Data Evaluation Report on the chronic toxicity of 7-hydroxy-pyroxsulam (7-hydroxy-XDE-742) to fresh water invertebrates – *Chironomus riparius*.
PMRA Submission Number 2006-4727; ID 1283200 EPA MRID Number 469085-09 APVMA ATS 40362

Data Requirement:

PMRA DATA CODE:	9.3.4
EPA DP Barcode:	D332116
OECD Data Point:	IIA 8.5.2
EPA Guideline:	Nonguideline

Test material: **7-OH metabolite of pyroxsulam** **Purity (%):** 99%

Common name: 7-OH metabolite of pyroxsulam or the 7-OH metabolite of XDE-742.

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

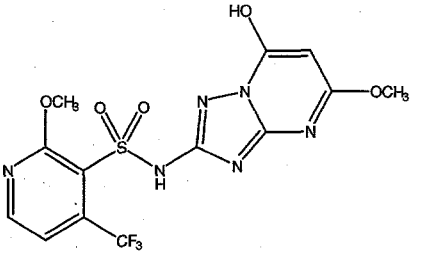
IUPAC: N-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

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, X11250641 (as reported in the Certificate of Analysis).

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:** 06 April 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

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

Émilie Larivière **Date:** 05 July 2007
Environmental Assessment Directorate, PMRA

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

CITATION: Putt, A. E. 2006. 7-OH Metabolite of XDE-742- Chironomid Toxicity Test with Midge (*Chironomus riparius*) Under Static Conditions using Spiked Water. Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Springborn Smithers Study No.

12550.6412, Sponsor Protocol/Project No. 050166. The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences, Indianapolis, Indiana 46268. 15 May 2006. Unpublished report.

Note: The PMRA suggests using 'transformation product' instead of 'metabolite' throughout the DER. The US EPA has commented, with respect to this proposal that, " ... some reviewers prefer particular terminology. Thus, whether the term metabolite, degradate or transformation product is used, depends on the individual reviewer. It is my understanding that metabolite is typically used to refer to a product of biotic degradation, where degradate can apply to either abiotic or biotic processes. The term transformation product is ... where the compound may not be "degraded" but simply conjugated with say a glucuronide. It is up to the team to decide which term to use; however, ideally, it should be used consistently throughout all of the DERs." DEW supports this approach which would mean that "metabolite" is retained for the pyroxsulam ecotoxicity studies and, that in future work share projects, a decision is made at an early stage whether to refer to metabolites or transformation degradation products.

EXECUTIVE SUMMARY:

The 28 day chronic midge spiked water sediment-water exposure study of the 7-hydroxy metabolite of pyroxsulam (7-OH metabolite of pyroxsulam; 99% purity) to instars of the midge, *Chironomus riparius*, was studied under static conditions without renewal. The study was conducted in accordance with OECD Guideline 219. Midge larvae were exposed to a control and the test chemical at nominal concentrations of 0, 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L. Overlying water concentrations were measured on day 0 (1 hour), day 7 and day 28 for the control and all treatment levels, while pore water and sediment concentrations were measured in the control, 7.5, 30 and 120 mg 7-OH metabolite of pyroxsulam/L treatment levels. Mean-measured concentrations in water ranged from 95-100% of nominal on day 0 (1 hour), from 83-92% of nominal on day 7 and from 56-73% of nominal on day 28. Results are reported in terms of nominal water concentrations. Midge emergence (date and number of emerged midges), the sex of emerged midges and abnormal behaviour (such as avoidance of sediment) were monitored daily. Emergence in all treatments was greater than 50%.

No concentration resulted in ≥50% reduction in either emergence numbers or development rate, thus the 28-day EC_{50} for emergence and development was empirically set, based on the study's results, at >120 mg 7-OH metabolite of pyroxsulam, the highest nominal concentration tested. Over the sampling period, pore water concentrations averaged ~42% of nominal and the reviewer calculated EC_{50} based on the adjusted pore water concentration would be >50.4 mg/L.

The study's 28 day NOEC based on combined male and female emergence numbers was 120 mg 7-OH metabolite of pyroxsulam/L or, based on an adjusted mean pore water concentration, 50.4 mg/L, as calculated by the reviewer.

The study reported the 28 day NOEC based on male development rate as 120 mg 7-OH metabolite of pyroxsulam/L, or, based on an adjusted mean pore water concentration, 50.4 mg/L, again as calculated by the reviewer. Similarly, the 28 day NOEC based on female development rate was reported as 30 mg 7-OH metabolite of pyroxsulam/L with the 28-day LOEC for emergence set at 60 mg 7-OH metabolite of pyroxsulam/L. (or, as calculated by the reviewer and based on an adjusted mean pore water concentration, respectively 12.6 and 25.2 mg/L).

As well as reporting the male and the female development rate results separately, the study also reported the combined male and female development rates. Because there is difference between the two development rates, the reporting of the combined rate could be considered both biologically inappropriate and confusing as to which value to choose for risk assessment. The NOEC for the female development rate is considered to be the most sensitive endpoint.

The 7-OH metabolite of pyroxsulam is considered to be very slightly toxic (NOEC >1 mg/L) .to the instars of the midge, *C. riparius* with the most sensitive endpoint a measured NOEC of 30 mg/L for female development rate or, as calculated by the reviewer based on an adjusted mean pore water concentration, 12.6 mg/L.

This toxicity study is classified as acceptable and is considered consistent with the guideline requirement for a sediment-water toxicity test using spiked water study on the larvae of the midge, *Chironomus riparius*.

Results Synopsis

Test Organism Age (e.g. 1st instar):	First instar (two days old at test initiation) of the midge, *Chironomus riparius.*
Test Type: Static, non-renewal	
28 day NOEC (combined male and female emergence numbers, i.e. proportion of larvae emerged):	120 mg 7-OH metabolite of pyroxsulam/L or, based on an adjusted mean pore water concentration as calculated by the reviewer, 50.4 mg/L
28 day NOEC (male development rate):	120 mg 7-OH metabolite of pyroxsulam/L, or, based on an adjusted mean pore water concentration as calculated by the reviewer, 50.4 mg/L
28 day NOEC (female development rate):	30 mg 7-OH metabolite of pyroxsulam/L, or, based on an adjusted mean pore water concentration as calculated by the reviewer, 12.6 mg/L
28 day LOEC (female development rate):	60 mg 7-OH metabolite of pyroxsulam/L, or, based on an adjusted mean pore water concentration as calculated by the reviewer, 25.2 mg/L
Endpoint(s) affected:	female midge development rates

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The methods used for the study were reported as based on the Organisation for Economic Cooperation and Development (OECD) "Full Life-Cycle Toxicity Test with Sediment Dwelling Midges (*Chironomus riparius*) under Static Conditions" draft Guideline 219, Sediment-Water Toxicity Test Using Spiked Water (2001) and Springborn Smithers Laboratories Protocol No.: 022403/OECD/Midge-FLC/Spiked water. A copy of the study protocol was provided.

COMPLIANCE:

The data and report presented were reported to have been produced and compiled in accordance with all pertinent OECD (OECD, 1998) Good Laboratory Practice regulations with the following exceptions: routine food, water and sediment screening analyses were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. Since the analyses were conducted following standard validated methods, these exceptions were considered by the study report to have had no adverse 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

Test Substance Number: TSN105384 (Lot 35172-56 with the test substance identified as SSL No. 115-19) and TSN105232 (Lot E2008-46 with the test substance identified as SSL No. 112-85).

SSL No. 115-19 was used to prepare test solutions and calibration standards while SSL No. 112-85 was used to prepare quality control samples.

Purity: Certificates of analysis reported purities of 99% (Lot 35172-56) and 96% (Lot E2008-46).

Stability of Compound under Test Conditions: Measured and calculated mean concentrations (as mg 7-OH pyroxsulam/L, see page 16 of this DER) over the 28 days of exposure indicate that the 7-OH metabolite of pyroxsulam was relatively stable in the test waters with measured concentrations at day 28 being 56 to 73% of the nominal starting concentrations in overlying waters.

Storage conditions of test chemicals: Upon receipt at Springborn Smithers, the test substances (SSL No. 115-19 and SSL No. 112-85) were stored at room temperature in their original containers in a dark ventilated cabinet.

Physicochemical properties of 7-OH metabolite of pyroxsulam.

Parameter	Values	Comments
Water solubility at 20°C	This information was not provided in the study report.	This information will be inserted into the DER on its receipt from the assessors conducting the chemical assessment section of this joint agency exercise.
Vapour pressure		
UV absorption		
pKa		
Kow		

Note: The company's study profile template (Putt, 2006) reported that physicochemical properties were not available at the time of publication of the study profile template.

2. Test organism:

Species: *Chironomus riparius*

Age of the parental stock: Three to four days prior to test initiation, egg masses were reported as removed from culture aquaria and observed daily until hatching was complete (approximately 24 to 48 hours after release of egg masses by the female midge). The larvae were reared in culture bowls for two days after hatching to provide first-instar larvae for use during the exposure to 7-OH metabolite of pyroxsulam. The study protocol states that the midge larvae are to be first instars (2 to 3 days post hatch) at test initiation.

