Data Evaluation Report on the Toxicity of Atrazine to Sheepshead Minnow (*Cyprinodon variegates*), Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 466482-03

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	322057
	OECD Data Point	{.....}
	EPA MRID	466482-03
	EPA Guideline	§72-4a

Test Material: Atrazine Technical **Purity(%):** 97.1%
Common name: Atrazine
Chemical name: IUPAC: 6-Chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine
CAS name: 6-Chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine
CAS No.: 1912-24-9
Synonyms: G-30027

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Reference/Submission No.: {.....}

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Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Cafarella, M.A. 2005. Atrazine (G-30027) – Early life-stage toxicity test with sheepshead minnow (*Cyprinodon variegates*). Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Project No. 1781.6642. Study submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Study conducted from May 27 – June 29, 2005, and submitted September 8, 2005.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 33-day chronic toxicity of Atrazine to the early life stage of sheepshead minnow (*Cyprinodon variegates*) was studied under flow-through conditions. Fertilized embryos (160/level), <26 hours old, were exposed to Atrazine at nominal concentrations of 0 (dilution water control), 0.20, 0.40, 0.80, 1.6, and 3.2 mg ai/L. Mean-measured concentrations were <0.028 (<LOQ, control), 0.15, 0.30, 0.57, 1.1, and 2.2 mg ai/L. The test system was maintained at 24-27°C and a pH of 7.8-8.2.

No effect on pre- or post-hatch survival was observed, and no abnormal behavior or other clinical signs of toxicity were reported. At study termination (28-day post-hatch), the total length and wet weights were adversely affected at the 2.2 mg ai/L level as indicated by a statistically-significant reduction for these endpoints when compared to the dilution water control. Control fish averaged 22.9 cm long and 0.204 g. In comparison, fish from the 2.2 mg ai/L group averaged 18.9 cm long and 0.111 g. The most sensitive endpoint was growth (i.e., larval length and wet weight). The subsequent NOAEC is 1.1 mg ai/L.

This study is scientifically sound, but does not fulfill the guideline requirements for a fish early life-stage toxicity study (§72-4a) because only two replicate aquaria were maintained, the time-to-hatch endpoint was not assessed, and the study was terminated at 28 days post-hatch. This study is classified as SUPPLEMENTAL. Data obtained from this study are useful for risk assessment purposes.

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Embryos, <26 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

LOAEC: 2.2 mg ai/L
NOAEC: 1.1 mg ai/L
Endpoint(s) Affected: Total length and wet weight

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Subdivision E, §72-4 (1982); the U.S. EPA Standard Evaluation Procedure EPA 540/9-86-138 (1986); and the ASTM Standard Guide for Conducting Early Life-Stage Toxicity Test with Fishes (1992). Deviations from §72-4a include:

1. A physical description of the test substance was not provided.

2. Only two replicate aquaria/test level were maintained in this study, whereas four replicates/level are required.

3. Fish were maintained for only 28 days post-hatch, rather than 32 days as required.

4. Results of periodic analysis of the dilution water for select pesticides, PCBs, and toxic metals were not provided.

5. The pH range (7.8-8.2) was higher than recommended (7.2-7.6).

6. The time that hatching was complete was reported as Day 5, but the time-to-hatch was not statistically compared between test and control groups.

These deviations do not affect the validity of the study. However, this study does not fulfill guideline requirements because only two replicates were maintained and the time-to-hatch endpoint was not assessed.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) and OECD GLP standards with the exception of the collection of samples for routine water contaminant screening analyses.

A. MATERIALS:

1. Test Material	Atrazine Technical
Description:	Not reported
Lot No./Batch No. :	SG8029BA10
Purity:	97.1%
Stability of compound under test conditions:	The stability of the test substance in the dilution water during the course of the study was verified by analytical determinations on Days 0, 5, 12, 19, 26, and 33 (all test levels). Concentrations were stable, with reviewer-calculated high-low ratios of ≤1.3.
Storage conditions of test chemicals:	Room temperature in the dark

Physicochemical properties of [test chemical].

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species: Sheepshead minnow (*Cyprinodon variegates*)
EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.

Age /embryonic stage at test initiation: <26 hours old
EPA recommends fish embryos 2 to 24 hours old.

