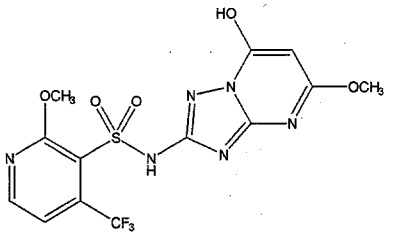
Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (XDE-742) to fresh water invertebrates - *Daphnia* sp.
PMRA Submission Number 2006-4727 ID 128-3191 EPA MRID Number 469084-26 APVMA ATS 40362

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:	**7-OH Metabolite of pyroxsulam (7-OH metabolite of XDE-742)**
Purity (%):	99% (ID No. TSN 105384, used to prepare test solutions and calibration standards), and 94% (ID No. TSN 105232, used to prepare quality control samples).
Common name/s:	7-OH metabolite of XDE-742, X11250641
Chemical name:	3-pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
IUPAC:	(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:	N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.:	Not available
Synonyms:	7-desmethyl XDE-742 metabolite

Chemical structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/02/08 **Date:** 14 March 2007
Australian Government Department of the Environment and Water Resources (DEW)

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

Thomas Steeger, Ph.D., Senior Biologist 4/13/08 **Date:** 9 April 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

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

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

CITATION: Sayers, L. E. (2006). 7-OH Metabolite of XDE-742 – Acute Toxicity to Water Fleas, *Daphnia magna*, Under Static Conditions. Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6410 and Sponsor Protocol/Project No. 050164. The Dow Chemical Company Midland, Michigan 48674 for Dow AgroSciences Indianapolis, Indiana 46268. 14 March 2006. Unpublished report.

Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (XDE-742) to fresh water invertebrates - *Daphnia* sp.
PMRA Submission Number 2006-4727 ID 128-3191 EPA MRID Number 469084-26 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: **7-OH Metabolite of pyroxsulam (7-OH metabolite of XDE-742)**
Purity (%): 99% (ID No. TSN 105384, used to prepare test solutions and calibration standards), and
94% (ID No. TSN 105232, used to prepare quality control samples).

Common name/s: 7-OH metabolite of XDE-742, XII250641

Chemical name: 3-pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-

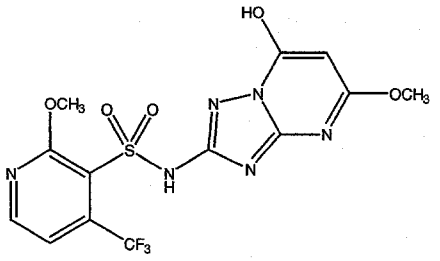
IUPAC: (7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

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

CAS No.: Not available

Synonyms: 7-desmethyl XDE-742 metabolite

Chemical structure:



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

Secondary Reviewer(s): Jack Holland **Date:** 14 March 2007
Australian Government Department of the Environment and Water Resources

Thomas Steeger, Ph.D., Senior Biologist **Date:** 9 April 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Catherine Evans Date: June 29, 2007
Environmental Assessment Directorate, PMRA

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

CITATION: Sayers, L. E. (2006). 7-OH Metabolite of XDE-742 – Acute Toxicity to Water Fleas, *Daphnia magna*, Under Static Conditions. Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6410 and Sponsor Protocol/Project No. 050164. The Dow

Chemical Company Midland, Michigan 48674 for Dow AgroSciences Indianapolis, Indiana 46268. 14 March 2006. Unpublished report.

EXECUTIVE SUMMARY:

The 48 hour acute static toxicity of the 7-hydroxy metabolite of pyroxsulam to neonate *Daphnia magna* was studied under static conditions. Daphnids were exposed to control and test chemical at nominal concentration of 0, 6.3, 13, 25, 50 and 100 mg 7-hydroxy metabolite of pyroxsulam/L for 48 hours. Mean-measured concentrations were <0.26 (LOQ, control), 6.1, 14, 28, 50 and 99 mg 7-hydroxy metabolite of pyroxsulam/L. Mortality/immobilization and sub-lethal effects were measured at 24 and 48 hours (test termination). No mortality/immobilization or other sub-lethal effects were observed throughout the study. The 48 hour EC_{50} for immobilisation was >99 mg 7-hydroxy metabolite of pyroxsulam/L (mean, measured concentration). The 48 hour NOECs based on immobilisation and sub-lethal adverse effects were 99 mg 7-hydroxy metabolite of pyroxsulam/L (mean, measured concentration).

