

Data Evaluation Report on the Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*)

PMRA Submission Number {.....}

EPA MRID Number 45831027

## Data Requirement:

## PMRA DATA CODE

EPA DP Barcode	D288160
OECD Data Point	
EPA MRID	45831027
EPA Guideline	§72-4a

Test material: XDE-638

Purity: 97.7%

Common name: Penoxsulam

Chemical name: IUPAC: Not reported

CAS name: 2-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-C]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide

CAS No.: Not reported

Synonyms: None reported

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*J. GOODYEAR**Goodyear*Secondary Reviewer(s):  
{EPA/OECD/PMRA}

Date:

Reference/Submission No.:

Company Code:

Active Code:

EPA PC Code: ~~19903T~~*119031*

Date Evaluation Completed:

CITATION: Marino, T.A., et. al. 2002. XDE-638: Toxicity to the Early Life Stages of the Fathead Minnow, *Pimephales promelas* Rafinesque. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Study No. 011018. Study submitted by Dow AgroSciences LLC, Indianapolis, IN. Study initiated February 7, 2001 and completed April 3, 2002.



## EXECUTIVE SUMMARY:

The chronic toxicity of XDE-638 (penoxsulam) to the early life-stage of Fathead minnow (*Pimephales promelas*) was studied under flow-through conditions for 36 days. Fertilized eggs/embryos (100 embryos/treatment), approximately 18-22 hours old, were exposed to XDE-638 at mean-measured concentrations of <0.08 (<LOQ, control), 0.802, 1.28, 2.09, 3.65, 6.19, and 10.2 ppm a.i.

Hatching commenced on Day 3 and was complete by Day 5, with no treatment-related differences observed in the day-to-mean hatch. No treatment-related effects on the percent hatch, or the survival of post-hatch larvae were observed. At test termination, all surviving larvae were normal, and no treatment-related effects on terminal growth (dry weight and length) were observed. Based on the results of this study, the NOAEC and LOAEC were 10.2 and >10.2 ppm a.i.

Since no endpoint was affected by treatment up to 10.2 ppm a.i., this study does not fulfill guideline requirements for an early life-stage toxicity study with the Fathead minnow (§72-4a) and is classified SUPPLEMENTAL, but it need not be repeated.

### Results Synopsis

Test Organism Size/Age (mean Weight or Length): Newly-fertilized embryos, 18-22 hours old  
Test Type (Flowthrough, Static, Static Renewal): Flow-through

#### Results Synopsis

##### % Embryo Hatch

NOAEC: 10.2 ppm a.i.  
LOAEC: >10.2 ppm a.i.

##### Days-to-Mean Hatch

NOAEC: 10.2 ppm a.i.  
LOAEC: >10.2 ppm a.i.

##### % Normal Larvae (Day 36)

NOAEC: 10.2 ppm a.i.  
LOAEC: >10.2 ppm a.i.

##### % Larvae Survival (Days 5-36)

NOAEC: 10.2 ppm a.i.  
LOAEC: >10.2 ppm a.i.

##### % Overall Survival (Days 0-36)

NOAEC: 10.2 ppm a.i.  
LOAEC: >10.2 ppm a.i.

##### Length

NOAEC: 10.2 ppm a.i.  
LOAEC: >10.2 ppm a.i.

##### Dry Weight

NOAEC: 10.2 ppm a.i.  
LOAEC: >10.2 ppm a.i.

Endpoints Affected: None

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The study protocol was based on procedures of the U.S. EPA Standard Evaluation Procedure: Fish Early Life-Stage (1986); and the OECD Guideline for Testing of Chemicals No. 210 (1992). Deviations from U.S. EPA FIFRA Guideline §72-4a:

- 1) The storage conditions of the test material were not reported.
- 2) The size and material of the embryo cups was not reported.
- 3) The flow-splitting accuracy was not reported.
- 4) Water hardness (40.0-66.7 mg/L as CaCO<sub>3</sub>) was slightly greater than recommended (40-48 mg/L).
- 5) The pH range (6.5-7.6) slightly exceeded recommendations (7.2-7.6).
- 6) No endpoint was adversely affected.

These deviations do not affect the validity of the study. However, this study does not fulfill guideline requirements.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the U.S. EPA, OECD, and EC Directive GLP standards (p. 3).

