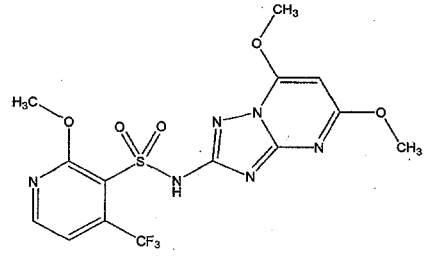
Data Evaluation Report on the chronic toxicity of pyroxsulam (XDE-742) to fresh water invertebrates - *Daphnia* sp.
PMRA Submission Number 2006-4727; ID 1283199 EPA MRID Number 69084-29 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.3.3
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.3.2.1
	EPA Guideline:	72-4 (OPPTS 850.1300)
Test material:	**Pyroxsulam (XDE-742)**	**Purity (%):** 98%
Common name:	Pyroxsulam or XDE-742	
Chemical name:	3-pyridinesulfonamide, N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)	
IUPAC:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
CAS name:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
CAS No.:	422556-08-9	
Synonyms:	XR-742, X666742	

Chemical Structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/08/08 **Date:** 14 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland 22/2/08 **Date:** 14 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Thomas Steeger, Ph.D., Senior Biologist 4/13/08 **Date:** 23 March 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Michelle Kivi for Michelle Kivi 05/03/08 **Date:** 01 May 2007
Pest Management Regulatory Agency

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

CITATION: Marino, T. A. McClymont, E. L. and Najar, J. R. 2005. XDE-742: A 21-Day Chronic Toxicity Study with the Daphnid, *Daphnia magna*. Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID 041023. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 31 January 2005. Unpublished report.

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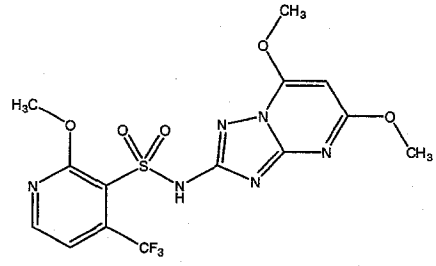

Primary Reviewer: Daryl Murphy **Date:** 14 March 2007
Australian Government Department of the Environment and Water Resources (DEW)

Secondary Reviewers: Jack Holland **Date:** 14 March 2007
Australian Government Department of the Environment and Water Resources

Thomas Steeger, Ph.D., Senior Biologist **Date:** 23 March 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Michelle Kivi **Date:** 01 May 2007
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CITATION: Marino, T. A. McClymont, E. L. and Najar, J. R. 2005. XDE-742: A 21-Day Chronic Toxicity Study with the Daphnid, *Daphnia magna.* Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID 041023. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 31 January 2005. Unpublished report.

EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of pyroxsulam (XDE-742) to *Daphnia magna* was studied under static renewal conditions. *D. magna* neonates were exposed to control and test chemical at nominal concentrations of 0 (water control), 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 mg pyroxsulam/L. Mean-measured concentrations over the 21 days were <0.0806 for the water control, 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg pyroxsulam/L.

The 21-day LC_{50} based on mortality and the 21 day EC_{50} based on sublethal effects (growth and reproduction) were both set at >10.4 mg pyroxsulam/L. The 21-day NOECs for survival, growth and reproduction were each set at 10.4 mg pyroxsulam/L. Production of offspring in the treated groups indicated that pyroxsulam did not have an effect on the reproduction at concentrations up to 10.4 mg pyroxsulam/L, the highest mean-measured concentration tested. The end points (mortality, growth and reproduction) determined were of equal sensitivity.

There was 100% survival of daphnids in the water control. There was also 100% survival at all test concentrations except at 5.27 mg pyroxsulam/L (mean-measured) where survival was 90% (1 mortality observed at test termination). The one mortality occurred at day 21 of the exposure period and was considered biologically insignificant.

The mean number of young produced per surviving female adult was 93.8 in the water control and 89.9, 83.3, 94.6, 84.4, 95.0 and 89.8 in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels, respectively and no statistically significant difference ($\alpha = 0.05$) in reproductive output was observed. The release of control and test first broods was on test day 9. No treatment level exhibited a reduction in reproductive output of greater than or equal to 50% when compared to the water control and no mortality/immobility of offspring was observed during the study. No sublethal effects were reported in any of the live young

The mean length per surviving female adult was 4.23 mm in the water control and 4.16, 4.08, 4.14, 4.20, 4.19 and 4.23 mm in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels, respectively with no statistically significant difference ($\alpha = 0.05$) in length observed. With respect to the mean weight (dry) per surviving female adult, the weights determined were 0.63 mg in the water control and 0.61, 0.59, 0.60, 0.63, 0.59 and 0.60 mg in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels. No statistically significant differences ($\alpha = 0.05$) in growth (weight) were observed in the treatments, with the exception of the 0.701 mg/L measured treatment level (0.59 mg dry weight). However, this was considered biologically insignificant since the greatest percent difference observed between the 0.701 mg/L test level and any remaining dose level or control was minimal at 6% and since no statistically significant differences in weight were observed in the dose levels above or below this test level.

The study is scientifically sound and is considered acceptable. The study's statistical analysis reported the presence of a statistically significant difference in the dry weight of the daphnids at the 0.701 mg pyroxsulam/L level. In contrast, the reviewer's statistical analysis did not confirm this but found such an effect in the daphnid length statistics for that same concentration. Despite this, both the study report and the reviewer have concluded that such effects are biologically insignificant and have not affected the study's reported endpoints. The study is also consistent with the guideline requirements for a chronic toxicity study with freshwater invertebrates.

Pyroxsulam is considered to be very slightly chronically toxic to *D. magna* with respect to survival, growth and reproduction based on the chronic toxicity classification scheme of the Australian Government Department of the Environment and Water Resources (NOEC > 1 mg/L).

Results Synopsis

Test Organism Age (e.g. 1st instar):	*Daphnia magna* neonates, <24 hours old
Test Type (Flowthrough, Static, Static Renewal):	Static renewal (48 hours)
21 day NOEC (survival, growth and reproduction):	10.4 mg pyroxsulam/L (mean, measured concentration)
21 day LOEC (survival, growth and reproduction):	>10.4 mg pyroxsulam/L (mean, measured concentration)
21 day EC (survival and reproduction)	>10.4 mg pyroxsulam/L (mean, measured concentration)
Endpoint(s) Effected:	None (no significant effects were observed)

Note: The template format used for this DER is based on the chronic daphnid toxicity and company study profile report (Henry, 2005) template formats.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study was reported as generally conforming to current procedures described by the following: Organisation for Economic Cooperation and Development (1998). *OECD Guideline for Testing Chemicals No. 211*, Daphnia magna Reproduction Test, Adopted 21 September, 1998; US Environmental Protection Agency (1982). *Pesticide Assessment Guidelines,* Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms, Guideline 72-4, EPA 540/9-82-024, Washington D.C.; and US Environmental Protection Agency (1987). Hazard Evaluation Division: Standard Evaluation Procedure, *Daphnia magna* Life Cycle (21-Day Renewal) Chronic Toxicity Test. EPA 540/9-86-141, Washington, D.C.

Some specific deviations with respect to OECD 211 and US EPA OPPTS 850.1300,. were identified. These are discussed in greater detail in the following sections, Study Deficiencies (page 28 of this DER) and Reviewer's Comments (page 29 of this DER). These deviations are not considered to have affected the validity of the study.

COMPLIANCE:

All facets of testing were reported as conducted following the OECD/EC Good Laboratory Practice Standards as described in: OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM (98) 17; and European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004) and the U.S. EPA Good Laboratory Practice Standard Environmental Protection Agency-FIFRA GLPs; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

The signed and dated Compliance with Good Laboratory Practice Standards statement was provided. The signed and dated Quality Assurance statement was provided. The signed and dated Statement of No Data Confidentiality Claims was provided.

A. MATERIALS:

1. Test Material XDE-742 (i.e. pyroxsulam)

Description: Solid

Lot No./Batch No. : E0952-52-01

Purity: 98%

Stability of Compound Under Test Conditions:
The 26-day stability of pyroxsulam in acetonitrile was reported as determined in a related acute study (Marino *et al.*, report in progress) with *Daphnia magna* by analysing a stock solution (nominal concentration 515 µg pyroxsulam/mL acetonitrile) that had been stored for 26 days at ~8 °C. The data provided an analysed concentration that was 104% of the expected concentration. The study report for this work has not been seen.

Also, in preparation for this study, the 21-day stability of pyroxsulam in laboratory dilution water was stated to have been evaluated. After 21 days at ambient temperature, a 100 mg pyroxsulam/L solution was measured to be 97% of the initial concentration. The study report for this work has not been seen.

Additionally, the study average and percent of target concentrations was also calculated for each dose level by averaging the day 0, 3, 7, 10, 14, 17, 19 and 21 daily values. Daily percent of target values ranged from 94.0 to 123%. Study average percent of target values ranged from 104 to 113%. The overall average percent of target and standard deviation values for the entire study (n=6) was 108 ± 3.83% (page 15 of this DER refers). These results show that the dose levels were prepared at their intended concentrations and the desired concentrations were maintained between renewal periods.

Storage conditions of test chemicals:
Information not located in study report. The company study profile template report (Marino, 2005) stated the pyroxsulam was stored at room temperature in the dark.