Based on the results of this study, the 7-hydroxy metabolite of pyroxsulam would be classified as, at worst, slightly toxic to the daphnid, *D. magna*, ($10<EC_{50}\leq100$ mg/L) in accordance with the classification systems of the Australian Government Department of the Environment and Water Resources, and of the US EPA

The EC_{50} is based on mean-measured concentrations of 7-OH metabolite of pyroxsulam adjusted for the purity of the 7-OH metabolite of pyroxsulam (99%).

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

Results Synopsis

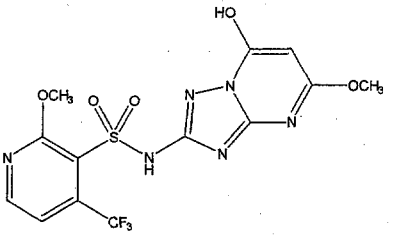
Test Organism, Age:	*Daphnia magna* neonates, <24 hours old
Test Type:	Static, 48 hours
48 hour EC_{50}:	>99 mg 7-OH metabolite of pyroxsulam/L (mean, measured concentration)
95% C.I.:	Not applicable
48 hour NOEC:	99 mg 7-OH metabolite of pyroxsulam/L (mean, measured concentration)
Probit Slope:	Not applicable
Endpoint(s) Effected:	None. 7-OH metabolite of pyroxsulam related immobility and other adverse effects on the exposed daphnids were not observed during the 48 hour exposure period of this study.

Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (XDE-742) to fresh water invertebrates - *Daphnia* sp.
PMRA Submission Number 2006-4727 ID 128-3191 EPA MRID Number 469084-26 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:	**7-OH Metabolite of pyroxsulam (7-OH metabolite of XDE-742)**
Purity (%):	99% (ID No. TSN 105384, used to prepare test solutions and calibration standards), and 94% (ID No. TSN 105232, used to prepare quality control samples).
Common name/s:	7-OH metabolite of XDE-742, X11250641
Chemical name:	3-pyridinesulfonamide, N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
IUPAC:	(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:	N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.:	Not available
Synonyms:	7-desmethyl XDE-742 metabolite

Chemical structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/02/08 **Date:** 14 March 2007
Australian Government Department of the Environment and Water Resources (DEW)

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

Thomas Steeger, Ph.D., Senior Biologist T. Steeger 4/3/08 **Date:** 9 April 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

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

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

CITATION: Sayers, L. E. (2006). 7-OH Metabolite of XDE-742 – Acute Toxicity to Water Fleas, *Daphnia magna*, Under Static Conditions. Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6410 and Sponsor Protocol/Project No. 050164. The Dow Chemical Company Midland, Michigan 48674 for Dow AgroSciences Indianapolis, Indiana 46268. 14 March 2006. Unpublished report.

Data Evaluation Report on the acute toxicity of the 7-OH metabolite of pyroxsulam (XDE-742) to fresh water invertebrates - *Daphnia* sp.
PMRA Submission Number 2006-4727 ID 128-3191 EPA MRID Number 469084-26 APVMA ATS 40362

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study report stated that the procedures used in the acute toxicity study followed those described in the Springborn Smithers protocol Springborn Smithers Laboratories Protocol No.: 042704/OECD/EC/FIFRA/Daphnid-STA/Dow (Appendix 1 of the study report).

The methods described in this protocol were stated to meet the testing requirements of:

- the Organization for Economic Co-operation and Development, OECD Guideline For Testing of Chemicals #202, Daphnia sp. Acute Immobilization Test (OECD, 2004),
- EC Guideline L383A, Method C.2, Acute Toxicity for Daphnia (EC, 1992) and
- the U.S. Environmental Protection Agency's Pesticide Assessment Guidelines (Subdivision E; Series 72-2; U.S. EPA, 1982).

Guidelines appear to have been generally followed with the exception of certain parameters (e.g. hardness, pH, supply of raw data). For further details see the relevant text entries below and Table 4. Summary of deficiencies/deviations from guidelines, page 17 of this DER.

COMPLIANCE:

The study report stated that the data and report presented were produced and compiled in accordance with all pertinent OECD and US EPA Good Laboratory Practice Regulations, namely -

- OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp., and
- U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

except for routine food and water screening analyses. These were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard US EPA procedures and are considered facility records under the relative Springborn Smithers Standard Operating Procedure (7.92). Because the analyses were conducted following standard validated methods, these exceptions were considered by the study report author to have had no impact on the study results.