Source: The midges used for the study were obtained from laboratory cultures maintained at Springborn Smithers.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study

Prior to initiating the definitive study, a preliminary range-finding exposure was reported as conducted at Springborn Smithers at nominal 7-OH pyroxsulam levels of 0.50, 1.0, 10, 50 and 100 mg/L and a control. Three replicates of twenty midges (three days old) were exposed to each treatment level and the control.

Following 26 days of exposure, the mean percent emergences among midge exposed to the nominal concentrations tested (0.50, 1.0, 10, 50 and 100 mg of the 7-OH metabolite of pyroxsulam/L) were 97, 95,

98, 92 and 93%, respectively. During the same period, mean percent emergence among the midge exposed to the control was 97%.

The mean development rate (male/female midge combined), after 26 days of exposure, for the nominal concentrations tested (0.50, 1.0, 10, 50 and 100 mg 7-OH pyroxsulam/L) was 0.0589, 0.0595, 0.0598, 0.0600 and 0.0600, respectively. During the same period, mean (male/female midge combined) development rate among the midge exposed to the control was 0.0613.

Based on these results and consultation with the Study Sponsor, the following nominal 7-OH metabolite of pyroxsulam concentrations were selected for the definitive study: 7.5, 15, 30, 60 and 120 mg 7-OH pyroxsulam/L.

The full report of this range finding study was not seen.

b) Definitive Study

Table 1. Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Parental acclimation:	In-house culture	Requirement considered met.
Period:	Three to four days prior to test initiation, egg masses were removed from culture aquaria and each individual egg mass was placed culture water. Hatching was complete approximately 24 to 48 hours after release of egg masses by the female midge. Hatched midge larvae were reared under static conditions in laboratory well water for two days after hatching. This provided first instar larvae for the exposure study.	OECD 219 states that four to five days before adding the test organisms to the test vessels, egg masses should be taken from the cultures and placed in small vessels in culture medium. OECD 219 notes that aged medium from the stock culture or freshly prepared medium may be used. If the latter is used, a small amount of food e.g. green algae and/or a few droplets of filtrate from a finely ground suspension of flaked fish food should be added to the culture medium.
Conditions: (same as test or not)	Culture conditions involved maintaining the hatched midges in a shallow bowl containing a litre of culture water (laboratory well water) for two days until study initiation at 21°C with a 16-hour light: 8-hour dark photoperiod. The dissolved oxygen ranged from 8.0 to 8.1 mg/L.	

Feeding:	The culture water in the glass bowl also contained 10 mL of *Ankistrodesmus falcatus* suspension (4 x 10^7 cells/mL). During rearing, the midge larvae were fed a finely-ground suspension of flaked fish food (i.e., 10 mg/mL). Midge larvae were fed daily during the culturing and rearing period. Prior to introducing the midge to the test vessels, 0.50 mL of finely ground flaked fish food suspension (10 mg/mL) was added to each test vessel. Test midge were fed 0.50 mL of finely ground flaked fish food suspension (10 mg/mL) daily, on test days -1 through 10. On days 11 through to termination, midge were fed 1.0 mL of finely ground flaked fish food suspension (10 mg/mL) daily.	
Health: (any mortality observed)	No mortality of midge larvae was observed 48 hours prior to test initiation. Records of midge culture are reported as retained in the Springborn Smithers Culture Log entitled "*Chironomus riparius* Culture Logbook," Volume 1 January 2006 to 31 December 2006.	
<u>Test conditions</u>: Renewal rate/procedure for static renewal	No renewal of either the 7-OH metabolite of pyroxsulam or test water occurred.	Requirement considered met. OECD 219 states (paragraph 30) that static systems are used.
Static renewal/flow through:	Static	
Type of dilution system- for flow through method.	Not applicable.	
Flow rate:	Not applicable.	

Aeration, if any	Test solutions were gently aerated (1 to 3 bubbles per second) seven days prior to addition of the test organisms and throughout the duration of the exposure period. At the time of addition of the midge larvae and for four hours after the test organisms were added, the aeration was suspended.	Requirement considered met. OECD 219 states the sediment-water system has to be aerated for 7 days before addition of the *C. riparius* instars. The Guideline also refers to gentle aeration of the overlying water in test vessels being supplied preferably 24 hours after addition of the larvae and pursued throughout the test with care taken that the dissolved oxygen concentration does not fall below 60 per cent of ASV. Aeration is provided through a glass Pasteur pipette fixed 2-3 cm above the sediment layer (with one or a few bubbles/sec).
Duration of the test	28 days	Requirement considered met. OECD 219 states that the test duration is in the range of 20-28 days for *C. riparius* with 28 days being the maximum exposure duration for this species.
<u>Test vessel</u> Material: (glass/stainless steel): Size (for growth and reproduction/survival test): Fill volume:	Clear glass beakers. 600-mL The total medium volume (sediment/water) was maintained at 375 mL and the ratio of sediment to water was 1:4. The initial water level in each test vessel was marked in order to evaluate evaporation. Each test vessel was covered with a clear plastic plate to minimize evaporation and to trap emerging adult midge.	See study deficiencies table of page 31 of this DER with respect to weight of wet sediment with and without pore water; OECD 219 states the study is conducted in glass 600 mL beakers measuring 8 cm in diameter. Other vessels are suitable, but they should guarantee a suitable depth of overlying water and sediment. The ratio of the depth of the sediment layer to the depth of the overlying water should be 1:4.

Depth of sediment and overlying water column:	A 75-mL (1.5-cm layer) aliquot of sediment was added to each test vessel with 300 mL (6 cm) of overlying water. The wet weight of the sediment in each jar averaged 151 g (98 g dry weight). A turbulence reducer composed of a modified plastic disk was used to minimize the disruption of the sediment layer during the introduction of the overlying water.	The Guideline also notes that the test report must provide the depth of sediment and overlying water and the "volume of overlying and pore water; weight of wet sediment with and without pore water;"
Source of dilution water Dilution water parameters pH Hardness Alkalinity Specific conductance	Laboratory well water which was characterized as a soft water 7.6 A typical total hardness of 58 mg/L as $CaCO_3$. Total alkalinity 34 mg/L as $CaCO_3$ 190 micromhos/cm.	Requirement considered met.
Overlying water parameters:	Overlying water used during this study was laboratory well water from the same source as the culture water. Total hardness, alkalinity, specific conductivity, and total ammonia of the test solutions were determined at test initiation and at test termination in a composite sample (replicates F through H at initiation, and A through D at termination) from the highest treatment level (120 mg/L) and the control solution.	See study deficiencies table of page 31 of this DER with respect to pesticide and PCB levels. OECD 219 states that at the start of the test, the total hardness should not be higher than 400 mg/L as $CaCO_3$.
Hardness	Water column hardness, as mg $CaCO_3$/L: **Nominal Concentration (mg/L)** / **Total Hardness (mg/L as $CaCO_3$)** Day 0 / Day 28 Control: 92 / 110 120: 76 / 150	

pH	Measurements of pH were made on the day the test organisms were added (day -l), the day of test substance application (day 0), and at test termination (day 28) in each exposure vessel. Individual pH results were not presented, summary data only provided. The pH values in the overlying water during the 28 days exposure were reported as: **Nominal Concentration (mg/L)** / **pH[a] Range** Control 7.2-8.1 7.5 7.3-8.2 15 7.2-8.1 30 7.3-8.1 60 7.1-8.1 120 6.8-8.2 **[a] N = 12, based on measurement of each replicate treatment level and the control on test day –1, test initiation and termination.**	OECD 219 states that at the start of the test, the pH of the test water should be between 6 and 9 and that this 6-9 pH range should be maintained for test validity.
Dissolved oxygen	Measurements of dissolved oxygen concentration were made on the day the test organisms were added (day -l), the day of test substance application (day 0), and at test termination (day 28) in each exposure vessel. In addition, dissolved oxygen concentration and temperature were measured daily in each replicate vessel of each treatment level and the control during the 28-day exposure. The dissolved oxygen content in the overlying water during the 28 days exposure was reported as: **Nominal Concentration (mg/L)** / **Dissolved Oxygen Concentration[ab] (mg/L) Range** Control 7.4-8.9 7.5 6.9-9.0 15 7.3-9.0 30 7.4-9.0 60 7.4-9.0 120 7.6-9.0 **[a] N = 120, based on daily measurement of each replicate treatment level and the control.** **[b] 60% of the air saturation value at 19°C = 5.6 mg/L and 60% of the air saturation value at 21°C = 5.3 mg/L.**	OECD 219 states that the oxygen concentration should be at least 60 per cent of the air saturation value (ASV) at the temperature used for the test to be valid.

