Data Requirement: PMRA DATA CODE
EPA DP Barcode D
OECD Data Point
EPA MRID 452995-04
EPA Guideline Special Study 70-1

Test material: Atrazine FL-931480 (Batch # SG 302011 BA) **Purity:** 98.2 % a.i.
Common name: Atrazine
Chemical names: IUPAC: 6-Chloro-N-ethyl-N-isopropyl-1,3,5-triazine-2,4-diamine
CAS name:
CAS No.: 1912 - 24 - 9
Synonyms: Aatrex, Atranex, Atratef, Crisazina, Gesaprim, Herbitrin, Posmil, Sanazin, Trac

Primary Reviewer: William S. Rabert *William S. Rabert* **Date:** *May 10, 2002*
US EPA, OPPTS, OPP, EFED, ERBIII

Secondary Reviewer(s): Thomas Steeger *Thomas M Steeger* **Date:** *5/13/02*
US EPA, OPPTS, OPP, EFED, ERBI

Reference/Submission No.:
Company Code:
Active Code:
EPA PC Code: **080803**
Date Evaluation Completed: 15 - 4 - 02

<u>**CITATION**</u>: Timothy J. Madsen. 2000. Effects of atrazine on the sex ratio of *Daphnia pulicaria*. Prepared by ABC Laboratories, Inc., Columbia, Missouri, ABC Study Number 45810; Submitted by Novartis Crop Protection, Inc., Greensboro, NC; October 2, 2000.

<u>EXECUTIVE SUMMARY</u>:

The 12-day partial chronic atrazine toxicity study with 3 to 4-day old *Daphnia pulicaria* was conducted under flow-through conditions. Daphnids were exposed to a control, solvent control, and mean measured atrazine concentrations of 0.93, 4.1, 8.7, 44, and 87 µg a.i/L. The concentration of the solvent dimethylformamide was not reported. The solvent control significantly differed ($p \leq 0.05$) from the control (i.e., more young per replicate and reduced adult body length). The 12-day LC_{50}/EC_{50} based on mortality/sublethal effect was > 87 µg a.i/L. The 12-day NOAEC based on reduced adult survival was 8.7 µg/L compared to the DMF Control. The sublethal effects included were reduced body length at 87 µg a.i/L compared to the solvent control. Production of offsprings in the treated groups indicated that atrazine had an indirect effect on the reproduction due to adult mortalities at concentrations greater than 8.7mg a.i/L. The most sensitive endpoint was adult survival (NOAEC 8.7 µg ai./L; 44 µg ai./L).

Although, it is implied that these data contradict the Dodson *et al.* (1999) study; the conditions for the test differ. Therefore, the test results are not comparable. The Dodson study employed stressful conditions intended to yield male daphnids. Therefore, test animals were intentionally crowded (8/30 ml), light cycles were short (6 hours light and 18 hours dark) and the artificial media was double strength. Further, young daphnids were not evaluated until after at least 3 days exposure. Also, the indices evaluated in the Dodson study were calculated differently from this study. The only comparable index measured was the sex ratio.

This study is classified as scientifically unsound and it does not satisfy guideline requirements for a chronic toxicity study with freshwater invertebrates. The test produced no male offspring. Since atrazine endocrine effects are typically demasculinization and the daphnids in this study are females, it is reasonable that this study did not have endocrine effects on *Daphnia pulicaria* females.

Results Synopsis

Test Organism Age (eg. 1st instar): 3- to 4-days old, Instar stage is unknown.
Test Type (Flow-through): Flow-through.
NOAEL: 8.7 µg a.i./L
LOAEC: 44 µg a.i./L Endpoint Effected: Adult survival.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study did not specify any particular guideline (Special Study 70-1)

1. The test species was *Daphnia pulicaria* rather than the required species, *Daphnia magna*.
2. The test began with daphnids 3- to 4-days old, rather than ≤ 24 hours old.
3. Duration of study was a subacute test of 12 days rather than the typical 21-day *Daphnia magna* life cycle study.
4. The dilution water was moderately hard (130-160 mg/L as $CaCO_3$) rather than the benchmark soft water (40-48 mg/L $CaCO_3$).
5. The dilution water was biologically aged and passed through a polypropylene cartridge filter and ultraviolet sterilizer before going to the proportional diluter. Total organic carbon and particulate matter levels in the dilution water were not reported. Fluoride levels were high (i.e., 0.55 mg/L).
6. The pH level ranged from 8.51 to 8.66 in the controls and 7.96 to 8.5 in the treatments which is higher than the recommended range of 7.2 to 7.6.

6\. Control mortality was 14 percent, which exceeds the limit of 10 percent mortality.

COMPLIANCE: The Good Laboratory Procedures, Quality Assurance, and Data Confidentiality statements were signed and dated.

A. MATERIALS:

1. Test Material Atrazine received from Novartis Crop Protectin, Inc., on Dec. 7, 1999.

Description: Atrazine (FL-931480) Chemical state of the test material was not reported.

