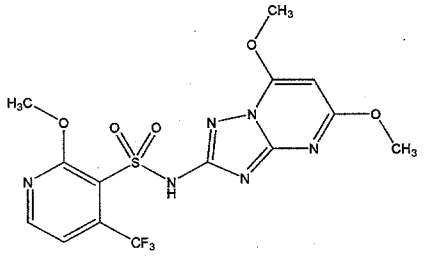
Data Evaluation Report on the toxicity of pyroxsulam (XDE-742) to fish, early life cycle

PMRA Submission Number 2006-4727; ID 1283215 EPA MRID Number 469084-30 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.5.3.1
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.2.4
	EPA Guideline:	72-4 (OPPTS 850.1400)

Test material: **Pyroxsulam (XDE-742)** **Purity (%):** 98%
Common name: Pyroxsulam or XDE-742
Chemical name: 3-pyridinesulfonamide, N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
IUPAC: *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name: *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.: 422556-08-9
Synonyms: X666742, XR-742
Test substance Number: TSN103826

Chemical Structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/02/08 **Date:** 12 December 2006
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewers: Jack Holland 22/2/08 **Date:** 15 December 2006
Australian Government Department of the Environment, Water, Heritage and the Arts

Thomas Steeger, Ph.D., Senior Biologist T Steeger 4/13/08 **Date:** 17 February 2007
Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Émilie Larivière Émilie Larivière 05/03/08 **Date:** 3 July 2007
Environmental Assessment Directorate, Pest Management Regulatory Agency

Company Code: [For PMRA]
Active Code: [For PMRA]
Use Site Category: [For PMRA]
EPA PC Code: 108702

CITATION: Marino, T. A. Hales, C. A. McClymont, E. L. and Yaroch, A. M. 2005. XDE-742: Toxicity to the Early-life Stages of the Fathead Minnow, *Pimephales promelas*. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID 051007. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 03 August 2005. Unpublished report.

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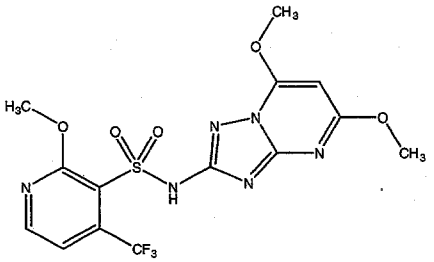

Primary Reviewer: Daryl Murphy **Date:** 12 December 2006
Australian Government Department of the Environment and Water Resources.

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EXECUTIVE SUMMARY:

The 40-day chronic toxicity of pyroxsulam) to early life stages of the fathead minnow, *Pimephales promelas*, was studied under flow-through conditions. Fertilized eggs/embryos (25 embryos/replicate, 4 replicates/control and test concentration with the embryos ≤24 hours old (tailbud stage)) of the fathead minnow were exposed to control, and nominal and mean measured concentrations of, respectively, 0.778, 1.30, 2.16, 3.60, 6.00 and 10.0 and 0.836, 1.28, 2.23, 3.62, 6.11, and 10.1 mg pyroxsulam/L. The test system was maintained at 25±2°C and a pH of 7.1-7.3 (controls) and 7.0 to 7.5 (test concentrations).

The sub-lethal effects examined for included were immobility, pale colouration, curved body/spine, bent spine (scoliosis), smallness, and edema. No treatment related effects on the number of embryos hatched, time to hatch, mortality of embryos, larvae, and juveniles and measurement of growth were observed. Small numbers of sub-lethal effects were seen from day 2 through to day 7 of the exposure in both the controls (in 2 of 99 hatched embryos at day 7) and the test concentrations (in 0 of 96 to 5 of 98 hatched at day 7).

The NOEC/NOAEC, LOEC/LOAEC and MATC values, based on mortality/sub-lethal effects (abnormal larvae or juvenile fish exhibiting lethal or sub-lethal effects, and growth (weight, dry and length) of surviving fish at test termination), were 10.1, >10.1 and >10.1 mg pyroxsulam/L, respectively
Based on the results of this study, pyroxsulam (98% active constituent) would be classified as very slightly toxic to fathead minnow in accordance with the chronic toxicity classification system of the Australian Government Department of the Environment and Water Resources (NOEC >1 mg/L).

This toxicity study is scientifically sound and classified as acceptable by the Australian Government Department of the Environment and Water Resources and the PMRA but, as it has not established a definitive LOAEC value, the US EPA finds that the study has not as such satisfied guideline study requirements and is classified as supplemental by that agency.

Results Synopsis

Test Type: Flow-through

Test Organism Size/Age

Mean dry weight: 7.33 mg (range 6.90 to 7.95 mg) in controls and 6.94 to 8.59 mg (range 6.53 to 10.8 mg) in test concentrations.

Mean length: 17.80 mm (range 17.75 to 19.24 mm) in controls and 17.75 to 19.24 mm (range 15.85 to 20.42 mm) in test concentrations.

Age: ≤24 hours (tailbud stage)

LOEC/LOAEC values for effects measured: All >10.1 mg pyroxsulam/L
NOEC/NOAEC values for effects measured: All 10.1 mg pyroxsulam/L

Endpoint(s) Effected: none (NOTE: there were no significant effects at any treatment level compared to controls, so no endpoints were effected.)

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study was said to generally conform to current procedures described in the following guidelines: Organisation for Economic Cooperation and Development (OECD) guideline for testing of chemicals No. 210 "Fish Early-Life Stage Toxicity Test", and the U.S. Environmental Protection Agency (U.S. EPA) Pesticide Assessment Guideline 72-4 and Standard Evaluation Procedure, "Fish Early Life-Stage".

Guidelines appear to have been generally followed with some minor deviations reported on occasion (see relevant text entries below and also the Study Deficiencies entry on page 39 of this DER).

COMPLIANCE:

All facets of testing were stated as conducted following the OECD/EC Good Laboratory Practice Standards and the U.S. EPA Good Laboratory Practice Standards, with the following referenced in the study report -

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM (98) 17; European Parliament and Council Directive 20041 101EC (0. J. No. L 50/44, 20/0212004); and U.S. Environmental Protection Agency - FIFRA GLPs; Title 40 CFR, Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

The signed and dated GLP Compliance Statement for the study was provided. The signed and dated Quality Assurance Statement for the study was provided. The signed and dated Statement of No Data Confidentiality for the study was provided.

A. MATERIALS:

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

Description: Solid and white powder (latter as stated in the Certificate of Analysis for XDE-742)

Lot No./Batch No. : E0952-52-01 and TSN103826 (latter as stated in Appendix A1, Certificate of Analysis for XDE-742)

Purity: 98%

Stability of Compound: The 26-day stability of XDE-742 in acetonitrile was reported as determined in a related pyroxsulam study with *Daphnia magna* (Marino *et al.,* 2004) by analyzing a stock solution (nominal concentration 515 µg pyroxsulam/mL acetonitrile) that had been stored for 26 days at ~8°C. The measured concentration after 26 days was 104% of nominal.

During the study's 40 day exposure phase, the mean measured concentrations of pyroxsulam in the test concentrations (0.778 to 10.0 mg pyroxsulam/L) ranged from 98.3 to 107% of target (nominal) concentrations, indicative of the pyroxsulam's being stable during the exposure (page 5 of this DER refers).

Storage conditions of

test chemicals: Room temperature in the dark (Marino, 2005)

Physicochemical properties of pyroxsulam

Parameter	Values	Comments
Water solubility at 20°C		
pH 4	0.0164 g/L	Turner (2004a)
pH 6	0.0626 g/L	Turner (2004a)
pH 7	3.2 g/L	Turner (2004a)
pH 9	13.7 g/L	Turner (2004a)
Vapour pressure	<1E-7	Madsen (2003)
UV absorption: Not available at the time of publication of the company's study profile template.		
pKa	4.670	Cathie (2004)
Kow		
pH 4	9.700E-02	Turner (2004b)
pH 7	2.400E-02	Turner (2004b)
pH 9	1.210E+01	Turner (2004b)

Note: The physicochemical properties of pyroxsulam were not reported in the study. The values recorded here come from the company's study profile template report (Dow Chemical Company study ID: 051007.SPT (Marino, 2005)).

2. Test organism:

Species: Fathead minnow (*Pimephales promelas*)

Age /embryonic stage at test initiation:
The embryos were ≤24 hours old (tailbud stage) based on microscopic observation.

Method of collection of the fertilized eggs:
The embryos were obtained from a commercial supplier.

Source: Aquatic BioSystems, Fort Collins, Colorado

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding study:
The study report stated that the conduct of a probe study was not necessary because a fathead minnow early-life stage test with pyroxsulam had previously been conducted by BASF (understood to be Bogi (2003) but not seen) and resulted in a NOEC of 3.2 mg/L and a LOEC of 10.0 mg/L, based on nominal concentrations. In addition, the study reported that pyroxsulam has been categorized as 'practically non-toxic' to fish on an acute basis (LC50 >100 mg/L) with the measured acute LC50 value with rainbow trout (*Oncorhynchus mykiss*) reported by the laboratory >100 mg/L (Marino and Yaroch, 2001, not seen). It was also noted in the study report that the OECD Guideline (No. 210, Fish Early-Life Stage Toxicity Test) states that concentrations of the substance higher than the 96-hour LC50 or 10 mg/L, whichever is the lower, need not be tested. Based on this information, the definitive study was conducted with the highest nominal concentration tested at 10.0 mg/L.

b) Definitive Study

Table 1. Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions: (same as test or not) Feeding (type, source, amount given, frequency): Health: (any mortality observed)	Parental acclimation information not provided, embryos were obtained from a commercial supplier.	See deficiencies/deviations table on page 39 of this DER. Only embryos appearing normal (stereomicroscope) were used to start the test. Abnormal or fungus infected embryos were discarded.
Number of fertilized eggs/embryos in each treatment at test initiation	4 replicates for control and test solutions, 25 embryos/replicate or 100 embryos per treatment at test initiation	See deficiencies/deviations table on page 39 of this DER. Number of fish/treatment for post-hatch exposure at day 6, day when hatching was complete in all vessels, ranged from 24-25 in controls and 22-25 in the test concentrations. *(EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure)*
Concentration of test material: Nominal:	0 (laboratory dilution water control), 0.788, 1.30, 2.16, 3.60, 6.00, and 10.0 mg pyroxsulam/L	See deficiencies/deviations table on page 39 of this DER. Note that the primary feedstock solution was prepared at a nominal concentration of 100 mg pyroxsulam/L without reference to correction to pure active constituent being made.

Parameter	Details	Remarks *Criteria*
Measured:	No pyroxsulam was detected in the water control solutions with the lowest level quantified (LLQ) of 0.08 mg/L. The mean measured exposure concentrations over 40 days were 0.836, 1.28, 2.23, 3.62, 6.11, and 10.1 mg/L, equivalent to 107, 98.3, 103, 100, 102 and 101% of target (nominal) concentrations. Analyses of test solutions were conducted on days 0, 7, 14, 21, 28, 35 and 40. A study average and percent of target were calculated for each dose level by averaging the daily measured concentrations. Weekly analyses of test solutions were also conducted.	*(EPA requires a minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.* *- Toxicant conc. must be measured in one tank at each toxicant level every week.* *- One concentration must adversely affect a life stage and one concentration must not affect any life stage.* *OECD requires 5 concentrations spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution must be within ± 20% of the mean measured values)*
Solvent (type, percentage, if used)	No solvent used.	Requirement met *(EPA requires that solvent should not exceed 0.1 mL/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD requires that solvent must have no effect on survival nor produce any other adverse effects; concentration should not be greater than 0.1 ml/L)*
Number of replicates Control: Solvent control: Treatments:	 4 Not applicable 4 per test concentration	Requirement met *(EPA requires 4 replicates per concentration* *EPA/OECD require solvent control when a solubilizing agent has been used)*
Test condition:		Requirement considered met.