Temperature	Measurements of temperature were made on the day the test organisms were added (day -1), the day of test substance application (day 0), and at test termination (day 28) in each exposure vessel. In addition, the temperature was continuously monitored in replicate H of the 7.5 mg/L treatment level throughout the study. The temperature in the overlying water during the 28 days exposure was reported as 19-21°C in all vessels. Continuous temperature monitoring reported the same temperature range over the exposure period.	OECD 219 states that the test is conducted at a constant temperature of 20°C (± 2°C). In the study, the test vessels were recorded as impartially positioned in a water bath containing circulating water designed to maintain the test solutions at a temperature of 20 ± 2 °C. For the test to be valid with respect to temperature, OECD 219 states that the the water temperature should not differ by more than ± 1.0°C. As OECD 219 states that the test is conducted at a constant temperature of 20°C (± 2°C), this validity criterion is considered met.
Total organic carbon	TOC content of the overlying water not identified in the study report.	OECD 219 recommends < 2 mg/L) as an acceptable value for dilution water. Putt (2006), in the company's study template reports that the overlying water had a total organic carbon content of 0.26 to 0.35 mg/L.
Particulate matter	Details on the particulate matter content of the overlying water were not identified in the study report.	OECD 219 recommends < 20 mg/L) as an acceptable value for dilution water. Putt (2006), in the company's study profile template reports that the overlying water had a particulate matter content "within normal limits"
Metals	Representative samples of the overlying 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 that were considered toxic in any of the water samples analysed, in agreement with ASTM (2002) standard practice. Putt (2006), in the company's study template reports that the overlying water had a metal content "within normal limits".	

Pesticides	See "Metals" above. Putt (2006), in the company's study template reports that the overlying water had a pesticides content "within normal limits".	
Chlorine	Details on the chlorine content of the overlying water were not identified in the study report. Putt (2006), in the company's study template reports that the overlying water had a chlorine content "within normal limits".	OECD 219 recommends as acceptable values for dilution water, a residual chlorine concentration of <10 µg/L.
Interval of water quality measurement	Measurements of dissolved oxygen concentration, temperature and pH were made on the day the test organisms were added (day -1), the day of test substance application (day 0), and at test termination (day 28) in each exposure vessel. In addition, dissolved oxygen concentration and temperature were measured daily in each replicate vessel of each treatment level and the control during the 28-day exposure. The temperature was continuously monitored in one replicate throughout the study. Total hardness, alkalinity, specific conductivity, and total ammonia of the test solutions were determined at test initiation (day 0) and at test termination (day 28) in a composite sample from the highest treatment level (120 mg7-OH metabolite of pyroxsulam/L) and the control solution.	OECD 219 states that, "at the end of the test, pH and the dissolved oxygen concentration should be measured in each vessel." and "Hardness and ammonia should be measured in the controls and one test vessel at the highest concentration at the start and the end of the test." And also "the water temperature should not differ by more than ± 1.0 °C. The water temperature could be controlled by isothermal room and in that case the room temperature should be confirmed in an appropriate time intervals."
Sediment parameters:	Artificial sediment prepared according to OECD 219 was used in this study.	See study deficiencies table on page 31 of this DER with respect to weight of wet sediment with and without pore water and particle size distribution. OECD 219 states, inter alia, that characteristics of the formulated sediment (e.g. organic carbon content, pH, moisture, etc.) be determined at the start of the test).

Composition	The sediment comprised approximately 74% sand, 20% kaolin clay, and 6% sphagnum peat (dry weight ratios). The artificial sediment (Springborn Smithers Batch No. 122305) was prepared according to OECD Guideline No. 219 (OECD, 2001) by blending the dry components together in a large-scale laboratory mixer with a small amount of overlying water added to moisten the sediment before it was added to the exposure vessels. The artificial sediment used in the study was characterized by Agvise Laboratories, Northwood, North Dakota.	OECD 219 states the sediment should be 4-5% peat, 75-76% quartz sand and 20% kaolinate clay. The sediment and overlying water were added to the test vessels seven days prior to test initiation.
Sediment organic carbon content	1.6%	OECD 219 states the organic carbon content of the sediment (adjusted by addition of sand and peat) should be 1.5 to 2.5% of the sediment by dry weight.
Particle size distribution	The study report referred to the artificial sediment having a particle size distribution of 79% sand, 6% silt and 15% clay.	OECD 219 makes recommendations for the particle size of the peat and quartz sand used to make up the sediment.
pH	7.3	OECD 219 refers to the sediment having a final pH of 6.5 to 7.5.
Water content	The moisture content at 1/3 bar (water holding capacity) 12.4%.	OECD 219 recommends sediment contains a water content of 30-50% of the sediment dry weight.
Number of replicates: Control (dilution water): Solvent control: Treatment:	Eight replicate exposure vessels were established for each treatment level and the control. Four replicate vessels (A through D) were established to monitor the biological results (i.e., percent emergence and development rate) of the exposed midge with four additional replicates (E through H) established to determine exposure concentrations of 7-OH metabolite of pyroxsulam in the overlying water, pore water and sediment. A solvent control not used. As for the control (4 replicates to monitor biological results and 4 to determine concentrations of 7-OH pyroxsulam.	Requirement considered met.

Number of organisms: For the emergence test:	One day prior to application of the test substance (day -1), 20 midge larvae were added to each replicate vessel (A through H). Replicates E to H were for chemical analysis and observations of midge emergence and water quality measurements were not made on these vessels.	Requirement considered met. Midges used to initiate the study were 2–3 days post-hatch. OECD 219 requires that twenty first instar larvae be allocated randomly to each test vessel and that first instar larvae (2-3 or 1-4 days post hatching) should be used in the test. Twenty-four hours after adding the larvae, the test substance is spiked into the overlying water column, and slight aeration is again supplied.

Treatment concentrations: Nominal:	One day after the organisms were added to the test vessels, the test substance was introduced by adding the appropriate volume of the 200 mg 7-OH pyroxsulam/L primary stock solution to the overlying water (0.30 L). Prior to addition of the stock solution to each vessel, an equal amount of overlying water was removed from each test vessel. The water columns were dosed at the following nominal concentrations : 0, 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L of overlying water. Note: for the nominal 15 mg 7-OH metabolite of pyroxsulam/L solution, 23 mL of a 200 mg/L stock solution was added to the test vessels and a final volume of 300 mL prepared. This is calculated as 15.3 mg 7-OH metabolite of pyroxsulam/L rather than the reported 15 mg/L. This difference is considered trivial and not to have affected the study or the calculations used. Eight dilution water control replicates were also prepared using only dilution water with no test substance. The control vessels were maintained under the same conditions as the treatment level vessels.	Requirement considered met. OECD 219 states that if the LOEC and NOEC are to be estimated, five test concentrations with at least four replicates should be used and the factor between concentrations should not be greater than two. The primary stock was prepared by adding 0.6060 g of 7-OH metabolite of pyroxsulam (0.5999 g as active ingredient, i.e. allowing for the purity of 99%) to 3 L of dilution water in a 4-L beaker. The stock solution was observed to be cloudy and white with undissolved test substance present at first observation. After sonication and overnight stirring, the resultant solution was clear and colourless with no visible undissolved test substance. Following the addition of the stock solution, each exposure solution was gently stirred to aid in the mixing of the test substance in the water column. All exposure solutions were observed to be clear and colourless with no visible undissolved test substance following preparation.

Measured:

Summary of results of analyses of the 7-OH metabolite of pyroxsulam in the overlying water samples:

Concentrations of 7-OH metabolite of pyroxsulam measured in overlying water samples during the full life-cycle exposure of midge (*Chironomus riparius*).

Nominal Concentration (mg/L)	Measured Concentration, mg/L (% of nominal concentration)		
	1 Hour[a]	Day 7[b]	Day 28[c]
Control	<0.22 (NA[d])	<0.27 (NA)	<0.28 (NA)
7.5	7.2 (95)	6.2 (83)	4.2 (56)
15	15 (100)	13 (86)	10(67)
30	29(96)	27(89)	20(65)
60	57(96)	55(92)	44(73)
120	120 (99)	100 (86)	87 (72)
Stock Solution[e]	250 (120)	NA	NA
QC[f]#1, 3.00	3.04 (101)	3.00 (100)	2.97 (98.9)
QC#2, 30.0	29.0 (96.7)	28.5 (95.0)	28.1 (93.6)
QC#3, 120	115 (95.9)	116 (97.0)	118 (98.5)

a Analytical samples were removed from replicate E of the additional exposure vessels.
b Analytical samples were removed from replicate F of the additional exposure vessels.
c Analytical samples were removed from replicate G of the additional exposure vessels.
d NA = Not Applicable.
e 200 mg 7-OH pyroxsulam/L
f QC = Quality Control sample. Measured concentration of each QC sample is presented with the percent recovery in parentheses.

Toxicity results are based on nominal water concentrations.

OECD 219 states that as a minimum, samples of the overlying water, the pore water and the sediment must be analysed at the start (preferably one hour after application of test substance) and at the end of the test, at the highest concentration and a lower one.

With respect to the control values, the US EPA commented that it was unclear why control values are reported as <0.20 mg/L when the level of quantitation is 0.0141 mg/L.

When asked about this, the applicant reported that, "LOQ values will vary slightly from one analysis interval to another, since this value is dependent on the regression of the calibration standards, the peak area of the low standards and the dilution factor. The value for the LOQ cited for the method validation, (e.g., 0.0141 mg ai/L in study 050164) was determined by analysis of standard solutions, corrected for a dilution factor of the standard solutions. … . The LOQ reported for the exposure phase of the experiment reflects that samples were diluted by a factor of approximately 20, therefore the LOQ of the overall analysis is approximately 0.25 mg/L ".

