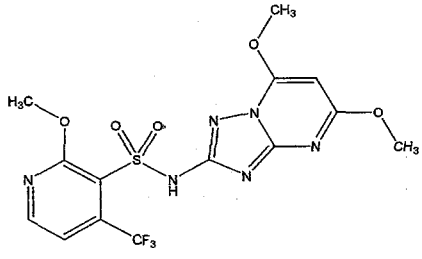
Data Evaluation Report on the acute toxicity of pyroxsulam (XDE-742) to fresh water invertebrates - *Daphnia* sp.

PMRA Submission Number 2006-4727; ID 1283197 EPA MRID Number 469084-28 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.3.2
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.3.1.1
	EPA Guideline:	FIFRA 72-2 (OPPTS 850.1010)

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-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 and XR-742	

Chemical Structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/02/07 **Date:** March 14, 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

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

Thomas Steeger, Ph.D., Senior Biologist 4/3/08 **Date:** April 3, 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Catherine Evans for Catherine Evans 05/03/08 **Date:** June 29, 2007
Environmental Assessment Directorate, PMRA

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. 2004. XDE-742: An Acute Toxicity Study with the Daphnid, *Daphnia magna*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674, Study ID 041022, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 22 December 2004. Unpublished report.

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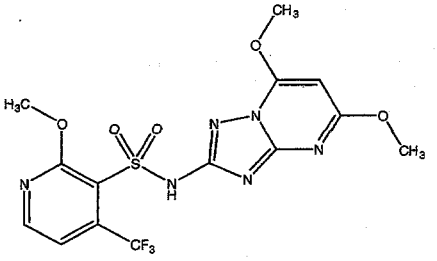

Primary Reviewer: Daryl Murphy **Date:** March 14, 2007
Australian Government Department of the Environment and Water Resources (DEW)

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EXECUTIVE SUMMARY:

The 48 hr acute static toxicity of pyroxsulam (XDE-742; 98%) to *Daphnia magna* was studied under static conditions, in accordance with the following guidelines: OECD Guideline 202, Daphnia sp., Acute Immobilization Test, Part 1; Official Journal of the European Communities. Annex to Commission Directive 92/69 EEC, C.2 Acute Toxicity for *Daphnia*; and US EPA *Pesticide Assessment Guidelines,* Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms, Guideline 72-2. Daphnids were exposed to control and the test chemical at mean-measured concentrations of <1.26 (LOQ, control), 12.2, 20.6, 34.9, 58.8 and 100 mg pyroxsulam/L for 48 hours. Mortality/immobilization and sub-lethal effects were recorded at 24 and 48 hours. The 48 hour EC_{50} was >100 mg pyroxsulam/L. The 48 hour NOEC based on immobilization and absence of sub-lethal adverse effects was 100 mg pyroxsulam/L. Only one daphnid at the 20.6 mg/L concentration showed immobility and no other sub-lethal effects were reported. The one dead daphnid appears to be an artifact and is not dose-related on an acute exposure basis.

Based on the results of this study, pyroxsulam would be classified as practically non-toxic to *Daphnia magna* in accordance with the classification systems of the Australian Government Department of the Environment and Water Resources (EC50 > 100 mg/L) and of the US EPA (EC50 >100 mg/L).

This study is classified as acceptable and is consistent with the guideline requirements for a 48 hour acute toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age:	<24-hour old instars
Test Type:	Static, 48 hours
48 h EC_{50}:	>100 mg pyroxsulam/L (mean-measured)
95% C.I.:	Not applicable
48 h NOEC:	100 mg pyroxsulam/L (mean-measured)
Probit Slope:	Not applicable
Endpoint(s) Effected:	None. No pyroxsulam related immobility and other sub-lethal adverse effects were noted during the exposure period of this study.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study was stated to generally conform to current procedures described by:

Organisation for Economic Cooperation and Development (1984). *OECD Guideline for Testing of Chemicals No. 202, Daphnia* sp., Acute Immobilization Test, Part 1. Adopted 4 April 1984,

Official Journal of the European Communities (1992). Annex to Commission Directive 92/69/EEC, C.2. Acute Toxicity Test for *Daphnia*. Vol. 35, 29 December 1992,

U.S. Environmental Protection Agency (1985). Hazard Evaluation Division: Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005. Washington D.C., and

U.S. Environmental Protection Agency (1982). *Pesticide Assessment Guidelines,* Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms, Guidelines 72-2.
EPA 540/9-82-020, Washington, D.C.