Parameter	Details	Remarks *Criteria*
Static renewal/flow through:	Flow through	*(EPA requires: intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used. Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%)*
Type of dilution system for flow through method: Flow rate:	An intermittent-flow proportional diluter system was used During the test, the diluter system provided an average of 16.5 volume turnovers in each test vessel during each 24-hour period.	The diluter was calibrated so that the concentration of the test substance in each treatment was approximately 60% of that in the next higher treatment level. System contained a diluter mixing chamber. Data to show the test solution is completely mixed before introduction into the test system was not located but may be inferred from the weekly analyses of the mixing chamber (9.68 to 10.7 mg pyroxsulam/L cf. a 10 mg pyroxsulam/L target) and dose stocks (92.7-104% of the 100 mg pyroxsulam/L target). Similarly, data on the flow splitting accuracy may be inferred from the measured test concentrations being 98.3 to 107% of nominal over the 40 days exposure. Fresh diluter feedstock was prepared on a weekly basis during the study.
Renewal rate for static renewal:	Not applicable	

Parameter	Details	Remarks *Criteria*
Aeration, if any	Not indicated as used	See deficiencies/deviations table on page 39 of this DER. Dilution water not indicated as aerated, aeration not used in the system. The group minimum dissolved oxygen contents were 6.2 to 6.7 mg/L over 40 days, taken as equivalent to 77 to 83% saturation. *(EPA requires: dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated)*
Duration of the test	40 days (34 days post-hatch, hatching complete by day 6)	See deficiencies/deviations table on page 39 of this DER. Exceeds EPA and OECD recommended test durations. *(EPA requires 32 days)*
<u>Embryo cups</u>, if used Type/material: (glass/stainless steel) Size: Fill volume:	 Glass (Marino, 2005) 7.5 cm high, 7 cm diameter, circular incubation cups. Cups suspended in a cylindrical glass test chamber, 8.5 cm high, 8.5 cm in diameter. Both cups and chambers had ~360 µm screen covered bottoms and were supported by 1 cm glass beads attached to the screen bottoms. Not applicable, the cups were placed within the test vessels supported by glass beads.	See deficiencies/deviations table on page 39 of this DER. Volumes of the cups are calculated as ~290 mL *(EPA requires 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen)*
<u>Test vessel</u> Type/material: (glass/stainless steel) Size:	 Double strength glass held together with clear silicone 15 cm X 10 cm X 9 cm	Requirement met

Parameter	Details	Remarks *Criteria*
Fill volume:	~850 mL	*(EPA/OECD requires all glass or glass with stainless steel frame)*
Source of dilution water	City of Midland Water Treatment Plant. The water was obtained from the upper Saginaw Bay of Lake Huron near Whitestone Point and was limed and flocculated with ferric chloride. The water is pumped to the laboratory prior to treatment for municipal use. Before laboratory use, the water is sand filtered, pH adjusted (carbon dioxide), carbon filtered and UV irradiated. For other testing see below.	Requirement considered met *(EPA requires natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. OECD accepts any water in which the test species show control survival at least as good as presented in SEP)*
Water parameters: Hardness: pH: Dissolved oxygen: Temperature(s) (record all the temperatures used for different life stages):	 58-82 (control water) and 60-78 (test waters) mg $CaCO_3$/L Control water 7.1-7.3 Test waters 7.0-7.5 Control water 6.7-8.1 mg/L Test waters 6.2-8.2 mg/L Percent oxygen saturation ≥ 77% of saturation over the 40-day exposure period. 25-26°C for all stages	See deficiencies/deviations table on page 39 of this DER. Hardness and pH slightly outside US EPA range. EPA requires hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended. OECD requires DO concentration between 60 - 90% saturation. DO must be measured at each conc. at least once a week; freshwater parameters in a control and one concentration must be analyzed once a week. Temperature depends upon test species; should not deviate by more than 2°C from appropriate temperature. Salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously)

Parameter	Details	Remarks *Criteria*
Photoperiod:	16-hour light/8-hour dark transitional photoperiod during testing.	
Salinity (for marine or estuarine species):	Not applicable	
Other measurements:	Light intensity range Control water 587-655 Lux Test waters 454-660 Lux	
Alkalinity	21-45 (control water) and 38-47 (test waters) mg $CaCO_3$/L	
Conductivity	175-242 (control water) and 174-192 (test waters) µmohs/cm	
Residual chlorine	20 ppb	
Total organic carbon	1100 µg/L	
Total suspended solids, metals and	Below detection levels (1 mg/L for	

Parameter	Details	Remarks *Criteria*
pesticides	total suspended solids) or levels of concern.	Note: The most recent analyses for selected inorganic and organic compounds presented in the study report indicated that pesticide etc. levels in the dilution water were generally satisfactory (See Table 3, Observations, Water Quality Acceptable entry for further details). The study report, in referring to analyses of selected organic species and pesticides in the laboratory dilution water, does not list the detection limits referred to. While this is not considered a problem for the overall assessment of this study as the detection limits are given in Marino *et al.* (2006), the study report on the acute toxicity of the ATSA metabolite of pyroxsulam to the rainbow trout. However, the absence is a deficiency. OECD 210 identifies acceptable total organophosphorus pesticide, organochlorine and polychlorinated biphenyls concentrations for dilution water.

Parameter	Details	Remarks *Criteria*
Interval of water quality measurements:	Dissolved oxygen, pH and temperature data were recorded on test days 0, 7, 14, 21, 28, 35 and 40 (test termination) in each replicate test vessel with surviving organisms; light intensity was measured from each test group location during the same time period. Water temperature was also continuously monitored from one test vessel (2.16 mg/L, replicate B) throughout the study with the continuously monitored temperature reported as ranging from 24 to 26°C. Water quality parameters such as alkalinity, hardness, pH, conductivity, and residual chlorine were measured from samples collected on test days 0, 7, 14, 21, 28, 35, and 40 from the water control group and the highest exposure group with surviving fish. Total organic carbon (TOC), total suspended solids (TSS), and selected inorganic and organic compounds, including pesticides, were typically monitored biannually.	
<u>Post-hatch details:</u> When the post-hatch period began: Number of hatched eggs (alevins)/ treatment released to the test chamber On what day, the alevins were released from the incubation cups to the test chamber	 Day 6, hatching complete in all control and test vessels All hatched eggs released at day 14. 83 to 92 larvae and fish Day 14, incubation cups removed	Requirement met (% of embryos hatched in controls, 80.0, 88.0, 92.0 and 95.8%). *(EPA requires % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup)*
Post-hatch Feeding: Start date: Type/source of feed:	 Within 2 days, following 90% hatch of the controls Newly hatched brine shrimp (*Artemia sp.*)	Requirement considered met. OECD 210 and US EPA OPPTS 850.1400 requirements

Parameter	Details	Remarks *Criteria*
Amount given: Frequency of feeding:	Equal amounts of concentrated brine shrimp were added to each vessel during each feeding with the daily amount varying (0.030 mL to 0.200 mL per feeding) and dependent on the age of the age and size of the fish. Twice daily with at least six hours between feedings. The fish were not fed during the 24 hours preceding test termination.	require feeding newly hatched larvae with newly hatched brine shrimp nauplii *ad libitum* from within 2 days of hatching.
<u>Recovery of chemical:</u> Frequency of measurement: LOQ: LOD: % of nominal (over 40 days)	 At test commencement and termination (days 0 and 40) and at weekly intervals between (days 7, 14, 21, 28 and 35) LOQ and LOD not reported. Lowest level quantified 0.08 mg pyroxsulam/L LDW. (Analyses were by HPLC/UV). Results from the weekly analysis of test solutions yielded percent of target values ranging from 93.8% to 113%. Study average percent of target values for pyroxsulam ranged from 98.3 to 107%.	Requirement considered met. OECD requires evidence must be available to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within ± 20% of the mean measured values. page 14 of this DER summarises the analytical results reported.
Positive control	Positive control not used	
<u>Fertilization success study</u>, if any Number of eggs used: On what day the eggs were removed to check the embryonic development:	 Not applicable Not applicable	
Other parameters, if any Biological loading	Not greater than 0.5 g fish/L of test solution passing through the test vessels in 24 hours and did not exceed 5 g fish/L of test solution at any time. Calculated dry weight measurements indicated mean and maximum loadings to be 0.010 and 0.011 g fish per litre of test solution passing through the test vessels in 24 hours (Mean Test Solution Volume: 16.5 L/vessel/day), and mean and	Requirement met. OECD 210 and US EPA 850.1400 state that for flow-through tests, a loading rate not exceeding 0.5 g/L/24 h and not exceeding 5 g/L of solution at any time has been recommended.

Parameter	Details	Remarks *Criteria*
	maximum loadings of 0.190 and 0.214 g fish/L of test solution at any time point (Test solution volume 0.85 L/vessel).	

A summary of the analytical results reported is given in Table 2.

Table 2. Summary of results from the analysis of test solutions of pyroxsulam.

Target or nominal concentration	Measured concentrations, mg pyroxsulam/L (% of target)							Mean[b] measured concentration
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 40	
0 (LDWControl)	<LLQ[a]	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	NA[c]
0.778	0.821 (106%) –0.839 (108%)	0.838 (108%)	0.848 (109%)	0.829 (107%)	0.812 (104%)	0.877 (113%)	0.831 (107%) – 0.840 (108%)	0.836 (107%)
1.30	1.26 (96.9%) – 1.29 (99.2%)	1.30 (100%)	1.22 (93.8%)	1.28 (98.5%)	1.24 (95.4%)	1.32 (102%)	1.28 (98.5%) – 1.29 (99.2%)	1.28 (98.3%)
2.16	2.20 (102%) – 2.27 (105%)	2.27 (105%)	2.12 (98.1%)	2.22 (103%)	2.17 (100%)	2.28 (106%)	2.22 (103%) – 2.26 (105%)	2.23 (103%)
3.60	3.62 (101%) – 3.67 (102%)	3.71 (103%)	3.42 (95.0%)	3.63 (101%)	3.46 (96.1%)	3.73 (104%)	3.60 (100%) – 3.65 (101%)	3.62 (100%)
6.00	6.12 (102%) – 6.17 (103%)	6.25 (104%)	5.79 (96.5%)	6.06 (101%)	5.89 (98.2%)	6.26 (104%)	6.14 (102%) – 6.15 (103%)	6.11 (102%)
10.0	10.1 (101%) – 10.3 (103%)	10.3 (103%)	9.60 (96%)	10.1 (101%)	9.82 (98.2%)	10.4 (104%)	10.2 (102%)	10.1 (101%)

a. <LLQ = less than the lowest level quantified, 0.08 mg pyroxsulam/L. b. Mean measured concentration = mean of day 0, 7, 14, 21, 28, 35 and 40 measured concentration values. c. NA = not applicable. Day 0 and day 40 results are the ranges reported for the four replicates analysed at those times. Days 7, 14, 21, 28 and 35 are results from analysis of one of the four replicates/control or test solution.

2. Observations:

Table 3. Observations.

Parameters	Details	Remarks Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	The embryos/larvae were observed and counted daily until hatching was complete. During each observation period, the total number of embryos that hatched, any dead embryos/larvae, and any abnormal larvae were recorded; dead or fungus-infected embryos/larvae were removed when recorded. Upon completion of hatching, the percent of embryos that hatched (embryo survival), percent normal larvae (exhibiting no lethal or sub-lethal effects) at hatch, and the day-to-mean hatch (time to hatch) for each replicate were calculated. Thereafter, larvae/juvenile fish were observed for mortality at least once daily and the number dead in each replicate recorded; dead larvae were removed when recorded. In addition, larvae/juvenile fish were observed at least once weekly and at test termination during the study with mortality and sub-lethal effects (abnormal behavior or appearance) recorded; dead larvae/juvenile fish were removed when recorded. At test termination, day 40, the percent larvae/juvenile fish survival (post-hatch survival), percent overall survival and the percent of normal juvenile fish (exhibiting no lethal or sub-lethal effects) at test termination were calculated for each replicate. At test termination, all surviving fish were sacrificed for dry weight (nearest 0.01 mg after ~44 hours at ~60°C) and total length (nearest 0.01 mm using a digital caliper) measurements.	All required parameters considered to have been addressed in study report. *(EPA minimally requires:* *- Number of embryos hatched; Time to hatch;* *- Mortality of embryos, larvae, and juveniles;* *- Time to swim-up (if approp.);* *- Measurement of growth;* *- Incidence of pathological or histological effects;* *- Observations of other effects or clinical signs.*

Parameters	Details	Remarks Criteria
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g. alevins): swim-up behavior: growth measurements: embryonic development: other sub-lethal effects	 Daily Daily Daily Not reported At test termination, day 40 At test initiation Daily to end of hatching and then weekly	Requirements considered met. OECD 210 and US EPA 850.1400 refer to "adequate intervals" for observations.
Water quality was acceptable (Yes/No)	Yes (Dissolved oxygen levels ranged from 6.2–8.2 mg/L and remained greater than or equal to 77% of saturation over the 40-day exposure period. Temperature ranged from 24-26°C, including the continuous monitoring of one replicate vessel (2.16 mg/L replicate B) during the study. The pH ranged from 7.0–7.5 and the light intensity ranged from 454-660 lux.)	Laboratory dilution water quality considered acceptable on the basis of the control results. Comparison of the inorganic analyses of the dilution water (Table 1, page 34 of the study report) and the analyses of pesticides etc in the dilution water (Table 2, page 35, *ibid.*) shows that Guideline levels for total organic carbon and particulate matter were met. Residual chlorine was <20 µg/L, the Guidelines specify <10 µg/L. Similarly the Guidelines refer to unionised ammonia being <1 µg/L, the reported concentration for ammonia was <100 µg/L. Organochlorine and pesticide residues were all below their respective detection limits (0.25 to 5 µg/L, as reported in Marino *et al.*, 2006).
Were raw data included?	Tabulated data included. Raw data reported as archived	Data provided were considered sufficient to allow statistical analysis as required by the Guidelines to be conducted.
Other observations, if any	None	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Embryo Survival

Embryo survival was defined for each test group as the percent of embryos that hatched (at day 6 when hatching was complete) with the reported results summarised in the following table. There were 25 embryos/replicate/test group at the commencement of the exposure period.

Table 4. Summary of Biological Observations for Embryo Survival (% Hatch).

Test Concentrations (mg/L) Mean Measured values	Embryo survival (% Hatch) at day 6 (when hatch was complete and based on 25 embryos/replicate/test concentration) Mean	Standard deviation	Range
0 (Water Control)	99.0%	2.00%	96.0-100%
0.836	99.0%	2.00%	96.0-100%
1.28	98.0%	2.31%	96.0-100%
2.23	98.0%	2.31%	96.0-100%
3.62	98.0%	2.31%	96.0-100%
6.11	96.0%	3.27%	92.0-100%
10.1	98.0%	2.31%	96.0-100%

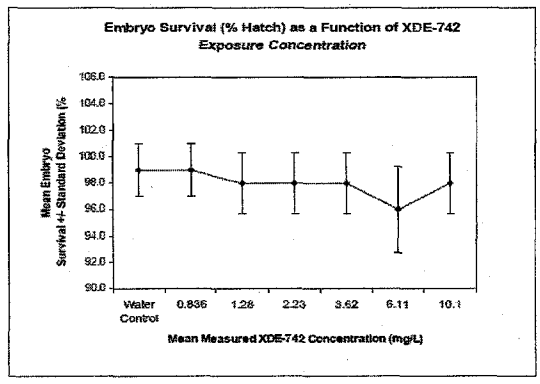
The hatching success in the control group (Table 4, 99 of 100 embryos successfully hatched) satisfied the minimum acceptable control value criteria of 66% according to the OECD 210 test guideline.

