.1688	2.1238
IC25	1.6213	1.1798	1.0672	6.6154	0.7458
IC40	4.8576	1.0151	0.0000	6.3560	-1.2186
IC50	5.7146	0.7344	2.3838	6.9633	-1.1295

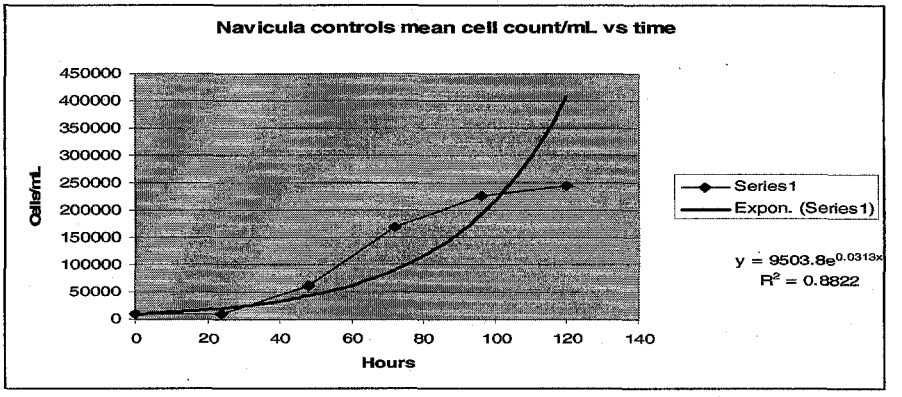


Exponential growth in the controls (page 20 of this DER refers)

To investigate the goodness of fit of the mean control and solvent control cell counts over time with exponential growth, the mean control and solvent control cell counts were separately plotted against time using the Microsoft Excel Chart Wizard function and the resultant curve fitted to an exponential curve. The data used and the Excel outputs are shown below:

Control results

Time (hours)	0	24	48	72	96	120
Mean cell count (cells/mL)	10000	10000	62500	169167	226667	243333



Solvent control results

Time (hours)	0	24	48	72	96	120
Mean cell count (cells/mL)	10000	5833	32500	205833	146667	197500