Guidelines appear to have been generally followed with some minor deviations (e.g. hardness and pH) reported on occasion (see relevant text entries below and also the Deviations from guidelines or other deficiencies identified table on page 16 of this DER).

COMPLIANCE:

All facets of testing were reported as conducted following:

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

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

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

The signed and dated GLP Compliance Statement for the study was provided.

The signed and dated Statement of the Quality Assurance Unit for the study was provided.

The signed and dated Statement of No Data Confidentiality for the study was provided.

A. MATERIALS:

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

Description: Solid

Lot No./Batch No.: E9052-52-01

Purity: 98% active ingredient

Stability of Compound

Under Test Conditions:

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

Additional details on the stability of pyroxsulam under the test conditions were not identified in the study report.

Recovery of pyroxsulam at 24 hours in the test solutions ranged from 94.6 to 101% of nominal while at 48 hours, the recoveries ranged from 93.8 to 100% of nominal (page 10 of this DER refers). These results indicate stability of the pyroxsulam under the test conditions.

Stable (the company Study Profile Template, Marino, 2005).

Storage conditions of test chemicals:

Information not identified in study report. The company Study Profile Template refers to "Room temperature in the dark" (Marino, 2005).

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 Pa	Madsen (2003)
UV absorption	NA	
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)

NA = not available at the time of publication of the study.

Note: The physicochemical properties of pyroxsulam were not reported in the study. The values recorded here come from the company's study profile template (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:	The freshwater daphnid, *Daphnia magna*
Age at test initiation:	<24-hour old instars
Source:	In-house cultures initially obtained from New England Bioassay, Inc., Manchester, Connecticut.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study:
A non-GLP acute screen test was reported conducted between 09 May and 11 May 2001 as a limit test with *Daphnia magna* exposed to pyroxsulam, over a 48-hour static exposure period. The results from this test indicated that the 48-hour EC50 value was greater than the nominal limit concentration tested of 100 mg/L. The information from this test was stated to have been used to set the range of concentrations for the definitive test.

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 acute 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

		Remarks
Parameter	**Details**	***Criteria***
Acclimation:		See Table 4 (deficiencies/deviations) on page 16 of this DER.
Period:	Not specifically identified in the study report, the Study Profile Template (Marino, 2005) states the period was >14 days.	*EPA requires 7 day minimum acclimation period*

Parameter	Details	Remarks *Criteria*
Conditions: (same as test or not)	Same as test	Requirement considered met. The information of the acclimation period, test conditions and daphnid health was provided in the company Study Profile Template report (Marino, 1995). The study report's reference to this period was not identified. Comparison of light intensity and temperature reported for the holding water (adjusted lab dilution water or ALDW) and the test solutions and of the reported water quality measurements of the control water (ALDW) and the 100 mg pyroxsulam/L bulk solution (Table 5 of the study report) would indicate conditions during acclimation were similar to those in the test vessels during the exposure period.
Feeding:	*Pseudokirchneriella subcapitata*, a freshwater green alga and YCT (yeast, Cerophyll, and trout chow suspension) five times weekly during rearing. Daphnia were not fed during the exposure period.	Requirement considered met. *EPA requires no feeding during study.*
Health: (any mortality observed)	No specific information identified. Results from the study indicate the control animals were healthy and in good condition	Requirement considered met.
Duration of the test	48 hours	Requirement met. *(EPA requires 96 hours, except daphnids which are 48 hours)*
Test conditions: Static/flow through	Static	Requirement considered met.
Type of dilution system- for flow through method	Not applicable	*(EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Flow rate	Not applicable	
Renewal rate for static renewal	Not applicable	
Aeration, if any	Not indicated as used in the exposure period. Daphnid water reported as being aerated for 24	Requirement considered met. OECD 202 states that the dilution water