The mean percent embryo survival or percent hatch (based on 4 replicates per test group) was 99.0% in the control group and ranged from 96.0 to 99.0% across all treatment groups (Table 4).

No statistically significant differences (alpha = 0.05) in percent embryo survival were observed in any of the treatment groups when compared to the control group.

A plot of mean embryo survival (% of hatch) as a function of the mean measured pyroxsulam concentration did not exhibit a dose-response relationship.

Dose-response Curve for Mean Embryo Survival (% Hatch) Versus Pyroxsulam Exposure Concentration (copied from the study report).



Post-Hatch Survival

Post-hatch survival was defined for each test group as the percent of surviving juvenile fish at test termination based on the number of embryos hatched. Reported results are summarised in Table 5.

Table 5. Summary of Effects of Pyroxsulam on Post-Hatch Survival.

Test Concentration (mg/L) Mean Measured values	Post-hatch survival (as determined from the number of embryos hatched and the number of larvae or fish alive after 40 days).		
	Mean	**Standard deviation**	**Range**
0 (Water Control)	89.0%	6.76%	80.0-95.8%
0.836	82.0%	15.43%	60.0-95.8%
1.28	90.7%	9.26%	79.2-100%
2.23	82.6%	2.31%	79.2-84.0%
3.62	80.8%	13.84%	60.0-88.0%
6.11	90.7%	3.97%	87.5-95.7%
10.1	86.8%	1.85%	84.0-88.0%

The mean percent post-hatch survival in the control group (Table 5, 89%, 88 live fish from 99 embryos) satisfied the minimum acceptable control value criteria of 70 and 80% according to the OECD 210 and FIFRA test guidelines, respectively.

The mean percent post-hatch survival (based on 4 replicates per test group) was 89.0% in the control group and ranged from 80.8-90.7% across all treatment groups (Table 5).

Percent post-hatch survival amongst the individual replicates across the test groups was relatively consistent, with the exception of two replicates, one in the 0.836 and the other in the 3.62 mg/L mean measured treatment groups. Survival in both these replicates was reduced (60% survival) and considered to be biologically insignificant as none of the remaining replicates within these test levels or any of the other treatment groups exhibited similar reductions in survival. The percentages of live fish from the numbers of embryos that hatched for these two concentrations after 40 days are shown in Table 6

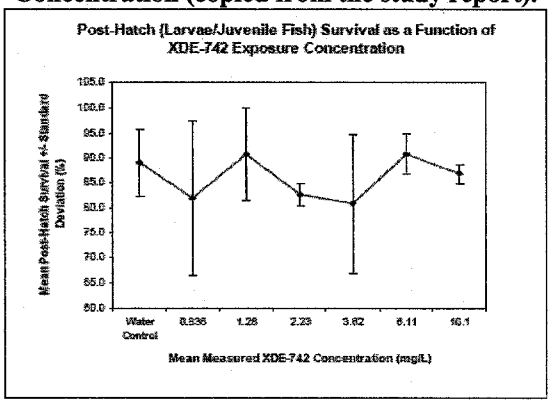
Table 6. Percent post-hatch survival in each of the 4 replicates in the 0.836 mg pyroxsulam/L and 3.62 mg pyroxsulam/L treatments.

Replicate:	A	B	C	D
0.836 mg pyroxsulam/L	88.0% (22 fish from 25 embryos)	84.0% (21 fish from 25 embryos)	95.8% (23 fish from 24 embryos)	60.0% (15 fish from 25 embryos)
3.62 mg pyroxsulam/L	88.0% (22 fish from 25 embryos)	87.5% (21 fish from 24 embryos)	87.5% (21 fish from 24 embryos)	60.0% (15 fish from 25 embryos)

No statistically significant differences (alpha = 0.05) in percent post-hatch survival were observed in any of the treatment groups when compared to the control group.

A plot of post-hatch (larvae/juvenile fish) as a function of the mean measured pyroxsulam concentration did not exhibit a dose-response relationship.

Dose-response Curve for Mean Post-Hatch Survival versus Pyroxsulam Exposure Concentration (copied from the study report).



Overall Survival

Overall survival was defined for each test group as the percent of surviving juvenile fish at test termination based on the initial number of embryos exposed at test initiation (4 replicates of 25 embryos per test group). The reported results are summarised in Table 7.

Table 7. Summary of Effects of Pyroxsulam on Overall Fathead Minnow Survival.

Test Concentration (mg/L) Mean Measured	Overall survival (based on the number of surviving juvenile fish and an initial number of 25 embryos/replicate/test group. Mean	Standard deviation	Range
0 (Water Control)	88.0%	5.66%	80.0-92.0%
0.836	81.0%	14.38%	60.0-92.0%
1.28	89.0%	11.02%	76.0-100%
2.23	81.0%	3.83%	76.0-84.0%
3.62	79.0%	12.81%	60.0-88.0%
6.11	87.0%	3.83%	84.0-92.0%
10.1	85.0%	2.00%	84.0-88.0%

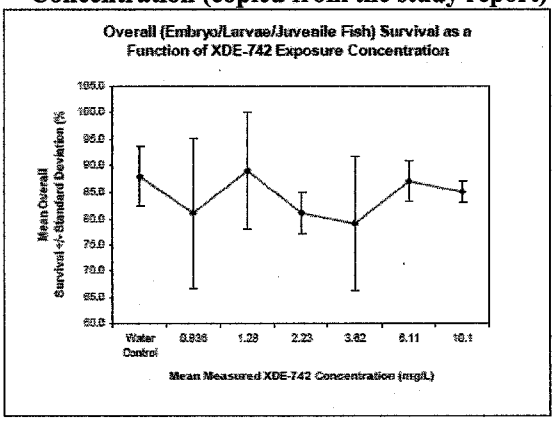
Percent overall survival amongst the individual replicates across the test groups was relatively consistent, with the exception of two replicates, one in the 0.836 and the other in the 3.62 mg/L mean measured treatment groups.

The overall survival rates of both replicates (both at 60%) were equivalent to their post-hatch survival rates and are considered biologically insignificant, as previously described above in Post-Hatch Survival (see previous section), as none of the remaining replicates within these treatment groups or any of the other treatment groups exhibited similar reduction in survival.

The mean percent overall survival (based on 4 replicates per test group) was 88.0% in the control group and ranged from 79.0 to 89.0% across all treatment groups (Table 7). No statistically significant differences (alpha = 0.05) in percent overall survival were observed in any of the treatment groups when compared to the control group.

A plot of mean overall survival as a function of pyroxsulam exposure concentration showed no dose-response relationship.

Dose-response Curve for Mean Overall Survival versus Pyroxsulam Exposure Concentration (copied from the study report)



Summary of mortality results

Table 8, based on the information in the previous three tables dealing with survival, summarises the effect of pyroxsulam on egg hatching and survival at the different life stages of the fathead minnow. The table also includes NOEC/NOAEC, LOEC/LOAEC and MATC values based on the data presented.

The table, taken from Marino (2005), was modified by that author from the Data Evaluation Report Template to better reflect the reporting of the study data presented in the study report.

Table 8. Effect of Pyroxsulam on Egg Hatching and Survival at Different Life Stage of Fish[a] (taken from Marino, 2005). NOEC/NOAEC, LOEC/LOAEC and MATC as mg pyroxsulam/L.

Treatment (mg pyroxsulam/L) [Measured/ Nominal Concentrations]	Egg Hatched/Embryo Viability (Embryo Survival) No. of Eggs at Study Start	Hatch/Embryo Viability Mean (SD)[b] % Hatch	Time to Hatch Mean (SD)[b] Day-to-Mean Hatch (see Table 9 below)	Post-Hatch Survival Mean (SD)[b] % Survival	Overall Survival Mean (SD)[b] % Survival
Negative control (dilution water only)	100	99.0 (2.00)	3.8 (0.50)	89.0 (6.76)	88.0 (5.66)
0.836/0.778	100	99.0 (2.00)	3.8 (0.50)	82.0 (15.43)	81.0 (14.38)
1.28/1.30	100	98.0 (2.31)	3.5 (0.58)	90.7 (9.26)	89.0 (11.02)
2.23/2.16	100	98.0 (2.31)	4.0 (0.00)	82.6 (2.31)	81.0 (3.83)
3.62/3.60	100	98.0 (2.31)	3.8 (0.50)	80.8 (13.84)	79.0 (12.81)
6.11/6.00	100	96.0 (3.27)	3.3 (0.50)	90.7 (3.97)	87.0 (3.83)
10.1/10.0	100	98.0 (2.31)	3.8 (0.50)	86.8 (1.85)	85.0 (2.00)
NOEC[c]	N/A	**10.1**	**10.1**	**10.1**	**10.1**
LOEC[c]	N/A	**>10.1**	**>10.1**	**>10.1**	**>10.1**
MATC[c]	N/A	**>10.1**	**>10.1**	**>10.1**	**>10.1**

a Table modified from the Data Evaluation Report Template to better reflect the reporting of the study data presented in the final report.

b Mean and Standard Deviation (SD) of the four individual replicates per test concentration.

c Results based on mean measured concentrations.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Day-to-Mean Hatch

The day-to-mean hatch was defined for each test group as the observation day in which •50% of the embryos had hatched and is summarized in Table 9.

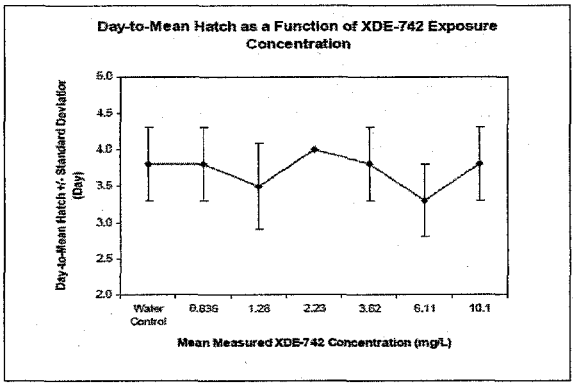
Table 9. Summary of Effects of Pyroxsulam on Day-to-Mean Hatch

Test Concentration (mg/L) Mean Measured	Day-to-mean hatch Mean	Standard deviation	Range
0 (Water Control)	3.8	0.50	3-4
0.836	3.8	0.50	3-4
1.28	3.5	0.58	3-4
2.23	4.0	0.00	4-4
3.62	3.8	0.50	3-4
6.11	3.3	0.50	3-4
10.1	3.8	0.50	3-4

The day-to-mean hatch (based on the mean of 4 replicates per test group) was 3.80 in the control group and ranged from 3.50 to 4.00 across all treatment groups (Table 9). No statistically significant differences (alpha = 0.05) in day-to-mean hatch were observed in any of the treatment groups when compared to the control group.

A plot of mean day-to-mean hatch against pyroxsulam exposure concentration did not show a dose/response relationship.

Dose-response Curve for Day-to-Mean Hatch versus Pyroxsulam Exposure Concentration (copied from the study report).



Hatching commenced at day 2 of the exposure period with the numbers of larvae/fish hatched at that time and at day 6 when hatching was complete described in Table 10.

Table 10. Number of living embryos on Day 2 and Day 6 of exposure.

	Control	0.836 mg/L	1.28 mg/L	2.23 mg/L	3.62 mg/L	6.11 mg/L	10.1 mg/L
Day 2	1 hatched	3 hatched	3 hatched	11 hatched	10 hatched	8 hatched	6 hatched
Day 6	97 hatched	97 hatched	97 hatched	92 hatched	93 hatched	94 hatched	95 hatched

Normal Larvae at Hatch

The normal larvae at hatch was defined for each test group as the percent of larvae exhibiting no lethal or sub-lethal effects (abnormal behaviour or appearance) after the completion of hatching (day 6; hatching complete in all replicate vessels) based on the number of embryos hatched. The results reported are summarised in Table 11.

Table 11. Summary of Effects of Pyroxsulam on Normal Larvae at Hatch.

Test Concentration (mg/L) Mean Measured	Normal at Hatch Mean	Standard Deviation	Range
0 (Water Control)	97.0%	2.04%	95.8-100%
0.836	93.0%	9.45%	80.0-100%
1.28	99.0%	2.10%	95.8-100%
2.23	89.8%	2.38%	87.5-100%
3.62	92.9%	3.81%	88.0-100%
6.11	97.9%	2.42%	95.8-100%
10.1	94.9%	1.94%	92.0-100%

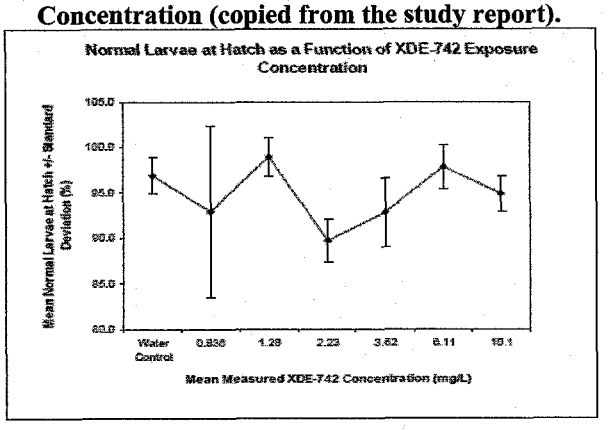
Abnormal larvae (larvae exhibiting lethal or sub-lethal effects) observed at termination of hatch were reported as minimal and sporadic across all test groups. Sub-lethal effects observed were reported to include such effects as pale coloration, curvature of the body, and scoliosis.

The mean percent normal larvae at hatch (based on 4 replicates per test group) was 97.0% in the control group and ranged from 89.8 to 97.9% across all treatment groups (Table 11).

No statistically significant differences (alpha = 0.05) in percent normal larvae at hatch were observed in any of the treatment groups when compared to the control group.

A plot of mean percent normal larvae at hatch as a function of pyroxsulam exposure concentration showed no dose-response relationship.