Lot No./Batch No. : SG 302011 BA

Purity: 98.2 %

Stability of Compound: Stability was not stated; the expiration date was cited as August 31, 2001.
Under Test Conditions:
(OECD requires water solubility (33 mg/L), stable in water, light half-life (335 days), pKa, Pow (87.78), vapor pressure of test compound (3×10^{-7})
Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:

Species: *Daphnia pulicaria* (*EPA/OECD require Daphnia magna*)
Age of the parental stock: Gravid females (16 to 23 days old).
Source: Supplied by University of Guelph, Canada

3. Study Objective: The primary objective was to determine the effect of atrazine on the sex ratio of young Daphnia pulicaria produced during a part ial life cycle exposure conducted under flow-through conditions. Additionally, the total number of offspring produced and the survival and growth of adult females were measured.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: No range-finding study.

b. Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: period: conditions: (same as test or not) Feeding: Health: (any mortality observed)	Not reported. Similar temperature and water hardness; other parameters not reported. Fed approx. 2 x 10^6 cells/mL (*Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*).	Acclimation period was not specified, but was at least 3 days.
Test condition: static renewal/flow through: Type of dilution system - for flow through method. Renewal rate for static renewal	Flow-through test. Two Hamilton syringe injectors into mixing chamber. 7.4 volumes per 24 hours. N/A	
Aeration, if any	Test solutions were not aerated during the test..	
Chemical properties	None reported.	None reported. *(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)*
Duration of the test	12 days	*21 days*: Shortened life cycle test.
Test vessel Material: (glass/stainless steel Size (for growth and reproduction/survival test): Fill volume:	Borosilicate beakers. 1 liter test chamber (10 cm diameter x 15 cm high with solution depth of 12 cm. 1,000 mL (1 daphnid/100 ml).	Okay. *1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50-100 ml of medium in each vessel.*

Parameter	Details	Remarks *Criteria*
Source of dilution water	Deep well water mixed with a portion from reverse osmosis, biologically aged and then filtered through polypropylene cartridge and ultraviolet sterilizer.	Biologically aged water is inappropriate source of dilution water, especially when water quality measurements are incomplete and inadequate. *Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 146 - 154 mg/L 7.96 - 8.66 3.89 - 8.47 mg O_2/L (DO dropped below 60% saturation in some atrazine treated beaker by Day 6). 20.5 to 21.7°C Not reported. Not reported. Levels of detection are inadequate for all metals. all less than 1.0 µg/L. Not reported. Fluoride conc.: 0.59 mg/L is too high. (Stephan, 1975; <100 µg/L)	Analysis is incomplete and many levels of detection are inadequate. Fluoride level is high. *EPA requires:* ***hardness** 160 to 180 mg/L as $CaCO_3$;* *OECD requires > 140 mg/L as $CaCO_3$* ***pH** 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH range 6 - 9 and should not vary more than 1.5 units in any one test.* ***Dissolved Oxygen** Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥ 60% throughout test.* ***Temperature**: 20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C* *OECD requires total organic carbon < 2 mg/L*
Experimental Design: Number of Treatments: Controls Number of replicates	 5 treatments. Negative and DMF controls. 8 Replicates.	Okay. *EPA requires Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.*
Number of organisms: For growth and reproduction: For survival test:	 8 replicates/treatment. 10 Daphnids per replicate. 80 Daphnids per treatment.	Okay. *22 daphnids/level;* *7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.*

Parameter	Details	Remarks / *Criteria*
Application rates nominal: measured:	Up to 4 lbs ai./A. 0, 1, 5, 10, 50 and 100 µg/L. < 0.05, 0.93, 4.1, 8.7, 44 and 87 µg/L.	Okay.
Solvent (type, percentage, if used)	Dimethylformamide – 100 µL/L.	Okay. *EPA requires:* *solvent not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.* *OECD requires ≤ 0.1 ml/L*
Lighting	16 hours light: 8 hours dark. 408 to 556 lux	Okay. *EPA/OECD requires: 16 hours light, 8 hours dark.*
Stability of chemical in the test system	Range of concentrations within 5% of mean concentration, except for the highest level – up to 14%.	Okay.
Other parameters, if any	About 7.4 volumes per 24 hours.	Okay. *Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.*

2. Observations:

Table X: Observations

Criteria	Details	Remarks / *Criteria*
Data end points measured (list)	Adult Survival Male to Female ratio Number of young Number of young per female reproductive day Day of first brood release Adult body length	Dry weight endpoint requirement not fulfilled. *EPA requires:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.*

Observation intervals	Observations of abnormal physical appearance, neonate production and mortality/ immobility were recorded daily, but only reported as of Day 12. Days 0, 6 and 12 for T, pH & DO.	Okay.
Were raw data included?	Yes.	Okay.
Other observations, if any	Sex determinations were made.	Okay.

II. <u>RESULTS AND DISCUSSION</u>

A. <u>MORTALITY</u>: Control mortalities (14 percent) were less than 30 percent limit. Mortalities occurred in all treatments, but they were erratic and showed no dose-related effect. Mortality at 44 and 87 µg ai/L was statistically significantly ($p < 0.05$) higher than the solvent control. The 12-day LC_{50} was greater than the highest test concentration, 87 µg/L. Few replicates produced 40 daphnids per adult, but the test was conducted for only 12 days, not the required 21 days.

Table 1: Effect of atrazine on growth and survival of *Daphnia pulicaria*.