### A. MATERIALS:

1. **Test Material** XDE-638 (penoxsulam)

**Description:** Off-white solid

**Lot No. :** B-765-44

**Purity:** 97.7%

**Stability:** To assess stability, samples of freshly-prepared and 7-day old aqueous stock solution were collected and analyzed (p. 18). Recoveries were 107% of nominal for the freshly-prepared solution and 104% of nominal for the 7-day old solution, indicating stability

of the test material in aqueous solution.

**Storage conditions of test chemical:** Not reported.

OECD requires water solubility, stability in water and light,  $pK_a$ ,  $P_{ow}$ , and vapor pressure of the test compound. OECD requirements were not reported.

## 2. Test organism:

**Species:** Fathead minnow (*Pimephales promelas* Rafinesque)

**Age/embryonic stage at test initiation:** Newly-fertilized, non-eyed embryos, approximately 18-22 hours old

**Method of collection of the fertilized eggs:** N/A

**Source:** Aquatic BioSystems, Fort Collins, CO.

## B. STUDY DESIGN:

### 1. Experimental Conditions

a. Range-finding study: A range-finding study was not conducted.

Definitive test levels were chosen based on information obtained from the acute toxicity studies conducted with Rainbow trout ( $LC_{50} > 102$  ppm), Bluegill sunfish ( $LC_{50} > 103$  ppm), Common carp ( $LC_{50} > 101$  ppm), and daphnia ( $EC_{50} > 98.3$  ppm), in addition to OECD (Method 210) guidance, which states that "concentrations of the substance higher than the 96-hour  $LC_{50}$  or 10 mg/L, whichever is lower, need not be tested."

b. Definitive Study

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
Parental acclimation.		

**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

Parameter	Details	Remarks
		Criteria
<u>if any</u> Period:  Conditions: (same as test or not)  Feeding (type, source, amount given, frequency):  Health: (any mortality observed)	N/A	
Number of fertilized eggs/embryos in each treatment at test initiation	100 embryos/treatment, divided into 25 embryos/cup, one cup/replicate, and four replicates/treatment.	
		<i>EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure</i>
<u>Concentration of test material:</u> nominal:  measured:	0 (negative control), 0.780, 1.30, 2.16, 3.60, 6.00, and 10.0 ppm  <0.08 (<LOQ, control), 0.802, 1.28, 2.09, 3.65, 6.19, and 10.2 ppm a.i.	Results of chemical analyses are provided in Table 3, p. 27.  High-low ratios were 1.3-1.4 for all test levels (reviewer-determined).
		<i>EPA requires a minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</i> - 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 $\pm 20\%$ of the mean measured values.
Solvent (type, percentage, if used)	N/A	
		<i>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.</i> OECD requires that solvent must have no effect on survival nor produce any

**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

Parameter	Details	Remarks
		Criteria
		<i>other adverse effects; concentration should not be greater than 0.1 ml/L.</i>
<u>Number of replicates</u> control: solvent control: treated ones:	4 N/A 4	EPA requires 4 replicates per concentration EPA/OECD require solvent control when a solubilizing agent has been used.
<u>Test condition:</u> Static renewal/flow through:  Type of dilution system for flow through method:  Flow rate:  Renewal rate for static renewal:	Flow-through  Intermittent-flow proportional diluter  8.7 volume turnovers/day  N/A	The diluter system was equipped with mixing/splitting chambers, and was calibrated prior to test initiation (pp. 14-15).  The flow-splitting accuracy was not reported.  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%.
Aeration, if any	Not reported.	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	36 days: 4 days hatching period and 32 days post-hatch	EPA requires 32 days post-hatch
<u>Embryo cups</u> , if used type/material: (glass/stainless steel)  size:	Not specified  Not specified	The embryo cups had mesh screen bottoms, and were suspended in a cylindrical glass incubation chamber (size not reported) within