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)
pH 9	13.7 g/L	Turner (2004a)
Vapour pressure	<1E-7	Madsen (2003)
UV absorption: Not available at the time of publication of the company's study profile template.		
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 physicochemical properties of pyroxsulam were not reported in the study. The values recorded here come from the company's study profile template report (Dow Chemical Company study ID: 041023.SPT (Marino, 2005)) with the exception that 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:

Species: *Daphnia magna* (neonates, <24 hours old were used for the study)

Note: OECD 211 (Section 57, pages 10/21 and 11/21) requires that the test report must include, *inter alia*, details on the clone (whether it has been genetically typed) while US EPA OPPTS 850.1300 (page 9) states detailed information about the daphnids used as brood stock, including the method of verification, should be provided. This specific detail on the clone does not appear to have been provided (See table on deviations and deficiencies, page 28 of this DER).

Age of the parental stock: 25 days old
Source: In-house culture, initially obtained from New England Bioassay, Inc., Manchester, Connecticut.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The study report stated that, "No preliminary chronic toxicity study was conducted. An acute toxicity test with *Daphnia magna* exposed to XDE-742 indicated that the 48-hour EC50 was greater than 100 mg/L and the NOEC was of 100 mg/L [10]. The OECD guideline for testing of chemicals No. 210 "Fish Early-Life Stage Toxicity Test" states that concentrations of the substance higher than the 96-hour LC50 or 10 mg/L, whichever is the lower, need not be tested [11]. This OECD guidance for the fish early-life stage test was used to determine the test levels for this *Daphnia magna* reproduction test and interpreted to be that concentrations of the substance higher than the 48-hour EC50 or 10 mg/L, whichever is the lower, need not be tested. Based on this information above and the results from the acute test referenced above, the definitive study was conducted with the highest nominal concentration tested at 10.0 mg XDE-742/L."

Note: reference 10 refers to Marino *et al.* (report in progress) while reference 11 refers to the OECD Guideline 210, Fish Early-Life Stage Toxicity Test, adopted 17 July 1992.

b) Definitive Study

In the following two tables' Criteria columns, entries in italics are those given in the PMRA's Draft Evaluation Report template for chronic toxicity to the freshwater invertebrate, *Daphnia magna*. 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 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation:		See the table of deficiencies, page 28 of this DER.
Period:	In-house culture, Information is not specifically cited in the study report. The Study Profile Template (Marino, 2005) refers to 25 days.	Requirement considered met. OECD 211 does not state specific requirements for acclimatisation but does require that the stock animals must be maintained in culture conditions (light, temperature, medium, feeding and animals per unit volume) similar to those to be used in the test. US EPA OPPTS 805.1300 refers to brood daphnids should be maintained in 100 percent dilution water at the test temperature for at least 48 h prior to the start of the test. This guideline also states that detailed information on the acclimation procedures should be submitted to the US EPA. Advice from the US EPA was that, typically, it is simply noted that the animals were obtained from in in-house culture and where the original strain was from.
Conditions: (same as test or not)	Mass cultures of *Daphnia magna* were reared in incubators under cool-white fluorescent lights (approximately 2050 ± 350 lux) and a 16-hour light:8-hour dark photoperiod at 20 ± 2°C. Conditions were considered comparable with the test conditions except for a higher light intensity in the acclimation periods.	Requirement considered met. By way of comparison, test solutions were conducted in the same water (Adjusted [for hardness] Laboratory Dilution water or ALDW) as the daphnid were cultured in. Test solution temperatures ranged from 19-21°C with a 16 hour light: 8 hour dark photoperiod but with lower light intensity (550-766 lux).

Parameter	Details	Remarks *Criteria*
Feeding:	The daphnid were fed a mixed diet of *Pseudokirchneriella subcapitata*, a freshwater green alga (formerly known as *Selenastrum capricornutum*) and YCT (yeast, Cerophyll and trout chow suspension) five times weekly. 0.4 mL of the algal suspension and 0.1 mL of the YCT were added to each test vessel. *Pseudokirchneriella subcapitata* and YCT suspensions had 0.232 mg C/mL and 0.737 mg C/mL, respectively. This resulted in a feeding ration level of 0.167 mg C/daphnid/day.	Requirement considered met. OECD 211 refers to research having shown that, for *Daphnia magna*, ration levels of between 0.1 and 0.2 mg C/*Daphnia*/day are sufficient for achieving the required number of offspring to meet the test validity criteria. US EPA OPPTS 850.1300 refers to suggested feeding rates of 5-7 mg food (dry weight)/L of dilution water for automatic feeders and 15 mg food (dry weight)/L of dilution water for manual, once a day feeding.
Health: (any mortality observed)	No specific information identified in the study report.	Requirement considered met. The raising of daphnids for 25 days and the 100% survival of the control daphnids over the 21 days of the test period indicate that the parental daphnid culture was healthy.
Test conditions:		
Static renewal/flow through:	Static renewal (every 48 hours)	Requirement considered met.
Type of dilution system- for flow through method.	Not relevant for static renewal.	
Flow rate	Not relevant for static renewal.	*(EPA requires consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Renewal rate/procedure for static renewal	Test solutions for the definitive study were prepared in bulk on day 0 and renewed every Monday, Wednesday and Friday thereafter. These bulk test solutions, in addition to control test solution (ALDW with no test material added and referred to as the water control), were apportioned (~ 90 mL) between ten replicate test vessels per dose level. *Daphnia* were introduced into test vessels containing fresh test solutions on day 0 and transferred (via a pipette) into vessels containing fresh (renewed) test solutions each Monday, Wednesday and Friday throughout the study.	Requirement considered met.

Parameter	Details	Remarks *Criteria*
Aeration, if any	None	Requirement met. OECD 211 states that the test vessels must not be aerated during the test. US EPA 850.1300 states that to maintain a dissolved oxygen concentration between 60 and 105 percent saturation, aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.
Duration of the test	21 days	Requirement met.
Test vessel		Requirement considered met.
Material: (glass/stainless steel)	Borosilicate (glass) jars	*EPA requires:* *1. Material: Glass, No. 316, stainless steel, or perfluorocarbon plastics* *2. Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 ml of medium in each vessel.* OECD 211 refers to Parent animals are maintained individually, one per test vessel, with 50 - 100 mL of medium in each vessel. US EPA OPPTS 850.1300 refers to 250 mL test chambers but only states that the volume of solution etc should be reported.
Size (for growth and reproduction/survival test):	120 mL	
Fill volume:	Approximately 90 mL of test solution. Vessels were covered with a sheet of Plexiglas® to reduce evaporation.	

Parameter	Details	Remarks *Criteria*
Source of dilution water	The laboratory water was Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant. The water was obtained from the upper Saginaw Bay of Lake Huron off Whitestone Point	Requirement considered met. The water from Lake Huron was limed and flocculated with ferric chloride and pumped to the laboratory prior to municipal treatment for human consumption. Before use in the laboratory, the water was sand-filtered, pH adjusted with gaseous carbon dioxide, carbon-filtered and UV-irradiated. Daphnid water (referred to as adjusted laboratory dilution water or ALDW) was prepared by adjusting laboratory water to a hardness of about 170 mg/L as $CaCO_3$ before autoclaving at 250°F (121°C) and 18 psi for 30 minutes, cooled and aerated for approximately 24 hours before use. *(EPA: Unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)*
Water parameters: Hardness	The ALDW's hardness was adjusted to about 170 mg $CaCO_3$/L. In the test solutions, hardness (as $CaCO_3$) ranged from 191-204 mg/L in the control water and 192-210 mg/L (as $CaCO_3$) in the high-dose (10 mg pyroxsulam/L) level over 19 days.	Considered met with exception of pesticides and chlorine parameters – see the table of deficiencies, page 28 of this DER. Hardness *EPA: 160 to 180 mg/L as $CaCO_3$* *OECD : > 140 mg/L as $CaCO_3$* For the test medium, OECD 211 states that a hardness above 140 mg/l (as $CaCO_3$) is recommended. US EPA OPPTS 850.1300 does not specify a hardnesss.

Parameter	Details	Remarks *Criteria*
pH	In fresh bulk solutions: Controls 7.4 (day 0) to 7.6 (day 14) Test solutions 7.6 to 7.7 over 19 days. In the spent test solutions over 21 days: Controls 7.2 to 7.8 Test solutions 7.4 to 7.9	*pH* *EPA: 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.* *OECD: pH range 6-9 and should not vary more than 1.5 units in any one test.* OECD 211 states that the pH should be within the range 6 - 9, and normally it should not vary by more than 1.5 units in any one test. No reference to a desirable pH range found in US EPA OPPTS 850.1300.
Dissolved oxygen	In fresh bulk solutions: Controls 8.3 (day 19) to 8.8 (day 7) Test solutions 8.2 (day 19) to 8.7 (day 7) In the spent test solutions over 21 days: Controls 8.1 (day 17) to 9.0 (day 0) Test solutions 8.0 (day 17) to 9.0 (day 0)	A summary of the test conditions in the study report stated that the dissolved oxygen range of 8.0–9.0 mg/L was equivalent to a % saturation of 89–101%. The study report's Summary referred to the percent oxygen saturation remaining at ≥ 90% throughout the study. *Dissolved Oxygen Renewal:* *EPA: must not drop below 50% for more than 48 hours.* *Flow-through:* *EPA: ≥ 60% throughout test.* OECD 211 states that the dissolved oxygen concentration should be above 3 mg/L at the beginning and during the test. US EPA OPPTS 850.1100 states that the dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.