Dose-response Curve for Mean Normal Larvae at Hatch versus Pyroxsulam Exposure Concentration (copied from the study report).



Larval abnormalities/sub-lethal effects were seen from day 2 to day 7. The abnormalities reported are summarised in Table 12 (page 24 of this DER refers). There were no abnormalities seen in the surviving fish at day 40.

Table 12. Sub-lethal effect of pyroxsulam on larval and juvenile fathead minnows between days 2 and 7 of the exposure period of 40 days.

Treatment (mg pyroxsulam/L) [record measured and nominal concentrations used]	Toxicity Symptoms/Behavioural Effects (specify) at days 2 to 7 (no toxicity symptoms/behavioural effects seen at day 1 or after day 7).					
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Negative control (dilution water only)	none	none	2 curved body	2 curved body	1 curved body	1 pale, 1 curved body
0.836/0.778	none	2 curved body	1 pale and immobile, 1 pale with curved body, 1 pale, 2 curved body	1 pale with curved body, 3 pale, 2 curved body	3 pale; 2 curved body	1 pale, 2 curved body
1.28/1.30	none	none	none	none	none	none
2.23/2.16	1 oedema	1 oedema, 1 pale, 2 curved body	1 pale with curved body, 2 curved body, 1 immobile	1 pale with curved body, 1 pale, 1 curved body	3 pale; 2 curved body	1 pale with curved body, 3 pale, 1 curved body
3.62/3.60	none	1 pale with curved body, 1 curved body	1 curved body, 2 bent spine	1 pale, 1 curved body	1 pale; 1 curved body	1 curved body
6.11/6.00	1 small, 1 immobile	1 pale	none	none	none	none
10.1/10.0	none	none	1 pale, 1 bent spine	1 pale, 1 bent spine	2 pale, 1 bent spine	1 pale with bent spine

Normal Juvenile Fish at Test Termination

The normal juvenile fish at test termination was defined for each test group as the percent of larvae exhibiting no lethal or sub-lethal effects (abnormal behaviour or appearance) at test termination (day 40) based on the number of embryos hatched. The results reported are summarised in Table 13. Note that the values in this table are the same as those reported in Table 5 (Post-hatch survival, page 18 of this DER) as a result of the two definitions being equivalent.

Table 13. Summary of Effects of Pyroxsulam on Normal Juvenile Fish at Test Termination.

Test Concentration (mg/L) Mean Measured	Normal at Hatch Mean	Normal at Hatch Standard Deviation	Normal at Hatch Range
0 (Water Control)	89.0%	6.76%	80.0-95.8%
0.836	82.0%	15.43%	60.0-95.8%
1.28	90.7%	9.26%	79.2-100%
2.23	82.6%	2.31%	79.2-84.0%
3.62	80.8%	13.84%	60.0-88.0%
6.11	90.7%	3.97%	87.5-95.7%
10.1	86.8%	1.85%	84.0-88.0%

The number of juvenile fish (fish exhibiting lethal or sub-lethal effects) observed at test termination was reported as minimal and sporadic across all test groups, with the exception of one replicate in each of the 0.836 and 3.62 mg/L mean measured treatment groups.

The reduction in the percent of normal fish in these replicates was stated to be due to the reduction in overall survival in both these replicates, which exhibited 60% survival. The effects observed in these replicates were considered biologically insignificant since none of the remaining replicates within these test levels, or any of the other treatment groups, exhibited similar reduction in survival.

Sub-lethal effects observed during the study were reported as including edema, small size, pale coloration, immobility, curvature of the body, and scoliosis, and were reported as no longer present following observations on exposure day 14 and for the remainder of the test.

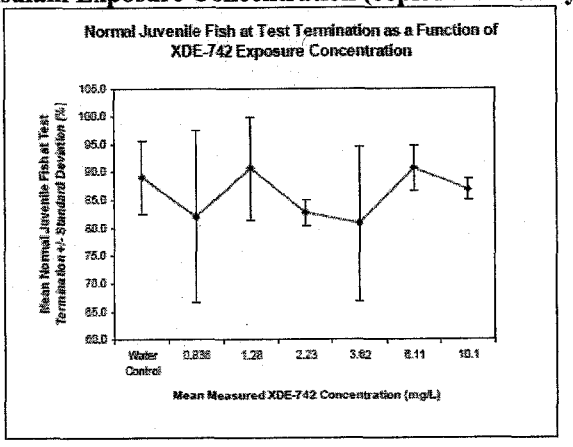
Therefore, survival of the larvae/juvenile fish during the study contributed entirely to the percent abnormal observed at test termination.

The mean percent normal juvenile fish at test termination (based on 4 replicates per test group) was 89.0% in the control group and ranged from 80.8 to 90.7% across all treatment groups (Table 13).

No significant differences (alpha = 0.05) in percent normal juvenile fish at test termination were observed in any of the treatment groups when compared to the control group.

A plot of mean percent normal juvenile fish at test termination as a function of pyroxsulam exposure concentration showed no dose-response relationship.

Dose-response Curve for Mean Normal Juvenile Fish at Test Termination Versus Pyroxsulam Exposure Concentration (copied from study report).



Growth (Length)

The body length (total length) of each surviving juvenile fish at test termination was recorded and the results reported are summarised inTable 14. Individual body length data for all replicates were presented in the study report.

Table 14. Summary of Effects of Pyroxsulam on Growth (Length).

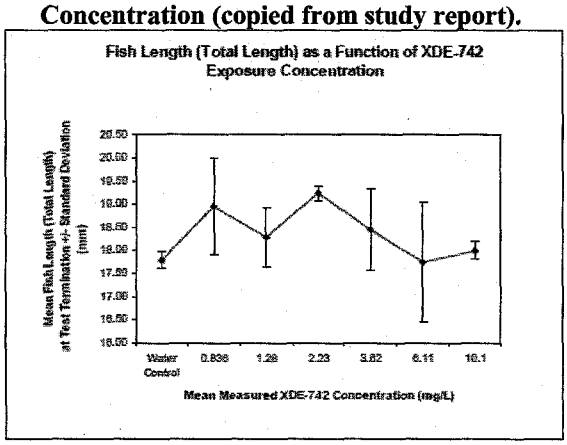
Test Concentration (mg/L) Mean Measured	Fish length (mm) at test termination Mean	Standard Deviation	Range	% of Control
0 (Water Control)	17.80	0.177	17.63-18.03	100.0%
0.836	18.96	1.050	18.16-20.42	106.5%
1.28	18.29	0.650	17.46-18.90	102.8%
2.23	19.24	0.150	19.07-19.42	108.1%
3.62	18.46	0.888	17.69-19.74	103.7%
6.11	17.75	1.285	15.85-18.69	99.7%
10.1	18.01	0.201	17.76-18.21	101.2%

The mean body length per individual fish (based on 4 replicate means per test group) was 17.80 mm in the control group and ranged from 17.75 to 19.24 mm (99.7 to 108.1% of the control group, respectively) across all treatment groups (Table 14).

No significant differences (alpha = 0.05) in growth (length) were observed in any of the treatment groups when compared to the control group.

A plot of mean fish length at test termination as a function of the mean measured pyroxsulam concentration did not exhibit a dose-response relationship.

Dose-response Curve for Mean Fish Length versus Pyroxsulam Exposure Concentration (copied from study report).



Growth (Weight, dry)

The body weight (dry weight; ~44 hours at ~60°C) for each surviving juvenile fish following test termination was recorded and the results presented summarised in (Table 15). Individual body weight (dry) data for all replicates was presented in the study report.

Table 15. Summary of Effects of Pyroxsulam on Growth (Weight, dry).

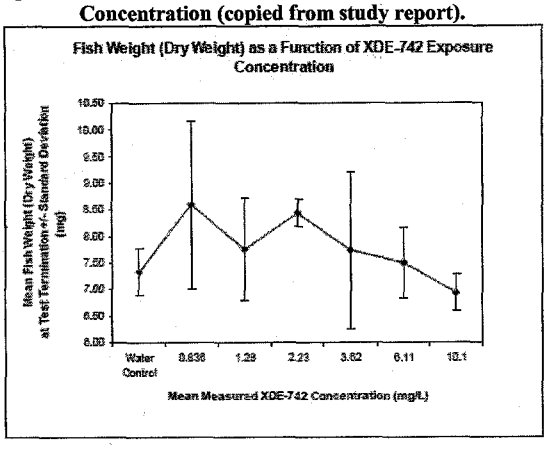
Test Concentration (mg/L) Mean Measured	Fish weight, dry (mg) at test termination			
	Mean	Standard Deviation	Range	% of Control
0 (Water Control)	7.33	0.442	6.90-7.95	100.0%
0.836	8.59	1.581	7.39-10.8	117.2%
1.28	7.76	0.960	6.73-8.60	105.9%
2.23	8.43	0.258	8.15-8.74	115.0%
3.62	7.73	1.476	6.78-9.92	105.5%
6.11	7.50	0.661	6.88-8.42	102.3%
10.1	6.94	0.333	6.53-7.21	94.7%

The mean dry body weight per individual fish (based on 4 replicate means per test group) was 7.33 mg in the control group and ranged from 6.94 to 8.59 mg (94.7 to 117.2% of the control group, respectively) across all treatment groups (Table 15).

No significant differences (alpha = 0.05) in growth (weight, dry) were observed in any of the treatment groups when compared to the control group.

A plot of mean fish weight, dry at test termination as a function of pyroxsulam exposure concentration showed no dose-response relationship.

Dose-response Curve for Mean Fish Weight versus Pyroxsulam Exposure Concentration (copied from study report).



Summary of effects of pyroxsulam on fish weight (dry) and length results

Table 16, taken from Marino (2005), is based on the information in the previous two tables dealing with fish weight, dry and length after 40 days, summarises the effect of pyroxsulam on fathead minnow lengths and dry weights. The table also includes NOEC/NOAEC, LOEC/LOAEC and MATC values based on the data presented.

Table 16. Effect of pyroxsulam on growth of juvenile fathead minnows[a] (taken from Marino, 2005). NOEC/NOAEC, LOEC/LOAEC and MATC values are as mg pyroxsulam/L.

Treatment (mg pyroxsulam/L) [measured and nominal concentrations used	Mean (SD)[b] Growth length (mm)	Mean (SD)[b] Growth dry weight (mg)
Negative control (dilution water only)	17.80 (0.177)	7.33 (0.442)
Solvent or positive control, if used	N/A	N/A
0.836/0.778	18.96 (1.050)	8.59 (1.581)
1.28/1.30	18.29 (0.650)	7.76 (0.960)
2.23/2.16	19.24 (0.150)	8.43 (0.258)
3.62/3.60	18.46 (0.888)	7.73 (1.476)
6.11/6.00	17.75 (1.285)	7.50 (0.661)
10.1/10.0	18.01 (0.201)	6.94 (0.333)
NOEC[c] (mg pyroxsulam/L)	**10.1**	**10.1**
LOEC[c] (mg pyroxsulam/L)	**>10.1**	**>10.1**
MATC[c] (mg pyroxsulam/L)	**>10.1**	**>10.1**

a Table modified from the Data Evaluation Report Template to better reflect the reporting of the study data presented in the final report.

b Mean and Standard Deviation (SD) of the four individual replicates per test concentration.

c Results based on mean measured concentrations.

Summary of sub-lethal effects of pyroxsulam on juvenile fathead minnow

Table 17, taken from Marino (2005) and based on the information in the two tables dealing with effects of pyroxsulam on normal larvae at hatch and on normal juvenile fish at test termination (Table 11 and Table 13) summarises the sub-lethal effect of pyroxsulam on fathead minnows. Table 17 also includes NOEC/NOAEC, LOEC/LOAEC and MATC values based on the data presented.

Table 17. Sub-lethal effects of pyroxsulam on juvenile fathead minnows[a]. Taken from Marino (2005). NOEC/NOAEC, LOEC/LOAEC and MATC are as mg pyroxsulam/L.

Treatment (mg pyroxsulam/L) [record measured and nominal concentrations used]	Mean (SD)[b] % Normal Larvae at Completion of Hatch (day 6)	Toxicity Symptoms /Behavioural Effects (specify) at Completion of Hatch (day 6)[d]	Mean (SD)[b] % Normal Fish at Test Termination	Toxicity Symptoms[d]/ Behavioural Effects (specify) at Test Termination
Negative control (dilution water only)	97.0 (2.04)	1 curved body	89.0 (6.76)	N/A
Solvent or positive control, if used	N/A	N/A	N/A	N/A
0.836/0.778	93.0 (9.45)	3 pale; 2 curved body	82.0 (15.43)	None
1.28/1.30	99.0 (2.10)	none	90.7 (9.26)	None
2.23/2.16	89.8 (2.38)	3 pale; 2 curved body	82.6 (2.31)	None
3.62/3.60	92.9 (3.81)	1 pale; 1 curved body	80.8 (13.84)	None
6.11/6.00	97.9 (2.42)	none	90.7 (3.97)	None
10.1/10.0	94.9 (1.94)	2 pale, 1 bent spine	86.8 (1.85)	None
NOEC/NOAEC[c]	**10.1**		**10.1**	
LOEC/LOAEC[c]	**>10.1**		**>10.1**	
MATC[c]	**>10.1**		**>10.1**	

a Table modified from the Data Evaluation Report Template to better reflect the reporting of the study data presented in the final report.
b Mean and Standard Deviation (SD) of the four individual replicates per test concentration. Normal defined as those organisms exhibiting no lethal or sub-lethal (abnormal behaviour or appearance) effects.
c Results based on mean measured concentrations.
d Toxicity symptoms/behavioural effects excluding mortality.
N/A = not applicable.