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Parameter	Details	Remarks
		Criteria
fill volume:	Not specified	the test vessel (p. 15). <i>EPA requires 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</i>
<u>Test vessel</u> type/material: (glass/stainless steel)  size:  fill volume:	Glass aquaria  15 x 10 x 9 cm  850 mL	<i>EPA/OECD requires all glass or glass with stainless steel frame.</i>
Source of dilution water	The dilution water was pumped to the laboratory from the upper Saginaw Bay of Lake Huron. The water was filtered (sand and carbon), pH-adjusted, and UV-irradiated prior to use.	<i>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.</i>
<u>Water parameters:</u> hardness:  pH:  TOC:  dissolved oxygen:  temperature:  photoperiod:  salinity (for marine or estuarine species):  other measurements:     interval of water quality measurements:	40.0-66.7 mg/L as CaCO <sub>3</sub>  6.5-7.6  <LOD (<1000 ng/mL)  6.2-8.1 mg/L (≥76% saturation)  24.3-25.4°C  16:8 light/dark hours  N/A  Residual chlorine - <100 ng/mL (<LOD); Total suspended solids: <1000 ng/mL (<LOD); Metals: See Table 1, p. 25; Pesticides: <LOD (Table 2, p. 26).  DO, pH, and temperature were determined weekly in each vessel. Temperature was	The water hardness was slightly greater than recommended.  The pH range exceeded recommendations.  Results from inorganic and organic analysis of the dilution water are provided in Tables 1 and 2, pp. 25-26.  <i>EPA requires hardness of 40 to 48 mg a.i./L as CaCO<sub>3</sub> and pH of 7.2 to 7.6 is recommended. 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. OECD requires DO concentration between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test.</i>

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Parameter	Details	Remarks
		Criteria
		<i>Temperature should be measured continuously.</i>
<u>Post-hatch details:</u> when the post-hatch period began:  number of hatched eggs (alevins)/ treatment released to the test chamber:  day that alevins were released from the incubation cups to the test chamber:	continuously recorded from one test vessel.  Day 5  All hatched larvae were released to the test chamber.  Released upon hatching.	All criteria were met for hatching success in control replicates (Appendix F, pp. 44-46).  <i>EPA requires % of embryos that  produce live fry must be <math>\geq 50\%</math> in each  control; % hatch in any control embryo  cup must be no more than 1.6 times  that in another control cup.</i>
<u>Post-hatch Feeding:</u> start date:  type/source of feed:  amount given:  frequency of feeding:	Day 5 (Day 0 post-hatch)  Newly-hatched brine shrimp ( <i>Artemia</i> sp.), supplemented with green algae ( <i>Selenastrum  capricornutum</i> ).  200-800 $\mu$ L shrimp/feeding and 0.5-1.0 mL algae/day  Shrimp were provided twice daily throughout the study, with increasing amounts. Algae was offered once daily beginning the day following first hatch to 4 days following the initiation of brine shrimp feeding.	
Stability of chemical in the test system	Verified. To assess stability, samples of freshly-prepared and 7-day old aqueous stock solution were collected and analyzed (p. 18). Recoveries were 107% of nominal for the freshly-prepared solution and 104% of nominal for the 7-day old solution, indicating stability of the test material in aqueous solution.	In addition, mean-measured concentrations, determined from weekly analyses, ranged from 96.8 to 103% of nominal concentrations (Table 3, p. 27).
Recovery of chemical:  Frequency of measurement:	78.7-113% of nominal  Days 0, 7, 14, 21, 28, and 36.	Based on measured concentrations at each interval (Table 3, p. 27). A separate procedural recovery



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Parameter	Details	Remarks
		Criteria
LOD:	Not reported	experiment was not conducted.
LOQ:	0.08 ppm a.i.	
Positive control {if used, indicate the chemical and concentrations}	N/A	
<u>Fertilization success study</u> , if any number of eggs used:  on what day the eggs were removed to check the embryonic development:	None conducted	
Other parameters, if any	N/A	

**2. Observations:**

**Table 2: Observations**

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	<ul style="list-style-type: none"> <li>- Hatching success</li> <li>- Day to mean hatch</li> <li>- Alevin and fry survival</li> <li>- Measurement of growth (length and dry weights)</li> <li>- Sub-lethal effects</li> </ul>	<p><i>EPA minimally requires:</i></p> <ul style="list-style-type: none"> <li>- Number of embryos hatched;</li> <li>- Time to hatch;</li> <li>- Mortality of embryos, larvae, and juveniles;</li> <li>- Time to swim-up (if approp.);</li> <li>- Measurement of growth;</li> <li>- Incidence of pathological or histological effects;</li> <li>- Observations of other effects or clinical signs.</li> </ul>
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	Daily Daily Daily N/A Day 36 Not determined Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes, adequate	
Other observations, if any	N/A	

## II. RESULTS AND DISCUSSION

### A. MORTALITY:

No treatment-related effects on the survival of embryos or post-hatch larvae were observed (Table 8, p. 32).