The signed and dated Good Laboratory Practice Compliance 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 — 7-OH metabolite of XDE-742 (i.e. 7-OH metabolite of pyroxsulam)

Description: Solid

Lot No./Batch No.: 35172-56 and E2008-46

ID No.: TSN 105384 and TSN 105232

Purity: Respectively 99 and 96%. The 99% material was used for preparation of the test and calibration standards (with the test concentrations adjusted for the 99% purity).

Storage conditions of test chemicals: Stored at room temperature in the original containers in a dark ventilated cabinet. The Certificates of Analysis for both lots of material refer to ambient storage in a locker.

Stability of Compound Under Test Conditions: Based on the measured mean concentrations at 0 and 48 hours being 96-110% of the nominal concentrations (page 11 of this DER), the 7-OH metabolite of pyroxsulam is considered to have shown stability under the test conditions.

Physicochemical properties of 7-OH metabolite of pyroxsulam (XDE-742).

Parameter	Values	Comments
Water solubility at 20°C	Not available	Physicochemical properties were stated by the company Study Profile Template (Sayers, 2006b) not to have been available at the time of publication of the Study Profile Template.
Vapour pressure		
UV absorption		
pKa		
Kow		

2. Test organism:

Species:	*Daphnia magna*
Age at test initiation:	<24 hours old
Source:	From laboratory cultures maintained at Springborn Smithers Laboratories.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study:
The study report stated that, "Prior to initiating the definitive study, a preliminary range-finding test was conducted at Springborn Smithers exposing daphnids under static conditions to nominal concentrations of 0.010, 0.10, 1.0, 10 and 100 mg a.i./L, and a dilution water control. Two replicate test vessels, containing five daphnids per replicate, were established for each treatment level and the control. All test solutions were clear and colorless with no visible undissolved test substance. Following 48 hours of exposure, no immobilization or adverse effects were observed among daphnids exposed to the treatment levels tested or the control. Based on these results and in consultation with the Study Sponsor, nominal concentrations of 6.3, 13, 25, 50 and 100 mg a.i./L were selected for the definitive exposure."

b) Definitive Study
In the following table's Criteria column, 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

Parameter	Details	Remarks *Criteria*
Acclimation: Period:	In-house culture. Culture conditions not specifically identified. Information indicates the daphnids were obtained from the laboratory cultures maintained at Springborn Smithers Laboratories but gave no detail on selection of the <24 hour old daphnids used for the test. The Study Profile Template (Sayers, 2006) provided no details on the acclimatisation process.	See deficiencies/deviations table on page 17 of this DER. *(EPA requires 7 day minimum acclimation period)*

Parameter	Details	Remarks *Criteria*
Conditions: (same as test or not)	The study protocol stated that the water used to culture the daphnids would be prepared in the same manner and have the same characteristics as described for dilution water. Conditions considered equivalent. Dilution water used for the study was from the same source as the water used to culture the daphnids The culture area had 16 hours light/8 hours darkness, 94-115 footcandles (1000-1200 lux) illumination in culture water (fortified well water) of total hardness and alkalinity as calcium carbonate of 160-180 mg/L and 110 mg/L respectively, a pH of 7.8-8.3, a temperature of 19-22°C, a dissolved oxygen content of 7.5-9.7 mg/L and a specific conductivity of 500 µmhos/cm.	Requirement considered met. Test conditions reported as: Light intensity 890-1100 lux with a 16 hour light/8 hour dark cycle. pH 7.2-8.0. Temperatures 19–21°C. Dissolved oxygen 8.3 mg/L-8.9 mg/L. (These values are similar to those reported for the daphnid culture medium).
Feeding:	During culture daphnids were fed various amounts based on age - 0 to 6 days old daphnids were fed 0.5 mL of algae and 0.5 mL of YCT suspension per vessel per day. Daphnids that were 7 to 10 days old were fed 1.0 mL of algae and 0.5 mL of YCT (yeast, Cerophyll, and trout chow) suspension per vessel per day. Daphnids that were >10 days old were fed 1.5 mL of algae and 0.5 mL of YCT suspension per vessel per day. Daphnids were not fed during the exposure period.	Requirement met. *EPA requires no feeding during study*
Health: (any mortality observed)	No information identified. Absence of deaths in the test's control daphnids indicates the parent population had had acceptable health.	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 met. *(EPA requires consistent flow rate of 5 -*