Parameter	Details	Remarks *Criteria*
Temperature	21°C in the bulk control and test solutions over 19 days. 19-21°C in the control and test solutions over 19 days.	*Temperature* *EPA: 20□C ±2□C. Must not deviate from 20□C by more than 5□C for more than 48 hours.* *OECD: range 18 - 22°C; temperature should not vary more than 2°C (within these limits)* OECD 211 states that the temperature of the test media should be within the range 18-22°C. US EPA OPPTS 850.1100 states the test temperature should be 20°C. Excursions from the test temperature should be no greater than ±1°C.
Total organic carbon (TOC)	1300 µg/L	Total organic carbon *OECD: < 2 mg/L)* OECD 211 refers TOC levels in the medium being below 2 mg/L. US EPA 850.1300 refers to dilution water as having a total organic carbon content 2 mg/L (understood as not to exceed this value).
Particulate matter	As total suspended solids, below the detection level of 1000 µg/L	US EPA 850.1300 refers to dilution water as having a particulate matter content of 20 mg/L.
Metals	A table of analysis of inorganic components in the ALDW showed most metallic ions were below their relevant detection levels as were anions such as bromide, chloride, nitrate etc. Where measurable concentrations were found (e.g. Al^{3+}, Ca^{2+}, Cl^- etc.), they were not identified in the study report as being of concern.	The levels of inorganic residues reported are probably consistent with the source of the water and, based on absence of immobility or sub-lethal effects in the control daphnids, not considered to have adversely affected the study.

Parameter	Details	Remarks *Criteria*
Pesticides	A table of analysis of selected organic species and pesticides in laboratory water indicated all analytes measured were below their relevant limits of detection (which ranged from 0.25 to 5 µg/L).	While all selected organic species and pesticides analysed were below their relevant limits of detection, it is not possible to know if the sum of the organophosphorus pesticides and the total organochlorine pesticides plus polychlorinated biphenyls are below the maxima set by OPPTS 850.1300 for these parameters, i.e. 50 ng/L in both cases. Again, absence of immobility or sub-lethal effects in the control daphnids indicates that the levels present had not adversely affected the study.
Chlorine	Residual chlorine levels were below the limit of detection of 10 µg/L.	US EPA 850.1300 states that dilution water should have a residual chlorine content of < 3 µg/L.
Interval of water quality measurement	Both laboratory and daphnid water were monitored weekly for pH, alkalinity, conductivity and hardness and twice yearly for total organic carbon (TOC), total suspended solids (TSS), selected inorganics and organic compounds. In the test solutions, dissolved oxygen, temperature and pH were measured in freshly prepared bulk solutions at days 0, 7, 14 and 19 and all their respective spent solution replicates at days 3, 10, 17 and 21. Water temperature was continuously monitored from one surrogate vessel throughout the study. Alkalinity, conductivity, hardness and residual chlorine were measured at least one time per week (at days 0, 7, 14 and 19) from fresh bulk test solutions for the water control and the highest test concentration with surviving organisms (10.0 mg pyroxsulam/L).	
<u>Number of replicates</u>: Control (dilution water):	 1 daphnid/replicate (1/test vessel)	Requirement considered met. Control and six test concentrations used with appropriate dilution/separation factors.

Parameter	Details	Remarks *Criteria*
Solvent control:	Not applicable	*(EPA requires Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2)*
Treatment:	1 daphnid/replicate (1/test vessel). The loading rate used was 1 daphnid/90 mL or, ~11 daphnid/L.	US EPA OPPTS 850.1300 refers to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, 64 mg/L). See "Treatment Concentrations: Nominal" below. US EPA OPPTS 850.1300 refers to a loading not exceeding 40 daphnid/L in static renewal systems.
<u>Number of organisms:</u>		Requirement considered met.
For growth and reproduction: For survival test:	For both parameters, 10 daphnids per control and per test concentration at day 0.	*(EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests.* *For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration)* Note: OECD 211 states that, for semi-static tests, at least 10 animals individually should be held at each test concentration and at least 10 animals should be individually held in the control series. US EPA OPPTS 850.1300 states that in static-renewal tests, 10 or more replicates of one daphnid each, for each concentration, should be used.

Parameter	Details	Remarks *Criteria*
Treatment concentrations: Nominal:	0.313, 0.625, 1.25, 2.50, 5.0 and 10.0 mg pyroxsulam/L. Dilution factor = 50% and separation factor = 2..	Requirement considered met. *EPA requires Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor* [preferably] *not exceeding 3.2.* US EPA OPPTS 850.1300 refers to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, 64 mg/L).
Measured:	Mean-measured concentrations over 21 days determined from the concentrations measured in the bulk dose solutions at days 0, 7, 14 and 19, the ten individual replicates/control or test concentration at days 3 and 21 (i.e. spent solutions) and pooled spent solutions at days 10 and 17. **Nominal** / **Mean-measured over 21 days** 0 (water control) / <LLQ* 0.313 / 0.353 (113%**) 0.625 / 0.701 (112%) 1.25 / 1.37 (110%) 2.50 / 2.66 (106%) 5.00 / 5.27 (105%) 10.0 / 10.4 (104%) *** Less than the lowest level of pyroxsulam quantified (0.0806 mg/L).** **** Percentage of the nominal concentration.** Daily percentages of the target concentrations ranged from 94% (10.0 mg pyroxsulam/L, day 3 mean of the 10 spent replicate values) to 123% (0.313 mg pyroxsulam/L, day 14 mean of the 10 spent replicate values). The overall average percent of target and standard deviation values for the entire study (n=6) was 108 ± 3.83%.	Analyses (HPLC/UV) indicated that, during the study, the daily test concentrations were 94.0 to 123% of the target or nominal values. The study report stated, "None of the analyses of the ALDW exhibited a peak eluting at the retention time of pyroxsulam at a concentration exceeding the lowest level quantified of 0.0806 mg/L ALDW … ." Typical chromatograms of a control, a standard and a bulk dose solution sample were presented and indicated absence of pyroxsulam in the control and ability to isolate and determine pyroxsulam concentrations at 0.0806 mg/L ALDW and above. The study report also stated, "The variability associated with the analytical method as well as solution homogeneity was assessed on day 0 of the study by collecting four replicate samples from the bulk dose solutions at nominal concentrations of 0.313 and 10.0 mg/L. Four repeated measurements (4 samples x 1 injection/sample) of the low and high concentration bulk dose solutions yielded percent RSD values of 0.418 and 1.37%, respectively (data not shown)." and "The HPLC/UV instrumentation exhibited

Parameter	Details	Remarks *Criteria*
	No evidence of incomplete dissolution of test material in either the bulk test solutions or the test solutions was observed following sonication.	a linear response over the concentration range extending from 0.0806 to 11.0 mg XDE-742/L ALDW." A standard curve was presented and showed linearity between peak area and pyroxsulam concentration over the test range.
Solvent (type, percentage, if used)	Test solutions were prepared without the use of solvent.	Requirement met. *(EPA requires solvent not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD requires 0.1 ml/L)*
Lighting	Mass cultures of *Daphnia magna* were reared in incubators under cool-white fluorescent lights (approximately 2050 ± 350 lux) and a 16-hour light:8-hour dark photoperiod. Light intensity (16-hour light/8-hour dark photoperiod) during the study ranged from 550-766 lux.	Requirement considered met. *(EPA/OECD require 16 hours light, 8 hours dark)*
<u>Recovery of the chemical</u>: Frequency of determination Level of Quantitation Level of Detection	 Pyroxsulam residues measured on day 0, 7, 14 and 19 (from the freshly prepared bulk solution), days 3 and 21 (from each replicate control and treatment spent solution) and day 10 and 17 (aliquots from each spent solution were pooled to provide one composite sample/dose level). Lowest level quantified was 0.0806 mg pyroxsulam/L. Not identified in the study report.	Requirement considered met. Pyroxsulam concentrations in the control and test solutions were determined by HPLC/UV with analytical standards over a concentration range of 0.0806 to 11.0 mg pyroxsulam/L prepared using ALDW.
Positive control {if used, indicate the chemical and concentrations}	No positive control used.	Requirement met.

Parameter	Details	Remarks *Criteria*
Other parameters, if any	Over 19 days the following water quality measurements were reported. Measurements were taken at days 0, 7, 14 and 19 from the bulk solutions. **ALDW (control) water** **Alkalinity, mg $CaCO_3$/L** 46-50 **Conductivity, µmhos/cm** 374-386 **10 mg pyroxsulam/L (nominal)** **Alkalinity, mg $CaCO_3$/L** 44-48 **Conductivity, µmhos/cm** 368-388	Requirement considered met. OECD 211 and US EPA OPPTS 850.1300 do not specify specific values for alkalinity or conductivity of the test waters.