Pore water samples were collected from the 7.5, 30 and 120 mg/L treatment levels and the control one hour after application on day 0 (replicate E), and on days 7 (replicate F), and 28 (replicate G).

Summary of results of analyses of pyroxsulam in pore water samples:

Concentrations of 7-OH metabolite of pyroxsulam measured in pore water samples during the full life-cycle exposure of midge (*Chironomus riparius*).

Nominal Concentration (mg/L)	Measured Concentration, mg/L (% of nominal concentration) 1 Hour[a]	Day 7[b]	Day 28[c]
Control	<0.22 (NA[d])	0.85[e] (NA)	<0.28 (NA)
7.5	1.1 (14)	4.1 (55)	4.3 (57)
30	2.5 (8.5)	17 (57)	18 (61)
120	6.9 (5.8)	62 (52)	83 (69)

a Analytical samples were removed from replicate E of the additional exposure vessels.
b Analytical samples were removed from replicate F of the additional exposure vessels.
c Analytical samples were removed from replicate G of the additional exposure vessels.
d NA = Not Applicable.
e Based on the absence of measured concentrations in the overlying water and sediment of the control sample on test day 7, the observed result was likely introduced into the sample during the sampling/handling process and is not believed to be representative of the exposure conditions.

Across each of the treatment groups, the mean measured pore water concentration is 3.2, 12.5 and 50.6 mg/L representing, 42.7%, 41.7% and 42.2% of nominal. The grand mean recovery would be ~42%.

Overlying water from each replicate vessel was decanted and its volume was measured.

Pore water samples were collected by removing the entire sediment sample and centrifuging for 30 minutes at approximately 10,000 *g*. Note that under "Protocol Deviation", the time of centrifugation is reported as 15 minutes.

The resulting pore water was removed from the centrifuge tube and its volume was measured.

The one reported protocol deviation was in relation to the centrifugation rate. The protocol referred to centrifugation at 1000 g for 15 to 30 minutes whereas 10,000 g were used.

The US EPA noted that the control day 7 value of 0.85 mg/L was suspect when viewed against the 1 hour and day 28 control results. The DEW and PMRA reviewers concur with this observation.

	Summary of results of analyses of 7-OH metabolite of pyroxsulam in sediment samples from surrogate vessels: **Concentrations of 7-OH metabolite of pyroxsulam measured in sediment samples during the full life-cycle exposure of midge (*Chironomus riparius*).** **Nominal Concentration (mg/L)** / **Measured Concentration, mg/kg: 1 Hour[a]** / **Day 7[b]** / **Day 28[c]** **Control**: <0.20 / <0.39 / <0.40 **7.5**: 0.29 / 0.79 / 1.6 **30**: 0.68 / 4.1 / 5.4 **120**: 1.5 / 17 / 25 **QC[d]#1, 2.00**: 1.79 (89.7%) / 1.55 (77.3%) / 1.62 (81.2%) **QC#2, 10.0**: 8.95 (89.5%) / 8.96 (89.6%) / 7.79 (77.9%) **QC#3, 400**: 377 (94.1%) / 336 (84.0%) / 312 (78.0%) **a Analytical samples were removed from replicate E of the additional exposure vessels.** **b Analytical samples were removed from replicate F of the additional exposure vessels.** **c Analytical samples were removed from replicate G of the additional exposure vessels.** **d QC = Quality Control sample in mg/L. Percent of nominal shown in brackets.**	Sediment samples were collected with a stainless steel spatula from the centrifuge tubes, following centrifugation and removal of the pore water sample (see above). Based on the analytical results of overlying water and sediment samples during this study, the study report stated only a small percentage of the 7-OH metabolite of pyroxsulam (<50%), applied to the overlying water partitioned to the sediment as evidenced by measured 7-OH metabolite of pyroxsulam concentrations in the sediment throughout the exposure The data presented in the study report support this conclusion.
Solvent (type, percentage, if used)	No solvent used.	Requirement met.
Lighting	A 16 hour light and 8 hour dark photoperiod was used. The test area was reported as illuminated with fluorescent bulbs at an intensity range of 540 to 710 lux. The actual measurements were not provided in the study report.	Requirement met. OECD 219 refers to a 16 hours photoperiod is used and the light intensity should be 500 to 1000 lux.

Recovery of the chemical:		Requirement considered met.
Frequency of determination	Stock solution analysed at day 0. During the in-life phase of the definitive study, water and sediment samples were removed from one replicate exposure vessel of each treatment level and the control one hour after the test substance was added on day 0 (replicate E), on test day 7 (replicate F), and on test day 28 (replicate G). Pore water samples were collected from the 7.5, 30 and 120 mg 7-OH pyroxsulam/L treatment levels and the control one hour after application on day 0 (replicate E), and on days 7 (replicate F), and 28 (replicate G). Three quality control (QC) samples were prepared at each sampling interval for each sample matrix (water and sediment) and stored and analysed with the set of study samples. These QC samples were prepared in overlying water and sediment at concentrations of 7-OH metabolite of pyroxsulam similar to the treatment level range.	All aqueous and sediment samples were analysed for 7-OH metabolite of pyroxsulam using high performance liquid chromatography with ultraviolet detection (HPLC/UV) procedures based on methodology validated at Springborn Smithers. The method validation studies were conducted prior to the initiation of the definitive test and established an average recovery of 105 ± 1.95% from 20X AAP medium, a freshwater algal medium and 83.7 ± 5.35% from artificial sediment. Method validation was by fortification of 20X AAP medium with 7-OH metabolite of pyroxsulam at concentrations of 0.05 and 100 mg/L. The quality control sample acceptance range from both 20X AAP medium and artificial sediment for subsequent studies was set at 80 to 120%. Conditions and procedures used throughout the analysis of exposure solutions and QC samples during study were stated as similar to those used in the method validation study Representative calibration, recovery and control chromatograms from determinations of the 7-OH metabolite of pyroxsulam in aqueous and sediment phases identified the presence of the metabolite in the calibration and recovery samples and its absence in the controls. Concentration versus response plots presented indicated a linear relationship with r^2 values of 0.99981 and 0.99992

Level of Quantitation Level of Detection	0.0141 mg 7-OH metabolite of pyroxsulam / L in the aqueous phase. 2.00 mg 7-OH metabolite of pyroxsulam / kg, the lowest fortified concentration, in the sediment. Not reported.	
Positive control {if used, indicate the chemical and concentrations}	A positive control was not used.	Requirement met.
Other parameters, if any	Other water quality measurements at Day 0 and at test completion: **Laboratory dilution water control** **Alkalinity, mg $CaCO_3$/L**: 68 (Day 0), 78 (Day 28) **Conductivity, µmho/cm**: 300, 360 **Ammonia, mg N/L**: 0.33, <0.10 **Highest Test Material Treatment (120 mg/L)** **Alkalinity, mg $CaCO_3$/L**: 38, 96 **Conductivity, µmho/cm**: 260, 350 **Ammonia, mg N/L**: <0.10, <0.10	Requirement considered met. OECD 219 does not specify alkalinity or conductivity parameters for the test waters but does state that acceptable dilution water would contain <1 µg unionized ammonia/L. The successful hatching of control midge is taken to indicate that these water parameters were acceptable.

2. Observations:

Table 2. Observations

Parameters	Details	Remarks *Criteria*
Data end points measured (list)	Abnormal behaviour (e.g. sediment avoidance); Emergence (date and number); Sex of the emerged midges; and Physical characteristics of the test solutions.	Requirement considered met. Male midges reported as identified by their plumose antennae.
Observation intervals	Replicate test vessels A through D were examined at test initiation and daily thereafter, until test termination (day 28). During the period of expected emergence (typically starting between day 10 through 16 and lasting until day 28), a daily check of emerged midges was made.	Requirement considered met.
Water quality was acceptable (Yes/No)	On the basis of the reported parameter values and the acceptable emergence of midge in the controls, the test water quality is considered to have been acceptable.	Requirement considered met.

Were raw data included?	No. Reported as archived.	Requirement considered met. While raw data (original laboratory reports) were not provided, results identified as "representative raw data" were. The data provided were the recording of the numbers of midge emerging (males and females) between days 10 and 28 of the exposure period for individual replicates from the control and 7-OH pyroxsulam test concentrations and calculated development rates for males and females and males and females combined per replicate control and 7-OH pyroxsulam test concentration. This was considered sufficient to use in the assessment of the study's findings. OECD 219 does not specify that raw data be provided. Rather, that only certain results must be provided (Paragraph 52 of OECD 219). These include, for example, the numbers of emerged male and female midges, percent emergence and mean development rates. The data provided is considered to have met the OECD 219 requirements in this regard. Advice from the US EPA on the inclusion of raw data was provided elsewhere in the assessment of the pyroxsulam ecotoxicity studies. Such advice indicated 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."
Other observations, if any	None made at this time.	Not applicable.

II. RESULTS AND DISCUSSION

A. PERCENTAGE EMERGENCE AND DEVELOPMENT RATES:

Number of midges emerged
The following table summarises the numbers of male and female midge which emerged in the control and pyroxsulam test vessels over the 28 days of the study.

Table 3. Effect of 7-OH metabolite of pyroxsulam (7-OH XDE-742) on emergence of *Chironomus riparius* adults – number of male and female midges that emerged between days 14 and 27 of the 28 day exposure period. Test vessels (replicates) each initially contained 20 midge larvae/replicate.