Parameter	Details	Remarks *Criteria*
Type of dilution system- for flow through method. Flow rate Renewal rate for static renewal	Not applicable Not applicable No renewal of test solutions	*10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Aeration, if any	No reference to aeration found.	Requirement considered met. OECD 202 states that the dilution water 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:	Glass beakers 250 mL 200 mL of test solution	Requirement considered met US EPA OPPTS 850.1010 refers to use of 250 mL beakers for static tests. *(EPA requires: size 20 mL or 3.9 L fill 200 mL)*
Source of dilution water	Well water.	Requirement considered met. US EPA OPPTS 850.1010 refers to dilution water being "Surface or ground water, reconstituted water or dechlorinated tap water are 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." *(EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water)*
Water parameters (for dilution water):		See deficiencies/deviations table on page 17 of this DER with respect to OC, OP and PCB concentrations and chlorine level.

Parameter	Details	Remarks *Criteria*
Hardness	160 mg $CaCO_3$/L (total hardness)	Dilution water hardness exceeds the template's requirement - *For hardness, the EPA requires 40 - 48 mg/L as $CaCO_3$.* OECD 202 refers a total hardness of 140-250 mg/L. US EPA OPPTS 850.1010 refers to water quality parameters of a maximum hardness of 180 mg/L, which was met on this occasion. Hardness is considered to have been acceptable.
pH	8.1 In the control and test vessels over 48 hours, the pH ranged from 7.2 to 8.2.	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* pH is considered to have been acceptable.
Dissolved oxygen	8.7-9.1 mg/L in the test vessels over the 48 hours exposure period (equivalent to >90% saturation, based on 100% saturation at 19°C being ~9.26 mg oxygen/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	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 (±1°C) (and for each single test, the temperature should be constant within ±1°C)*

Parameter	Details	Remarks *Criteria*
Total organic carbon	The TOC concentration of the dilution water source was 0.38 and 0.90 mg/L for the months of January and February 2006, respectively.	OECD 202 and US EPA OPPTS 850.1010 refer to dilution water having an acceptable TOC of <2 mg/L.
Particulate matter	Not reported. Culture water had been filtered through an Amberlite XAD-7 resin column.	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 and pesticides	Representative samples of the dilution water source were reported as analysed periodically for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were reported as having been detected at concentrations considered toxic in any of the water samples analysed, in agreement with ASTM (2002)	Metals: OECD 202 says measurements of heavy metals should be made. 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). With respect to absence of data on metals and pesticides, the study report states that several species of daphnids are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over multiple generations. The acceptable performance of the cultured daphnids, in combination with the previously mentioned analyses, confirmed the acceptability of this dilution water for bioassays.
Chlorine	Not reported	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.

Parameter	Details	Remarks *Criteria*
Intervals of water quality measurement	Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24 and 48 hours. The pH, dissolved oxygen concentration and temperature were measured at 0, 24 and 48 hours in replicate A of the treatment levels and the control. Continuous temperature monitoring was performed in replicate D of the 50 mg pyroxsulam/L (nominal) treatment level throughout the exposure period. Representative samples of the dilution water source were analysed monthly for total organic carbon concentration.	
Number of replicates: Control (dilution water): Solvent control: Treatments:	 4 Not applicable (no solvent control used) 4	Requirement met. US EPA OPPTS 850.1010 states that an equal number of daphnids should be placed in two or more replicates. OECD 202 states that at least 20 animals, preferably divided into four groups of five animals each, should be used at each test concentration and for the controls.
Number of organisms per replicate: Control (dilution water): Solvent control:	 5 Not applicable (no solvent control used)	Requirement met. OECD 202 and US EPA OPPTS 850.1010 refer to at least 20 animals, preferably divided into four groups of five animals each, being used at each test concentration and for the controls. *(EPA/OECD require 5 treatment levels plus control*

Parameter	Details	Remarks *Criteria*
Treatments:	5 For the biomass loading , there were 5 daphnid/200 mL or 25 daphnid/L.	*EPA requires a minimum of 20 daphnid per treatment. Biomass loading rate for static: 0.8 g/L at 17oC, 0.5 g/L at > 17oC; flow-through: 1 g/L/day).* 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:	Nominal concentrations were: 0 (control), 6.3, 13, 25, 50 and 100 mg of 7-OH metabolite of pyroxsulam/L. Nominal concentrations are ~50% of the next higher one with a separation factor of ~2. The template refers to nominal concentrations always being at least 60% of the next higher one.	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 Template requirement of each concentration being at least 60% of the next higher one not met. Because the OECD and US EPA OPPTS requirements are complied with, the treatment concentrations are considered acceptable.
Measured:	Reported concentrations of 7-OH	