2. Observations

Table 2. Observations

Parameters	Details	Remarks *Criteria*
Data end points measured (list)	Daphnid survival. Daphnid reproduction. Daphnid growth (length and (dry) weight). Observations with respect to daphnid behaviour and appearance.	These end points are considered appropriate and the requirement is considered met. *(EPA requires:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs)*
Observation intervals	Daily for daphnid survival, behaviour and appearance. Reproduction (counting of neonates, living and dead, at each test vessel renewal). Daphnid growth (measurement of lengths and dry weights at day 21, test termination). Daily observations with respect to daphnid behaviour and appearance (reported as "Normal" in the study report).	These intervals are considered appropriate and the requirement is considered met. Mortality was defined as when the animal was immobile, i.e., when it was not able to swim, or if there was no observed movement of the appendages or post-abdomen, within 15 seconds after gentle agitation of the test container. "Reproduction was evaluated by counting surviving and dead (if present) *Daphnia magna* neonates in each test vessel every renewal day (Monday, Wednesday, or Friday) and at test termination. This was performed at the same time as test solution renewal by first transferring the adult daphnid into a new test vessel, followed by sieving the spent solution from the old test vessel. The time (day) to production of first brood was recorded." At test termination (day 21), lengths (distance measured from apex of helmet to base of the shell spine using a dissecting scope with a calibrated ocular and converted to millimeters) and dry weights (dried at ~60 °C for ~ 3 days following length measurements) of all surviving parent *Daphnia* were measured (to the nearest 0.1 mm and 0.01 mg, respectively) and recorded." The individual length data presented in the study report noted that growth was measured as body length minus anal spine and that the daphnid dry weights were without foils.
Water quality was acceptable (Yes/No)	Dissolved oxygen content was 8.2 to 8.8 mg/L in the fresh bulk control and test solutions and in the spent test and control solutions over 21 days, 8.0 to 9.0 mg/L.	OECD TG 211 requires the dissolved oxygen concentration should be above 3 mg/L at the beginning and during the test. The pH should be within the range 6 - 9 and normally it should not vary by more than 1.5 units in any one test. Hardness above 140 mg/L (as

	pH in the fresh bulk control and test solutions was 7.4 to 7.7 and, in the spent control and test solutions over 21 days, 7.2 to 7.9. In the test solutions, hardness (as $CaCO_3$) ranged from 191-204 mg/L in the control water and 192-210 mg/L (as $CaCO_3$) in the high-dose (10 mg pyroxsulam/L) level over 19 days. Total organic carbon, particulate matter, metals, pesticides and residual chlorine levels considered acceptable.	$CaCO_3$) is recommended. However, the template states the US EPA requires a hardness of 160 to 180 mg/L as $CaCO_3$. Water quality is considered acceptable
Were raw data included?	No. Reported as archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Records Center, Midland, Michigan. Tabulated mortality, sub-lethal effects etc results presented.	[OECD 211 provides examples of data sheets which are to be provided with the study report. These sheets are considered equivalent to the data sheets provided in the study report. No specific requirement to supply raw (i.e. laboratory notes etc) data was identified in the OECD guideline. US EPA OPPTS 850.1300 states that the sponsor should submit to the EPA all data developed by the test that are suggestive or predictive of chronic toxicity and all associated toxicological manifestations. . However, the absence of "raw data" is not considered a deficiency as the US EPA has advised elsewhere that "Tabularized results are considered sufficient since they allow the reviewer to recalculate dose response if necessary. This is how the raw data are typically provided to EPA for analysis." In its comments on this DER, the US EPA also noted that the presentation of tabulated mortality etc. data is fairly common with "raw" data from laboratory notebooks not typically received.
Other observations, if any	None.	

II. RESULTS AND DISCUSSION

A. MORTALITY, 29

There was 100% survival of daphnids in the water control. There was also 100% survival at all test concentrations except at 5.27 mg pyroxsulam/L (mean-measured) where survival was 90% (1 dead observed at test termination).

The one mortality occurred at day 21 of the exposure period. This result was considered biologically insignificant.

Because no dose response relationship was found, the 21 day survival NOEC was set at 10.4 mg pyroxsulam/L (the highest mean-measured concentration). Similarly, the 21 day LOEC, MATC and survival EC50 were each set at >10.4 mg pyroxsulam/L. Mortality, reproduction and growth (length and dry weight) results are summarized in the following table which also shows the 21 day NOECs, LOECs and MATCs:

Table 3, page 20 of this DER summarises the effect of pyroxsulam on growth and survival of *Daphnia* sp. reported in the study.

Table 3. Effect of pyroxsulam on growth and survival of *Daphnia* sp.

Treatment (mg pyroxsulam/L) [record nominal and measured concentrations used]		F_0 (parent) daphnid mortality (dead or immobile) after 21 days		Average Cumulative Progeny Per Surviving Female Adult (mean ± SD)	Length in Millimeters (mean ± SD)	Dry Weight in Milligrams (mean ± SD)
Nominal	Mean-measured	No Dead	%			
Negative control (dilution water)	<LLQ[a]	0	0	93.8 ± 8.1	4.23 ± 0.12	0.63 ± 0.04
Positive control (if used)	Not used.	Not applicable				
0.313	0.353	0	0	89.9 ± 9.5	4.16 ± 0.11	0.61 ± 0.04
0.625	0.701	0	0	83.3 ± 13.9	4.08 ± 0.10	*0.59 ± 0.06
1.25	1.37	0	0	94.6 ± 7.2	4.14 ± 0.12	0.60 ± 0.08
2.50	2.66	0	0	84.4 ± 15.0	4.20 ± 0.07	0.63 ± 0.03
5.0	5.27	1	10	95.0 ± 8.5	4.19 ± 0.15	0.59 ± 0.08
10.0	10.4	0	0	89.8 ± 11.1	4.23 ± 0.15	0.60 ± 0.07
Survival, Reproduction and Growth (Length and Weight) 21 day NOEC	10.4 mg/L, measured					
Survival, Reproduction and Growth (Length and Weight) 21 day LOEC	>10.4 mg/L, measured					
Survival, Reproduction and Growth (Length and Weight) 21 day MATC	>10.4 mg/L, measured					
Survival and Reproduction 21 day EC50	>10.4 mg/L, measured					

[a]LLQ = 0.0806 mg pyroxsulam/L.
*The study report stated that "Differences in growth (dry weight) statistically significant (• = 0.05) when compared to the control group, but is considered biologically insignificant since the greatest percent difference observed between the 0.701 mg/L test level and any remaining dose level or control was minimal at 6% and since no statistically significant differences in weight were observed in the dose levels above or below this test level."

B. EFFECT ON REPRODUCTION AND CHRONIC/SUBLETHAL EFFECTS:

The study report stated that the mean number of young produced per surviving female adult was 93.8 in the water control and 89.9, 83.3, 94.6, 84.4, 95.0 and 89.8 in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels, respectively and that no statistically significant difference ($\alpha = 0.05$) in reproductive output was observed.

The release of control and test first broods was on test day 9.

No treatment level exhibited a reduction in reproductive output of greater than or equal to 50% when compared to the water control and no mortality/immobility of offspring was observed during the study.

The total numbers of progeny in the control and test replicates after 21 days are given in Table 5, page 25 of this DER.

No sublethal effects were reported in any of the live young (all were described as normal).

OECD 211 states that, "Generally in a well-run test, the coefficient of variation around the mean number of living offspring produced per parent animal in the control(s) should be ≤ 25% and this should be reported for test designs using individually held animals." (Point 38, page 7/21 of OECD 211). The reported coefficients of variation for the controls and exposed daphnids were as shown in Table 4 and the OECD 211 requirement is complied with.

Table 4. Coefficients of variation for the numbers of progeny at day 21 after exposure to pyroxsulam.

Concentration, mg/L (mean-measured)	Control	0.353	0.701	1.37	2.66	5.27	10.4
Coefficient of variation	8.6%	10.6%	16.7%	7.6%	17.8%	8.9%	12.4%

Note: Coefficients of variation are determined from the reported standard deviations and means for the total number of progeny at day 21. Mean and standard deviation values used to determine the coefficients of variation are given on page 25 of this DER.

Similarly, it was reported that the mean length per surviving female adult was 4.23 mm in the water control and 4.16, 4.08, 4.14, 4.20, 4.19 and 4.23 mm in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels, respectively with no statistically significant difference ($\alpha = 0.05$) in length observed. Individual replicate growth (length) data were reported and are reproduced on page 26 of this DER.

With respect to the mean weight (dry) per surviving female adult, the weights determined were 0.63 mg in the water control and 0.61, 0.59, 0.60, 0.63, 0.59 and 0.60 mg in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels. No statistically significant differences ($\alpha = 0.05$) in growth (weight) were observed in the treatments, with the exception of the 0.701 mg/L measured treatment level (0.59 mg dry weight). However, this was considered biologically insignificant since the greatest percent difference observed between the 0.701 mg/L test level and any remaining dose level or control was minimal at 6% and since no statistically significant differences in weight were observed in the dose levels above or below this test level. Individual replicate growth (weight) data were reported and are reproduced on page 27 of this DER.

The 21 day NOECs for survival, growth and reproduction were each set at 10.4 mg pyroxsulam/L (mean-measured concentration). The 21 day LOECs for these parameters were set at >10.4 mg pyroxsulam/L.

C. REPORTED STATISTICS:

The following details on statistics used in the study are based on information provided in the study report:

With respect to statistical analysis, as only one adult daphnid died during the conduct of the 21-day study, therefore, the statistical evaluation of a 21-day EC50, NOEC and LOEC value for parent survival was not performed.

Similarly, a statistical evaluation of a 21-day EC50 value for reproductive output was not performed, since reduction in reproduction greater than 50% when compared to the controls was not observed at any of the dose levels tested.

For the NOEC and LOEC values for reproduction (total young) and growth (length), the raw data were first tested for normality using the Shapiro-Wilk's test at a Type I error rate of 0.01. If the data were not normally distributed, the logarithmic, inverse and square root transformations were tested sequentially to search for a normalizing transformation. Next, the data and the same transformed variables were tested for homogeneity of variance using Bartlett's test at a Type I error rate of 0.01. If the raw data, or a transformed variable, were both normal and homogeneous, a parametric analysis was conducted using a Dunnett's test to compare each treated group with the control. A one-tailed Dunnett's test, looking for a significant decrease from the control group, was conducted at a Type I error rate of 0.05. The no-observed-effect concentration (NOEC) was defined as the highest dose group not significantly different compared to the control. Data that were not normally distributed and/or not homogeneous were analyzed nonparametrically with a Steel's Many-One Rank Test if the number of replicates in each treatment group were the same. A Kruskal-Wallis test was used if the number of replicates was different. Steel's Many-One Rank Test is one-sided and the Kruskal-Wallis test is two-sided; both have a Type I error rate of 0.05. A significant result in the Kruskal-Wallis test leads to a pairwise comparison of each treatment with the control using the Wilcoxon procedure having a Type I error rate of 0.01 (one-sided). Both the Steel's test and the Wilcoxon test lead to the determination of a NOEC.