Treatment (µg a.i./L) [record measured and nominal conc. used]		Mortality (Dead or Immobile)		Mean Days to First Brood	Total Number of Young (Y/Rep)	Male: Female Ratio	Mean Daphnid Length (mm)
		No. Dead	%				
DMF Control (0.1 ml/L)		3	3.8	5	2834 (354)	0.0	2.1
Negative control, if used		11[1]	14[1]	5	2132[2] (355)	0.0	2.4
1.0	0.93	7	8.8	4	2854 (357)	0.0	2.1
5.0	4.1	4	5.0	5	3472 (434)	0.0	2.2
10	8.7	6	7.5	5	3708 (464)	0.0	2.2
50	44	21[3]	26	5	2475 (309)	0.0	2.1
100	87	12[3]	15	5	2390 (299)	0.0	2.0
NOAEC		8.7 µg/L		87 µg/L	87 µg/L	87 µg/L	44 µg/L
LOAEC		44 µg/L		> 87 µg/L	> 87 µg/L	> 87 µg/L	87 µg/L

[1] Negative controls with statistically more mortalities than DMF controls, therefore analyses of treatments were made with the solvent control.
[2] The count for two control replicates were uncertain and therefore they were not reported.
[3] Treatment mortality statistically lower than solvent controls.

B. <u>EFFECT ON REPRODUCTION:</u>

No male daphnid offspring were produced in any of the treatments; therefore the male:female ratio is zero.
The lower number of offspring at the two highest test concentrations appear to be due to higher mortality but the differences were not significantly different from the solvent control due to high variability in the replicates The NOAEC is 87 µg ai./L and the LOAEC is > 87 µg ai./L.

The mean adult body length at 87 µg ai./L was statistically different ($p < 0.05$) from the solvent control (NOAEC, 44 µg ai./L and the LOAEC, 87 µg ai./L). This is a special study 70-1. Since the adult dry body weight was not measured and the test was limited to 12 days, this study would no fulfill the OPP test guideline 72-4(b).

Based on the solvent control, the most sensitive endpoint was adult mortality (NOAEC, 8.7 µg ai./L and the LOAEC, 44 µg ai./L).

C. <u>REPORTED STATISTICS</u>: The following endpoints were measured and analyzed for statistical significance: adult mortality, adult body length, and the number of young. The NOAEC and LOAEC values are listed at the bottom of the columns in the above table.

D. <u>VERIFICATION OF STATISTICAL RESULTS</u>:

Statistical Method: ANOVA and Williams Test

12-day LC_{50}	> 87 µg ai./L	95% C.I.:	N/A
NOAEC:	8.7 µg ai. /L	LOAEC:	44 µg ai./L (Adult mortality)
Probit Slope:	N/A	95% C.I.:	N/A

E. <u>STUDY DEFICIENCIES</u>: The use of "biologically aged water" as the dilution water is unacceptable, because the water quality of the dilution water is unknown (i.e., TOC, particulate matter and other contaminant levels were not reported for the biologically-aged, dilution water. The test was conducted for 12 days rather than the required 21 days. Adult dry body weight was not measured as an endpoint as required. The above items are major deficiencies which render the test results incomplete and uncertain. This study is invalid.

F. <u>REVIEWER'S COMMENTS</u>: Compared to the solvent control and most treatments, the blank control had unexplained high mortality (14 percent). The collection of young from two replicates of the blank controls were mixed and had to be omitted from the statistical analysis of the number of young. Dose-response curves were erratic for adult mortality and the total number of young.

G. <u>CONCLUSIONS</u>: This study was not performed using good scientific practices. The "biologically aged water" is inappropriate as the dilution water, unless all water quality criteria are measured for each batch of aged water. The study showed no evidence of endocrine effects, but if atrazine has a mode of action of demasculinization, the effects would not be found in this daphnid study with all females. If the intent of this study was to 'refute' the Dodson *et al.* (1999) study, the test conditions were not similar (i.e., the daphnids were not tested with the environmental stresses employed in the Dodson *et al.* study).

This special study was not intended to and does not meet the test requirement for an aquatic invertebrate life cycle test for the following reasons. The test period was of insufficient duration (12 days rather than 21 days) and the required endpoint for adult dry body weight was not measured.

The atrazine concentrations were measured using the Insite™ atrazine plate kit (Beacon Analytical Systems, Portland, ME. The limit of quantification for atrazine in the control and solvent control was 0.05 µg/L.

III. <u>REFERENCES</u>: [Provide references that were cited in the study report: studies in the open literature, references to other study reports in the submission or other studies conducted by the proponent . Do not include references to standard guidelines or methodologies.]

Dodson, S. I., C. M. Merritt, L. Torrentera, J. Shannahan and C. M. Shults. 1999. Low exposure concentrations of atrazine increase male production in *Daphnia pulicaria*. Environmental Toxicology and Chemistry 18(7):1568-1573.

Dodson, S. I., C. M. Merritt, L. Torrentera, K. M. Winter, C. K. Tonehl and K. Girvin. 1999. Toxicology and Industrial Health 15:192-199.

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The Reviewer's analyses of the test results are presented below.