C. REPORTED STATISTICS:

The statistical analyses performed were described in the study report as follows:

The percent embryos hatched (embryo survival), days-to-mean hatch, percent post-hatch survival, percent overall survival, the percent normal larvae at hatch, percent normal juvenile fish at test termination, and the growth parameters (weight, dry and length) data were statistically evaluated to determine the NOEC, LOEC, and MATC (the MATC was defined in the study report as the theoretical threshold or allowable chronic concentration; it is the geometric mean of the NOEC and LOEC values).

The day-to-mean hatch and growth (length and weight, dry) raw data were first tested for normality using the Shapiro-Wilk's test at a type I error rate of 0.01. If the data were not normally distributed, the logarithmic inverse and square root transformations were tested sequentially to search for a normalizing transformation. Next, the data and transformed variables were tested for homogeneity of variance using Bartlett's test at a type I error rate of 0.01. If the raw data or a transformed variable were both normal and homogeneous, a parametric analysis was conducted using a one-tailed Dunnett's test (for growth) and a two-tailed Dunnett's test (for day-to-mean hatch) to compare each treated group with the control at a type I error rate of 0.05.

Data that was not normally distributed and/or not homogeneous was analysed non-parametrically with a Steel's Many-one rank test if the number of replicates in each treatment group was the same, or with a Kruskal-Wallis test if the number of replicates was different. Steel's Many-one rank test is one-sided and the Kruskal-Wallis test is two-sided, both with a type I error rate of 0.05. A significant result in the Kruskal Wallis test leads to a pairwise

comparison of each treatment with the control(s) using the Wilcoxon procedure at a type I error rate of 0.01 (one-sided). Both the Steel's test and the Wilcoxon test will lead to the determination of a NOEC.

The percent of embryos that hatched, percent normal larvae at hatch, percent normal juvenile fish at test termination, percent post-hatch survival, and percent overall survival parameters were arcsine square root transformed and analysed by a parametric analysis of variance followed by a one-tailed Dunnett's test at a type I error rate of 0.05. The final toxicological interpretation of the data also considered other factors such as dose-response, biological plausibility and consistency.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

ANOVA calculations were conducted using the Microsoft Excel Data Analysis function while the TidePool Scientific Software program, ToxCalc (v5.0.23A) was used for tests for normality, equality of variance, comparisons of means, estimation of NOEC values etc.

Embryo survival (at day 6)
The total numbers and percentages of embryos hatched by day 6, when hatching was complete in all test vessels, are shown in Table 18.

Table 18. Total numbers and percentages of embryos hatched by day 6.

Total number hatched day 6	Mean measured pyroxsulam concentration (mg/L)						
	Control	**0.836**	**1.28**	**2.23**	**3.62**	**6.11**	**10.1**
Replicate A	25 (100%)	25 (100%)	25 (100%)	24 (96%)	25 (100%)	24 (96%)	24 (96%)
Replicate B	25 (100%)	25 (100%)	25 (100%)	24 (96%)	24 (96%)	25 (100%)	24 (96%)
Replicate C	24 (96%)	24 (96%)	24 (96%)	25 (100%)	24 (96%)	24 (96%)	25 (100%)
Replicate D	25 (100%)	25 (100%)	24 (96%)	25 (100%)	25 (100%)	23 (92%)	25 (100%)
Mean	24.75 (99%)	24.75 (99%)	24.5 (98%)	24.5 (98%)	24.5 (98%)	24 (96%)	24.5 (98%)
Standard deviation	0.5 (2%)	0.5 (2%)	0.58 (2.31%)	0.58 (2.31%)	0.58 (2.31%)	0.82 (3.27%)	0.58 (2.31%)

The percentage mean and standard deviations are based on an initial 25 embryos/replicate/test concentration with the reviewer calculated mean and standard deviation results the same as those in the study report.

A two factor ANOVA (without replication) of these data indicated no statistically significant differences of the numbers of embryos hatched either between the concentrations tested or the individual replicates.

ToxCalc analysis of the total numbers of hatched embryos at day 6 indicated the data were had a non-normal distribution (Shapiro-Wilk's Test, $p \leq 0.01$) and that variances were equal (Bartlett's Test, $p = 0.99$). Dunnett's Test indicated no differences between the mean numbers of hatched embryos in the pyroxsulam solutions compared to the mean number hatched in the controls and that the NOEC/NOAEC and LOEC/LOAEC were, respectively, 10.1 and >10.1 mg pyroxsulam/L.

The ToxCalc analysis of the percentage hatched values was done with an arcsine square root transformation with the Shapiro-Wilk's Test indicating a non-normal distribution ($p \leq 0.01$) and the Bartlett's Test that variances were equal ($p = 1.00$). Steel's Many-One Rank Test indicted no difference between the treatments and the controls with the NOEC and LOEC, respectively, 10.1 and >10.1 mg pyroxsulam/L.

The ANOVA results and summary of the ToxCalc results are provided on page 46 of this DER.

The reviewer's recalculation of the numbers and percentages of embryos hatched at day 6 indicate no statistically significant differences between any of the treatment groups compared to the control group. This result confirms the study report's conclusion of an absence of effects.

Post-hatch survival (Total number of larvae/fish alive at day 6 - not determined in the study report)
The total numbers of larvae/fish alive at day 6 are shown in Table 19.

Table 19. Total number of larvae/fish alive at day 6.

Total # of larvae/fish alive at day 6	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	24	25	25	23	24	23	23
Replicate B	24	25	25	22	23	25	24
Replicate C	24	24	23	24	23	23	24
Replicate D	25	23	24	23	23	23	24
Mean	24.25 (97%)	24.25 (97%)	24.25 (97%)	23 (92%)	23.25 (93%)	23.5 (94%)	23.75 (%)
Standard deviation	0.5 (2%)	0.96 (3.8%)	0.96 (3.8%)	0.82 (3.3%)	0.5 (2%)	1 (4%)	0.5 (%)

A two factor ANOVA (without replication) of these data indicated no statistically significant differences of the numbers of embryos hatched either between the concentrations tested or the individual replicates.

ToxCalc analysis of the total numbers of larvae/fish alive at day 6 indicated the data were normally distributed (Shapiro-Wilk's Test, $p > 0.01$) and that variances were equal (Bartlett's Test, $p = 0.77$). Dunnett's Test indicated no differences between the mean numbers of larvae/fish alive in the pyroxsulam solutions compared to the mean number in the controls and that the NOEC and LOEC were, respectively, 10.1 and >10.1 mg pyroxsulam/L.

The ANOVA results and summary of the ToxCalc results are provided on page 48 of this DER.

Post hatch survival at day 40
Post-hatch survival was defined as the percent of surviving juvenile fish at test termination base on the numbers of embryos hatched. The numbers of fish alive at day 40 are shown in Table 20.

Table 20. Numbers of fish alive at day 40.

Total # alive day 40	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	22	22	24	20	22	21	21
Replicate B	20	21	25	19	21	23	21
Replicate C	23	23	19	21	21	21	22
Replicate D	23	15	21	21	15	22	21

The percentages of the total numbers of embryos hatched at day 6 (see Embryo Survival above) are shown in Table 21.

Table 21. Total numbers of embryos hatched at day 6 expressed as percentages.

Total number alive day 40	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	88%	88%	96%	83.3%	88%	87.5%	87.5%
Replicate B	80%	84%	100%	79.2%	87.5%	92%	87.5%
Replicate C	95.8%	95.8%	79.2%	84%	87.5%	87.5%	88%
Replicate D	92 %	60%	87.5%	84%	60%	95.7%	84%
Mean	89.0%	82.0%	90.7%	82.6%	80.8%	90.7%	86.8%
Standard deviation	6.76%	15.43%	9.26%	2.31%	13.84%	3.97%	1.85%

The percentage mean and standard deviations calculated by the reviewer are the same as those in the study report.

A two factor ANOVA (without replication) of the numbers of larvae/fish alive at day 40 indicated no statistically significant differences of the numbers of fish surviving at day 40 either between the concentrations tested or the individual replicates.

ToxCalc analysis of the total numbers of fish alive at day 40 indicated the data were normally distributed (Shapiro-Wilk's Test, $p > 0.01$) and that variances were equal (Bartlett's Test, $p = 0.03$). Dunnett's Test indicated no differences between the mean numbers of fish alive in the pyroxsulam solutions at day 40 compared to the mean number of living fish in the controls at that time and that the NOEC and LOEC were, respectively, 10.1 and >10.1 mg pyroxsulam/L.

The ToxCalc analysis of the percentage post-hatch values was done with an arcsine square root transformation with the Shapiro-Wilk's Test indicating a normal distribution ($p > 0.01$) and the Bartlett's Test that variances were equal ($p = 0.02$). Dunnett's Test indicted no difference between the treatments and the controls with the NOEC/NOAEC and LOEC/LOAEC, respectively, 10.1 and >10.1 mg pyroxsulam/L.

The ANOVA results and summary of the ToxCalc results are provided on page 50 of this DER.

The reviewer's recalculation of the numbers and percentages of fish alive at day 40 indicate no statistically significant differences between any of the treatment groups compared to the control group. This result supports the study report's conclusion of there being no statistically significant differences in percent post-hatch survival were observed in any of the treatment groups when compared to the control group.

Overall survival

As noted previously, overall survival was defined for each test group as the percent of surviving juvenile fish at test termination based on the initial number of embryos exposed at test initiation (4 replicates of 25 embryos per test group). The reported results (as numbers of surviving fish at day 40) were the same values as reported above and as shown in Table 22.

Table 22. Overall survival of juvenile fish at test termination.

Total # alive day 40	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	22	22	24	20	22	21	21
Replicate B	20	21	25	19	21	23	21
Replicate C	23	23	19	21	21	21	22
Replicate D	23	15	21	21	15	22	21

As percentages based on the total numbers of embryos/replicate/test concentration (25), these values are shown in Table 23.

Table 23. Overall survival as the percent of surviving juvenile fish at test termination.

% of surviving fish at day 40, based on an initial 25 embryos/replicate	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	88%	88%	96%	80%	88%	84%	84%
Replicate B	80%	84%	100%	76%	84%	92%	84%
Replicate C	92%	92%	76%	84%	84%	84%	88%
Replicate D	92%	60%	84%	84%	60%	88%	84%
Mean:	88%	81%	89%	81%	79%	87%	85%
Standard deviation	5.66%	14.38%	11.02%	3.83%	12.81%	3.83%	2.00%

The percentage mean and standard deviations calculated by the reviewer are the same as those given in the study report.

The ToxCalc analysis of the percentage post-hatch values (based on an initial 25 embryos/replicate/test concentration) was done with an arcsine square root transformation with the Shapiro-Wilk's Test indicating a normal distribution ($p > 0.01$) and the Bartlett's Test that variances were equal ($p = 0.05$). Dunnett's Test indicted no difference between the treatments and the controls with the NOEC/NOAEc and LOEC/LOAEC, respectively, 10.1 and >10.1 mg pyroxsulam/L.

A summary of the ToxCalc analysis of the percentage survival values is provided on page 52 of this DER.

The reviewer's recalculation of the percentages of overall survival of the hatched at day 40 (based on the initial 25 embryo/replicate/test concentration) indicate no statistically significant differences between any of the treatment groups compared to the control group. The study report's conclusion of there being no statistically significant differences in percent overall survival in any of the treatment groups when compared to the control group is in accord with the reviewer's recalculations.

Day-to-Mean Hatch

The day-to-mean hatch was defined for each test group as the observation day in which •50% of the embryos had hatched with the results reported and recalculated by the reviewer for this DER are summarised in Table 24.

Table 24. Day-to-mean hatch.

Test Concentration (mg/L) Mean Measured	Reported day-to-mean hatch	Reviewer calculated day-to-mean hatch/replicate				Reviewer calculated day-to-mean hatch (standard deviation)
	Mean (standard deviation)	A	B	C	D	
0 (Water Control)	3.8 (0.50)	4	4	4	3	3.75 (0.50)
0.836	3.8 (0.50)	4	4	3	4	3.75 (0.50)
1.28	3.5 (0.58)	4	4	3	3	3.50 (0.58)
2.23	4.0 (0.00)	4	4	4	4	4.00 (0.0)
3.62	3.8 (0.50)	4	4	4	3	3.75 (0.50)
6.11	3.3 (0.50)	3	4	3	4	3.50 (0.58)
10.1	3.8 (0.50)	4	4	4	4	4.00 (0.0)

The recalculated means are equivalent to those reported in the study report except for the 6.11 and 10.1 mg/L results where the reported means and standard deviations were, respectively 3.3 (0.50) and 3.8 (0.50).

The day 3 and day 4 total numbers of embryos hatched for the 10.1 mg/L results reported in the study report were as follows (Table 25) and the reason for the study report's reporting of the mean as 3.8 is not clear.

Table 25. Total numbers of embryos hatched

Total number of embryos hatched/replicate in the 10.1 mg/L exposure group Day 3	Total number of embryos hatched/replicate in the 10.1 mg/L exposure group Day 4	Day-to-mean Hatch
8	22	4
12	21	4
10	20	4
5	19	4
	Mean:	4 (cf. reported mean of 3.8)

A single factor ANOVA was conducted on the calculated day-to-mean hatch results with the results indicating no significant difference exists between the treatment levels. The ANOVA results are shown in Table 26.

Table 26. ANOVA of day to mean hatch data.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0 (Water Control)	4	15	3.75	0.25
0.836	4	15	3.75	0.25
1.28	4	14	3.5	0.333333
2.23	4	16	4	0
3.62	4	15	3.75	0.25
6.11	4	14	3.5	0.333333
10.1	4	16	4	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1	6	0.166667	0.823529	0.564441	2.572712
Within Groups	4.25	21	0.202381			
Total	5.25	27				

The reviewer's recalculation of the day to mean hatch indicate no statistically significant differences between any of the treatment groups compared to the control group. Apart from a number of minor differences between the reviewers recalculated results and the reported results, the study report's conclusion of there being no statistically significant differences in the day to mean hatch in any of the treatment groups when compared to the control group is in accord with the reviewer's recalculations.