Parameter	Details	Remarks *Criteria*
	hours before use.	may be aerated prior to the test but not during the test. US EPA OPPTS 850.1010 specifies that aeration not take place.
Test vessel: Material: *(glass/stainless steel)* Size: Fill volume:	 Borosilicate beakers . 250-mL Approximately 200 mL of control or test solution. Beakers covered to reduce evaporation	Requirement considered met. *(EPA requires: size 20 mL or 3.9 L fill 200 mL)* OECD 202 refers to at least 2 ml of test solution should be provided for each animal (i.e. a volume of 10 ml for five daphnids per test vessel). US EPA 850.1010 refers to use of 250 mL beakers.
Source of dilution water	Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plan	Requirement considered met. Before use in the laboratory, the water was sand-filtered, pH adjusted with gaseous CO_2, carbon-filtered, and UV-irradiated. Daphnid water (referred to as adjusted lab dilution water or ALDW) was prepared by adjusting laboratory water to a hardness of about 170 mg/L as $CaCO_3$ before autoclaving. After adjusting hardness, the water was autoclaved at 250°F (121°C) and 18 psi for 30 minutes, cooled, and aerated for approximately 24 hours before use.
		(EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water) OECD 202 and US EPA OPPTS 850.1010 indicate that dilution water is acceptable if daphnids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress.
Water parameters:		See Table 4 (deficiencies/deviations) on page 16 of this DER with respect to hardness and OC, OP and PCB

Parameter	Details	Remarks *Criteria*
		concentrations.
Hardness	123 mg $CaCO_3$/L in control water and 138 mg $CaCO_3$/L in bulk test solution.	The hardness values are greater than the template EPA values listed below and just fall below the OECD 202 requirements (also given below). *For hardness, the EPA requires 40 - 48 mg/L as $CaCO_3$.* OECD 202 refers to a recommended total hardness of 140-250 mg/L for *D. magna.*. US EPA OPPTS 850.1010 refers to water quality parameters of a maximum hardness of 180 mg/L, which was met in this study.
pH	7.2-7.7 in day 0 bulk solutions and 7.5-8.0 in day 2 spent solutions. [In the day 0 control bulk solution, 7.7. In the two control replicates at day 2, 7.9 and 8.0].	The pH range exceeds the upper US EPA template range limit specified below but was within the OECD range of 6 to 9. US EPA OPPTS 850.1010 does not state a range but requires the pH to be measured at the start and end of the test *For pH, the EPA requires 7.2 - 7.6*
Dissolved oxygen	Dissolved oxygen range (mg/L) 8.8-8.9 in day 0 bulk solutions and 8.3-8.5 in the day 2 spent test solutions. [In the day 0 control bulk solution, 8.8 mg/L. In the two control replicates at day 2, 8.5 and 8.5] Percent oxygen saturation stated to have remained ≥ 93% throughout the exposure, based on a theoretical value of 8.9 mg/L.	*Dissolved oxygen:* *EPA requires Static: ≥ 60% during 1st 48 hr and ≥40% during 2nd 48 hr* *Flow-through: ≥ 60%)* US EPA 850.1010 requires dissolved oxygen content to between 60 and 105 percent saturation. OECD 202 states that the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.
Temperature	Temperature range (°C): In bulk solutions (Day 0) and individual test vessels (Day 2): 20-21°C. [Continuous monitoring: 19-20°C]	*Temperature:* *EPA requires 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > ±1°C;* *OECD requires range of 18-22°C (and*

Parameter	Details	Remarks *Criteria*
		for each single test, the temperature should be constant within ±1°C)
Total organic carbon	1300 µg/L (1.3 mg/L)	Total organic carbon etc values from the reported inorganic analyses of laboratory water results were given in Table 1 of the study report. OECD 202 and US EPAOPPTS 850.1010 refer to dilution/testing water having an acceptable TOC of <2 mg/L.
Particulate matter	No specific value reported, total suspended solids, below limit of detection (1000 µg/L)	OECD 202 and US EPA OPTTS 850.1010 refers to dilution /testing water having, *inter alia*, a maximum particulate matter concentration of 20.0 mg/L.
Metals	Metal concentrations in the laboratory water were reported	Metals: OECD 202 says measurements of heavy metals should be made. The quantifiable metals (and pesticide) residues in the laboratory water were indicated as not causing adverse effects in the controls. The water's ongoing use in the testing facility also points to its suitability of the testing undertaken.
Pesticides	Specific pesticide levels were reported with all results below the relevant detection limits (which ranged from 0.25 to 5 µg/L).	Pesticides: OECD 202 and US EPA OPPTS 850.1010 refer to the maximum total organophosphorus pesticide level and the total organochlorine pesticides plus polychlorinated biphenyls each being <50 ng/L (OECD) or 50 ng/L (US EPA).
Chlorine	<10 µg/L (as residual chlorine, detection level = 10 µg/L)	OECD 202 refers to a total residual chlorine value of <10 µg/L while US EPA OPPTS 850.1010 refers to residual chlorine being <3 µg/L.
Intervals of water quality measurement	Dissolved oxygen, pH, and temperature data were recorded from each bulk dose solution at test initiation (day 0) and from all test vessels (spent test solutions) at test termination (day 2). Water temperature was continuously monitored from one surrogate vessel throughout the study; Water quality parameters such as alkalinity, hardness, residual chlorine, and conductivity were measured from the day 0 bulk solutions of the control water and the highest test level.	Requirement considered met.