Method of collection of the fertilized eggs: N/A (purchased).

Source: Aquatic Biosystems, Fort Collins, CO

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 14-day preliminary flow-through study was conducted with 20 sheepshead minnow larvae per level at nominal concentrations of 0 (dilution water control), 0.20, 0.40, 0.80, 1.6, and 3.2 mg ai/L. Following 14 days of exposure, larval survival in the control and all treatment levels tested was 100%, with no clinical signs of toxicity observed. Larval growth, however, appeared to be adversely affected at the 3.2 mg ai/L level.

In addition, an analytical pilot study was conducted in which a 25 mg ai/L Atrazine diluter stock solution and a sample of the high test concentration (3.2 mg ai/L) were sampled and analyzed along with one QC sample (3.00 mg ai/L). Measured concentrations for the 25 and 3.2 mg ai/L solutions were 68 and 62% of nominal, respectively. Analysis of the QC sample resulted in a recovery of 98.5% of nominal. It was reported that based on these results, the functional water solubility of Atrazine could be maintained under the test conditions.

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)	N/A	
Number of fertilized eggs/embryos in each treatment at test initiation	120 embryos/treatment level, divided into 60 embryos/cup, 1 cup/aquarium, and 2 replicate aquaria/treatment	On Day 5, larvae were thinned to 80 organisms per treatment level (40 per replicate). *Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)*
Concentration of test material nominal: measured:	0 (dilution water control), 0.20, 0.40, 0.80, 1.6, and 3.2 mg ai/L <0.028 (<LOQ, control), 0.15, 0.30, 0.57, 1.1, and 2.2 mg ai/L	Mean-measured concentrations were determined on study Days 0, 5, 12, 19, 26, and 33. Values were consistently low, with overall mean-measured values averaging 68-75% of nominal concentrations. Concentrations were adjusted for the purity of the substance. *A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.* *- Toxicant concentration should be measured in one tank at each toxicant level every week.* *- One concentration should adversely affect a life stage and one concentration should not affect any life stage.* *OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within ± 20% of the mean measured values.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	None used.	*The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol. OECD recommends that the solvent not have an 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: treated ones:	 2 N/A 2	*Number of replicates should be 4 per concentration. A solvent control should be used in conjunction with a solubilizing agent.*
Test condition 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 6.8 volume additions/day N/A	The diluter was calibrated prior to test initiation and confirmed at test termination. Diluter function was monitored for normal operation daily and a visually checked twice/day. *Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water. 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 preferred; 2) Aeration should not be used for mixing; 3) The test solution should be completely mixed before introduction into the test system; 4) Flow splitting accuracy should be within 10%.*

Parameter	Details	Remarks *Criteria*
Aeration, if any	None reported	*Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	33 days (28 days post-hatch)	*Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.*
Embryo cups, if used type/material (glass/stainless steel): size: fill volume:	 Round glass jars with 475 µm mesh screen bottoms 5-cm diameter, 8-cm height Not reported	The embryo cups were suspended in the water column and gently oscillated at 2 rpm. *Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
Test vessel type/material: (glass/stainless steel) size: fill volume:	 Glass 39 x 29 x 25 cm 15 L	*Recommended test vessel is all glass or glass with stainless steel frame.*
Source of dilution water	Natural filtered seawater pumped from the Cape Cod Canal, Bourne, MA and UV sterilized prior to use.	The dilution water was analyzed for pesticides, PCBs and toxic metals, and it was reported that none of these compounds were detected at concentrations that are considered toxic (actual results not provided). *Source of dilution water should be 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.*