TITLE: Atrazine – Daphnia pulicaria Partial Life Cycle – Adult Survival

Transform: LOG BASE 10(Y)

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GROUP1 (SOLVENT CONTROL) MEAN = 0.9822 CALCULATED t VALUE = 1.7484
GROUP2 (BLANK CONTROL) MEAN = 0.9302 DEGREES OF FREEDOM = 14
DIFFERENCE IN MEANS = 0.0520

TABLE t VALUE (0.05 (2),14) = 2.145 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),14) = 2.977 NO significant difference at alpha=0.01

TRANSFORM: LOG BASE 10(Y) NUMBER OF GROUPS: 7

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	DMF Control	1	10.0000	1.0000
1	DMF Control	2	10.0000	1.0000
1	DMF Control	3	10.0000	1.0000
1	DMF Control	4	10.0000	1.0000
1	DMF Control	5	9.0000	0.9542
1	DMF Control	6	8.0000	0.9031
1	DMF Control	7	10.0000	1.0000
1	DMF Control	8	10.0000	1.0000
2	Control	1	6.0000	0.7782
2	Control	2	10.0000	1.0000
2	Control	3	8.0000	0.9031
2	Control	4	10.0000	1.0000
2	Control	5	8.0000	0.9031
2	Control	6	8.0000	0.9031
2	Control	7	10.0000	1.0000
2	Control	8	9.0000	0.9542
3	0.93	1	10.0000	1.0000
3	0.93	2	10.0000	1.0000
3	0.93	3	10.0000	1.0000
3	0.93	4	9.0000	0.9542
3	0.93	5	10.0000	1.0000
3	0.93	6	10.0000	1.0000
3	0.93	7	6.0000	0.7782
3	0.93	8	8.0000	0.9031
4	4.1	1	10.0000	1.0000
4	4.1	2	9.0000	0.9542
4	4.1	3	9.0000	0.9542
4	4.1	4	9.0000	0.9542
4	4.1	5	10.0000	1.0000
4	4.1	6	10.0000	1.0000
4	4.1	7	9.0000	0.9542
4	4.1	8	10.0000	1.0000
5	8.7	1	10.0000	1.0000
5	8.7	2	10.0000	1.0000
5	8.7	3	9.0000	0.9542

5	8.7	4	8.0000	0.9031
5	8.7	5	10.0000	1.0000
5	8.7	6	9.0000	0.9542
5	8.7	7	9.0000	0.9542
5	8.7	8	9.0000	0.9542
6	44	1	7.0000	0.8451
6	44	2	5.0000	0.6990
6	44	3	8.0000	0.9031
6	44	4	9.0000	0.9542
6	44	5	7.0000	0.8451
6	44	6	9.0000	0.9542
6	44	7	4.0000	0.6021
6	44	8	10.0000	1.0000
7	87	1	9.0000	0.9542
7	87	2	10.0000	1.0000
7	87	3	10.0000	1.0000
7	87	4	9.0000	0.9542
7	87	5	9.0000	0.9542
7	87	6	3.0000	0.4771
7	87	7	9.0000	0.9542
7	87	8	9.0000	0.9542

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	DMF Control	8	0.903	1.000	0.982
2	Control	8	0.778	1.000	0.930
3	0.93	8	0.778	1.000	0.954
4	4.1	8	0.954	1.000	0.977
5	8.7	8	0.903	1.000	0.965
6	44	8	0.602	1.000	0.850
7	87	8	0.477	1.000	0.906

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	DMF Control	0.001	0.036	0.013
2	Control	0.006	0.076	0.027
3	0.93	0.006	0.079	0.028
4	4.1	0.001	0.024	0.009
5	8.7	0.001	0.034	0.012
6	44	0.019	0.137	0.048
7	87	0.030	0.175	0.062

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.106	0.018	2.000
Within (Error)	49	0.450	0.009	
Total	55	0.556		

Critical F value = 2.34 (0.05,6,40); Since F < Critical F FAIL TO REJECT Ho: All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	DMF Control	0.982	9.625		
2	Control	0.930	8.625	1.095	
3	0.93	0.954	9.125	0.585	
4	4.1	0.977	9.500	0.106	
5	8.7	0.965	9.250	0.362	
6	44	0.850	7.375	2.779	*
7	87	0.906	8.500	1.605	

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

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	DMF Control	8			
2	Control	8	0.112	1.2	1.000
3	0.93	8	0.112	1.2	0.500
4	4.1	8	0.112	1.2	0.125
5	8.7	8	0.112	1.2	0.375
6	44	8	0.112	1.2	2.250
7	87	8	0.112	1.2	1.125

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	DMF Control	8	9.625	0.982	0.982
2	Control	8	8.625	0.930	0.957
3	0.93	8	9.125	0.954	0.957
4	4.1	8	9.500	0.977	0.957
5	8.7	8	9.250	0.965	0.957
6	44	8	7.375	0.850	0.878
7	87	8	8.500	0.906	0.878

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
DMF Control	0.982				
Control	0.957	0.531		1.68	k= 1, v=49
0.93	0.957	0.531		1.76	k= 2, v=49
4.1	0.957	0.531		1.79	k= 3, v=49
8.7	0.957	0.531		1.80	k= 4, v=49
44	0.878	2.169	*	1.80	k= 5, v=49
87	0.878	2.169	*	1.81	k= 6, v=49

s = 0.096; Note: df used for table values are approximate when v > 20.