Concentration of 7-OH metabolite of pyroxsulam, mg/L, in the overlying water				Replicate 1		Replicate 2		Replicate 3		Replicate 4		Treatment average
Nominal concentrations mg/L	Measured concentrations, mg/L			Male	Female	Male	Female	Male	Female	Male	Female	Total males and females emerged (%)
	1 Hour	Day 7	Day 28									
Control	<0.22	<0.27	<0.28	10	6	8	8	9	9	14	5	86%
7.5	7.2	6.2	4.2	8	10	9	10	13	5	6	13	93%
15	15	13	10	7	11	7	7	8	8	9	8	81%
30	29	27	20	9	8	11	6	7	10	11	8	88%
60	57	55	44	9	8	6	9	11	8	5	10	83%
120	120	100	87	12	5	7	7	9	7	9	10	83%
Solvent control, if used				No solvent control used								
Positive control, if used				No positive control was used								

The study report stated that, following 28 days of exposure, midge percent emergence for males and females combined in the control was 86% which met the minimum standard criteria established by the OECD guideline emergence of •70% and mean emergence between test day 12 and test day 23. As demonstrated by the performance of the control organisms, the exposure system provided conditions which were appropriate for promoting emergence and development of the test species. The study report also stated that at test termination (day 28), the mean percent emergence observed among the midge exposed to nominal treatment levels of 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L was 93, 81, 88, 83 and 83%, respectively and that statistical analysis (Williams' Test) determined no significant difference in mean percent emergence among midge exposed to any of the treatment levels tested as compared to the control.

OECD 219 states that at 18-22°C adults will begin to emerge from the larval rearing vessels after approximately 13-15 days and that males are easily distinguished by having plumose antennae. The observed results are consistent with such statements.

Based on the nominal concentrations of applied test substance and on numbers of midge emerged, the 28-day EC_{50} value was empirically estimated in the study report to be >120 mg 7-OH metabolite of pyroxsulam/L, the highest nominal concentration tested.

The 28-day NOEC and LOEC values for the numbers of midge emerged were not specifically identified in the study report but indicated to be, respectively, 120 and >120 mg 7-OH metabolite of pyroxsulam/L. The William's test statistical treatments for numbers of male and females (separately and combined) indicated the 28 day NOECs and LOECs for numbers of males emerged, numbers of females emerged and numbers of males and females emerged were each respectively, 120 and >120 mg 7-OH metabolite of pyroxsulam/L (page 16 of this DER refers).

Male and female and combined male and female midge development rates
Male and female midge development rates were also calculated over the 28 days of the study with the mean development rates reported (as midges/day) in the study report shown in the following table. Separated rates were determined for male, female and total (combined male and female results) development rates.

The mean development rates for male and female midges and for the males and females combined were as reported in the following table.

Table 4. Mean male, female and combined (male and female) development rates following exposure of first instars of the midge, *Chironomus riparius*, to 7-OH metabolite of pyroxsulam over a 28 day period.

Nominal Concentration, mg of 7-OH pyroxsulam/L	Mean midge development rates		
	Male midge	Female midge	Male and female midge
0 (water control)	0.0617	0.0577	0.0599
7.5	0.0640	0.0590	0.0612
15	0.0587	0.0526	0.0556
30	0.0631	0.0574	0.0606
60	0.0586	0.0524[a]	0.0555[a]
120	0.0625	0.0517[a]	0.0578[a]

a. Statistically significantly different from the control, based on William's test.

Based on the nominal concentrations of applied test substance and on midge development rate (female and male/female combined), the 28-day EC_{50} value was empirically estimated in the study report to be to be >120 mg 7-OH metabolite of pyroxsulam/L, the highest nominal concentration tested. The Lowest-Observed-Effect Concentration (LOEC) [for development rate] was established to be 60 mg 7-OH metabolite of pyroxsulam/L and the No-Observed-Effect Concentration (NOEC) [for development rate] was established to be 30 mg 7-OH metabolite of pyroxsulam/L.

B. EFFECT ON PERCENTAGE EMERGENCE AND DEVELOPMENT RATES OF THE MIDGE LARVAE:

All 7-OH metabolite of pyroxsulam concentrations in the overlying water decreased to be 56 to 73% of the day 0 concentrations and the study report used the day 0 nominal concentrations in the estimation of statistical parameters.

The study report did not indicate that abnormal behaviour of the larvae was observed nor did it indicate that any behavioural abnormalities or other signs of toxicity in the emerged midges were seen, although such behaviour was looked for in the daily observations of the test vessels.

As noted above, the 28 day NOEC and LOEC for emergence in the male, female and combined male and female midges were established as, respectively, 120 and >120 mg 7-OH metabolite of pyroxsulam.

For midge development rates, the male 28 day NOEC and LOEC would respectively be set at 120 and >120 mg 7-OH metabolite of pyroxsulam while the female and combined male and female results had 28 day NOEC and LOEC values of, respectively, 30 and 60 mg 7-OH metabolite of pyroxsulam/L.

The 28 day EC_{50} for midge emergence and development rate (female and male and female combined) was set at >120 mg 7-OH pyroxsulam/L.

C. REPORTED STATISTICS:

With respect to test endpoints and statistical analyses, the study report stated the following:

During the period of expected emergence (typically starting between day 10 through 16 and lasting until day 28), a daily check of emerged midge was made. The sex and number of adult midge that emerged daily were recorded. Male midge were identified by their plumose antennae.

The development rate of male, female and male and female midge combined was determined in each exposure vessel. Mean development time represents the mean time span between the application of the test substance (day 0) and the emergence of the midge.

The development rate is the reciprocal of the development time (unit: 1/day) and represents that portion of larval development which takes place per day. In accordance with the OECD guideline for conducting a life-cycle exposure with *C. riparius*, the development rate is preferred for the evaluation of this type of sediment study as its variance is lower, more homogeneous and is closer to normal distribution as compared to development time. Therefore, powerful parametric statistical procedures may be applied with development rate rather than development time. For the calculation of development rate, the number of midge observed on inspection day x are assumed to be emerged at the mean of the time interval between day x and day x-1 (inspection interval = 1 day). The mean development rate per vessel is calculated according to the following calculation:

$$\text{Mean development rate} = \sum_{i=1}^{m} (F_i * X_i) \div N_e$$

where:

m = maximum number of inspection intervals
i = index of inspection interval
Fi = number of midge emerged in the inspection interval i
Ne = total number of midge emerged until the end of the study (= •Fi)
Xi = development rate of the midge emerged in the interval i

$$X_i = 1 / [day_i - (L_i / 2)]$$

where:
day_i = inspection day (days since application of test substance)
Li = length of inspection interval i (i = 1 day)

Determination of LOEC and NOEC Values

At the termination of the study, data obtained on midge emergence and development rate (as male, female and male/female combined) were statistically analysed to identify significant treatment-related effects. The lowest test concentration that showed a statistically significant effect (Lowest-Observed-Effect Concentration, LOEC) and the highest test concentration that showed no statistically significant effect (No-Observed-Effect Concentration, NOEC) were determined. Analyses were performed using the mean replicate organism response in each treatment group rather than individual response values. All statistical analyses were conducted at the 95% level of certainty except in the case of the Shapiro-Wilks' Test and the Bartlett's Test, in which the 99% level of certainty was applied. The 99% level of certainty is preferred for these qualifying tests. The following procedures were used:

1. Determination of adverse effects on the percent emergence was determined after transformation (e.g., arcsine square-root percentage) of the data.

2. Shapiro-Wilks' Test for normality (Weber *et al.*, 1989) was conducted to compare the observed sample distribution with a normal distribution for all endpoints. The assumption that observations are normally distributed must be validated before subsequent analyses, following parametric

procedures, can be performed. If the data are not normally distributed, then a non-parametric procedure is used for subsequent analyses. Analysis of midge emergence and development rate (as male, female and male/female combined) data met this assumption of normal distribution.

4. As a check on the assumption of homogeneity of variance implicit in parametric statistics, data for each endpoint were analysed using Bartlett's Test (Sokal and Rohlf, 1981). Midge emergence and development rate (as male, female and male/female combined) data passed Bartlett's Test for homogeneity.

5. Williams' Test (Williams, 1971, 1972) is a parametric procedure and is preferred for evaluating data obtained during toxicity tests. For this study, the midge emergence and development rate (as male, female and male/female combined) data met the assumptions for normal distribution and homogeneity, therefore, Williams' Test was used to establish treatment effects on these endpoints.

TOXSTAT® version 3.5 (Gulley *et al.*, 1996) was used to perform the computations. The results were used to establish, at the 95% level of certainty, the lowest test concentration that showed a statistically significant effect (Lowest-Observed-Effect Concentration, LOEC) and the highest test concentration that showed no statistically significant difference (No-Observed-Effect Concentration, NOEC) from the control data.