Individual replicate survival and reproduction data were reported in the study report. The NOEC and LOEC concentrations for survival, the number of progeny (total) per surviving female adult (reproduction) and individual length and weight (growth) were determined using standard statistical methodology. The number of progeny per surviving female adult and the length data met the normality and homogeneity of variance, so the NOEC for reproduction was determined by the Dunnett's test. This procedure maintained $\alpha = 0.05$ while making multiple comparisons to the control group.

Weight data did not meet the homogeneity assumptions. These data were analyzed using Steel's Many-One Rank test.

A statistical analysis for survival was not undertaken since there was only one adult daphnid that did not survive during the study, at any dose level. Survival was 90% (1 dead observed at test termination) in the 5.27 mg/L mean-measured treatment level and 100% in the water control and all remaining treatment levels. Therefore, since only a single mortality was observed during the conduct of the study, it was considered biologically insignificant and the resulting endpoint values empirically derived.

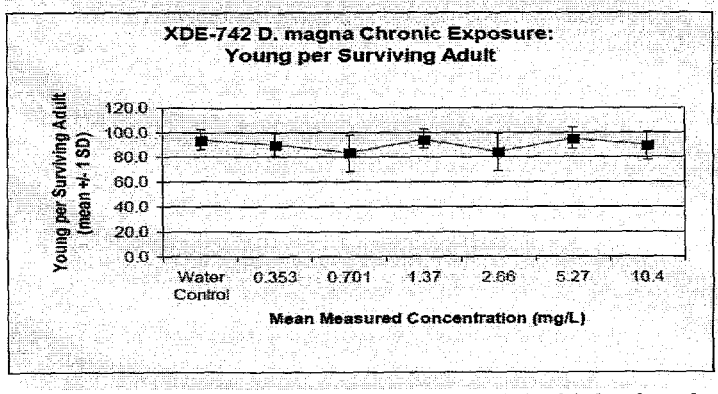
The NOEC for survival was thus 10.4 mg/L and the LOEC and MATC greater than 10.4 mg/L, the highest mean-measured concentration tested. No treatment level exhibited mortality (including immobility) of greater than or equal to 50%, therefore, the survival 21-day EC50 value was greater than the highest measured test concentration of 10.4 mg/L.

Since the one organism that died during the study died on day 21 and since progeny was also observed in the test vessel, data from this replicate were included in the statistical evaluation of the data. This DER notes that OECD 211 states that, "If, in any replicate the parent animal dies during the test or turns out to be male, then the replicate is excluded from the analysis. The analysis will then be based on a reduced number of replicates." (Section 51, page 9/21 of the Guideline) and, consequently, the number of replicates used for the concentration in which the death occurred (5.00 mg/L nominal) could have been taken as 9 rather than 10. This DER considers the use of 10 as the number of replicates is acceptable under the circumstances.

The mean number of young produced per surviving female adult was 93.8 in the water control and 89.9, 83.3, 94.6, 84.4, 95.0 and 89.8 in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels, respectively. No statistically significant difference ($\alpha = 0.05$) in reproductive output was observed. Therefore, the statistically derived NOEC for reproduction was 10.4 mg/L and the LOEC and MATC greater than 10.4 mg/L, the highest mean-measured concentration tested. The coefficient of variation for the water control reproduction was 8.6% and the release of their first brood was on test day 9. No treatment level exhibited a reduction in reproductive

output of greater than or equal to 50% when compared to the water control, therefore, the reproductive 21-day EC50 value was greater than the highest mean-measured test concentration of 10.4 mg/L. No mortality/immobility of offspring was observed during the study.

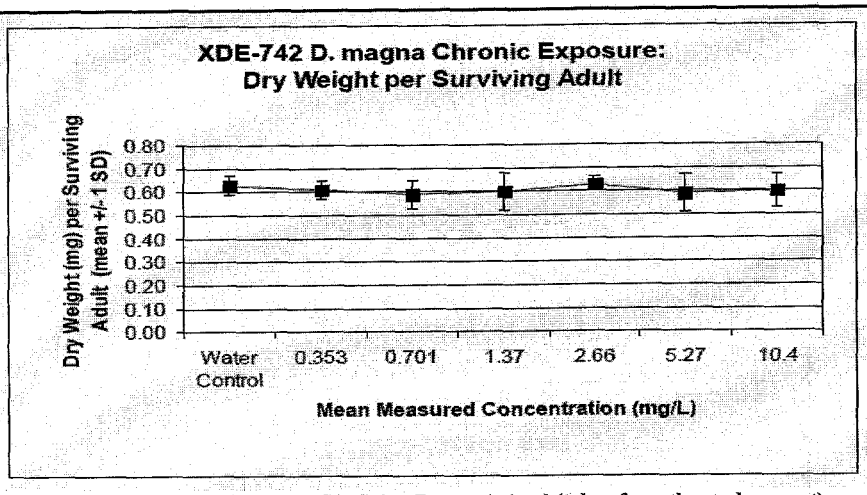
A plot of the total number of living offspring per parent animal was provided and is reproduced below:



Plot of Total Number of Living Offspring per Surviving Parent Animal (taken from the study report)

The mean length per surviving female adult was 4.23 mm in the water control and 4.16, 4.08, 4.14, 4.20, 4.19 and 4.23 mm in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels, respectively. No statistically significant difference ($\alpha = 0.05$) in length was observed. Therefore, the statistically derived NOEC for growth (length) was 10.4 mg/L and the LOEC and MATC greater than 10.4 mg/L, the highest mean-measured concentration tested. Individual replicate growth (length) data were reported.

The mean weight (dry) per surviving female adult was 0.63 mg in the water control and 0.61, 0.59, 0.60, 0.63, 0.59 and 0.60 mg in the 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L mean-measured treatment levels. No statistically significant differences ($\alpha = 0.05$) in growth (weight) was observed in the treatments, with the exception of the 0.701 mg/L measured treatment level. However, this was considered biologically insignificant since the greatest percent difference observed between the 0.701 mg/L test level and any remaining dose level or control was minimal at 6% and since no statistically significant differences in weight were observed in the dose levels above or below this test level. Therefore, the absence of a dose-response indicates that the NOEC for growth (weight) was 10.4 mg/L and the LOEC and MATC were both greater than 10.4 mg/L, the highest measured concentration tested. Individual replicate growth (weight) data were reported and a plot of the mean weight per parent animal was provided and is copied below:



Plot of Mean Dry Weight per Surviving Parent Animal (taken from the study report)

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Mortality
Although there was only one mortality in the daphnia (5.27 mg pyroxsulam/L, mean-measured) of the 21 days and statistical assessment of mortality was not performed in the study, the mortality data have been assessed by the reviewer using the TidePool Scientific Software program ToxCalc (version 5.0.23j).

The ToxCalc analysis of these data (total numbers of surviving daphnia/replicate/test concentration (Arcsine square root transformation)) reported a non-normal distribution (Kolmogorov D Test, $p \leq 0.01$) with equality of variance not being able to be confirmed. Dunnett's Test indicated no difference between the treatments and the controls with the NOEC and LOEC, respectively, 10.4 and >10.4 mg pyroxsulam/L (mean-measured). The ToxCalc analysis also reported the EC50 (21 day, survival) as >10.4 mg pyroxsulam/L using its linear interpolation method.

A summary of the ToxCalc results are provided on page 34 of this DER.

The study report found that since only a single mortality was observed during the conduct of the study, it was considered biologically insignificant and the resulting endpoint values empirically derived with the NOEC for survival being 10.4 mg/L and the LOEC and MATC greater than 10.4 mg/L, the highest mean-measured concentration tested. The reviewer's statistical examination of the survival data has confirmed these conclusions of the study report.

With respect to the 21 day EC50, the study report stated that no treatment level exhibited mortality (including immobility) of greater than or equal to 50% and therefore, the survival 21-day EC50 value was greater than the highest measured test concentration of 10.4 mg/L. The reviewer's statistical examination of the survival data has also confirmed this conclusion of the study report.

Daphnid reproduction/progeny
The total numbers of live, normal neonates reported are shown in **Table 5**.

Table 5. Number of progeny (neonates) per control and test replicate after 21 days exposure to pyroxsulam.

	Number of neonates (total progeny)/replicate after 21 days exposure to either control water or various pyroxsulam concentrations						
Replicate	**Control**	**0.313**	**0.625**	**1.25**	**2.5**	**5**	**10**
1	102	86	101	94	95	86	100
2	99	96	79	89	75	90	75
3	84	88	87	96	80	104	108
4	96	106	95	99	82	100	92
5	78	97	97	82	110	96	80
6	96	76	84	85	97	102	80
7	100	99	61	99	83	97	100
8	88	78	93	103	54	84	82
9	102	85	65	96	89	107	84
10	93	88	71	103	79	84	97
Mean (progeny/surviving daphnid)	93.8	89.9	83.3	94.6	84.4	95	89.8
Standard Deviation	8.1	9.5	13.9	7.2	15.0	8.5	11.1

The ToxCalc analysis of this data (total numbers of progeny at day 21/replicate/test concentration (untransformed)) reported a normal distribution (Kolmogorov D Test, $p > 0.01$) and equal variances (Bartlett's Test, $p = 0.23$). This result verifies the study report statement that the number of progeny per surviving female adult data met the normality and homogeneity of variance (and consequently, the NOEC for reproduction was determined by the Dunnett's test).