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td></td><td>metabolite of pyroxsulam (mg/L) were:
<table>
<tr><td colspan="5">Mean, measured, 0 hours</td></tr>
<tr><td>6.2</td><td>16</td><td>32</td><td>50</td><td>100</td></tr>
<tr><td colspan="5">Mean, measured, 48 hours</td></tr>
<tr><td>5.9</td><td>13</td><td>25</td><td>50</td><td>97</td></tr>
<tr><td colspan="5">Mean, measured over 48 hours</td></tr>
<tr><td>6.1</td><td>14</td><td>28</td><td>50</td><td>99</td></tr>
<tr><td colspan="5">Percentage of nominal over 48 hours</td></tr>
<tr><td>96</td><td>110</td><td>110</td><td>100</td><td>99</td></tr>
</table>
With respect to these reported values, the study report stated that the mean-measured concentration and percent of nominal were calculated using actual analytical data and not the rounded (2 significant figures) data presented in the table.

Control water had <0.26 mg 7-OH metabolite of pyroxsulam/L (taken as the lowest level quantified in the study).

As noted previously, the concentrations were adjusted for the 99% purity of the 7-OH metabolite of pyroxsulam.

Quality control samples analysed at 0 and 48 hours gave the following results:
<table>
<tr><td colspan="3">Nominal concentrations</td></tr>
<tr><td>3.00</td><td>20.0</td><td>100</td></tr>
<tr><td colspan="3">Measured, 0 hours</td></tr>
<tr><td>3.08
(103%)</td><td>19.9
(99.7%)</td><td>99.8
(99.8%)</td></tr>
<tr><td colspan="3">Mean, measured, 48 hours</td></tr>
<tr><td>3.02
(101%)</td><td>19.2
(95.9%)</td><td>96.1
(96.1%)</td></tr>
</table>
Percent of nominal presented in brackets.</td><td>Analyses were by HPLC/UV. This method was reported validated by fortification of 20X AAP medium with 7-OH metabolite of pyroxsulam at concentrations of 0.0500 and 100 mg/L.

Recoveries averaged 105 ± 1.95% with a limit of quantitation (LOQ) of 0.0141 mg/L for the method validation.
The quality control sample range for subsequent studies was set at 80 to 120%.

The linear regression analysis for the concentration of the 7-hydroxy metabolite against detector response had an r^2 value of 0.99981.</td></tr>
<tr><td>Solvent (type, percentage, if</td><td>No solvent used.</td><td>Requirement met.</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
used)		*(EPA requires solvents not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests)*
Lighting	Photoperiod of 16 hours of light and 8 hours of darkness. The test area was illuminated with fluorescent bulbs at an intensity range of 83 to 98 footcandles (890 to 1100 lux) at the solutions' surface.	Requirement 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 Limit of Quantitation Level of Detection	 At 0 and 48 hours exposure. 0.0141 mg 7-OH metabolite of pyroxsulam/L (from the method validation). In the control solutions at 0 and 48 hours, the measured concentrations of the analyte were, respectively, <0.26 and <0.25 mg/L. The latter value is taken as the level of quantification for the study. Not identified.	Requirement considered met. A representative chromatogram from a 0.250 mg 7-OH metabolite of pyroxsulam/L calibration standard was presented in the study report with the analyte peak clearly visible. A control sample chromatogram showed no equivalent peak.
Positive control {if used, indicate the chemical and concentrations}	Positive control not used.	Requirement met.
Other parameters, if any	None identified.	Requirement considered met.