Parameter	Details	Remarks *Criteria*
Water parameters hardness: pH: dissolved oxygen: temperature (s) (record all the temperatures used for different life stages): photoperiod: salinity (for marine or estuarine species): other measurements: interval of water quality measurements:	 Not reported 7.8-8.2 5.2-7.4 mg/L (≥75% saturation) 24-27°C 16:8 light:dark cycle maintained throughout exposure (540-940 lux) 29-31‰ TOC was <2.0 mg/L in dilution water for the months of May and June 2005 DO, pH, salinity, and temperature were measured in each aquarium daily. Temperature was also continuously monitored in one negative control replicate.	Water hardness was not reported. The pH was greater than recommended. *Recommended hardness: 40-48 mg/L as $CaCO_3$;* *Recommended pH:* 7.2 to 7.6 *Dissolved Oxygen (DO) should be measured at each concentration at least once a week;* *Freshwater parameters in a control and one concentration should be analyzed once a week.* *Temperature depends upon test species and should not deviate by more than 2 °C from appropriate temperature.* *OECD recommends that DO concentration be 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. Temperature should be measured continuously.*
Post-hatch details 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 5, when no more than 10% unhatched viable embryos remained in any embryo incubation cup. Newly hatched larvae were thinned to 80 organisms per treatment level (40/replicate) on Day 5. Day 5	 *Percentage of embryos that produce live fry should be ≥ 50% in each control; percentage of hatch in any control embryo cup should not be more than 1.6 times that in another control cup.*

Parameter	Details	Remarks *Criteria*
Post-hatch Feeding start date: type/source of feed: amount given: frequency of feeding:	 Day 5 Live brine shrimp nauplii (*Artemia salina*) *Ad libitum* Three times daily on weekdays and at least twice daily on weekends. No feeding for the 24 hours prior to test termination.	
Stability of chemical in the test system	Verified by analytical measurements conducted on Days 0, 5, 12, 19, 26, and 33. Although recoveries were consistently low, the test material was stable during the 33-day test, with reviewer-calculated high-low ratios of 1.1-1.3.	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	83.4-104% of nominal Days 0, 5, 12, 19, 26, and 33 Not reported 0.020-0.028 mg ai/L	Based on QC (matrix spike) samples fortified at 0.200, 0.800, or 4.00 mg ai/L and analyzed concurrently with the test samples.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	 No separate fertilization study was conducted.	Prior to test initiation, a representative sub-sample of approximately 100 eggs was examined to estimate the percent of successfully fertilized embryos. Based on visible developmental stages, it was estimated that 78% of the eggs were viable.
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- Number of embryos hatched - Larval survival - Measurement of growth (length and wet weight) - Clinical signs of toxicity or abnormal behavior	Time to hatch was defined as Day 5, but was not statistically monitored. *Recommended parameters measured include:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and Juveniles:* *- Time to swim-up (if appropriate);* *- Measurement of growth;* *- Incidence of pathological or Histological effects;* *- Observations of other effects or clinical signs.*
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 Twice weekly N/A Day 33 (day 28 post-hatch) Not determined Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No treatment-related effect on the survival was observed. At hatch on Day 5, an average 78% of control organisms hatched, compared to 71-79% for the treatment groups. At study termination 28-day following hatch, survival of control organisms was 100%, compared to 98-100% for the treatment groups. The NOAEC for embryo and larvae survival was 2.2 mg ai/L, the highest concentration tested.

Table 3: Effect of Atrazine on egg hatching and survival at different life stage of fish.

Treatment (mg ai/L) Measured (and nominal) concentrations	Egg hatched/embryo viability			Time to hatch[1]			Juvenile-survival on Day 33 (28 days post-hatch)	
	No. of eggs at study initiation	hatch/embryo viability		Day 3	Day 4	Day 5	No. dead	% mortality
		No.	%					
Control (dilution water only)	120	94	78				0/80	0
0.15 (0.20)	120	89	74				1/80	1
0.30 (0.40)	120	95	79				1/80	1
0.57 (0.80)	120	85	71				2/79	1
1.1 (1.6)	120	91	76				2/80	2
2.2 (3.2)	120	89	74				0/80	0
NOAEC		2.2 mg ai/L		No data provided			2.2 mg ai/L	
EC_{50}		>2.2 mg ai/L		No data provided			>2.2 mg ai/L	
Positive control, if used mortality: EC_{50}: NOAEC	N/A							

[1] The time-to-hatch was not assessed.

Table 4: Effect of Atrazine on Growth of Juvenile Fish

Treatment (mg ai/L) Measured (and nominal) concentrations	Growth -length (cm)	Growth-wet weight (g)
Control (dilution water only)	22.9	0.204
0.15 (0.20)	22.7	0.197
0.30 (0.40)	22.8	0.196
0.57 (0.80)	22.9	0.202
1.1 (1.6)	22.2	0.182
2.2 (3.2)	18.9*	0.111*
NOAEC	1.1 mg ai/L	1.1 mg ai/L
LOAEC	2.2 mg ai/L	2.2 mg ai/L
EC_{50}	Not reported	Not reported
Positive control, if used mortality: EC_{50}: NOAEC	N/A	N/A

* Significantly different from control based on Williams Test.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

No behavioral abnormalities or other signs of toxicity were reported.