Normal Larvae at Hatch

The normal larvae at hatch was defined for each test group as the percent of larvae exhibiting no lethal or sub-lethal effects (abnormal behaviour or appearance) after the completion of hatching (day 6; hatching complete in all replicate vessels) based on the number of embryos hatched. The numbers of normal larvae at day 6 are shown in Table 27.

Table 27. Numbers of normal larvae at hatch.

Numbers of normal larvae at day 6	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	24	25	25	22	24	23	23
Replicate B	24	23	25	21	22	25	23
Replicate C	23	24	23	23	23	23	24

Replicate D	**25**	**20**	**24**	**22**	**22**	**23**	**23**

Expressed as a percentage of the numbers of embryo hatched (see under "Embryo survival (at day 6)" above), these values are shown in Table 28.

Table 28. Percentages of normal larvae at hatch.

Percentages of normal larvae at day 6	**Mean measured pyroxsulam concentration (mg/L)**						
	Control	**0.836**	**1.28**	**2.23**	**3.62**	**6.11**	**10.1**
Replicate A	**96%**	**100%**	**100%**	**91.7%**	**96%**	**95.8%**	**95.8%**
Replicate B	**96%**	**92 %**	**100%**	**87.5%**	**91.7%**	**100%**	**95.8%**
Replicate C	**95.8%**	**100%**	**95.8%**	**92%**	**95.8%**	**95.8%**	**96%**
Replicate D	**100%**	**80%**	**100%**	**88%**	**88%**	**100%**	**92%**
Mean	**97.0%**	**93.0%**	**99%**	**89.8%**	**92.9%**	**97.9%**	**94.9%**
Standard deviation	**2.04%**	**9.45%**	**2.10%**	**2.38%**	**3.81%**	**2.42%**	**1.94%**

The percentage mean and standard deviations recalculated by the reviewer are the same as those in the study report.

A two factor ANOVA (without replication) of these percentage data indicated no statistically significant differences between the percentages of normal larvae hatched based on the numbers of larvae hatched either between the concentrations tested or the individual replicates.

The ToxCalc analysis of the percentage normal larvae at hatch (based on the numbers of embryos/replicate/test concentration hatched) was done with an arcsine square root transformation with the Shapiro-Wilk's Test indicating a normal distribution ($p > 0.01$) and the Bartlett's Test that variances were equal ($p = 0.09$). Dunnett's Test indicated no difference between the treatments and the controls with the NOEC/NOAEC and LOEC/LOAEC, respectively, 10.1 and >10.1 mg pyroxsulam/L.

The ANOVA results and summary of the ToxCalc results are provided on page 53 and following of this DER.

The study report's finding of no statistically significant differences in percent normal larvae at hatch were observed in any of the treatment groups when compared to the control group are considered to be in accord with the results obtained in the reviewer's recalculation of the normal larvae at hatch data.

Normal Juvenile Fish at Test Termination

The normal juvenile fish at test termination was defined for each test group as the percent of larvae (fish?) exhibiting no lethal or sub-lethal effects (abnormal behaviour or appearance) at test termination (day 40) based on the number of embryos hatched. The numbers of normal fish at day 40 are shown in Table 29.

Table 29. Numbers of normal juvenile fish at test termination.

Numbers of normal fish at day 40	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	22	22	24	20	22	21	21
Replicate B	20	21	25	19	21	23	21
Replicate C	23	23	19	21	21	21	22
Replicate D	23	15	21	21	15	22	21

As percentages of the embryos hatched, these values were recalculated as shown in Table 30.

Table 30. Numbers of normal fish hatched at day 40 as percentages of the embryos hatch.

Numbers of normal fish at day 40	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	88%	88%	96%	83.3%	88%	87.5%	87.5%
Replicate B	80%	84%	100%	79.2%	87.5%	92%	87.5%
Replicate C	95.8%	95.8%	79.2%	84%	87.5%	87.5%	88%
Replicate D	92%	60%	87.5%	84%	60%	95.7%	84%
Mean	89.0%	82.0%	90.7%	82.6%	80.8%	90.7%	86.8%
Standard deviation	6.76%	15.43%	9.26%	2.31%	13.84%	3.97%	1.85%

The percentage mean and standard deviations recalculated by the reviewer are the same as those given in the study report.

A two factor ANOVA (without replication) of these percentage data indicated no statistically significant differences between the percentages of normal fish at day 40 based on the numbers of larvae hatched either between the concentrations tested or the individual replicates.

The ToxCalc analysis of the percentage normal juvenile fish at test termination (based on the total numbers of embryos/replicate/test concentration hatched) was done with an arcsine square root transformation with the Shapiro-Wilk's Test indicating a normal distribution ($p > 0.01$) and the Bartlett's Test that variances were equal ($p = 0.02$). Dunnett's Test indicted no difference between the treatments and the controls with the NOEC/NOAEC and LOEC/LOAEC, respectively, 10.1 and >10.1 mg pyroxsulam/L.

The ANOVA results and summary of the ToxCalc results are provided on page 55 of this DER.

The study report's finding of no statistically significant differences in the percent normal fish at test termination being observed in any of the treatment groups when compared to the control group are considered to be in accord with the results obtained in the reviewer's recalculation of the numbers of normal fish at test termination data.

Growth (Length)

The individual body length (total length) of each surviving juvenile fish at test termination was presented in the study report together with calculated means and standard deviations/replicate/test concentration.

The mean lengths per replicate and test concentration were reported as shown in Table 31.

Table 31. Mean fish lengths (mm)/replicate at day 40.

Conc.-mg/L	Replicate			
	A	B	C	D
Control	17.700	17.850	17.630	18.030
0.836	18.230	19.030	18.160	20.420
1.28	18.090	17.460	18.900	18.700
2.23	19.180	19.420	19.290	19.070
3.62	18.150	17.690	18.260	19.740
6.11	15.850	18.310	18.690	18.130
10.1	18.130	18.210	17.760	17.940

The reported means etc. from these data are shown in Table 32.

Table 32. Descriptive statistics for fish lengths at day 40

Test Concentration (mg/L) Mean Measured	Fish Length (mm) Mean	St. Dev.	Range of means	% of Control
0 (Water Control)	17.80	0.177	17.63 - 18.03	100.0%
0.836	18.96	1.050	18.16 - 20.42	106.5%
1.28	18.29	0.650	17.46 - 18.90	102.8%
2.23	19.24	0.150	19.07 - 19.42	108.1%
3.62	18.46	0.888	17.69 - 19.74	103.7%
6.11	17.75	1.285	15.85 - 18.69	99.7%
10.1	18.01	0.201	17.76 - 18.21	101.2%

The reported means and standard deviations, ranges and percentages of control results were verified as correct by recalculation from the mean length data reported in the study.

The ToxCalc analysis of the mean fish lengths/replicate/test concentration (without transformation) reported a normal distribution (Shapiro-Wilk's Test, $p > 0.01$). Bartlett's Test indicated that the variances were unequal ($p = 4.13E-03$). Dunnett's and Steel's Many-One Rank Tests indicted no difference between the treatments and the controls with the NOEC/NOAEC and LOEC/LOAEC, respectively, 10.1 and >10.1 mg pyroxsulam/L. Reciprocal and log transformations did not result in the variances being made equal.

A summary of the ToxCalc results are provided on page 56 of this DER.

The study report's finding of no statistically significant differences in the lengths of fish at test termination being observed in any of the treatment groups when compared to the control group are considered to be in accord with the results obtained in the reviewer's recalculation of the mean fish length data.

Growth (Dry weight)

The body dry weight (after ~44 hours at ~60°C) of each surviving juvenile fish at test termination was presented in the study report together with calculated means and standard deviations/replicate/test concentration.

The mean fish weights per replicate and test concentration reported are shown in Table 33.

Table 33. Mean fish weights/replicate and test concentration at day 40.

	Replicate			
Conc.-mg/L	**A**	**B**	**C**	**D**
B-Control	7.2400	7.9500	6.9000	7.2300
0.836	7.5100	8.6700	7.3900	10.7600
1.28	7.1500	6.7300	8.5500	8.6000
2.23	8.5200	8.7400	8.1500	8.3000
3.62	6.7800	6.9100	7.3200	9.9200
6.11	7.4700	6.8800	8.4200	7.2200
10.1	7.2100	7.2100	6.5300	6.8000

The means etc. from these data are shown in Table 32.

Table 34. Descriptive statistics for fish lengths at day 40

Test Concentration (mg/L)	**Fish Weight(mg)**			
Mean Measured	Mean	St.Dev.	Range	% of Control
0(Water Control)	7.33	0.442	6.90 - 7.95	100.0%
0.836	8.59 (8.58)*	1.581 (1.56)*	7.39- 10.8	117.2%
1.28	7.76	0.960	6.73- 8.60	105.9%
2.23	8.43	0.258	8.15- 8.74	115.0%
3.62	7.73	1.476	6.78- 9.92	105.5%
6.11	7.50	0.661	6.88- 8.42	102.3%
10.1	6.94	0.333	6.53- 7.21	94.7%

*** Reviewer calculated results.**

The reported values were verified as correct by recalculation from the mean weight data reported in the study (the 1.28 mg/L calculated mean and standard deviation results were slightly different from those reported – see table above).

The ToxCalc analysis of the mean fish weights/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 ($p = 0.03$). Dunnett's Test indicted no difference between the treatments and the controls with the NOEC/NOAEC and LOEC/LOAEC, respectively, 10.1 and >10.1 mg pyroxsulam/L.

A summary of the ToxCalc results are provided on page 57 of this DER.

The study report's finding of no statistically significant differences in the mean dry weights of the fish at test termination being observed in any of the treatment groups when compared to the control group are considered to be in accord with the results obtained in the reviewer's recalculation of the mean fish dry weight data.

Determination of the MATC

The MATC is defined in the study report as the geometric mean of the NOEC and LOEC values. With a LOEC/LOAEC of >10.1 mg pyroxsulam/L, the reported MATC values are determined as >10.1 mg pyroxsulam/L without further calculation.

Endpoints
The study's endpoints are shown in Table 35.

Table 35. Effect endpoints for the effect of pyroxsulam on the early life stage of the fathead minnow.

Effect Endpoint	NOEC/NOAEC[a] (mg/L)	LOEC/LOAEC[a] (mg/L)	MATC[a] (mg/L)
Percent Embryos Hatched (at day 6)	10.1	>10.1	>10.1
Days-to-Mean Hatch (based on day-6 observations; day which hatching was observed to be complete in all replicates of each test level)	10.1	>10.1	>10.1
Percent Post-Hatch Survival (at day 6)	10.1	>10.1	>10.1
Percent Overall Survival (at day 40)	10.1	>10.1	>10.1
Percent Normal Larvae at Hatch (at day 6)	10.1	>10.1	>10.1
Percent Normal Juvenile Fish at Test Termination (at day 40)	10.1	>10.1	>10.1
Length (mm) (at day 40)	10.1	>10.1	>10.1
Weight, dry (mg) (at day 40)	10.1	>10.1	>10.1

a. Based on mean analysed pyroxsulam concentrations.

E. STUDY DEFICIENCIES:
Table 36 summarises the deviations from guidelines or deficiencies that were noted. These were not considered to have significantly affected the study's conduct or outcome with priority given to the OECD or US EPA OPPTS guideline requirements, rather than to the template's reference to unidentified US/OECD guidelines:

Table 36. Summary of deficiencies/deviations from guidelines.

Parameter	Study report result	Template reference to US/OECD Guideline	US EPA OPPTS 850.1400 Fish Early-Life Stage Toxicity Test, EPA 712-C-96-121, April 1996	OECD Guideline for Testing Chemicals, Fish, Early-Life Stage Toxicity Test, 210, adopted 17/07/92
Parental acclimation, if any Period: Conditions: (same as test or not) Feeding (type, source, amount given, frequency): Health: (any mortality observed)	Parental acclimation information not provided, embryos were obtained from a commercial supplier.	*Not referred to in guideline.*	Refers to provision of, *inter alia,* strain, source and method of collection of the fertilized eggs, and subsequent handling.	Refers to provision of, *inter alia,* strain, source and method of collection of the fertilised eggs and subsequent handling.
Number of fertilized eggs/embryos in each treatment at test initiation	Number of fish/treatment for post-hatch exposure at day 6, day when hatching was complete in all vessels, ranged from 24-25 in controls and 22-25 in the test	*EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure.*	At least 60 eggs divided equally between at least two replicate test chambers.	At least 60 eggs divided equally between at least two replicate test chambers.

	concentrations.			
Test concentrations	All considered met except for one concentration having to adversely affect a life stage and one concentration not affecting a life stage – no concentrations affected a life stage adversely.	*EPA requires ... . - One concentration must adversely affect a life stage and one concentration must not affect any life stage.*	Equivalent requirement not located.	Equivalent requirement not located.
Aeration	Dilution water not indicated as aerated, aeration not used in the system.	*(EPA requires: dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.* The group minimum dissolved oxygen contents were 6.2 to 6.7 mg/L over 40 days, taken as equivalent to 77 to 83% saturation.	Equivalent requirement not located. Guideline states that the loading rate (biomass per volume of test solution) should be low enough in order that a dissolved oxygen concentration of at least 60 percent of the air saturation value (ASV) can be maintained without aeration.	Equivalent requirement not located. Guideline states that the loading rate (biomass per volume of test solution) should be low enough in order that a dissolved oxygen concentration of at least 60 percent of the air saturation value (ASV) can be maintained without aeration.
Duration of the test	40 days (34 days post-hatch, hatching complete by day 6)	*EPA requires 32 days*	32 days from start of test (or 28 days post-hatch)	32 days from start of test (or 28 days post-hatch)
Embryo cups, Size:	Volumes of the cups are ~290 mL	*EPA requires 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen*	Equivalent requirement not located.	Equivalent requirement not located.
Water parameters: Hardness and pH	58-82 (control water) and 60-78 (test waters) mg $CaCO_3$/L Control water pH 7.1-7.3 Test waters pH 7.0-7.5	*EPA requires hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended.*	Equivalent requirement not located. Parameters to be measured but no values proposed.	Equivalent requirement not located. Parameters to be measured but no values proposed.
Pesticides	Specified polycyclic aromatic	*Not specified.*	Total organophosphorus	Total organophosphorus

	hydrocarbons, organophosphate insecticides, chlorphenoxy herbicides and organochlorine pesticides all identified as less than respective detection limits (0.25 to 5 µg/L, as reported in Marino *et al.*, 2006 but not given in the study report).	pesticides should be <50 ng/L; total organochlorine pesticides plus polychlorinated biphenyls should be less than 50 ng/L. Absence of mortalities and adverse effects in controls indicates dilution water was acceptable for these and the following two parameters.	pesticides should be <50 ng/L; total organochlorine pesticides plus polychlorinated biphenyls should be less than 50 ng/L. Absence of mortalities and adverse effects in controls indicates dilution water was acceptable for these and the following two parameters.
Residual chlorine	<20 ppb (limit of detection)	<10 µg/L	<20 ppb
Ammonia (taken as being unionized)	<100 ppb (limit of detection)	<1 µg/L (ppb)	<1 µg/L (ppb)

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. While certain deviations from the guidelines or deficiencies have been identified, they are not considered to have adversely affected the conduct or outcome of the study. Some minor differences in the statistical results reported in the study report and those recalculated from the data presented were identified but, again, the differences are not considered to have identified any significant adverse biological effects on the fathead minnow embryos, larvae or fish at the pyroxsulam concentrations tested.