2. Observations:

Table 2. Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects	Number of immobilized daphnids at 24 and 48 hours; biological observations ("adverse effects") and observations of the physical characteristics of each replicate test solution were made at 0, 24 and 48 hours (pH, dissolved oxygen concentration and temperature were measured in one replicate of the treatment levels and control. Continuous temperature measurement was conducted in a replicate of the 50 mg/L nominal test concentration. Observations of test organisms were accompanied by observations of the characteristics of the test solutions at those times, i.e. presence of precipitated material, cloudiness etc.	Requirement considered 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.
Observation intervals	At 0, 24 and 48 hours	Requirement considered met. OECD 202 and US EPA OPPTS 850.1010 refer to checking for immobilized daphnids with the OECD standard also referring to checking any abnormal behaviour or appearances at 24 and 48 hours after the beginning of the test.
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 mortality and sublethal effects results were presented. All original raw data, the protocol and the original final report produced during this study are archived are archived at 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	All test solutions were reported as clear and colourless with no visible undissolved test substance.	

II. RESULTS AND DISCUSSION

A. Mortality/Immobililization:

Following 48 hours of exposure (test termination), no immobilisation was observed among daphnids exposed to the treatment levels tested or the control (Table 3). Note that, as is usual, immobilisation was the effect measured in lieu of mortality itself.

Table 3. Effect of 7-OH metabolite of pyroxsulam on mortality/immobilisation of *Daphnia magna*.

Treatment (mg 7-OH metabolite of pyroxsulam /L) [mean-measured (0-48 hours) and nominal conc. used]	No. of organisms	Observation period			
		Day 1 (24 hours)		Day 2 (48 hours)	
		No. Immobilised	% immobilisation	No. Immobilised	% immobilisation
Control (dilution water only), if used	20 (5 daphnids X 4 replicates)	0	0	0	0
Solvent control, if used	**No solvent control used.**				
6.1/6.3 mg/L	20	0	0	0	0
14/13 mg/L	20	0	0	0	0
28/25 mg/L	20	0	0	0	0
50/50 mg/L	20	0	0	0	0
99/100 mg/L	20	0	0	0	0
NOEC (immobilisation), mean-measured concentration		99 mg 7-OH metabolite of pyroxsulam/L		99 mg 7-OH metabolite of pyroxsulam/L	
EC_{50} (immobilisation), mean-measured		>99 mg 7-OH metabolite of pyroxsulam/L		>99 mg 7-OH metabolite of pyroxsulam/L	

Treatment (mg 7-OH metabolite of pyroxsulam /L) [mean-measured (0-48 hours) and nominal conc. used]	No. of organisms	Observation period			
		Day 1 (24 hours)		Day 2 (48 hours)	
		No. Immobilised	% immobilisation	No. Immobilised	% immobilisation
concentration					
Positive control, if used	No positive control used.				

B. OTHER SUB-LETHAL TOXICITY ENDPOINTS:

Following 48 hours of exposure (test termination), adverse effects were not observed in any of the daphnids exposed to the treatment levels tested or in the controls.

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 concluded that, "Since no concentration tested resulted in >50% immobilization, the 48-hour EC50 value for *Daphnia magna* exposed to 7-OH metabolite of XDE-742 was empirically estimated to be >99 mg a.i./L, the highest mean-measured concentration tested. The No-Observed-Effect Concentration (NOEC) was determined to be 99 mg a.i./L. The lowest concentration producing 100% immobilization was >99 mg a.i./L. The highest concentration producing 0% immobilization was 99 mg a.i./L."

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

The absence of immobilisation or adverse effects in the control and test solutions and the acceptability of the measured concentrations, pH, oxygen content and temperature values support the study report's decision not to conduct a statistical analysis of the data.

The 48 hour NOECs and LC50s for immobilisation and adverse effects can be estimated from a visual inspection of the results presented for these parameters.

Statistical Method: Not conducted as a result of the study's results. Consequently, the LC50 and its 95% confidence limits, the NOEC and the probit slope with its 95% confidence limits were not calculated by the reviewer using statistical methodology.

E. STUDY DEFICIENCIES:

The following deficiencies or deviations from guidelines were noted but not considered to have significantly affected the study's conduct or outcome.

Table 4. Summary of deficiencies/deviations from guidelines.