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle – Number of Young

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.618	13.068	20.628	13.068	3.618
OBSERVED	4	12	26	8	4

Calculated Chi-Square goodness of fit test statistic = 3.5324
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Shapiro-Wilks test for normality
******** Shapiro-Wilks Test is aborted ********
This test can not be performed because total number of replicates is greater than 50.
Total number of replicates = 54

Hartley test for homogeneity of variance
Calculated H statistic (max Var/min Var) = 19.31
Closest, conservative, Table H statistic = 20.0 (alpha = 0.01)
Used for Table H ==> R (# groups) = 7, df (# reps-1) = 7
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 6.71 (average df used)
Data PASS homogeneity test. Continue analysis.
NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Bartletts test for homogeneity of variance
Calculated B statistic = 13.57
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 6.71
Used for Chi-square table value ==> df (#groups-1) = 6
Data PASS homogeneity test at 0.01 level. Continue analysis.
NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

GROUP1 (SOLVENT CONTROL) MEAN = 354.2500 CALCULATED t VALUE = 2.5236
GROUP2 (BLANK CONTROL) MEAN = 252.8333 DEGREES OF FREEDOM = 12
DIFFERENCE IN MEANS = 101.4167
TABLE t VALUE (0.05 (2),12) = 2.179** SIGNIFICANT DIFFERENCE at alpha = 0.05
TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha = 0.01

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle – Number of Young (cont.)

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	DMF Control	1	319.0000	319.0000
1	DMF Control	2	301.0000	301.0000
1	DMF Control	3	493.0000	493.0000
1	DMF Control	4	354.0000	354.0000
1	DMF Control	5	185.0000	185.0000
1	DMF Control	6	341.0000	341.0000
1	DMF Control	7	453.0000	453.0000
1	DMF Control	8	388.0000	388.0000
2	Control	1	241.0000	241.0000
2	Control	2	245.0000	245.0000
2	Control	3	220.0000	220.0000
2	Control	4	262.0000	262.0000
2	Control	5	298.0000	298.0000
2	Control	6	251.0000	251.0000
3	0.93	1	370.0000	370.0000
3	0.93	2	341.0000	341.0000
3	0.93	3	512.0000	512.0000
3	0.93	4	373.0000	373.0000
3	0.93	5	304.0000	304.0000
3	0.93	6	374.0000	374.0000
3	0.93	7	241.0000	241.0000
3	0.93	8	339.0000	339.0000
4	4.1	1	375.0000	375.0000
4	4.1	2	463.0000	463.0000
4	4.1	3	403.0000	403.0000
4	4.1	4	406.0000	406.0000
4	4.1	5	561.0000	561.0000
4	4.1	6	413.0000	413.0000
4	4.1	7	393.0000	393.0000
4	4.1	8	458.0000	458.0000
5	8.7	1	492.0000	492.0000
5	8.7	2	558.0000	558.0000
5	8.7	3	503.0000	503.0000
5	8.7	4	442.0000	442.0000
5	8.7	5	570.0000	570.0000
5	8.7	6	405.0000	405.0000
5	8.7	7	381.0000	381.0000
5	8.7	8	357.0000	357.0000
6	44	1	240.0000	240.0000
6	44	2	116.0000	116.0000
6	44	3	373.0000	373.0000
6	44	4	414.0000	414.0000
6	44	5	334.0000	334.0000
6	44	6	396.0000	396.0000
6	44	7	189.0000	189.0000
6	44	8	413.0000	413.0000
7	87	1	164.0000	164.0000
7	87	2	283.0000	283.0000
7	87	3	483.0000	483.0000
7	87	4	343.0000	343.0000
7	87	5	343.0000	343.0000
7	87	6	118.0000	118.0000

7	87	7	301.0000	301.0000
7	87	8	355.0000	355.0000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	DMF Control	8	185.000	493.000	354.250
2	Control	6	220.000	298.000	252.833
3	0.93	8	241.000	512.000	356.750
4	4.1	8	375.000	561.000	434.000
5	8.7	8	357.000	570.000	463.500
6	44	8	116.000	414.000	309.375
7	87	8	118.000	483.000	298.750

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	DMF Control	9005.929	94.900	33.552
2	Control	681.367	26.103	10.657
3	0.93	5940.500	77.075	27.250
4	4.1	3556.286	59.635	21.084
5	8.7	6382.571	79.891	28.246
6	44	12934.268	113.729	40.209
7	87	13158.500	114.711	40.556

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	248610.273	41435.046	5.406
Within (Error)	47	360253.208	7664.962	
Total	53	608863.481		

Critical F value = 2.34 (0.05,6,40); Since F > Critical F REJECT Ho: All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	DMF Control	354.250	354.250		
2	Control	252.833	252.833	2.145	
3	0.93	356.750	356.750	-0.057	
4	4.1	434.000	434.000	-1.822	
5	8.7	463.500	463.500	-2.496	
6	44	309.375	309.375	1.025	
7	87	298.750	298.750	1.268	

Bonferroni T table value = 2.50 (1 Tailed Value, P=0.05, df=40,6)

BONFERRONI T-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	DMF Control	8			
2	Control	6	118.158	33.4	101.417
3	0.93	8	109.393	30.9	-2.500
4	4.1	8	109.393	30.9	-79.750
5	8.7	8	109.393	30.9	-109.250
6	44	8	109.393	30.9	44.875
7	87	8	109.393	30.9	55.500

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	DMF Control	8	354.250	354.250	378.553
2	Control	6	252.833	252.833	378.553
3	0.93	8	356.750	356.750	378.553
4	4.1	8	434.000	434.000	378.553
5	8.7	8	463.500	463.500	378.553
6	44	8	309.375	309.375	309.375
7	87	8	298.750	298.750	298.750

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
DMF Control	378.553				
Control	378.553	0.514		1.68	k= 1, v=47
0.93	378.553	0.555		1.76	k= 2, v=47
4.1	378.553	0.555		1.79	k= 3, v=47
8.7	378.553	0.555		1.80	k= 4, v=47
44	309.375	1.025		1.80	k= 5, v=47
87	298.750	1.268		1.81	k= 6, v=47

s = 87.550; Note: df used for table values are approximate when v > 20.