Dunnett's Test indicated no difference between the treatments and the controls with the NOEC and LOEC, respectively, 10.4 and >10.4 mg pyroxsulam/L (mean-measured). The ToxCalc analysis also reported the EC50 (21 day, total number of progeny) as >10.4 mg pyroxsulam/L using its linear interpolation method.

The mean reproduction value per test concentration ranged from 83.3 to 95 progeny/survivor, values which are all greater than 50% of the mean control value of 93.8 progeny/survivor.

A summary of the ToxCalc results are provided on page 35 of this DER.

The study report had stated that no statistically significant difference ($\alpha = 0.05$) in reproductive output was observed (Dunnett's Test) and, consequently, the statistically derived NOEC for reproduction was 10.4 mg/L and the LOEC and MATC greater than 10.4 mg/L, the highest mean-measured concentration tested.

With respect to the 21 day reproductive EC50, the study report noted that no treatment level exhibited a reduction in reproductive output of greater than or equal to 50% when compared to the water control, therefore, the reproductive 21 day EC50 value was greater than the highest mean-measured test concentration of 10.4 mg/L.

The reviewer's calculation of these endpoints gave the same values as determined in the study.

Daphnid growth – length
The individual lengths of the daphnids after 21 days exposure period are shown in Table 6. The table includes the length of the daphnid which died at day 21 in one of the 5.00 mg/L (nominal) test vessels. The table also shows the means and standard deviations as determined using the Descriptive Statistics function of the Excel Data Analysis function. The means and standards deviations calculated were the same as those reported in the study report.

Table 6. Daphnid lengths after 21 days

	Concentration:	Length (mm) of parent daphnids after 21 days exposure to either control water or various pyroxsulam concentrations						
Replicate	Nominal:	Control	0.313	0.625	1.25	2.5	5	10
	Mean-measured:	Control	0.353	0.701	1.37	2.66	5.27	10.4
1		4.2	4.3	4.1	4.1	4.2	4.1	4.3
2		4.3	4.3	4.2	4.2	4.1	4.3	4.2
3		4.4	4.1	4.0	4.1	4.2	4.3	4.2
4		4.0	4.2	4.0	4.3	4.1	4.2	4.3
5		4.3	4.2	4.2	4.3	4.2	4.3	4.5
6		4.3	4.0	4.0	3.9	4.2	4.4	4.2
7		4.1	4.2	3.9	4.1	4.3	4.0	4.2
8		4.3	4.2	4.2	4.2	4.2	4.2	3.9
9		4.2	4.1	4.1	4.1	4.2	4.2	4.3
10		4.2	4.0	4.1	4.1	4.3	3.9	4.2
Mean length, mm		4.23	4.16	4.08	4.14	4.20	4.19	4.23
Standard deviation		0.12	0.11	0.10	0.12	0.07	0.15	0.15

The ToxCalc analysis of this data (daphnid lengths at day 21/replicate/test concentration (untransformed)) reported a normal distribution (Kolmogorov D Test, $p > 0.01$) with equality of variances confirmed (Bartlett's Test, $p = 0.33$). Dunnett's Test indicated no difference between the treatments and the controls with the NOEC and LOEC, respectively, 10.4 and >10.4 mg pyroxsulam/L. The ToxCalc analysis also reported the EC50 (21 day, daphnid length) as >10.4 mg pyroxsulam/L using its linear interpolation method.

These findings are in accord with the study report's stating that the length data met the normality and homogeneity of variance criteria, so the NOEC for reproduction was determined by the Dunnett's test.

However, the ToxCalc analysis of the length data also reported an interrupted dose-response had been detected (at 0.701 mg/L) and offered the choice of using the first significant treatment as the LOEC. If this choice is accepted, the normal distribution and equal variances are reconfirmed with Dunnett's Test indicating the NOEC is 0.353 mg pyroxsulam/L and the LOEC, 0.701 mg pyroxsulam/L. If the choice is not accepted, the NOEC and LOEC are as reported above, namely, 10.4 and >10.4 mg pyroxsulam/L. It is the reviewer's opinion that the correct NOEC and LOEC are the higher values as the significant dose response at 0.701 mg/L was not seen at any higher concentrations.

A summary of the ToxCalc results are provided on page 36 of this DER.

The study report had stated that no statistically significant difference ($\alpha = 0.05$) in mean length per surviving daphnid was observed and, consequently, the statistically derived NOEC for growth (length) was 10.4 mg/L and the LOEC and MATC greater than 10.4 mg/L, the highest mean-measured concentration tested.

The reviewer's calculation of these endpoints gave the same endpoints as determined in the study when the ToxCalc message with respect to an interrupted dose response was ignored. The study report did not identify the 0.701 mg pyroxsulam/L results as being dose interrupted with regard to daphnid length (in contrast to the weight results, see below). Because the effect was not seen in the higher test concentrations, it is not considered to be of biological significance by the reviewer.

Daphnid growth – dry weight

The individual dry weights of the daphnids which survived to 21 days exposure period are shown in Table 7. The table includes the dry weight of the daphnid which died at day 21 in one of the 5.00 mg/L (nominal) test vessels. The table also shows the means and standard deviations as determined using the Descriptive Statistics function of the Excel Data Analysis function. The means and standards deviations calculated were the same as those reported in the study report.

Table 7. Daphnid dry weights after 21 days.

Replicate	Concentration: Nominal: Mean-measured:	Dry weight (mg) of parent daphnids after 21 days exposure to either control water or various pyroxsulam concentrations						
		Control	0.313	0.625	1.25	2.5	5	10
		Control	0.353	0.701	1.37	2.66	5.27	10.4
1		0.67	0.56	0.67	0.63	0.62	0.58	0.49
2		0.66	0.57	0.58	0.66	0.56	0.6	0.63
3		0.59	0.62	0.54	0.65	0.65	0.62	0.63
4		0.58	0.61	0.62	0.51	0.66	0.66	0.51
5		0.61	0.58	0.65	0.55	0.64	0.63	0.64
6		0.66	0.58	0.52	0.43	0.64	0.62	0.53
7		0.66	0.6	0.58	0.61	0.6	0.65	0.7
8		0.58	0.69	0.68	0.65	0.64	0.58	0.64
9		0.66	0.64	0.54	0.62	0.63	0.37	0.61
10		0.67	0.63	0.55	0.64	0.62	0.59	0.57
Mean weight, mg		0.63	0.61	0.59	0.60	0.63	0.59	0.60
Standard deviation		0.04	0.04	0.06	0.08	0.03	0.08	0.07

The study report noted that no statistically significant differences ($\alpha = 0.05$) in growth (weight) was observed in the treatments, with the exception of the 0.701 mg/L measured treatment level. However, this was considered biologically insignificant since the greatest percent difference observed between the 0.701 mg/L test level and any remaining dose level or control was minimal at 6% and since no statistically significant differences in weight were observed in the dose levels above or below this test level. As a result, the absence of a dose-response was taken in the study to indicate that the NOEC for growth (weight) was 10.4 mg/L and the LOEC and MATC were both greater than 10.4 mg/L, the highest measured concentration tested.

The study report also noted that the weight data did not meet the homogeneity of variance criteria and were analysed by Steel's Many-one Rank Test.

The ToxCalc analysis of these data (daphnid dry weights at day 21/replicate/test concentration (untransformed)) reported a normal distribution (Kolmogorov D Test, $p > 0.01$) with equality of variances confirmed (Bartlett's Test, $p = 0.03$ at the 0.01 level of significance). Dunnett's Test indicated no difference between the treatments and the controls with the NOEC and LOEC, respectively, 10.4 and >10.4 mg pyroxsulam/L. The ToxCalc analysis also reported the EC50 (21 day, daphnid length) as >10.4 mg pyroxsulam/L using its linear interpolation method.

Because of the study report's reference to the dry weight data showing homogeneity of variance, the ToxCalc calculations were re-run using an α of 0.05 for the equality of variance. In this situation, the Kolmogorov D Test again indicated a normal distribution ($p > 0.01$) but Bartlett's Test indicated unequal variances ($p = 0.03$). These results confirm the study report's findings. In this case, the ToxCalc analysis using a non-parametric rank analysis on the untransformed data reported no statistically significant differences between any of the treatments and the controls when using Steel's Many-one Rank Test. The NOEC and LOEC were, respectively, 10.4 and >10.4 mg pyroxsulam/L, the values proposed in the study report.

The recalculation of the statistics is considered to have confirmed the study report's findings except with respect to the study report's finding that the 0.701 mg pyroxsulam/L result for mean dry weight of surviving daphnids was statistically significantly different from the mean control weight. This difference was not identified in the ToxCalc recalculation of the dry weight results.

A summary of the ToxCalc results are provided on page 38 of this DER.

Determination of the MATC
The MATC is defined in the study report as the theoretical threshold or allowable chronic concentration and it is the geometric mean of the NOEC and LOEC.

With a LOEC of >10.4 mg pyroxsulam/L determined for survival, reproduction and growth (length and dry weight), the reported MATC values are determined as >10.4 mg pyroxsulam/L without further calculation. The study report's reported MATC values were all >10.4 mg pyroxsulam/L, consistent with the value obtained by the reviewer.