Parameter	Details	Remarks *Criteria*
	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.	
Number of replicates: Control (dilution water): Solvent control: Treatments:	 2 Solvent not used. 2	Requirement considered met. OECD 202 refers to use of 20 daphnid, preferably divided into four groups of 5 daphnids for each test concentration and the controls but the Guideline does not appear to specify the number of replicates. US EPA OPPTS 850.1010 refers to 2 or more replicates.
Number of organisms per replicate: Control (dilution water): Solvent control: Treatments:	 10 Not applicable 10 (Control and 5 treatment levels were tested). For the biomass loading, there were 10 daphnid/200 mL of solution or 50 daphnids/L.	See Table 4 (deficiencies/deviations) on page 16 of this DER with respect to biomass. *(EPA/OECD require 5 treatment levels plus control* *EPA requires a minimum of 20 daphnid per treatment. Biomass loading rate for static 0.8 g/L at 17°C, 0.5 g/L at > 17°C; flow-through: 1 g/L/day).* OECD 202 and US EPA OPPTS 850.1010 refer to a minimum of 20 daphnid per concentration. US EPA OPPTS 850.1010 advises, that with respect to loading, that there should not exceed 40 daphnids per litre of test solution in the static system.
Treatment concentrations: Nominal:	 The concentration of pyroxsulam in the test solutions was confirmed by analyzing a sample from each bulk dose solution on day 0 and each replicate test solution on day 2 with analysis by HPLC/UV. **Nominal test concentrations, as mg pyroxsulam/L, at test start (day 0).**	Requirement considered met. *(EPA requires a geometric series with each concentration being at least 60% of the next higher one)* OECD 202 refers to a geometric series with a separation factor preferably not exceeding 2.2. US EPA OPPTS 850.1010 refers to a geometric series in which the ratio is between 1.5 and 2.0

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	Not applicable, solvent not used.	Requirement met. *(EPA requires solvents not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests)*
Lighting	Rearing conditions used illumination (cool-white fluorescent) of 2050 ± 350 lux and a 16-hour light/8-hour dark photoperiod. In the test solutions, the light intensity ranged from 1770 to 1988 lux. A 16 hour light/8 hour dark photoperiod in the exposure period was considered implied in the company study profile template (Marino, 2005).	Requirements considered met. *(EPA requires 16 hours light, 8 hours dark; OECD : optional light-dark cycle or complete darkness)* OECD 202 and US EPA OPPTS 850.1010 recommend a 16 hours light and 8 hour dark cycle.
Recovery of chemical: Frequency of determination Level of Quantitation Level of Detection	Day 0 and Day 2 1.26 mg pyroxsulam/L of ALDW (Adjusted laboratory dilution water) and based on lowest standard analysed being multiplied by the dilution factor. Not reported.	Requirement considered met. Methodology used appears satisfactory. Chromatograms presented confirmed the absence of detectable pyroxsulam in a control ALDW sample and an identifiable peak corresponding to pyroxsulam (retention time 3.618 minutes) in a standard containing 1.01 mg pyroxsulam/L ALDW and in a bulk dose solution containing 13.0 mg pyroxsulam/L ALDW (retention time 3.640 minutes). A typical response curve from the HPLC/UV analysis of peak area against concentration was linear over the range of 0 to 100 mg pyroxsulam/L.
Positive control {if used, indicate the chemical and concentrations}	Positive control not used.	Requirement considered met.
Other parameters, if any	None identified.	