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle – Adult Body Length

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.752	13.552	21.392	13.552	3.752
OBSERVED	5	15	16	16	4

Calculated Chi-Square goodness of fit test statistic = 2.3875
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Shapiro-Wilks test for normality
******** Shapiro-Wilks Test is aborted ********

This test can not be performed because total number of replicates is greater than 50.
Total number of replicates = 56

Hartley test for homogeneity of variance
Calculated H statistic (max Var/min Var) = 14.71
Closest, conservative, Table H statistic = 20.0 (alpha = 0.01)
Used for Table H ==> R (# groups) = 7, df (# reps-1) = 7
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 7.00
Data PASS homogeneity test. Continue analysis.
NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Bartletts test for homogeneity of variance
Calculated B statistic = 15.52
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 6
Data PASS homogeneity test at 0.01 level. Continue analysis.
NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN
GROUP1 (SOLVENT CRTL) MEAN = 2.1125 CALCULATED t VALUE = -8.0643
GROUP2 (BLANK CRTL) MEAN = 2.4125 DEGREES OF FREEDOM = 14
DIFFERENCE IN MEANS = -0.3000
TABLE t VALUE (0.05 (2),14) = 2.145** SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2),14) = 2.977** SIGNIFICANT DIFFERENCE at alpha=0.01

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle – Adult Body Length (cont.)

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	DMF Control	1	2.1000	2.1000
1	DMF Control	2	2.1000	2.1000
1	DMF Control	3	2.1000	2.1000
1	DMF Control	4	2.2000	2.2000
1	DMF Control	5	1.9000	1.9000
1	DMF Control	6	2.2000	2.2000
1	DMF Control	7	2.2000	2.2000
1	DMF Control	8	2.1000	2.1000
2	Control	1	2.4000	2.4000
2	Control	2	2.4000	2.4000
2	Control	3	2.4000	2.4000
2	Control	4	2.4000	2.4000
2	Control	5	2.4000	2.4000
2	Control	6	2.5000	2.5000
2	Control	7	2.4000	2.4000
2	Control	8	2.4000	2.4000
3	0.93	1	2.1000	2.1000
3	0.93	2	2.2000	2.2000
3	0.93	3	2.2000	2.2000
3	0.93	4	2.2000	2.2000
3	0.93	5	2.1000	2.1000
3	0.93	6	2.2000	2.2000
3	0.93	7	2.0000	2.0000
3	0.93	8	2.1000	2.1000
4	4.1	1	2.1000	2.1000
4	4.1	2	2.3000	2.3000
4	4.1	3	2.0000	2.0000
4	4.1	4	2.2000	2.2000
4	4.1	5	2.2000	2.2000
4	4.1	6	2.1000	2.1000
4	4.1	7	2.2000	2.2000
4	4.1	8	2.1000	2.1000
5	8.7	1	2.2000	2.2000
5	8.7	2	2.3000	2.3000
5	8.7	3	2.3000	2.3000
5	8.7	4	2.2000	2.2000
5	8.7	5	2.2000	2.2000
5	8.7	6	2.2000	2.2000
5	8.7	7	2.2000	2.2000
5	8.7	8	2.2000	2.2000
6	44	1	1.9000	1.9000
6	44	2	2.0000	2.0000
6	44	3	2.2000	2.2000
6	44	4	2.2000	2.2000
6	44	5	2.1000	2.1000
6	44	6	2.2000	2.2000
6	44	7	2.0000	2.0000
6	44	8	2.2000	2.2000
7	87	1	1.8000	1.8000
7	87	2	2.0000	2.0000
7	87	3	2.2000	2.2000
7	87	4	2.0000	2.0000

7	87	5	2.0000	2.0000
7	87	6	1.9000	1.9000
7	87	7	2.0000	2.0000
7	87	8	2.2000	2.2000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	DMF Control	8	1.900	2.200	2.112
2	Control	8	2.400	2.500	2.413
3	0.93	8	2.000	2.200	2.138
4	4.1	8	2.000	2.300	2.150
5	8.7	8	2.200	2.300	2.225
6	44	8	1.900	2.200	2.100
7	87	8	1.800	2.200	2.013

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	DMF Control	0.010	0.099	0.035
2	Control	0.001	0.035	0.012
3	0.93	0.006	0.074	0.026
4	4.1	0.009	0.093	0.033
5	8.7	0.002	0.046	0.016
6	44	0.014	0.120	0.042
7	87	0.018	0.136	0.048

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.769	0.128	14.222
Within (Error)	49	0.420	0.009	
Total	55	1.189		