E. STUDY DEFICIENCIES:

Table 8 summarises deficiencies and deviations from OECD 211 and US EPA OPPTS 850.1300 Guidelines s. These deficiencies and deviations were not considered to have significantly affected the study's outcome. With regard to the absence of acclimatisation information, the US EPA comments (*q.v.*) and the successful survival, growth and reproduction of the parent daphnids are taken to indicate that acclimatisation had been properly conducted.

Table 8. Deviations from Guidelines and other deficiencies.

Parameter	Study reported results	OECD 211 *Daphnia magna* Reproduction Test	US EPA OPPTS 850.1300 Daphnid Chronic Toxicity Test
Parental acclimation: Period	In-house culture. The period of acclimatisation was not located in the study report but is assumed to be satisfactory for in-house cultures. The Study Profile Template (Marino, 2005) refers to 25 days.	OECD 211 does not state specific requirements for acclimatisation but does require that the stock animals must be maintained in culture conditions (light, temperature, medium, feeding and animals per unit volume) similar to those to be used in the test. It also states, under "Test report", that culture conditions must be supplied.	US EPA OPPTS 805.1300 refers to brood daphnids should be maintained in 100 percent dilution water at the test temperature for at least 48 h prior to the start of the test. This guideline also states that detailed information on the acclimation procedures should be submitted to the US EPA. In its comments on this DER, US EPA advised that in-house cultures are considered to have adjusted for acclimation.
Water parameters:			
Total organo-phosphorus and organochlorine pesticides and polychlorinated biphenyls	All below their respective individual detection limits (0.25 to 5 µg/L)	No specific requirement identified.	US EPA 850.1300 Total organophosphorus pesticides 50 ng/L Total organochlorine pesticides and polychlorinated biphenyls 50 ng/L
Residual chlorine	Residual chlorine levels were below the limit of detection of 10 µg/L.	OECD 211 does not refer to a residual chlorine limit.	US EPA 850.1300 states that dilution water should have a residual chlorine content of <3 µg/L.

Information on the daphnids used	Identified as *Daphnia magna* neonates for in-house cultures.	OECD 211 (Section 57, pages 10/21 and 11/21) requires that the test report must include, *inter alia*, details on the clone (whether it has been genetically typed).	This guideline (page 9) states detailed information about the daphnids used as brood stock, including the method of verification, should be provided.

F. REVIEWERS' COMMENTS:

The study was satisfactorily conducted using controls and pyroxsulam at nominal concentrations of 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 mg pyroxsulam/L (mean-measured concentrations of 0.353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg/L (corrected for purity of the pyroxsulam)) and its results generally considered as scientifically sound. Pyroxsulam, as the active constituent, is considered very slightly chronically toxic to the daphnid, *Daphnia magna* with a 21 day NOEC (mortality, growth and reproduction) of 10.4 mg pyroxsulam/L (NOEC > 1 mg/L).

The definitive toxicity test was conducted from 23 April to 14 May 2004.

With respect to validity criteria of the Guidelines used, OECD 211 specifies a number of criteria that are to be met for the test to be valid. Although the study was not conducted to US EPA OPPTS 850.1300, Daphnid chronic

toxicity test, that US EPA document also gives test validity criteria. The mortality, mean number of live offspring/parent and absence of ephippia requirements are considered to have been met by the study.

Other parameters, e.g. pH, temperature and dissolved oxygen content, specified in these two guidelines are considered to have been satisfactorily maintained or met over the study exposure period.

The recalculation of the study's statistics confirmed the study report's conclusions with respect to the NOEC and LOEC proposed for survival, reproduction and growth, namely 10.4 and >10.4 mg pyroxsulam/L. Confirmation of the proposed 21 day EC50 for survival and reproduction being >10.4 mg pyroxsulam/L also occurred in the recalculation of the study's statistics. The study report's finding of a statistically significant difference in dry weight of the daphnids exposed to 0.701 mg pyroxsulam/L was not confirmed – such effect was, however, determined in the daphnid length analysis. These results are not considered to have affected the study's outcome or the endpoints determined and may be associated with differences in the statistical packages used.

Stock solution preparation does not appear to be reported correctly in the study report. Report states that a 50 mg/L bulk solution was prepared by delivering diluting 10 mg in 2 liters. This would yield a 5 mg/L solution consistent with the stated treatment concentration as opposed to a 50 mg/L solution. The reference to 50 mg/L is presumed to by a typographical error on the part of the study authors.

The study report's finding that there was a statistically significantly different result in the mean dry weight of the surviving daphnids at day 21 at the 0.701 mg/L test level was not confirmed in the statistical verification of the reported data. Instead, a statistically significant result was identified by the reviewer as occurring at the 0.701 mg/L level in the verification of the daphnid length statistics.

The deviations from guidelines and deficiencies identified are considered to be of such a nature as not to have adversely affected the study's performance or outcomes.

The PMRA concurs with the results and conclusions reached by the APVMA.

G. CONCLUSIONS:

The study is scientifically sound and is considered acceptable. The reviewer's inability to reproduce the statistical analysis reported with respect to the presence of a statistically significant difference in the dry weight of the daphnids at the 0.701 mg pyroxsulam/L and the reviewer's finding of such an effect in the daphnid length statistics not being identified in the study report are attributed to differences in the statistical approaches used. The differences are not considered to have invalidated the study's results.

The 21 day chronic static toxicity study resulted in a NOEC for mortality, growth and reproduction of pyroxsulam as the technical grade material in the daphnid, *D. magna*, of 10.4 mg pyroxsulam/L (as mean analytically determined concentrations over 21 days). The LOEC for these parameters was established as >10.4 mg pyroxsulam/L.

Pyroxsulam is considered to be very slightly chronically toxic to *D. magna* with respect to survival, growth and reproduction based on the chronic toxicity classification scheme of the Australian Government Department of the Environment and Water Resources (NOEC > 1 mg/L).

III. REFERENCES:

Note: for the purpose of this parallel process work, references to standard guidelines or methodologies have been included at this time in the list of references.

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Note: This study report, in its finalised version, was presented with the data package for the pyroxsulam assessment as has been assessed as part of the data package (see relevant DER for the study "XDE-742: An Acute Toxicity Study with the Daphnid, *Daphnia magna*, authors Marino, McClymont and Najar, report dated 22 December 2004).

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Approved 04/01/01 C.K.

Attachment 1
Daphnid survival over 21 days

The numbers of daphnid which survived over the 21 days exposure to pyroxsulam are discussed on page 24 of this DER.

ToxCalc analysis of the numbers of surviving daphnids (concentrations are mean, measured values):

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
B-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.353	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.701	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.37	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
2.66	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
5.27	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	1.0000
10.4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
B-Control	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10			
0.353	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	0.000	2.347	0.0657
0.701	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	0.000	2.347	0.0657
1.37	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	0.000	2.347	0.0657
2.66	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	0.000	2.347	0.0657
5.27	0.9000	0.9000	0.9948	0.5236	1.0472	16.644	10	1.871	2.347	0.0657
10.4	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	10	0.000	2.347	0.0657

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)					4.10827		1.035		-7.2108	57.9173
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	10.4	>10.4			0.05888	0.07851	0.00392	0.00392	0.43336	6, 63
Treatments vs B-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL	Skew
IC05	>10.4			
IC10	>10.4			
IC15	>10.4			
IC20	>10.4			
IC25	>10.4			
IC40	>10.4			
IC50	>10.4			

Attachment 2
Daphnid reproduction over 21 days

The numbers of daphnid produced over the 21 days exposure to pyroxsulam are discussed on page 24 of this DER.

ToxCalc analysis of the numbers of surviving daphnids (concentrations are mean, measured values):

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
B-Control	102.000	99.000	84.000	96.000	78.000	96.000	100.000	88.000	102.000	93.000
0.353	86.000	96.000	88.000	106.000	97.000	76.000	99.000	78.000	85.000	88.000
0.701	101.000	79.000	87.000	95.000	97.000	84.000	61.000	93.000	65.000	71.000
1.37	94.000	89.000	96.000	99.000	82.000	85.000	99.000	103.000	96.000	103.000
2.66	95.000	75.000	80.000	82.000	110.000	97.000	83.000	54.000	89.000	79.000
5.27	86.000	90.000	104.000	100.000	96.000	102.000	97.000	84.000	107.000	84.000
10.4	100.000	75.000	108.000	92.000	80.000	80.000	100.000	82.000	84.000	97.000

			Transform: Untransformed					Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
B-Control	93.800	1.0000	93.800	78.000	102.000	8.629	10	93.800	1.0000
0.353	89.900	0.9584	89.900	76.000	106.000	10.559	10	89.900	0.9584
0.701	83.300	0.8881	83.300	61.000	101.000	16.731	10	89.420	0.9533
1.37	94.600	1.0085	94.600	82.000	103.000	7.610	10	89.420	0.9533
2.66	84.400	0.8998	84.400	54.000	110.000	17.735	10	89.420	0.9533
5.27	95.000	1.0128	95.000	84.000	107.000	8.959	10	89.420	0.9533
10.4	89.800	0.9574	89.800	75.000	108.000	12.320	10	89.420	0.9533

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.54719	1.035	-0.2663	0.19453
Bartlett's Test indicates equal variances (p = 0.23)	8.17276	16.8119		

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL	Skew
IC05	>10.4			
IC10	>10.4			
IC15	>10.4			
IC20	>10.4			
IC25	>10.4			
IC40	>10.4			
IC50	>10.4			

Attachment 3
Daphnid growth (length) over 21 days

The lengths of surviving daphnids after the 21 days exposure to pyroxsulam are discussed on page 25 of this DER.