Percent hatch averaged 90% for the dilution water control, and 88-93% for the treatment groups. Larval survival averaged 86.8% for the dilution water control, and 89.7-94.3% for the treatment groups. Overall survival (relative to 100 initial embryos) averaged 78% for the control group, and 81-86% for the treatment groups.

Table 3: Effect of XDE-638 on Egg Hatching and Survival of Fathead minnow (*Pimephales promelas*)

Treatment, ppm a.i., measured (and nominal) concentrations	No. of eggs at study initiation <sup>1</sup>	% Embryos Hatched (Day 5) <sup>2</sup>	% Time to Hatch <sup>2,3</sup>			% Larval Survival	
			Day 3	Day 4	Day 5	Post-hatch (Days 5-36)	Overall (Days 0-36)
Negative control	100	90	10	99	100	86.8	78
0.802 (0.780)	100	90	4	99	100	89.7	81
1.28 (1.30)	100	87	10	98	100	94.3	82
2.09 (2.16)	100	88	1	98	100	94.3	83
3.65 (3.60)	100	88	1	94	100	92.9	82
6.19 (6.00)	100	93	9	98	100	92.4	86
10.2 (10.0)	100	89	16	96	100	91.2	81
NOAEC, ppm a.i.		10.2	10.2			10.2	10.2
LOAEC, ppm a.i.		>10.2	>10.2			>10.2	>10.2
MATC, ppm a.i.		>10.2	>10.2			>10.2	>10.2
Positive control, if used mortality: EC <sub>50</sub> :		N/A	N/A			N/A	

<sup>1</sup> Larvae were not thinned following hatching.

<sup>2</sup> Raw data provided in Appendix F, pp. 44-46.

<sup>3</sup> Reviewer-calculated, relative to total number of hatched embryos/level.

## B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

Hatching commenced on Day 3 and was complete by Day 5, with no treatment-related differences observed in the day-to-mean hatch (Table 8, p. 32). Furthermore, no treatment-related effects were observed on the percent of normal larvae at test termination (100% for control and all treatment groups), or on terminal growth (dry weight and length). For all control and test levels, dry weights ranged from 7.80 to 9.64 mg and lengths ranged from 14.4 to 17.8 mm.

Table 4: Effect of XDE-638 on Growth (mean±SD) of Juvenile Fathead minnow (*Pimephales promelas*).

Treatment, ppm a.i. measured (and nominal) concentrations	Length (mm)	Dry Weight (mg)
Negative control	15.2 ± 0.3	9.64 ± 1.05
0.802 (0.780)	14.8 ± 0.8	8.41 ± 1.28
1.28 (1.30)	15.0 ± 0.2	8.98 ± 0.82
2.09 (2.16)	14.8 ± 0.6	8.05 ± 0.87
3.65 (3.60)	14.7 ± 0.8	8.63 ± 2.06
6.19 (6.00)	14.4 ± 0.3	7.80 ± 0.68
10.2 (10.0)	17.8 ± 3.8	8.12 ± 0.83
NOAEC, ppm a.i.	10.2	10.2
LOAEC, ppm a.i.	>10.2	>10.2
MATC, ppm a.i.	>10.2	>10.2
EC <sub>50</sub> , ppm a.i.	>10.2	>10.2
Positive control, if used mortality: EC <sub>50</sub> :	N/A	N/A

### C. REPORTED STATISTICS:

Endpoints that were statistically analyzed included the percent of hatched embryos, days-to-mean hatch, percent of normal larvae at test termination, percent larvae survival (Days 5-36), percent overall survival (Days 0-36), Day 36 length, and Day 36 dry weight. Percent data were arcsine transformed prior to analysis. Endpoints were assessed for normality and homogeneity assumptions, then the NOAEC values were determined by parametric ANOVA followed by Dunnett's test (except for days-to-mean hatch). The NOAEC for the days-to-mean hatch data was determined by the Steel's Many-one Rank test. Mean-measured concentrations were used in all estimations.

### D. VERIFICATION OF STATISTICAL RESULTS:

Embryo hatch, length, and dry weight data were determined to be normally distributed and the variances were homogeneous. The NOAEC and LOAEC for these endpoints were determined using ANOVA. These analyses were conducted using TOXSTAT statistical software. The NOAEC and LOAEC for days to hatch, % normal larvae at test termination, % larval survival, and % overall survival could be visually determined, as there were obviously no differences from control.