EC_{50} Calculation
The nominal concentrations tested were used to estimate the median effective concentration (EC_{50}) and 95% confidence intervals. The EC_{50} is the estimated nominal concentration of the test substance which produces a 50% reduction in either emergence rate or development time of the test organisms. During this study, no concentration tested resulted in • 50% reduction in either emergence rate or development rate, therefore, the EC_{50} value was empirically estimated to be > 120 mg 7-OH metabolite of pyroxsulam/L, the highest nominal concentration tested.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Statistical Methods:

Midge emergence
The numbers of midge which emerged (from day 10 to 28 of the exposure period) were provided in the study report as numbers of males and females emerged per day and are reproduced in Table 3 on page 23 of this DER.

Mean percentage emergence values
Average percentage emergence values were determined by the reviewer from the results provided in the study report and were equivalent to the mean percentage emergence values reported.

Calculated values (from Table 3 on page 23 of this DER) are shown in Table 5:

Table 5. Mean percentage emergence values (males and female values combined) as reported and as calculated by the reviewer.

Nominal concentration, mg pyroxsulam/L:	0 (Control)	7.5	15	30	60	120
Mean % emergence (males and females combined) reported in study report:	86	93	81	88	83	83
Reviewer calculated mean % emergence	86	93	81	88	83	83

Validity of combining of male and female midge emergence numbers
Using the Microsoft Excel Tools Data Analysis function, a t-Test (Two Samples assuming unequal variances) was calculated from the control results with the analysis indicating that numbers of male and female midge that emerged in the controls and pyroxsulam test solutions were marginally statistically significantly different but, with the overall decision by the reviewer that biologically the numbers of emerged males and females were not significantly different.

The data used for the calculations are as follows:

Control replicate values	Control numbers of midge emerged	
	Male	Female
Replicate 1	10	6
Replicate 2	8	8
Replicate 3	9	9
Replicate 4	14	5

The Microsoft Excel calculation determined a t value of 2.03.

As the t-statistic determined, 2.03, only marginally exceeds the critical one-tail t value of 2.02 and is less than the two tail critical t-value of 2.57, no practical (biological) difference between the numbers of emerged males and females in the control replicates is considered to have been shown.

The results of the separate re-analyses of the numbers of male and female emerged midges support the study report's decision to combine the numbers of male and female midges for the statistical treatments conducted.

The study report analysed the numbers of male and female midges emerged as a single entity and did not separately analyse the numbers of male midges emerged/test concentration. OECD 219 allows pooling of male and female results where appropriate (paragraph 42 of the guideline states, "If there are no indications of statistically different sensitivities of sexes, male and female results may be pooled for statistical analyses.").

The reviewer has similarly considered only the combined or total numbers of male and female midges which emerged.

Numbers of male and female midges emerged
The combined numbers of male and female midges emerged are obtained from Table 3 on page 23 of this DER and are as follows (with the number expressed as a percentage based on 20 larvae/replicate also shown): Table 6 shows the combined numbers and percentages of emerged male and female midges.

Table 6. Combined numbers of male and female midges that emerged (plus the percentage equivalent based on 20 midge larvae/replicate at the start of the study.

Concentration mg/L:	Control	7.7	15	30	60	120
Replicate A	16 (80%)	18 (90%)	18 (90%)	17 (85%)	17 (85%)	17 (85%)
Replicate B	16 (80%)	19 (95%)	14 (70%)	17 (85%)	15 (75%)	14 (70%)
Replicate C	18 (90%)	18 (90%)	16 (80%)	17 (85%)	19 (95%)	16 (80%)
Replicate D	19 (95%)	19 (95%)	17 (85%)	19 (95%)	15 (75%)	19 (95%)

The ToxCalc analysis of this data (total numbers of male and female midges/replicate/test concentration (without transformation)) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$). Bartlett's Test indicated that the variances were equal ($p = 0.46$). William's Test indicated no difference between the treatments and the controls with the NOEC and LOEC, respectively, 120 and >120 mg 7-OH metabolite of pyroxsulam/L (nominal). The use of the Dunnett's Test produced the same results. When expressed as percentages, the ToxCalc analysis of the data used an Arcsine Square Root transformation and reported a normal distribution (Shapiro-Wilk's Test, $p>0.01$) and equal variances (Bartlett's Test, $p=0.71$). The William's Test again returned NOEC and LOEC values of, respectively, 120 and >120 mg 7-OH metabolite of pyroxsulam/L.

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

The study report's finding that statistical analysis (William's Test) at day 28 determined no significant difference in mean percent emergence among midge exposed to any of the treatment levels tested as compared to the control results is consistent with the reviewer's re-analysis of the combined numbers and percentages of emerged male and female midges.

Because emergence in all treatments was greater than 50%, the reviewer agrees with the study report's decision to set the 28 day EC_{50} for emergence is greater that 120 mg 7-OH metabolite of pyroxsulam/L (or, as calculated by the reviewer based on adjusted pore water concentration, 120 X 0.42 = 50.4 mg/L).

Midge development rates

The reported development rates for male and female midges were verified by recalculation using an Excel spreadsheet procedure based on the reported emergence data (number of male and/or female midges emerged/day between days 10 and 28) and the expression:

$$\text{Mean development rate} = \sum_{i=1}^{m} (F_i * X_i) \div N_e$$

as reported under "C. REPORTED STATISTICS" on page 24 of this DER.

The reported mean development rate (per sex) for each replicate in the controls and each test concentration were also verified by recalculation (by summation of the individual emergence values over the 10 to 28 day period).

Similarly, the reported mean development rates per treatment (control, 7.5 mg/L etc) were verified by recalculation as the average of the four male, female and male and female replicates.

The study report used the William's test to analyse the male development rate data and reported no statistically significant difference between the mean development rates for the 7.5 to 120 mg/L results and the mean control development rate. For the female midge development rate, the William's test indicated a significant difference in the mean development rates among female midge exposed to 60 and 120 mg 7-OH pyroxsulam/L compared to the female mean control development rate. A similar result was reported for the male and female midge combined mean development rates for midge exposed to the 60 and 120 mg 7-OH pyroxsulam/L concentrations, again

compared to the mean male and female combined midge development rate.

Consequently, the reviewer's statistical analysis was conducted on the mean male, female and combined male and female development rates.

The mean development rates used for the statistical re-analysis are shown in Table 7.

Table 7. Mean male, female and combined male and female midge development rats based on emergences from day 10 to 28

	Mean male midge development rates				Mean female midge development rates				Combined mean male and femal midge development rates			
Replicate:	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Control	0.0631	0.0595	0.0623	0.0620	0.0570	0.0558	0.0562	0.0616	0.0608	0.0577	0.0593	0.06
7.5	0.0641	0.0634	0.0600	0.0685	0.0573	0.0586	0.0580	0.0619	0.0604	0.0609	0.0594	0.06
15	0.0552	0.0609	0.0582	0.0607	0.0522	0.0534	0.0501	0.0547	0.0534	0.0572	0.0541	0.05
30	0.0615	0.0632	0.0660	0.0618	0.0572	0.0547	0.0601	0.0576	0.0595	0.0602	0.0626	0.06
60	0.0630	0.0538	0.0580	0.0595	0.0554	0.0536	0.0479	0.0529	0.0594	0.0537	0.0538	0.05
120	0.0581	0.0681	0.0654	0.0584	0.0469	0.0566	0.0513	0.0518	0.0548	0.0623	0.0593	0.05
Expressed as percentages of the control results, these rates are:												
Control	100	100	100	100	100	100	100	100	100	100	100	10
7.5	102	107	96	110	101	105	103	100	99	106	100	10
15	87	96	93	98	92	96	89	89	88	99	91	9
30	97	104	106	100	100	98	107	94	98	104	106	9
60	100	85	93	96	97	96	85	86	98	93	91	8
120	92	127	105	94	82	101	91	84	90	108	100	8

Because development rates in all treatments were greater than 50% of control values, the reviewer agrees with the study report's decision to set the 28 day EC_{50} for development rate as greater that 120 mg 7-OH metabolite of pyroxsulam / L.

Mean male development rates – ToxCalc re-analysis
The ToxCalc analysis of this data (mean male midge development rates/replicate/test concentration (without transformation)) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$). Bartlett's Test indicated that the variances were equal ($p = 0.49$). William's Test indicated no difference between the treatments and the controls with the NOEC and LOEC, respectively, 120 and >120 mg 7-OH metabolite of pyroxsulam/L (nominal).

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

The study report's findings that the male midge development rates (Mean development rates of male midge in the 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L treatment levels were 0.0640, 0.0587, 0.0631, 0.0586 and 0.0625, respectively) in the 7-OH metabolite of pyroxsulam treatments were not statistically significantly different from that of the control mean value of 0.0617 (William's Test, alpha = 0.05), and that the NOEC and LOEC values for male development rate were, respectively, 120 and >120 mg 7-OH metabolite of pyroxsulam/L, the highest concentration tested, are consistent with the reviewer's re-analysis of the mean male midge development rates.

Mean female development rates
The ToxCalc analysis of this data (mean female midge development rates/replicate/test concentration (without transformation)) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$). Bartlett's Test indicated that the variances were equal ($p = 0.82$). William's Test indicated statistically significant differences between the treatments at the 60 and 120 mg 7-OH metabolite of pyroxsulam/L levels.

A summary of the ToxCalc results are provided on page 37 of this DER.