ToxCalc analysis of the numbers of surviving daphnids (concentrations are mean, measured values):

1. **Results from <u>not</u> accepting the choice to identify the first significant treatment as the LOEC as a result of the analysis detecting an interrupted dose-response.**

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
B-Control	4.2000	4.3000	4.4000	4.0000	4.3000	4.3000	4.1000	4.3000	4.2000	4.2000
0.353	4.3000	4.3000	4.1000	4.2000	4.2000	4.0000	4.2000	4.2000	4.1000	4.0000
0.701	4.1000	4.2000	4.0000	4.0000	4.2000	4.0000	3.9000	4.2000	4.1000	4.1000
1.37	4.1000	4.2000	4.1000	4.3000	4.3000	3.9000	4.1000	4.2000	4.1000	4.1000
2.66	4.2000	4.1000	4.2000	4.1000	4.2000	4.2000	4.3000	4.2000	4.2000	4.3000
5.27	4.1000	4.3000	4.3000	4.2000	4.3000	4.4000	4.0000	4.2000	4.2000	3.9000
10.4	4.3000	4.2000	4.2000	4.3000	4.5000	4.2000	4.2000	3.9000	4.3000	4.2000

			Transform: Untransformed					1-Tailed		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
B-Control	4.2300	1.0000	4.2300	4.0000	4.4000	2.741	10			
0.353	4.1600	0.9835	4.1600	4.0000	4.3000	2.584	10	1.313	2.347	0.1251
*0.701	4.0800	0.9645	4.0800	3.9000	4.2000	2.531	10	2.814	2.347	0.1251
1.37	4.1400	0.9787	4.1400	3.9000	4.3000	2.835	10	1.688	2.347	0.1251
2.66	4.2000	0.9929	4.2000	4.1000	4.3000	1.587	10	0.563	2.347	0.1251
5.27	4.1900	0.9905	4.1900	3.9000	4.4000	3.637	10	0.750	2.347	0.1251
10.4	4.2300	1.0000	4.2300	3.9000	4.5000	3.533	10	0.000	2.347	0.1251

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)					1.01377		1.035		-0.5332	0.72431
Bartlett's Test indicates equal variances (p = 0.33)					6.88227		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs B-Control	10.4	>10.4			0.12511	0.02958	0.02895	0.01421	0.07354	6, 63

			Linear Interpolation (200 Resamples)	
Point	**mg/L**	**SD**	**95% CL**	**Skew**
IC05	>10.4			
IC10	>10.4			
IC15	>10.4			
IC20	>10.4			
IC25	>10.4			
IC40	>10.4			
IC50	>10.4			

Attachment 3 (continued)
Daphnid growth (length) over 21 days

2. **Results from accepting the choice to identify the first significant treatment as the LOEC as a result of the analysis detecting an interrupted dose-response.**

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
B-Control	4.2000	4.3000	4.4000	4.0000	4.3000	4.3000	4.1000	4.3000	4.2000	4.2000
0.353	4.3000	4.3000	4.1000	4.2000	4.2000	4.0000	4.2000	4.2000	4.1000	4.0000
0.701	4.1000	4.2000	4.0000	4.0000	4.2000	4.0000	3.9000	4.2000	4.1000	4.1000
1.37	4.1000	4.2000	4.1000	4.3000	4.3000	3.9000	4.1000	4.2000	4.1000	4.1000
2.66	4.2000	4.1000	4.2000	4.1000	4.2000	4.2000	4.3000	4.2000	4.2000	4.3000
5.27	4.1000	4.3000	4.3000	4.2000	4.3000	4.4000	4.0000	4.2000	4.2000	3.9000
10.4	4.3000	4.2000	4.2000	4.3000	4.5000	4.2000	4.2000	3.9000	4.3000	4.2000

			Transform: Untransformed					1-Tailed		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
B-Control	4.2300	1.0000	4.2300	4.0000	4.4000	2.741	10			
0.353	4.1600	0.9835	4.1600	4.0000	4.3000	2.584	10	1.313	2.347	0.1251
*0.701	4.0800	0.9645	4.0800	3.9000	4.2000	2.531	10	2.814	2.347	0.1251
1.37	4.1400	0.9787	4.1400	3.9000	4.3000	2.835	10	1.688	2.347	0.1251
2.66	4.2000	0.9929	4.2000	4.1000	4.3000	1.587	10	0.563	2.347	0.1251
5.27	4.1900	0.9905	4.1900	3.9000	4.4000	3.637	10	0.750	2.347	0.1251
10.4	4.2300	1.0000	4.2300	3.9000	4.5000	3.533	10	0.000	2.347	0.1251

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)					1.01377		1.035		-0.5332	0.72431
Bartlett's Test indicates equal variances (p = 0.33)					6.88227		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	0.353	0.701	0.49745		0.12511	0.02958	0.02895	0.01421	0.07354	6, 63
Treatments vs B-Control										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL	Skew
IC05	>10.4			
IC10	>10.4			
IC15	>10.4			
IC20	>10.4			
IC25	>10.4			
IC40	>10.4			
IC50	>10.4			

Attachment 4
Daphnid growth (dry weight) over 21 days

The dry weights of the daphnids which survived the 21 days exposure to pyroxsulam are discussed on page 27 of this DER.

ToxCalc analysis of the numbers of surviving daphnids (concentrations are mean, measured values):

1. Using an α of 0.01 for the equality of variance.

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
B-Control	0.6700	0.6600	0.5900	0.5800	0.6100	0.6600	0.6600	0.5800	0.6600	0.6700
0.353	0.5600	0.5700	0.6200	0.6100	0.5800	0.5800	0.6000	0.6900	0.6400	0.6300
0.701	0.6700	0.5800	0.5400	0.6200	0.6500	0.5200	0.5800	0.6800	0.5400	0.5500
1.37	0.6300	0.6600	0.6500	0.5100	0.5500	0.4300	0.6100	0.6500	0.6200	0.6400
2.66	0.6200	0.5600	0.6500	0.6600	0.6400	0.6400	0.6000	0.6400	0.6300	0.6200
5.27	0.5800	0.6000	0.6200	0.6600	0.6300	0.6200	0.6500	0.5800	0.3700	0.5900
10.4	0.4900	0.6300	0.6300	0.5100	0.6400	0.5300	0.7000	0.6400	0.6100	0.5700

			Transform: Untransformed					t-Stat	1-Tailed Critical	MSD
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N			
B-Control	0.6340	1.0000	0.6340	0.5800	0.6700	6.140	10			
0.353	0.6080	0.9590	0.6080	0.5600	0.6900	6.431	10	0.989	2.347	0.0617
0.701	0.5930	0.9353	0.5930	0.5200	0.6800	9.835	10	1.559	2.347	0.0617
1.37	0.5950	0.9385	0.5950	0.4300	0.6600	12.633	10	1.483	2.347	0.0617
2.66	0.6260	0.9874	0.6260	0.5600	0.6600	4.593	10	0.304	2.347	0.0617
5.27	0.5900	0.9306	0.5900	0.3700	0.6600	13.908	10	1.674	2.347	0.0617
10.4	0.5950	0.9385	0.5950	0.4900	0.7000	11.323	10	1.483	2.347	0.0617

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)					0.96		1.035		-1.2287	2.90065
Bartlett's Test indicates equal variances (p = 0.03)					14.2989		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	10.4	>10.4			0.06171	0.09734	0.00309	0.00346	0.5046	6, 63
Treatments vs B-Control										

2. Using an α of 0.05 for the equality of variance.

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
B-Control	0.6700	0.6600	0.5900	0.5800	0.6100	0.6600	0.6600	0.5800	0.6600	0.6700
0.353	0.5600	0.5700	0.6200	0.6100	0.5800	0.5800	0.6000	0.6900	0.6400	0.6300
0.701	0.6700	0.5800	0.5400	0.6200	0.6500	0.5200	0.5800	0.6800	0.5400	0.5500
1.37	0.6300	0.6600	0.6500	0.5100	0.5500	0.4300	0.6100	0.6500	0.6200	0.6400
2.66	0.6200	0.5600	0.6500	0.6600	0.6400	0.6400	0.6000	0.6400	0.6300	0.6200
5.27	0.5800	0.6000	0.6200	0.6600	0.6300	0.6200	0.6500	0.5800	0.3700	0.5900
10.4	0.4900	0.6300	0.6300	0.5100	0.6400	0.5300	0.7000	0.6400	0.6100	0.5700

			Transform: Untransformed					Rank Sum	1-Tailed Critical
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N		
B-Control	0.6340	1.0000	0.6340	0.5800	0.6700	6.140	10		
0.353	0.6080	0.9590	0.6080	0.5600	0.6900	6.431	10	85.50	74.00
0.701	0.5930	0.9353	0.5930	0.5200	0.6800	9.835	10	84.00	74.00
1.37	0.5950	0.9385	0.5950	0.4300	0.6600	12.633	10	84.50	74.00
2.66	0.6260	0.9874	0.6260	0.5600	0.6600	4.593	10	92.00	74.00
5.27	0.5900	0.9306	0.5900	0.3700	0.6600	13.908	10	84.50	74.00
10.4	0.5950	0.9385	0.5950	0.4900	0.7000	11.323	10	84.50	74.00

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)					0.96	1.035	-1.2287	2.90065
Bartlett's Test indicates unequal variances (p = 0.03)					14.2989	12.5916		
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**				
Steel's Many-One Rank Test	10.4	>10.4						
Treatments vs B-Control								