**Results Synopsis**

**% Embryo Hatch**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Days-to-Mean Hatch**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**% Normal Larvae (Day 36)**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**% Larvae Survival (Days 5-36)**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**% Overall Survival (Days 0-36)**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Length**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Dry Weight**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Endpoints Affected:** None

**E. STUDY DEFICIENCIES:**

For early-life stage studies, a minimum of five toxicant levels are required, one of which must adversely affect a life stage and one concentration must not affect any life stage. In this study, the toxicity of XDE-638 was assessed at six toxicant levels, ranging from mean-measured values of 0.802 to 10.2 ppm a.i., with no adverse effects observed on any required endpoints. As a result, this study does not fulfill guideline requirements for a fish early life-stage toxicity study (§72-4a) and is classified SUPPLEMENTAL, but it need not be repeated.

**F. REVIEWER'S COMMENTS:**

The reviewer's conclusions were identical to the study authors'. There were no effects of XDE-638 on the early life-stage of the Fathead minnow.

The study authors reported that definitive test levels were chosen based on information obtained from the acute toxicity studies conducted with Rainbow trout ( $LC_{50} > 102$  ppm), Bluegill sunfish ( $LC_{50} > 103$  ppm), Common carp ( $LC_{50} > 101$  ppm), and daphnia ( $EC_{50} > 98.3$  ppm), in addition to OECD (Method 210) guidance, which states that "concentrations of the substance higher than the 96-hour  $LC_{50}$  or 10 mg/L, whichever is lower, need not be tested."

**G. CONCLUSIONS:**

This study is scientifically sound, but does not satisfy the guideline requirements for an early life-stage toxicity test with the Fathead minnow [§72-4(a)] because no endpoint was adversely affected by the toxicant range (mean-measured levels of 0.780-10.2 ppm a.i.) used in this study. This study provides useful information, and is classified SUPPLEMENTAL, but it need not be repeated.

**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

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**Results Synopsis**

**% Embryo Hatch**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Days-to-Mean Hatch**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**% Normal Larvae (Day 36)**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**% Larvae Survival (Days 5-36)**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**% Overall Survival (Days 0-36)**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Length**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Dry Weight**

NOAEC: 10.2 ppm a.i.

LOAEC: >10.2 ppm a.i.

**Endpoints Affected: None**

**III. REFERENCES:**

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- Winer, B.J. 1971. *Statistical Principles in Experimental Design* (2<sup>nd</sup> Ed.). McGraw-Hill Book Company, New York, NY.
- Steel, R.G.D. 1959. A Multiple Comparison Rank Sum Test: Treatments Versus Control *Biometrics* 15: 560-572.

**APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

embryo hatched

File: 1027h Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	5.857	0.976	0.222
Within (Error)	21	92.250	4.393	
Total	27	98.107		

Critical F value = 2.57 (0.05,6,21)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All groups equal

embryo hatched

File: 1027h Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	22.500	22.500		
2	0.802	22.500	22.500	0.000	
3	1.28	21.750	21.750	0.506	
4	2.09	22.000	22.000	0.337	
5	3.65	22.000	22.000	0.337	
6	6.19	23.250	23.250	-0.506	
7	10.2	22.250	22.250	0.169	

Dunnett table value = 2.46 (1 Tailed Value,  $P=0.05$ ,  $df=20,6$ )

embryo hatched

File: 1027h Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	4		
2	0.802	4	3.646	16.2
3	1.28	4	3.646	16.2
4	2.09	4	3.646	16.2
5	3.65	4	3.646	16.2
6	6.19	4	3.646	16.2
7	10.2	4	3.646	16.2



**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

embryo hatched

File: 1027h Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	22.500	22.500	22.500
2	0.802	4	22.500	22.500	22.500
3	1.28	4	21.750	21.750	22.250
4	2.09	4	22.000	22.000	22.250
5	3.65	4	22.000	22.000	22.250
6	6.19	4	23.250	23.250	22.250
7	10.2	4	22.250	22.250	22.250

embryo hatched

File: 1027h Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE DEGREES OF WILLIAMS	FREEDOM
control	22.500				
0.802	22.500	0.000	1.72	k= 1, v=21	
1.28	22.250	0.169	1.80	k= 2, v=21	
2.09	22.250	0.169	1.83	k= 3, v=21	
3.65	22.250	0.169	1.84	k= 4, v=21	
6.19	22.250	0.169	1.85	k= 5, v=21	
10.2	22.250	0.169	1.85	k= 6, v=21	

s = 2.096

Note: df used for table values are approximate when v > 20.