2. Observations:

Table 2. Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects	Daphnia were observed for immobility (inability to swim within 15 seconds after gentle agitation of the test container) at 24 and 48 hours of exposure. Any abnormal behavior or appearance was reported.	Requirement considered met. OECD 202 and US EPA OPPTS 850.1010 refer to immobilisation as the effect measured but also state that any adverse effects, including abnormal behaviour or appearance, should be reported.
Observation intervals	At 24 and 48 hours of exposure.	Requirement met. OECD 202 and US EPA OPPTS 850.1010 refer to checking for immobilized daphnids at 24 and 48 hours after the beginning of the test. OECD 202 also refers to checking for any abnormal behaviour or appearances at those times.
Water quality was acceptable (Yes/No)	Yes	Requirement considered met. Water quality considered acceptable on the basis of the 100% survival of daphnids in the control solutions. OECD 202 and US EPA OPPTS 850.1010 refer to dilution water being acceptable as dilution water if daphnids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress.
Were raw data included?	No, tabulated results were presented. The data, protocol, protocol changes/revisions, and final report are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.	OECD 202 makes no comment on supply of raw data and allows for presentation in a summarised, tablular form. The absence of raw data is not considered a deficiency even though US EPA OPPTS 850.1010 states the sponsor must submit to the EPA all data developed by the test that are suggestive or predictive of acute toxicity and all concomitant gross toxicological manifestations. This decision on the absence of a deficiency is on the basis of advice from the US EPA that tabulated results are considered sufficient as they allow recalculation of dose response if necessary.
Other observations, if any	No evidence of incomplete dissolution of test material in the	

	test solutions was observed.	

II. RESULTS AND DISCUSSION

A. MORTALITY/IMMOBILITY:

A summary of the immobility observed in the study is provided in Table 3. Note that in this study, the effect criterion was set as immobility (rather than mortality *per se*) with this defined as the inability to swim within 15 seconds after gentle agitation of the test container.

The 48 hour NOEC was determined based on the highest mean-measured concentration tested exhibiting no *Daphnia* immobility or change in behaviour or appearance. The immobile *Daphnia* observed in the nominal 21.6 mg pyroxsulam/L test level (20.6 mg/L, mean-measured) was believed, by the study authors, to be incidental, since no *Daphnia* immobility was observed in any of the remaining treatment levels and no additional immobility was observed in the 21.6 mg pyroxsulam/L test level during the study conduct. The study report reviewer concurs with this explanation.

Table 3. Effect of pyroxsulam (XDE-742) on mortality (as immobility) of *Daphnia magna.*

Treatment (mg pyroxsulam/L) [record measured and nominal conc. used]	Number of organisms (10 daphnids X 2 replicates)	Observation period			
		Day 1 (24 hours)		Day 2 (48 hours)	
		No. Immobile	% immobility	No. Immobile	% immobility
Control (dilution water only), if used	20	0	0%	0	0%
13.0 mg/L nominal or 12.2 mg/L, mean-measured	20	0	0%	0	0%
21.6 mg/L nominal or 20.6 mg/L, mean-measured	20	1	5%	1	5%
36.0 mg/L nominal or 34.9 mg/L, mean-measured	20	0	0%	0	0%
60.0 mg/L nominal or 58.8 mg/L, mean-measured	20	0	0%	0	0%
100.0 mg/L nominal or 100 mg/L, mean-measured	20	0	0%	0	0%
48-hour NOEC (mobility/immobility)	Not applicable	100 mg pyroxsulam/L		100 mg pyroxsulam/L	
48-hour LC50/EC50	Not applicable	>100 mg pyroxsulam/L Taking the 1 immobile daphnid as being dead,		>100 mg pyroxsulam/L Taking the 1 immobile daphnid as being dead,	
Positive control, if used	Not applicable	Not applicable, no positive control used.		Not applicable, no positive control used.	

B. OTHER SUB-LETHAL TOXICITY ENDPOINTS:

No other sub-lethal effects were observed.

C. **REPORTED STATISTICS**: Parameters analysed were: immobility and adverse effects in the daphnids in the test and control solutions and pH, oxygen content, temperature and physical appearance of the control and test solutions. No statistical tests were performed.

The study report stated that "Insufficient adverse effects to the Daphnia exposed to pyroxsulam during this study were observed (only one immobile Daphnia observed, which was in the 21.6 mg/L dose level, and no changes in behavior or appearance were observed at any dose level) to statistically evaluate the data. Therefore, no statistical analysis programs were used for the data analysis and the statistical determination of the EC50 and NOEC values were not attempted. All endpoint values were empirically determined and were based on mean-measured concentrations."

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: The reviewer agrees with the study report's reasons for not using statistical analysis in view of only one daphnid being immobile and the lack of sub-lethal/adverse effects. NOEC and EC50 values can be estimated from visual inspection of the results presented for these parameters.

Statistical Method: Not conducted as a result of the study's results. Consequently, the 48 hours EC50 and the NOEC were not calculated by the reviewer using statistical methodology.

E. STUDY DEFICIENCIES:

The deviations/deficiencies from OECD 202 and US EPA OPPTS 850.1010 Guidelines shown in Table 4 were noted but not considered to have significantly affected the study's outcome.