The study report's findings that the female midge development rates (Mean development rates of female midge in the 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L treatment levels were 0.0590, 0.0526, 0.0574, 0.0524 and 0.0517, respectively) in the 7-OH metabolite of pyroxsulam treatments were statistically significantly different from that of the control mean value of 0.0577 (William's Test, alpha = 0.05) at the 60 and 120 mg 7-OH metabolite of pyroxsulam/L treatments, are consistent with the reviewer's re-analysis of the mean male midge development rates (i.e. a NOEC of 30 mg 7-hydroxy metabolite of pyroxsulam/L is indicated).

Mean combined male and female development rates
While the statistical analysis of the combined development rate data has been conducted by the study, the previous analyses which indicated the male and female development rates were different provides an indication that the pooling is probably not appropriate in this case.

The ToxCalc analysis of this data (mean combined male and female midge development rates/replicate/test concentration (without transformation)) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$). Bartlett's Test indicated that the variances were equal ($p = 0.69$). William's Test indicated statistically significant differences between the treatments at the 60 and 120 mg 7-OH metabolite of pyroxsulam/L levels.

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

The study report's findings that the combined male and female midge development rates (mean development rates of female midge in the 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L treatment levels were 0.0612, 0.0556, 0.0606, 0.0555 and 0.0578, respectively) in the 7-OH metabolite of pyroxsulam treatments were statistically significantly different from that of the control mean value of 0.0599 (William's Test, alpha = 0.05) at the 60 and 120 mg 7-OH metabolite of pyroxsulam/L treatments, are consistent with the reviewer's re-analysis of the mean combined male and female midge development rates.

The study report's conclusion that, based on the nominal concentrations of applied test substance and on midge emergence and development rate (female and male/female combined), the 28 day EC_{50} value was empirically estimated to be >120 mg 7-OH metabolite of pyroxsulam/L, the highest nominal concentration tested, the LOEC at 60 mg 7-OH metabolite of pyroxsulam/L and the NOEC at 30 mg 7-OH metabolite of pyroxsulam/L are considered to have been confirmed by the reviewer's statistical reanalysis of the data presented using the William's test for the analysis. .

Because development rates in all treatments was greater than 50%, the reviewer agrees with the study report's decision to set the 28 day EC_{50} for development rate at greater that 120 mg 7-OH metabolite of pyroxsulam/L.

E. STUDY DEFICIENCIES:

The following table identifies deviations from OECD 219 and other deficiencies remarked upon in the preparation of this DER. These deficiencies are not considered to have adversely affected either the study's conduct or its outcomes.

Table 8. Deviations from OECD 219 and other study deficiencies

Parameter	Study reported results	Guideline value/comment
Overlying water parameters, total organic carbon content, particulate matter, metals and chlorine contents	Numerical values for these parameters were not supplied in the test report.	While OECD 219 recommends various values for some of these parameters, the study report's statement that, "Midge were maintained in water from the same source as the overlying water utilized in this study and have successfully survived and reproduced over several generations. The acceptable performance of the midge cultures, in combination with the previously mentioned analyses, confirmed the acceptability of this overlying water for use during the conduct of bioassays." is indicative that the values of these parameters were such that the test's conduct and results are considered valid.
Overlying water parameters, pesticides	The study report noted that representative samples of the overlying water source were analysed periodically for the presence of pesticides, PCBs and toxic metals and that none of these compounds have been detected at concentrations that are considered toxic in any of the water samples analyzed. No actual results were presented.	OECD 219 recommends as acceptable values for dilution water: Total organophosphorus pesticides <50 ng/L and Total organochlorine pesticides plus polychlorinated biphenyls <50 ng/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 OECD 219. With an average 97.5% emergence of midges in the control solutions, the levels of pesticide residues present are not considered to have adversely affected the study.
Sediment	The wet weight of the sediment in each jar averaged 151 g (98 g dry weight).	The OECD Guideline notes that the test report must provide the ".... weight of wet sediment with and without pore water; ....". While specific detail on the weight of wet sediment with and without pore water was not identified in the study, this is not considered a significant deficiency, especially as details on water and sediment volumes and heights were provided.
	Particle size distribution not reported.	OECD 219 makes recommendations for the particle size of the peat and quartz sand used to make up the sediment, e.g. the peat particle size should be <1 mm and the quartz sand should be made predominantly of fine sand with more than 50% of the particles between 50 and 200 µm.
Specific OECD 219 requirements not addressed elsewhere in this DER		
OECD 219 states (paragraph 52) that the test report must at least provide, *inter alia,* the following information:		
Test substance:		
structural formula	Not provided.	Not considered a serious deficiency because of the structure being provided in other documentation provided by the applicant.

Results:

- replacement of evaporated test water, if any.	Study report did state that evaporation was monitored and water levels were brought back to original levels as necessary.	Not considered a serious deficiency on the basis that evaporation was not identified as excessive, the test vessels were loosely covered and any water addition was to bring the level/s back to the original levels.

These deviations and/or deficiencies are not considered to have significantly affected the conduct of the study or the validity of the results obtained.

F. REVIEWER'S COMMENTS:

The study was satisfactorily conducted using controls and the 7-OH metabolite of pyroxsulam at concentrations of 7.5, 15, 30, 60 and 120 mg 7-OH metabolite of pyroxsulam/L (corrected for the 99% purity of the active constituent).

Emergence in all treatments was greater than 50% and an EC_{50} value for emergence was empirically estimated as >120 mg 7-OH metabolite of pyroxsulam/L, the highest nominal concentration tested. Similarly, because no concentration tested resulted in • 50% reduction in development rate, therefore, the EC_{50} value for this parameter was empirically estimated as the same value, i.e. >120 mg/L.

The 28 day NOECs based on male and female emergence numbers and on combined male and female numbers were each 120 mg 7-OH metabolite of pyroxsulam/L based on use of William's Test for comparison of the means of the relevant test (exposed) and control means.

The 28 day NOEC based on male development rate was 120 mg 7-OH metabolite of pyroxsulam/L (nominal, corrected for purity of the 7-OH metabolite of pyroxsulam). The 28 day NOECs based on female and on combined (male and female) development rates were each 30 mg 7-OH metabolite of pyroxsulam/L with the 28 day LOECs for emergence both 60 mg 7-OH metabolite of pyroxsulam/L.

Consequently, the 7-OH metabolite of pyroxsulam, as the active constituent, is considered very slightly chronically toxic to the instars of the midge, *Chironomus riparius* (NOEC > 1 mg/L).

Based on an adjusted mean pore water concentration, the reviewer calculated the above NOEC and LOEC values as:

28 day EC50 for combined male and female emergence numbers: and separate male and female development rates	>50.4 mg/L (>120 X 0.42)
28 day NOEC for combined male and female emergence numbers:	50.4 mg/L
28 day NOEC for the male development rate:	50.4 mg/L
28 day NOEC for female development rate:	12.6 mg/L.

The validity criteria for OECD 219 are considered to have been met by the study and the study deficiencies or deviations from the guidelines identified are not considered to have adversely affected the study or its outcomes.

The recalculated statistical analysis of the data presented gave results in accord with those reported in the study report.

Definitive test dates for the study were 14 March to 11 April 2006.

The PMRA has verified whether the development rate in the control varied between males and females. A t-test detected a significant difference between the development rates of both sexes ($p<0.05$). This supports

the notion that the development rates of midges vary between sexes. The PMRA will not consider the results of the development rates for both sexes combined. The most sensitive endpoint used for risk assessment purposes is the NOEC of 30 mg 7-OH metabolite of pyroxsulam/L, based on female development rate. The output of the statistical verification of the PMRA reviewer is below.

t-test Thursday, July 05, 2007, 10:55:34

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.645)

Group Name	N	Missing	Mean	Std Dev	SEM
male	4	0	0.0617	0.00165	0.000825
female	4	0	0.0577	0.00268	0.00134

Difference 0.00402

t = 2.558 with 6 degrees of freedom. (P = 0.043)

95 percent confidence interval for difference of means: 0.000174 to 0.00788

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.043).

Power of performed test with alpha = 0.050: 0.503

The power of the performed test (0.503) is below the desired power of 0.800.
Consequently, the interpretation of the negative findings should be done with caution.

G. CONCLUSIONS:

This study is classified as acceptable and considered consistent with the guideline requirement for a sediment-water toxicity test using spiked water study on the larvae of the midge, *Chironomus riparius*.

The 28 day NOEC based on combined male and female emergence numbers was 120 mg 7-OH metabolite of pyroxsulam/L or, based on adjusted pore water concentrations, 50.4 mg/L (as calculated by the reviewer).

The 28 day NOEC based on male development rate was 120 mg 7-OH metabolite of pyroxsulam/L. The 28 day NOECs based on female development rates was 30 mg 7-OH metabolite of pyroxsulam/L with the 28 day LOEC for emergence being 60 mg 7-OH metabolite of pyroxsulam/L. Based on adjusted pore water concentrations, these results have been calculated by the reviewer as equivalent to, respectively, 50.4, 12.6 and 25.2 mg/L.

Consequently, the 7-OH metabolite of pyroxsulam, as the active constituent, is considered very slightly chronically toxic to the instars of the midge, *Chironomus riparius* (NOEC > 1 mg/L).

III. REFERENCES:

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Approved 04/01/01 C.K.