length

File: 1027l Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1.464	0.244	0.875
Within (Error)	21	5.850	0.279	
Total	27	7.314		

**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

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 Critical F value = 2.57 (0.05,6,21)  
 Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All groups equal

length  
 File: 1027I Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	15.200	15.200		
2	0.802	14.825	14.825	1.004	
3	1.28	15.050	15.050	0.402	
4	2.09	14.775	14.775	1.138	
5	3.65	14.725	14.725	1.272	
6	6.19	14.450	14.450	2.008	
7	10.2	14.675	14.675	1.406	

-----  
 Dunnett table value = 2.46 (1 Tailed Value,  $P=0.05$ ,  $df=20,6$ )

length  
 File: 1027I Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	4		
2	0.802	4	0.919	6.0 0.375
3	1.28	4	0.919	6.0 0.150
4	2.09	4	0.919	6.0 0.425
5	3.65	4	0.919	6.0 0.475
6	6.19	4	0.919	6.0 0.750
7	10.2	4	0.919	6.0 0.525

length  
 File: 1027I Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	15.200	15.200	15.200

**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

2	0.802	4	14.825	14.825	14.938
3	1.28	4	15.050	15.050	14.938
4	2.09	4	14.775	14.775	14.775
5	3.65	4	14.725	14.725	14.725
6	6.19	4	14.450	14.450	14.563
7	10.2	4	14.675	14.675	14.563

length

File: 1027l Transform: NO TRANSFORMATION

**WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2**

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	15.200				
0.802	14.938	0.703	1.72	k= 1, v=21	
1.28	14.938	0.703	1.80	k= 2, v=21	
2.09	14.775	1.139	1.83	k= 3, v=21	
3.65	14.725	1.273	1.84	k= 4, v=21	
6.19	14.563	1.708	1.85	k= 5, v=21	
10.2	14.563	1.708	1.85	k= 6, v=21	

s = 0.528

Note: df used for table values are approximate when v > 20.

dry weight

File: 1027dw Transform: NO TRANSFORMATION

**ANOVA TABLE**

SOURCE	DF	SS	MS	F
Between	6	9.515	1.586	1.160
Within (Error)	21	28.699	1.367	
Total	27	38.213		

Critical F value = 2.57 (0.05,6,21)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

dry weight

File: 1027dw Transform: NO TRANSFORMATION

**DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment**

TRANSFORMED MEAN CALCULATED IN

**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

GROUP	IDENTIFICATION	MEAN	ORIGINAL UNITS	T STAT	SIG
1	control	9.635	9.635		
2	0.802	8.413	8.413	1.479	
3	1.28	8.983	8.983	0.789	
4	2.09	8.045	8.045	1.923	
5	3.65	8.628	8.628	1.219	
6	6.19	7.803	7.803	2.217	
7	10.2	8.123	8.123	1.829	

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

dry weight

File: 1027dw Transform: NO TRANSFORMATION

**DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment**

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	4		
2	0.802	4	2.034	21.1
3	1.28	4	2.034	21.1
4	2.09	4	2.034	21.1
5	3.65	4	2.034	21.1
6	6.19	4	2.034	21.1
7	10.2	4	2.034	21.1

dry weight

File: 1027dw Transform: NO TRANSFORMATION

**WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2**

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	9.635	9.635	9.635
2	0.802	4	8.413	8.413	8.698
3	1.28	4	8.983	8.983	8.698
4	2.09	4	8.045	8.045	8.336
5	3.65	4	8.628	8.628	8.336
6	6.19	4	7.803	7.803	7.963
7	10.2	4	8.123	8.123	7.963

dry weight

File: 1027dw Transform: NO TRANSFORMATION

**Toxicity of XDE-638 to the Early Life Stage of Fathead Minnow (*Pimephales promelas*) MRID 45831027**

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG	WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	9.635					
0.802	8.698	1.134	1.72	k= 1, v=21		
1.28	8.698	1.134	1.80	k= 2, v=21		
2.09	8.336	1.571	1.83	k= 3, v=21		
3.65	8.336	1.571	1.84	k= 4, v=21		
6.19	7.963	2.023	*	1.85	k= 5, v=21	
10.2	7.963	2.023	*	1.85	k= 6, v=21	

s = 1.169

Note: df used for table values are approximate when v > 20.