The definitive test dates were 26 January 2005 to 07 March 2005.

This flow-through study was conducted to determine the toxicity of pyroxsulam to the early-life stages of the fathead minnow, *P. promelas*.

In the study under assessment, on each of the sampling days between days 0 and 40, a single replicate aquarium was sampled from each exposure level. Replicate aquaria having the same identifiers (A, B, C, and D) were sampled across all exposure levels for a given sampling date and the pyroxsulam concentration determined by HPLC with UV detection. Weekly analyses of the mixing chamber and dose stocks were also conducted.

None of the controls exhibited a peak eluting at the retention time of pyroxsulam at a concentration exceeding the lowest level quantified of ~0.08 mg pyroxsulam/L LDW, which was the concentration of analyte in the lowest standard analysed times the lowest dilution factor. Typical chromatograms of a LDW control and two pyroxsulam standards were presented which confirmed the absence of pyroxsulam in the controls and clearly identified its presence in the standards. It is noted that the study report referred to a typical chromatogram of a sample being provided, but the actual chromatogram given was labelled as being for a 0.778 mg pyroxsulam/L test solution.

The HPLC/UV instrumentation exhibited a linear response over a concentration range of ~0 to ~15 mg of pyroxsulam/L.

The validity criteria for OECD 210 (adopted 17.07.92) and US EPA OPPTS 850.1400 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 PMRA does not share the same acceptability classification scheme as the US EPA. The study is classified as acceptable to the PMRA.

G. CONCLUSIONS:

This study is scientifically sound, however it failed to establish a definitive LOEC/LOAEC value for any of the measurement endpoints, including:

Percent Embryos Hatched
Days-to-Mean Hatch
Percent Post-Hatch Survival
Percent Overall Survival
Percent Normal Larvae at Hatch
Percent Normal Juvenile Fish at Test Termination
Length (mm)
Weight, dry (mg)

The NOEC/NOAEC, LOEC/LOAEC and MATC were, respectively, 10.1, >10.1 and >10.1 mg pyroxsulam for each of these endpoints. The NOEC/NOAEC, LOEC/LOAEC and MATC values are based on mean measured concentrations over the 40 days of the study.

Pyroxsulam or XDE-742 is considered to be very slightly toxic to the early life stage of the fathead minnow (NOEC >1 mg/L), based on the chronic toxicity classification scheme of the Australian Government Department of the Environment and Water Resources. It is classified as acceptable by the Australian Government Department of the Environment and Water Resources and the PMRA.

However, as it has not established a definitive LOAEC, and as US EPA guideline testing requirements state that an LOAEC must be established, the study does not fulfill guideline requirements. Consequently, the US EPA has classified the study as supplemental.

III. REFERENCES:

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

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Approved 04/01/01 C.K.

Attachment 1
Reviewer calculated statistical results.
Embryo survival (numbers hatched at day 6)
The numbers of embryos hatched at day 6 are discussed on page 30 of this DER.

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Replicate A	7	172	24.57143	0.285714
Replicate B	7	172	24.57143	0.285714
Replicate C	7	170	24.28571	0.238095
Replicate D	7	172	24.57143	0.619048
Control	4	99	24.75	0.25
0.836	4	99	24.75	0.25
1.28	4	98	24.5	0.333333
2.23	4	98	24.5	0.333333
3.62	4	98	24.5	0.333333
6.11	4	96	24	0.666667
10.1	4	98	24.5	0.333333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.428571	3	0.142857	0.363636	0.780034	3.159908
Columns	1.5	6	0.25	0.636364	0.699884	2.661305
Error	7.071429	18	0.392857			
Total	9	27				

Attachment 1 (Continued)

ToxCalc results for (1) the analysis of the numbers of embryos hatched at day 6, the day hatching was complete in all vessels (with the total numbers of embryos hatched shown) and (2) analysis of the percentages hatched at day 6:

Numbers of embryos hatched at day 6:

Conc- mg/L	1	2	3	4
Control	25.000	25.000	24.000	25.000
0.836	25.000	25.000	24.000	25.000
1.28	25.000	25.000	24.000	24.000
2.23	24.000	24.000	25.000	25.000
3.62	25.000	24.000	24.000	25.000
6.11	24.000	25.000	24.000	23.000
10.1	24.000	24.000	25.000	25.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc- mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	24.750	1.0000	24.750	24.000	25.000	2.020	4				24.750	1.0000
0.836	24.750	1.0000	24.750	24.000	25.000	2.020	4	0.000	2.451	1.036	24.750	1.0000
1.28	24.500	0.9899	24.500	24.000	25.000	2.357	4	0.592	2.451	1.036	24.500	0.9899
2.23	24.500	0.9899	24.500	24.000	25.000	2.357	4	0.592	2.451	1.036	24.500	0.9899
3.62	24.500	0.9899	24.500	24.000	25.000	2.357	4	0.592	2.451	1.036	24.500	0.9899
6.11	24.000	0.9697	24.000	23.000	25.000	3.402	4	1.775	2.451	1.036	24.250	0.9798
10.1	24.500	0.9899	24.500	24.000	25.000	2.357	4	0.592	2.451	1.036	24.250	0.9798

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.89046	0.896	-0.2043	-1.1722
Bartlett's Test indicates equal variances (p = 0.99)	0.97206	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test Treatments vs B-Control	10.1	>10.1		9.90099	1.03592	0.04186	0.25	0.35714	0.6527	6, 21

Percentages hatched at day 6, based on an initial 25 embryos/replicate/test concentration.

Conc-%	1	2	3	4
Control	1.0000	1.0000	0.9600	1.0000
0.836	1.0000	1.0000	0.9600	1.0000
1.28	1.0000	1.0000	0.9600	0.9600
2.23	0.9600	0.9600	1.0000	1.0000
3.62	1.0000	0.9600	0.9600	1.0000
6.11	0.9600	1.0000	0.9600	0.9200
10.1	0.9600	0.9600	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	
Control	0.9900	1.0000	1.4453	1.3694	1.4706	3.501	4			
0.836	0.9900	1.0000	1.4453	1.3694	1.4706	3.501	4	18.00	10.00	0.10275
1.28	0.9800	0.9899	1.4200	1.3694	1.4706	4.114	4	16.00	10.00	0.10275
2.23	0.9800	0.9899	1.4200	1.3694	1.4706	4.114	4	16.00	10.00	0.10275
3.62	0.9800	0.9899	1.4200	1.3694	1.4706	4.114	4	16.00	10.00	0.10275
6.11	0.9600	0.9697	1.3734	1.2840	1.4706	5.556	4	13.50	10.00	0.10275
10.1	0.9800	0.9899	1.4200	1.3694	1.4706	4.114	4	16.00	10.00	0.10275

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.88081	0.896	-0.1594	-1.3401
Bartlett's Test indicates equal variances (p = 1.00)	0.65031	16.8119		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test Treatments vs B-Control	10.1	>10.1		9.90099

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

Attachment 2

Post-survival (Total number of larvae/fish alive at day 6) – not determined in the study report

The total numbers of larvae/fish alive at day 6 are discussed on page 31 of this DER.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Replicate A	7	167	23.85714	0.8095238
Replicate B	7	168	24	1.3333333
Replicate C	7	165	23.57143	0.2857143
Replicate D	7	165	23.57143	0.6190476
Control	4	97	24.25	0.25
0.836	4	97	24.25	0.9166667
1.28	4	97	24.25	0.9166667
2.23	4	92	23	0.6666667
3.62	4	93	23.25	0.25
6.11	4	94	23.5	1
10.1	4	95	23.75	0.25

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.9642857	3	0.321429	0.4909091	0.692984	3.159908
Columns	6.5	6	1.083333	1.6545455	0.189752	2.661305
Error	11.785714	18	0.654762			
Total	19.25	27				

Attachment 2 (Continued)

ToxCalc results for the analysis of the numbers of embryos alive at day 6, the day hatching was complete in all vessels.

Conc-mg/L	1	2	3	4
Control	24.000	24.000	24.000	25.000
0.836	25.000	25.000	24.000	23.000
1.28	25.000	25.000	23.000	24.000
2.23	23.000	22.000	24.000	23.000
3.62	24.000	23.000	23.000	23.000
6.11	23.000	25.000	23.000	23.000
10.1	23.000	24.000	24.000	24.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	24.250	1.0000	24.250	24.000	25.000	2.062	4				24.250	1.0000
0.836	24.250	1.0000	24.250	23.000	25.000	3.948	4	0.000	2.451	1.351	24.250	1.0000
1.28	24.250	1.0000	24.250	23.000	25.000	3.948	4	0.000	2.451	1.351	24.250	1.0000
2.23	23.000	0.9485	23.000	22.000	24.000	3.550	4	2.269	2.451	1.351	23.375	0.9639
3.62	23.250	0.9588	23.250	23.000	24.000	2.151	4	1.815	2.451	1.351	23.375	0.9639
6.11	23.500	0.9691	23.500	23.000	25.000	4.255	4	1.361	2.451	1.351	23.375	0.9639
10.1	23.750	0.9794	23.750	23.000	24.000	2.105	4	0.907	2.451	1.351	23.375	0.9639

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.94941		0.896		0.13828	-0.3788
Bartlett's Test indicates equal variances (p = 0.77)					3.31531		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	10.1	>10.1			1.35067	0.0557	1.08333	0.60714	0.15113	6, 21

Attachment 3
Post hatch survival (day 40)
The total numbers of larvae/fish alive at day 40 are discussed on page 31 of this DER.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Replicate A	7	152	21.714286	1.571429
Replicate B	7	150	21.428571	3.952381
Replicate C	7	150	21.428571	1.952381
Replicate D	7	138	19.714286	10.90476
Control	4	88	22	2
0.836	4	81	20.25	12.91667
1.28	4	89	22.25	7.583333
2.23	4	81	20.25	0.916667
3.62	4	79	19.75	10.25
6.11	4	87	21.75	0.916667
10.1	4	85	21.25	0.25

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	17.5714286	3	5.8571429	1.212818	0.333646	3.159908
Columns	23.3571429	6	3.8928571	0.806081	0.5783	2.661305
Error	86.9285714	18	4.8293651			
Total	127.857143	27				

Attachment 3 (Continued)

ToxCalc results for (1) the analysis of the numbers of surviving juvenile fish at day 40 and (2) analysis of these numbers as percentages based on the numbers of embryos hatched at day 6:

Total numbers of larvae/fish alive at day 40.

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	Test ID: 55	Sample ID:	No. of living larvae/fish at day 40
End Date:	Lab ID:	Sample Type:	
Sample Date:	Protocol: EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:			

Conc-mg/L	1	2	3	4
Control	22.000	20.000	23.000	23.000
0.836	22.000	21.000	23.000	15.000
1.28	24.000	25.000	19.000	21.000
2.23	20.000	19.000	21.000	21.000
3.62	22.000	21.000	21.000	15.000
6.11	21.000	23.000	21.000	22.000
10.1	21.000	21.000	22.000	21.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	22.000	1.0000	22.000	20.000	23.000	6.428	4				22.000	1.0000
0.836	20.250	0.9205	20.250	15.000	23.000	17.748	4	1.109	2.451	3.867	21.250	0.9659
1.28	22.250	1.0114	22.250	19.000	25.000	12.377	4	-0.158	2.451	3.867	21.250	0.9659
2.23	20.250	0.9205	20.250	19.000	21.000	4.728	4	1.109	2.451	3.867	20.750	0.9432
3.62	19.750	0.8977	19.750	15.000	22.000	16.210	4	1.426	2.451	3.867	20.750	0.9432
6.11	21.750	0.9886	21.750	21.000	23.000	4.402	4	0.158	2.451	3.867	20.750	0.9432
10.1	21.250	0.9659	21.250	21.000	22.000	2.353	4	0.475	2.451	3.867	20.750	0.9432

Auxiliary Tests	Statistic	Critical	Skew	Kurt

Percentages of surviving juvenile fish at test termination, based on the numbers of embryos hatched.