Attachment 1.1

Total numbers of male and female chironomids emerged

The numbers of emerged male and female chironomids are discussed on page 27 of this DER.
ToxCalc analysis of the numbers of male and female midge emerged/replicate/test concentration gave the following results:

Conc-mg/L	1	2	3	4
B-Control	16.000	16.000	18.000	19.000
7.5	18.000	19.000	18.000	19.000
15	18.000	14.000	16.000	17.000
30	17.000	17.000	17.000	19.000
60	17.000	15.000	19.000	15.000
120	17.000	14.000	16.000	19.000

			Transform: Untransformed					1-Tailed		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
B-Control	17.250	1.0000	17.250	16.000	19.000	8.696	4			
7.5	18.500	1.0725	18.500	18.000	19.000	3.121	4	-1.137	1.730	1.902
15	16.250	0.9420	16.250	14.000	18.000	10.510	4	0.341	1.820	2.001
30	17.500	1.0145	17.500	17.000	19.000	5.714	4	0.341	1.850	2.034
60	16.500	0.9565	16.500	15.000	19.000	11.605	4	0.682	1.860	2.045
120	16.500	0.9565	16.500	14.000	19.000	12.616	4	0.682	1.870	2.056

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96082	0.884	0.15044	-0.5478
Bartlett's Test indicates equal variances (p = 0.46)	4.6368	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs B-Control	120	>120			2.05558	0.11916	2.86667	2.41667	0.35458	5, 18

When calculated as total percentage emergence (based on 20 larvae/replicate at test initiation), the ToxCalc analysis is:

Conc-mg/L	1	2	3	4
B-Control	0.8000	0.8000	0.9000	0.9500
7.5	0.9000	0.9500	0.9000	0.9500
15	0.9000	0.7000	0.8000	0.8500
30	0.8500	0.8500	0.8500	0.9500
60	0.8500	0.7500	0.9500	0.7500
120	0.8500	0.7000	0.8000	0.9500

			Transform: Arcsin Square Root					1-Tailed		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
B-Control	0.8625	1.0000	1.2022	1.1071	1.3453	9.693	4			
7.5	0.9250	1.0725	1.2972	1.2490	1.3453	4.283	4	-1.180	1.730	0.1393
15	0.8125	0.9420	1.1301	0.9912	1.2490	9.670	4	0.360	1.820	0.1466
30	0.8750	1.0145	1.2161	1.1731	1.3453	7.079	4	0.360	1.850	0.1490
60	0.8250	0.9565	1.1532	1.0472	1.3453	12.239	4	0.602	1.860	0.1498
120	0.8250	0.9565	1.1542	0.9912	1.3453	12.819	4	0.602	1.870	0.1506

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94643	0.884	0.44356	-0.6227
Bartlett's Test indicates equal variances (p = 0.72)	2.86798	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs B-Control	120	>120			0.11639	0.13375	0.01481	0.01297	0.37471	5, 18

Note: The replicate values shown are percentage values expressed as fractions.

Attachment 1.2
Midge emergence and development rates

Males
The mean development rates of male midges are discussed on page 29 of this DER.

ToxCalc analysis of the mean male development rate/replicate/test concentration:

Conc-mg/L	1	2	3	4
D-Control	0.0631	0.0595	0.0623	0.0620
7.5	0.0641	0.0634	0.0600	0.0685
15	0.0552	0.0609	0.0582	0.0607
30	0.0615	0.0632	0.0660	0.0618
60	0.0630	0.0538	0.0580	0.0595
120	0.0581	0.0681	0.0654	0.0584

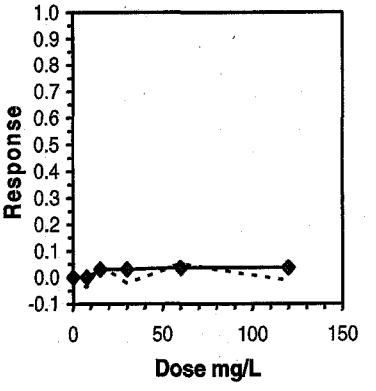
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.0617	1.0000	0.0617	0.0595	0.0631	2.518	4				0.0629	1.0000
7.5	0.0640	1.0369	0.0640	0.0600	0.0685	5.459	4	-0.972	1.730	0.0041	0.0629	1.0000
15	0.0588	0.9518	0.0588	0.0552	0.0609	4.539	4	0.336	1.820	0.0043	0.0609	0.9694
30	0.0631	1.0227	0.0631	0.0615	0.0660	3.255	4	0.336	1.850	0.0043	0.0609	0.9694
60	0.0586	0.9490	0.0586	0.0538	0.0630	6.506	4	0.507	1.860	0.0044	0.0605	0.9630
120	0.0625	1.0126	0.0625	0.0581	0.0681	8.050	4	0.507	1.870	0.0044	0.0605	0.9630

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9674	0.884	0.06146	-0.6876
Bartlett's Test indicates equal variances (p = 0.49)	4.44926	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	120	>120			0.00438	0.07094	2.1E-05	1.1E-05	0.14437	5, 18

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>120			
IC10	>120			
IC15	>120			
IC20	>120			
IC25	>120			
IC40	>120			
IC50	>120			



Attachment 1.3
Midge emergence and development rates
Females
The mean development rates of female midges are discussed on page 29 of this DER.

ToxCalc analysis of the mean female development rate/replicate/test concentration:

Conc-mg/L	1	2	3	4
D-Control	0.0570	0.0558	0.0562	0.0616
7.5	0.0573	0.0586	0.0580	0.0619
15	0.0522	0.0534	0.0501	0.0547
30	0.0572	0.0547	0.0601	0.0576
60	0.0554	0.0536	0.0479	0.0529
120	0.0469	0.0566	0.0513	0.0518

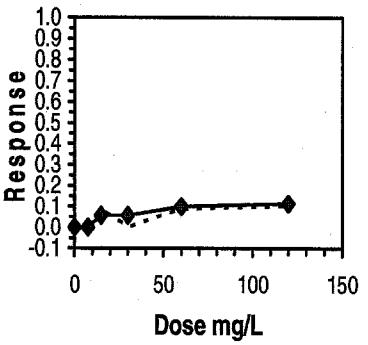
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.0577	1.0000	0.0577	0.0558	0.0616	4.649	4				0.0583	1.0000
7.5	0.0590	1.0225	0.0590	0.0573	0.0619	3.456	4	-0.663	1.730	0.0034	0.0583	1.0000
15	0.0526	0.9124	0.0526	0.0501	0.0547	3.716	4	1.352	1.820	0.0036	0.0550	0.9434
30	0.0574	0.9957	0.0574	0.0547	0.0601	3.851	4	1.352	1.850	0.0036	0.0550	0.9434
*60	0.0525	0.9098	0.0525	0.0479	0.0554	6.122	4	2.653	1.860	0.0036	0.0525	0.8997
*120	0.0517	0.8959	0.0517	0.0469	0.0566	7.680	4	3.062	1.870	0.0037	0.0517	0.8859

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97743	0.884	0.04345	-0.1153
Bartlett's Test indicates equal variances (p = 0.82)	2.1826	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	30	60	42.4264		0.00366	0.06357	4.1E-05	7.7E-06	0.00333	5, 18

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	14.125	13.270	6.719	77.544	1.2029
IC10	59.765				
IC15	>120				
IC20	>120				
IC25	>120				
IC40	>120				
IC50	>120				



Attachment 1.4
Midge development rates
Combined male and female rates
The mean development rates of the combined male and female midge results are discussed on page 29 of this DER.

ToxCalc analysis of the combined mean male and female development rate/replicate/test concentration:

Conc-mg/L	1	2	3	4
D-Control	0.0608	0.0577	0.0593	0.0619
7.5	0.0604	0.0609	0.0594	0.0639
15	0.0534	0.0572	0.0541	0.0578
30	0.0595	0.0602	0.0626	0.0600
60	0.0594	0.0537	0.0538	0.0551
120	0.0548	0.0623	0.0593	0.0549

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.0599	1.0000	0.0599	0.0577	0.0619	3.048	4				0.0605	1.0000
7.5	0.0612	1.0204	0.0612	0.0594	0.0639	3.167	4	-0.725	1.730	0.0029	0.0605	1.0000
15	0.0556	0.9282	0.0556	0.0534	0.0578	3.951	4	1.080	1.820	0.0031	0.0581	0.9597
30	0.0606	1.0108	0.0606	0.0595	0.0626	2.281	4	1.080	1.850	0.0031	0.0581	0.9597
*60	0.0555	0.9262	0.0555	0.0537	0.0594	4.824	4	1.930	1.860	0.0031	0.0567	0.9360
*120	0.0578	0.9650	0.0578	0.0548	0.0623	6.307	4	1.930	1.870	0.0032	0.0567	0.9360

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94222	0.884	0.53744	-0.6214
Bartlett's Test indicates equal variances (p = 0.69)	3.06455	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Williams' Test Treatments vs D-Control	30	60	42.4264		0.00316	0.05275	2.5E-05	5.7E-06	0.00905	5, 18

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	42.300			
IC10	>120			
IC15	>120			
IC20	>120			
IC25	>120			
IC40	>120			
IC50	>120