The time to hatch endpoint was not assessed. Total length and wet weights were adversely affected at the 2.2 mg ai/L level as indicated by a statistically-significant reduction for these endpoints when compared to the dilution water control. Control fish averaged 22.9 cm long and 0.204 g. In comparison, fish from the 2.2 mg ai/L group averaged 18.9 cm long and 0.111 g. The most sensitive endpoint was growth. The subsequent NOAEC is 1.1 mg ai/L.

C. REPORTED STATISTICS:

Endpoints that were analyzed statistically included survival at hatch (Day 5), larval survival (Day 33), and larval growth (Day 33). Analyses were performed using the mean organism response in each replicate vessel rather than individual response values. Survival data were arcsine square-root transformed prior to analysis. The data were checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test. The Williams' test was then used to identify treatments statistically different than the dilution water control for all endpoints, at a 95% level of certainty. The NOAEC and LOAEC were based on significance data. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. A computer program (West and Gulley, 1996) was used to perform the statistical analyses, and mean-measured values were used in all estimations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Survival of organisms at hatch, larval survival, total length, and wet weight endpoints were statistically analyzed to determine NOAEC and LOAEC values. With the exception of larval survival, all endpoints satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). These assumptions were tested using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett's tests for homogeneity of variances. The NOAEC and LOAEC for these endpoints were determined using ANOVA, followed by Dunnett's test. The NOAEC and LOAEC for larval survival were verified using the non-parametric Kruskal-Wallis test. These analyses were conducted using TOXSTAT statistical software.

EC_{50}:N/A 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 1.1 mg ai/L
LOAEC: 2.2 mg ai/L

E. STUDY DEFICIENCIES:

This study is scientifically sound. However, only two replicate aquaria were maintained during the study (four are required), the time-to-hatch endpoint was not assessed, and the study was terminated at 28 days post-hatch, instead of the required 32 days. Therefore, this study does not fulfill the guideline requirements for a fish early life-stage toxicity study (§72-4a).

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. Growth parameters were the most sensitive endpoints. Significant reductions in total length and wet weight were observed at the highest treatment level.

The biomass loading did not exceed 0.083 g/L of flowing test solution per day in any replicate exposure aquarium.

In a method validation study conducted in May 2002 and provided as an appendix, fresh water was fortified with Atrazine at 0.0100, 0.500, or 24.0 mg ai/L. Recoveries for all samples averaged 100 ± 2.27%. The LOQ was 0.00382 mg ai/L.

G. CONCLUSIONS:

This toxicity study is scientifically sound, but does not fulfill the guideline requirements for a fish early life-stage study (§72-4a) because only two replicate aquaria were maintained (four are required), and the time-to-hatch endpoint was not assessed. This study is classified as SUPPLEMENTAL. No treatment-related effect on either pre- or post-hatch survival was observed. Growth, however, was adversely affected at the 2.2 mg ai/L level, as indicated by statistically-significant reductions in total length and wet weights when compared to controls. The NOAEC and LOAEC were 1.1 and 2.2 mg ai/L, respectively.

III. REFERENCES:

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APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

survival at hatch
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	93.417	18.683	0.439
Within (Error)	6	255.500	42.583	
Total	11	348.917		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

survival at hatch
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	77.500	77.500		
2	0.15	74.000	74.000	0.536	
3	0.30	79.000	79.000	-0.230	
4	0.57	70.500	70.500	1.073	
5	1.1	76.000	76.000	0.230	
6	2.2	73.500	73.500	0.613	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

survival at hatch
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.15	2	18.467	23.8	3.500
3	0.30	2	18.467	23.8	-1.500
4	0.57	2	18.467	23.8	7.000
5	1.1	2	18.467	23.8	1.500
6	2.2	2	18.467	23.8	4.000