Table 4. Summary of deviations from guidelines or other deficiencies identified.

Parameter	Study report result	US EPA OPPTS 850.1010, April 1996	OECD Guideline 202, 13 April 2004
Acclimation: Period	Not specifically stated.	Refers to "At the initiation of the test, daphnids which have been cultured and acclimated in accordance with the test design …" and 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." Additionally, the data records of the culture, acclimation, and test temperatures must be submitted by the sponsor to the EPA. Also, "During culturing and acclimation, daphnids should be observed carefully for ephippia and other signs of stress, physical damage, and mortality." Advice from the US EPA was that with in-house cultures, the test organisms are typically assumed to have been adequately acclimatised.	The stock animals must be maintained in culture conditions (light, temperature, medium) similar to those to be used in the test.
Water parameters: Hardness	123 mg $CaCO_3$/L in control water and 138 mg $CaCO_3$/L in bulk test solution.	US EPA OPPTS 850.1010 states not greater than180 mg/L	OECD 202 states a value of between140 and 250 mg/l (as $CaCO_3$) is recommended for *D. magna*.
Organophosphorus and organochlorine pesticides and polychlorinated biphenyls	All below their respective individual detection limits (0.25 to 5 µg/L)	US EPA OPPTS 850.1010 states that the total organophosphorus pesticides be <50 ng/L. Total organochlorine pesticides and polychlorinated biphenyls are to be <50 ng/L.	OECD 202 states that the total organophosphorus pesticides content be <50 ng/L. Total organochlorine pesticides and polychlorinated biphenyls are to be <50 ng/L.

Parameter	Study report result	US EPA OPPTS 850.1010, April 1996	OECD Guideline 202, 13 April 2004
Number of organisms per replicate: Loading (biomass)	There were 10 daphnid/200 mL of solution or 50 daphnids/L.	US EPA OPPTS 850.1010 advises, that with respect to loading, that there should not exceed 40 daphnids per litre of test solution in the static system.	No reference found in OECD 202 to a value for the loading (biomass).

F. REVIEWERS' COMMENTS:

The study was satisfactorily conducted using controls and pyroxsulam at mean-measured concentrations of 12.2, 20.6, 34.9, 58.8 and 100 mg/L (corrected for purity of the pyroxsulam). Pyroxsulam, as the active constituent, is considered practically non-toxic to the daphnid, *Daphnia magna* with a 48 hour EC50 of >100 mg pyroxsulam/L.

The in-life portion of the definitive toxicity test was conducted from 16 March 2004 to 18 March 2004.

With respect to validity criteria of the Guidelines used, OECD 202 states that the test is valid if, in the control, not more that 10 per cent of the daphnids have been immobilised (or show other signs of disease or stress, for example, discoloration or unusual behaviour such as trapping at the surface of water) and the dissolved oxygen concentration at the end of the test should be ≥3 mg/l in control and test vessels.

US EPA 850.1010 states that the test is unacceptable if more than 10 percent of the control organisms are immobilized during the 48 h test period and a dissolved oxygen content between 60 and 105 percent saturation.

The study's results show these parameters (and also the acclimation requirements) were met and the study is considered acceptable (No immobility was seen in the controls over 48 hours, the dissolved oxygen content was 8.8-8.9 mg/L in day 0 bulk solutions and 8.3-8.5 mg/L in the day 2 spent test solutions and the daphnids were not reported as showing signs of disease or stress).

Consequently, the validity criteria for OECD 202 and US EPA OPPTS 850.1010 were considered to have been met by the study.

The PMRA reviewer agrees with the conclusions of the reviewer from the Australian Government Department of the Environment and Water Resources. This study is acceptable to the PMRA.

G. CONCLUSIONS:

This study is acceptable.

The 48 hour acute static toxicity study resulted in an EC50 of pyroxsulam as the technical grade material in the daphnid, *D. magna*, of >100 mg pyroxsulam/L (as nominal and mean analytically determined concentrations over 48 hours).

The 48 hour NOECs for immobilisation and sub-lethal effects were both 100 mg pyroxsulam/L (for both nominal concentrations and mean analytically determined concentration over 48 hours).

Based on the results of this study, pyroxsulam would be classified as practically non-toxic to *Daphnia magna* in accordance with the classification system of the Australian Government Department of the Environment and Water Resources (EC50 > 100 mg/L), as well as that of the US EPA.

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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Approved 04/01/01 C. K.