Critical F value = 2.34 (0.05,6,40); Since F > Critical F REJECT Ho: All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	DMF Control	2.112	2.112		
2	Control	2.413	2.413	-6.325	
3	0.93	2.138	2.138	-0.527	
4	4.1	2.150	2.150	-0.791	
5	8.7	2.225	2.225	-2.372	
6	44	2.100	2.100	0.264	
7	87	2.013	2.013	2.108	

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

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	DMF Control	8			
2	Control	8	0.112	5.3	-0.300
3	0.93	8	0.112	5.3	-0.025
4	4.1	8	0.112	5.3	-0.038
5	8.7	8	0.112	5.3	-0.112
6	44	8	0.112	5.3	0.012
7	87	8	0.112	5.3	0.100

BONFERRONI T-TEST - TABLE 1 OF 2 Ho: Control < Treatment

TRANSFORMED MEAN CALCULATED T STAT

GROUP	IDENTIFICATION	MEAN	ORIGINAL	IN UNITS	SIG
1	DMF Control	2.112	2.112		
2	Control	2.413	2.413	-6.325	
3	0.93	2.138	2.138	-0.527	
4	4.1	2.150	2.150	-0.791	
5	8.7	2.225	2.225	-2.372	
6	44	2.100	2.100	0.264	
7	87	2.013	2.013	2.108	

Bonferroni T table value = 2.50 (1 Tailed Value, P=0.05, df=40,6)

BONFERRONI T-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	DMF Control	8			
2	Control	8	0.119	5.6	-0.300
3	0.93	8	0.119	5.6	-0.025
4	4.1	8	0.119	5.6	-0.038
5	8.7	8	0.119	5.6	-0.112
6	44	8	0.119	5.6	0.012
7	87	8	0.119	5.6	0.100

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	DMF Control	8	2.112	2.112	2.263
2	Control	8	2.413	2.413	2.263
3	0.93	8	2.138	2.138	2.171
4	4.1	8	2.150	2.150	2.171
5	8.7	8	2.225	2.225	2.171
6	44	8	2.100	2.100	2.100
7	87	8	2.013	2.013	2.013

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
DMF Control	2.263				
Control	2.263	3.241	*	1.68	k= 1, v=49
0.93	2.171	1.260		1.76	k= 2, v=49
4.1	2.171	1.260		1.79	k= 3, v=49
8.7	2.171	1.260		1.80	k= 4, v=49
44	2.100	0.270		1.80	k= 5, v=49
87	2.013	2.160	*	1.81	k= 6, v=49

s = 0.093; Note: df used for table values are approximate when v > 20.

Data Requirement: PMRA DATA CODE
EPA DP Barcode D
OECD Data Point
EPA MRID 452995-04
EPA Guideline ~~72-4b~~ 70-1

Test material: Atrazine FL-931480 (Batch # SG 302011 BA) **Purity:** 98.2 % a.i.
Common name: Atrazine
Chemical names: IUPAC: 6-Chloro-N-ethyl-N-isopropyl-1,3,5-triazine-2,4-diamine
CAS name:
CAS No.: 1912 - 24 - 9
Synonyms: Aatrex, Atranex, Atratef, Crisazina, Gesaprim, Herbitrin, Posmil, Sanazin, Trac

Primary Reviewer: William S. Rabert William S. Rabert **Date:** May 7, 2002
US EPA

Secondary Reviewer(s): Harry Craven **Date:**
US EPA

Although it is implied that these data contradict the Dodson et al (1999) study; the conditions for the test differ. Therefore, the test results are not comparable. The Dodson study employed conditions intended to yield male daphnids. The test animals were intentionally crowded (8/30ml), light cycles were short (6 hrs light 18 dark) and artificial media was double strength. Further, young daphnids were not evaluated until after 2 days of exposure. Also, the indices evaluated in the Dodson study are calculated differently from this study (all but the sex ratio - perhaps the only comparable index measured)

Reference/Submission No.:
Company Code:
Active Code:
EPA PC Code: 080803
Date Evaluation Completed: 15 - 4 - 02

CITATION: Timothy J. Madsen. 2000. Effects of atrazine on the sex ratio of *Daphnia pulicaria*. Prepared by ABC Laboratories, Inc., Columbia, Missouri, ABC Study Number 45810; Submitted by Novartis Crop Protection, Inc., Greensboro, NC; October 2, 2000.

EXECUTIVE SUMMARY:

The 12-day partial chronic atrazine toxicity study with 3 to 4-day old *Daphnia pulicaria* was conducted under flow-through conditions. Daphnids were exposed to a control, solvent control, and mean measured atrazine concentrations of 0.93, 4.1, 8.7, 44, and 87 µg a.i/L. The concentration of the solvent dimethylformamide was not reported. The solvent control significantly differed ($p \leq 0.05$) from the control (i.e., more young per replicate and reduced adult body length). The 12-day LC_{50}/EC_{50} based on mortality/sublethal effect was > 87 µg a.i/L. The 12-day NOAEC based on reduced adult survival was 8.7 µg/L compared to the DMF Control. The sublethal effects included were reduced body length at 87 µg a.i/L compared to the solvent control. Production of offsprings in the treated groups indicated that atrazine had an indirect effect on the reproduction due to adult mortalities at concentrations greater than 8.7mg a.i/L. The most sensitive endpoint was adult survival (NOAEC 8.7 µg ai./L; 44 µg ai./L).