Parameter	Study report result	US EPA OPPTS 850.1010, April 1996	OECD Guideline 202, 13 April 2004
Acclimation: Conditions: (same as test or not)	In-house culture. Period of acclimatisation not identified in the study report but assumed to be satisfactory for in-house cultures.	No specific time identified but the data records of the acclimation (and test temperatures) must be submitted to the EPA. At the initiation of the test, daphnids have been cultured and acclimated in accordance with the test design 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. 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.	No specific time identified. The stock animals must be maintained in culture conditions (light, temperature, medium) similar to those to be used in the test.
Organophosphorus and organochlorine pesticides and polychlorinated biphenyls	All reported as below their respective limits of detection of 0.25 to 5 µg/L.	Total organophosphorus pesticides <50 ng/L and total organochlorine pesticides plus polychlorinated biphenyls <50 ng/L.	Total organophosphorus pesticides <50 ng/L and total organochlorine pesticides plus polychlorinated biphenyls <50 ng/L.
Chlorine	Not reported	Residual chlorine <3 µg/L.	Total residual chlorine <10 µg/L

F. REVIEWER'S COMMENTS:

The study report, the data it provided and the internal consistency of the study results are considered to show the study was conducted satisfactorily and that its results are sound.

This study has estimated the toxicity of the 7-OH metabolite of pyroxsulam to *Daphnia magna* neonates over the nominal concentration range of 6.3 to 100 mg 7-OH metabolite of pyroxsulam/L (6.1 to 99 mg 7-OH metabolite of pyroxsulam/L, mean-measured concentrations over 48 hours). The 48 hour EC50 (immobility) and the 48 hour EC50 (adverse effects other than immobility) were determined at >99 mg 7-OH metabolite of pyroxsulam/L (mean-measured concentration).

Consequently, the 7-OH metabolite of pyroxsulam is considered as, at worst, slightly toxic to the daphnid, *D. magna*, on an acute exposure basis ($10<EC50s\leq100$ mg 7-OH metabolite of pyroxsulam/L) based on mean-measured concentrations of 7-OH metabolite of pyroxsulam.

The 48-hour definitive test was conducted from 31 January to 2 February 2006.

The validity criteria for OECD 202 (adopted 13 April 2004) and US EPA OPPTS 850.1010 (April 1996) 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:

The study is classified as acceptable and is consistent with recommended study guidelines.

Based on mean-measured concentrations over 48 hours exposure, the 48 hour EC50s for immobilisation and adverse effects were both set at >99 mg 7-OH metabolite of pyroxsulam/L.

The 48 hour NOECs for immobilisation and adverse effects were both set at 99 mg 7-OH metabolite of pyroxsulam.

Based on the results of this study, the 7-hydroxy metabolite of pyroxsulam would be classified as, at worst, slightly toxic to the daphnid, *D. magna*, (96 hour EC50 >10 but less than or equal to 100 mg/L) in accordance with the classification systems of the Australian Government Department of the Environment and Water Resources, and of the US EPA.

III. REFERENCES:

(Note: for the purpose of this work sharing program, the reference list is based on that in the study report and does refer to standard guidelines or methodologies.)

ASTM (2002). Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

EC (Official Journal of the European Communities). 1992. Commission Directive 92/69/EEC of 31 July 1992. Part C: Methods for the Determination of Ecotoxicity. Method C.2, Acute Toxicity for Daphnia. L383 A Volume 35, 29 December 1992.

EC (Official Journal of the European Communities). March 8, 1999. 1999/12/EEC, Adapting to technical progress for the second time the Annex to Council Directive 88/320/EEC on the inspection the principals of good laboratory practice (GLP).

EC (1997). Official Journal of the European Communities. January 1997. Annex V. Part C: Methods for the Determination of Ecotoxicity. Method C.2, Acute toxicity for Daphnids.

OECD (1998). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp.

OECD (2004). Guideline for Testing of Chemicals. *Daphnia sp.*, Acute Immobilization Test. Guideline #202. Adopted 13 April 2004.

Sayers L (2006a). Study Profile Template (SPT) for: 7-OH Metabolite of XDE-742 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6411.SPT, Dow Study No. 050165.SPT. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences, Indianapolis, Indiana 46268. April 5, 2006. Unpublished report.

Sayers L (2006b). 7-OH Metabolite of XDE-742 – Acute Toxicity to Water Fleas, *Daphnia magna,* Under Static Conditions. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Springborn Smithers Study No. 12550.6410.SPT, Dow Study No. 050164.SPT. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences, Indianapolis, Indiana 46268. April 5, 2006. Unpublished report.

Note: The company STP refers to a report completion date of March 14, 2005. This is considered an error as the study completion date according to the study report was March 14, 2006. Consequently, the STP is referenced as "Sayers L (2006b)" rather than "Sayers L (2005)".

U.S. EPA (1975). Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009). 61 pp.

U.S. EPA (1982). Office of Pesticide Programs. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-82-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.

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

Approved 04/01/01 C. K.