survival at hatch
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	77.500	77.500	77.500
2	0.15	2	74.000	74.000	76.500
3	0.30	2	79.000	79.000	76.500
4	0.57	2	70.500	70.500	73.333
5	1.1	2	76.000	76.000	73.333
6	2.2	2	73.500	73.500	73.333

survival at hatch
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	77.500				
0.15	76.500	0.153		1.94	k= 1, v= 6
0.30	76.500	0.153		2.06	k= 2, v= 6
0.57	73.333	0.639		2.10	k= 3, v= 6
1.1	73.333	0.639		2.12	k= 4, v= 6
2.2	73.333	0.639		2.13	k= 5, v= 6

s = 6.526
Note: df used for table values are approximate when v > 20.

larval survival
File: 8203s Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	100.000	100.000	18.000
2	0.15	99.000	99.000	12.000
3	0.30	99.000	99.000	12.000
4	0.57	99.000	99.000	12.000
5	1.1	98.000	98.000	6.000
6	2.2	100.000	100.000	18.000

Calculated H Value = 5.343 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

larval survival
File: 8203s Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 5 3 4 2 1 6
5	1.1	98.000	98.000	\
3	0.30	99.000	99.000	. \
4	0.57	99.000	99.000	. . \
2	0.15	99.000	99.000	. . . \
1	control	100.000	100.000	. . . . \
6	2.2	100.000	100.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.090

total length
File: 82031 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	25.240	5.048	20.032
Within (Error)	6	1.510	0.252	
Total	11	26.750		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

total length
File: 82031 Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	22.900	22.900		
2	0.15	22.700	22.700	0.398	
3	0.30	22.750	22.750	0.299	
4	0.57	22.900	22.900	-0.000	
5	**1.1**	**22.200**	**22.200**	**1.394**	
6	2.2	18.850	18.850	8.068	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

total length
File: 82031 Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.15	2	1.421	6.2	0.200
3	0.30	2	1.421	6.2	0.150
4	0.57	2	1.421	6.2	-0.000
5	1.1	2	1.421	6.2	0.700
6	2.2	2	1.421	6.2	4.050

total length
File: 82031 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	22.900	22.900	22.900
2	0.15	2	22.700	22.700	22.783
3	0.30	2	22.750	22.750	22.783
4	0.57	2	22.900	22.900	22.783
5	1.1	2	22.200	22.200	22.200
6	2.2	2	18.850	18.850	18.850

total length
File: 82031 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	22.900				
0.15	22.783	0.233		1.94	k= 1, v= 6
0.30	22.783	0.233		2.06	k= 2, v= 6
0.57	22.783	0.233		2.10	k= 3, v= 6
1.1	22.200	1.395		2.12	k= 4, v= 6
2.2	18.850	8.073	*	2.13	k= 5, v= 6

s = 0.502

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

wet weight
File: 8203w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0126	0.0025	12.500
Within (Error)	6	0.0010	0.0002	
Total	11	0.0136		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

wet weight
File: 8203w Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.205	0.205		
2	0.15	0.197	0.197	0.495	
3	0.30	0.197	0.197	0.566	
4	0.57	0.202	0.202	0.212	
5	**1.1**	**0.182**	**0.182**	**1.591**	
6	2.2	0.111	0.111	6.576	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

wet weight
File: 8203w Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.15	2	0.040	19.6	0.007
3	0.30	2	0.040	19.6	0.008
4	0.57	2	0.040	19.6	0.003
5	1.1	2	0.040	19.6	0.023
6	2.2	2	0.040	19.6	0.093

wet weight
File: 8203w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	0.205	0.205	0.205
2	0.15	2	0.197	0.197	0.198
3	0.30	2	0.197	0.197	0.198
4	0.57	2	0.202	0.202	0.198
5	1.1	2	0.182	0.182	0.182
6	2.2	2	0.111	0.111	0.111

wet weight
File: 8203w 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	0.205				
0.15	0.198	0.460		1.94	k= 1, v= 6
0.30	0.198	0.460		2.06	k= 2, v= 6
0.57	0.198	0.460		2.10	k= 3, v= 6
1.1	0.182	1.726		2.12	k= 4, v= 6
2.2	0.111	7.133	*	2.13	k= 5, v= 6

s = 0.013
Note: df used for table values are approximate when v > 20.