Control	0.8800	0.8000	0.9583	0.9200
0.836	0.8800	0.8400	0.9583	0.6000
1.28	0.9600	1.0000	0.7917	0.8750
2.23	0.8333	0.7917	0.8400	0.8400
3.62	0.8800	0.8750	0.8750	0.6000
6.11	0.8750	0.9200	0.8750	0.9565
10.1	0.8750	0.8750	0.8800	0.8400

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.8896	1.0000	1.2434	1.1071	1.3652	8.780	4			
0.836	0.8196	0.9213	1.1569	0.8861	1.3652	17.314	4	0.962	2.451	0.2203
1.28	0.9067	1.0192	1.2866	1.0968	1.4706	12.905	4	-0.481	2.451	0.2203
2.23	0.8263	0.9288	1.1414	1.0968	1.1593	2.631	4	1.135	2.451	0.2203
3.62	0.8075	0.9077	1.1305	0.8861	1.2171	14.417	4	1.256	2.451	0.2203
6.11	0.9066	1.0192	1.2659	1.2094	1.3607	5.715	4	-0.251	2.451	0.2203
10.1	0.8675	0.9752	1.1988	1.1593	1.2171	2.218	4	0.496	2.451	0.2203

Auxiliary Tests				Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)				0.94337		0.896		-0.6907	0.79672
Bartlett's Test indicates equal variances (p = 0.02)				14.6404		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	10.1	>10.1		9.90099	0.16771	0.18705	0.01584	0.01615	0.46237	6, 21
Treatments vs B-Control										

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

Attachment 4

Overall survival of fish/larvae is discussed on page 32 of this DER.

The ToxCalc treatment of the percentage of surviving juvenile fish at day 40, based on the initial number of embryos exposed at test commencement (25/replicate/test concentration), gave the following results:

Conc-%	1	2	3	4
Control	0.8800	0.8000	0.9200	0.9200
0.836	0.8800	0.8400	0.9200	0.6000
1.28	0.9600	1.0000	0.7600	0.8400
2.23	0.8000	0.7600	0.8400	0.8400
3.62	0.8800	0.8400	0.8400	0.6000
6.11	0.8400	0.9200	0.8400	0.8800
10.1	0.8400	0.8400	0.8800	0.8400

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.8800	1.0000	1.2231	1.1071	1.2840	6.826	4			
0.836	0.8100	0.9205	1.1366	0.8861	1.2840	15.364	4	1.013	2.451	0.2091
1.28	0.8900	1.0114	1.2645	1.0588	1.4706	14.926	4	-0.486	2.451	0.2091
2.23	0.8100	0.9205	1.1211	1.0588	1.1593	4.305	4	1.195	2.451	0.2091
3.62	0.7900	0.8977	1.1054	0.8861	1.2171	13.456	4	1.379	2.451	0.2091
6.11	0.8700	0.9886	1.2049	1.1593	1.2840	4.927	4	0.213	2.451	0.2091
10.1	0.8500	0.9659	1.1737	1.1593	1.2171	2.461	4	0.578	2.451	0.2091

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.9448		0.896		-0.7172	0.62657
Bartlett's Test indicates equal variances (p = 0.05)					12.7602		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs B-Control	10.1	>10.1		9.90099	0.16323	0.18467	0.01363	0.01456	0.49026	6, 21

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

Attachment 5
Normal larvae at hatch (day 6)
The numbers of normal larvae at hatch is discussed on page 34 of this DER.
The ANOVA results for the analysis of the number of normal larvae at day 6 are:

Numbers of normal larvae at day 6	Mean measured pyroxsulam concentration (mg/L)						
	Control	0.836	1.28	2.23	3.62	6.11	10.1
Replicate A	24	25	25	22	24	23	23
Replicate B	24	23	25	21	22	25	23
Replicate C	23	24	23	23	23	23	24
Replicate D	25	20	24	22	22	23	23

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Replicate A	7	166	23.71429	1.238095
Replicate B	7	163	23.28571	2.238095
Replicate C	7	163	23.28571	0.238095
Replicate D	7	159	22.71429	2.571429
Control	4	96	24	0.666667
0.836	4	92	23	4.666667
1.28	4	97	24.25	0.916667
2.23	4	88	22	0.666667
3.62	4	91	22.75	0.916667
6.11	4	94	23.5	1
10.1	4	93	23.25	0.25

ANOVA

Source of Variatio	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	3.535714	3	1.178571	0.894578	0.463006	3.159908
Columns	14	6	2.333333	1.771084	0.161977	2.661305
Error	23.71429	18	1.31746			
Total	41.25	27				

The percentage of normal larvae at day 6 are:

Replicate:	A	B	C	D
Control	96.0	96.0	95.8	100.0
0.8	100.0	92.0	100.0	80.0
1.3	100.0	100.0	95.8	100.0
2.2	91.7	87.5	92.0	88.0
3.6	96.0	91.7	95.8	88.0
6.1	95.8	100.0	95.8	100.0
10.1	95.8	95.8	96.0	92.0

Attachment 5 (continued)

With the ANOVA analysis of these values:

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
B-Control	4	387.8333	96.95833	4.118056
0.836	4	372	93	89.33333
1.28	4	395.8333	98.95833	4.340278
2.23	4	359.1667	89.79167	5.618056
3.62	4	371.5	92.875	14.58102
6.11	4	391.6667	97.91667	5.787037
10.1	4	379.6667	94.91667	3.787037
1	7	675.3333	96.47619	8.207672
2	7	663	94.71429	21.24735
3	7	671.3333	95.90476	5.34127
4	7	648	92.57143	60.95238

ANOVA

ource of Variati	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	254.4444	6	42.40741	2.385062	0.071397	2.661305
Columns	62.64683	3	20.88228	1.174453	0.347107	3.159908
Error	320.0476	18	17.78042			
Total	637.1389	27				

Normal larvae at hatch (day 6)

Page 34 of this DER refers.

ToxCalc analysis of the percentages of normal larvae at day 6:

Conc-mg/L	1	2	3	4
Control	0.9600	0.9600	0.9583	1.0000
0.836	1.0000	0.9200	1.0000	0.8000
1.28	1.0000	1.0000	0.9583	1.0000
2.23	0.9167	0.8750	0.9200	0.8800
3.62	0.9600	0.9167	0.9583	0.8800
6.11	0.9583	1.0000	0.9583	1.0000
10.1	0.9583	0.9583	0.9600	0.9200

			Transform: Arcsin Square Root					1-Tailed		
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.9696	1.0000	1.3937	1.3652	1.4706	3.683	4			
0.836	0.9300	0.9592	1.3326	1.1071	1.4706	13.050	4	1.045	2.451	0.1433
1.28	0.9896	1.0206	1.4438	1.3652	1.4706	3.627	4	-0.857	2.451	0.1433
*2.23	0.8979	0.9261	1.2471	1.2094	1.2840	3.153	4	2.507	2.451	0.1433
3.62	0.9288	0.9579	1.3074	1.2171	1.3694	5.624	4	1.476	2.451	0.1433
6.11	0.9792	1.0099	1.4169	1.3652	1.4706	4.210	4	-0.396	2.451	0.1433
10.1	0.9492	0.9789	1.3460	1.2840	1.3694	3.072	4	0.816	2.451	0.1433

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.93796		0.896		-0.7202	2.38713
Bartlett's Test indicates equal variances (p = 0.09)					11.015		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs B-Control	10.1	>10.1			0.06816	0.07034	0.01846	0.00683	0.04199	6, 21

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

Attachment 6

Normal juvenile fish at test termination (day 40).

The numbers of normal juvenile fish at day 40 is discussed on page 35 of this DER.

The ANOVA results for the analysis of the number of normal larvae at day 6 are:

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Replicate A	7	618.3	88.32857	14.30571
Replicate B	7	610.2	87.17143	52.18905
Replicate C	7	617.8	88.25714	35.85952
Replicate D	7	563.2	80.45714	212.8795
Control	4	355.8	88.95	45.74333
0.836	4	327.8	81.95	238.1433
1.28	4	362.7	90.675	85.68917
2.23	4	330.5	82.625	5.3225
3.62	4	323	80.75	191.4167
6.11	4	362.7	90.675	15.7225
10.1	4	347	86.75	3.416667

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	298.2068	3	99.40226	1.227057	0.328786	3.159908
Columns	433.2471	6	72.20786	0.89136	0.521664	2.661305
Error	1458.156	18	81.00865			
Total	2189.61	27				

ToxCalc analysis of the percentages of normal fish at day 40:

Conc-mg/L	1	2	3	4
Control	0.8800	0.8000	0.9583	0.9200
0.836	0.8800	0.8400	0.9583	0.6000
1.28	0.9600	1.0000	0.7917	0.8750
2.23	0.8333	0.7917	0.8400	0.8400
3.62	0.8800	0.8750	0.8750	0.6000
6.11	0.8750	0.9200	0.8750	0.9565
10.1	0.8750	0.8750	0.8800	0.8400

			Transform: Arcsin Square Root					1-Tailed		
Conc-mg/L	**Mean**	**N-Mean**	**Mean**	**Min**	**Max**	**CV%**	**N**	**t-Stat**	**Critical**	**MSD**
Control	0.8896	1.0000	1.2434	1.1071	1.3652	8.780	4			
0.836	0.8196	0.9213	1.1569	0.8861	1.3652	17.314	4	0.962	2.451	0.2203
1.28	0.9067	1.0192	1.2866	1.0968	1.4706	12.905	4	-0.481	2.451	0.2203
2.23	0.8263	0.9288	1.1414	1.0968	1.1593	2.631	4	1.135	2.451	0.2203
3.62	0.8075	0.9077	1.1305	0.8861	1.2171	14.417	4	1.256	2.451	0.2203
6.11	0.9066	1.0192	1.2659	1.2094	1.3607	5.715	4	-0.251	2.451	0.2203
10.1	0.8675	0.9752	1.1988	1.1593	1.2171	2.218	4	0.496	2.451	0.2203

Auxiliary Tests	**Statistic**	**Critical**	**Skew**	**Kurt**
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94337	0.896	-0.6907	0.79672
Bartlett's Test indicates equal variances (p = 0.02)	14.6404	16.8119		

Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs B-Control	10.1	>10.1			0.16771	0.18705	0.01584	0.01615	0.46237	6, 21

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

Attachment 7
Growth (length) of fish at test termination (day 40).

The lengths of the fish at day 40 is discussed on page 36 of this DER.

ToxCalc analysis of the mean lengths of fish (per replicate per test concentration) at day 40:

Conc-mg/L	1	2	3	4
Control	17.700	17.850	17.630	18.030
0.836	18.230	19.030	18.160	20.420
1.28	18.090	17.460	18.900	18.700
2.23	19.180	19.420	19.290	19.070
3.62	18.150	17.690	18.260	19.740
6.11	15.850	18.310	18.690	18.130
10.1	18.130	18.210	17.760	17.940

			Transform: Untransformed						1-Tailed	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	17.803	1.0000	17.803	17.630	18.030	0.996	4			
0.836	18.960	1.0650	18.960	18.160	20.420	5.540	4	-2.149	2.451	1.320
1.28	18.288	1.0272	18.288	17.460	18.900	3.557	4	-0.901	2.451	1.320
2.23	19.240	1.0807	19.240	19.070	19.420	0.779	4	-2.669	2.451	1.320
3.62	18.460	1.0369	18.460	17.690	19.740	4.812	4	-1.221	2.451	1.320
6.11	17.745	0.9968	17.745	15.850	18.690	7.240	4	0.107	2.451	1.320
10.1	18.010	1.0117	18.010	17.760	18.210	1.119	4	-0.385	2.451	1.320

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.952		0.896		-0.2874	1.8076
Bartlett's Test indicates unequal variances (p = 4.13E-03)					19.0202		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs B-Control	10.1	>10.1			1.32018	0.07416	1.30744	0.58004	0.07775	6, 21

n.b. The ToxCalc analysis using the EPA Methods option reported that Steel's Many-One Rank Test indicated no statistically significant difference between the controls and test concentrations with the same NOEC and LOEC values reported for Dunnett's Test.

Attachment 8
Growth (weight) of fish at test termination (day 40).

The weights of the fish at day 40 are discussed on page 37 of this DER.

ToxCalc analysis of the mean weights of fish (per replicate per test concentration) at day 40:

Conc-%	1	2	3	4
Control	7.2400	7.9500	6.9000	7.2300
0.836	7.5100	8.6700	7.3900	10.7600
1.28	7.1500	6.7300	8.5500	8.6000
2.23	8.5200	8.7400	8.1500	8.3000
3.62	6.7800	6.9100	7.3200	9.9200
6.11	7.4700	6.8800	8.4200	7.2200
10.1	7.2100	7.2100	6.5300	6.8000

			Transform: Untransformed					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	7.3300	1.0000	7.3300	6.9000	7.9500	6.037	4			
0.836	8.5825	1.1709	8.5825	7.3900	10.7600	18.202	4	-1.860	2.451	1.6511
1.28	7.7575	1.0583	7.7575	6.7300	8.6000	12.370	4	-0.635	2.451	1.6511
2.23	8.4275	1.1497	8.4275	8.1500	8.7400	3.060	4	-1.629	2.451	1.6511
3.62	7.7325	1.0549	7.7325	6.7800	9.9200	19.093	4	-0.598	2.451	1.6511
6.11	7.4975	1.0229	7.4975	6.8800	8.4200	8.814	4	-0.249	2.451	1.6511
10.1	6.9375	0.9465	6.9375	6.5300	7.2100	4.806	4	0.583	2.451	1.6511

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.90632		0.896		1.13187	1.587
Bartlett's Test indicates equal variances (p = 0.03)					13.5677		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs B-Control	10.1	>10.1		9.90099	1.65114	0.22526	1.36847	0.90732	0.22386	6, 21