This study is classified as scientifically unsound and it does not satisfy guideline requirements for a chronic toxicity study with freshwater invertebrates. The test produced no male offspring. Since atrazine effects are typically demasculinization and the daphnids in this study are females, it is reasonable that this study did not have endocrine effects on *Daphnia pulicaria* females.

Results Synopsis

Test Organism Age (eg. 1[st] instar): 3- to 4-days old, Instar stage is unknown.
Test Type (Flow-through): Flow-through.
NOAEL: 8.7 µg a.i./L
LOAEC: 44 µg a.i./L Endpoint Effected: Adult survival.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study did not specify any particular guideline. Special study 70-1

1. The test species was *Daphnia pulicaria* rather than the required species, *Daphnia magna.*
2. The test began with daphnids 3- to 4-days old, rather than ≤ 24 hours old.
3. Duration of the study was limited to 12 days rather than the typical 21-day study with *Daphnia magna.* ∴ subacute
4. The dilution water was moderately hard (130-160 mg/L as $CaCO_3$) rather than the benchmark soft water (40-48 mg/L $CaCO_3$).
5. The dilution water was biologically aged and passed through a polypropylene cartridge filter and ultraviolet sterilizer before going to the proportional diluter. Total organic carbon and particulate matter levels in the dilution water were not reported. Fluoride levels were high (i.e., 0.55 mg/L). pH ranged from Control 8.51-8.66 Treat 7.96-8.5 and is higher than the recommende range of 7.2-7.6
6. Control mortality was 14 percent, which exceeds the limit of 10 percent mortality.

COMPLIANCE: The Good Laboratory Procedures, Quality Assurance, and Data Confidentiality statements were signed and dated.

A. MATERIALS:

1. Test Material Atrazine received from Novartis Crop Protectin, Inc., on Dec. 7, 1999.

Description: Atrazine (FL-931480) Chemical state of the test material was not reported.

Lot No./Batch No. : SG 302011 BA

Purity: 98.2 %

Stability of Compound: Under Test Conditions: Stability was not stated; the expiration date was cited as August 31, 2001.

(OECD requires water solubility (33 mg/L), stable in water, light half-life (335 days), pKa, Pow (87.78), vapor pressure of test compound (3×10^{-7})

Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:

Species: *Daphnia pulicaria* (*EPA/OECD require Daphnia magna*)
Age of the parental stock: Gravid females (16 to 23 days old).
Source: Supplied by University of Guelph, Canada

3. Study Objective: ~~To determine if atrazine~~ has endocrine ~~effects on the~~ sex ratio of *Daphnia pulicaria.* The primary objective was to determine the effect of Atrazine on the sex ratio of young Daphnia pulicaria produced during a partial life cycle exposure conducted under flow-through conditions. Additionally, the Total number of offspring produced and the survival and growth of adult females were measured

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: No range-finding study.

b. Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: period: conditions: (same as test or not) Feeding: Health: (any mortality observed)	Not reported. Similar temperature and water hardness; other parameters not reported. Fed approx. 2 x 10^6 cells/mL (*Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*).	Acclimation period not reported. (implied 3 days)
Test condition: static renewal/flow through: Type of dilution system - for flow through method. Renewal rate for static renewal	Flow-through test. Two Hamilton syringe injectors into mixing chamber. N/A	Flow rate in replicates not reported. 100 mL/cycle 7.4 volume replacements/24 hrs
Aeration, if any	~~Not reported.~~ Test solutions not aerated during test	~~Not reported.~~
Chemical properties	None reported.	None reported. *(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)*
Duration of the test	12 days	*21 days*: Shortened life cycle test.
Test vessel Material: (glass/stainless steel Size (for growth and reproduction/survival test): Fill volume:	Borosilicate beakers. 1 liter test chamber. 1,000 mL 10 cm diameter x 15 cm high ≥ solution depth 12 cm ∴ 1 daphnid / 100 mL	Okay. *1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Size: 250 ml with 200 ml fill volume is preferred;* *100 ml with 80 ml fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50-100 ml of medium in each vessel.*
Source of dilution water	Deep well water mixed with a portion from reverse osmosis, biologically aged and then filtered through polypropylene cartridge and ultraviolet sterilizer.	Biologically aged water is inappropriate source of dilution water, especially when water quality measurements are incomplete and inadequate. *Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*

Parameter	Details	Remarks / *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 146 - 154 mg/L 7.96 - 8.66 3.89 - 8.47 mg O_2/L 20.5 to 21.7°C Not reported. Not reported. Levels of detection are inadequate for all metals. all less than 1.0 µg/L. Not reported. Fluoride conc.: 0.59 mg/L is too high. (Stephan, 1975; <100 µg/L) D.O. dropped below 60% saturation in some of the atrazine treated cultures by Day 6	Analysis is incomplete and many levels of detection are inadequate. Fluoride level is high. --- *EPA requires:* ***hardness*** *160 to 180 mg/L as $CaCO_3$;* *OECD requires > 140 mg/L as $CaCO_3$* ***pH*** *7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH range 6 - 9 and should not vary more than 1.5 units in any one test.* ***Dissolved Oxygen*** *Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥ 60% throughout test.* ***Temperature****: 20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C* *OECD requires total organic carbon < 2 mg/L*
Experimental Design: Number of Treatments: Controls Number of replicates	 5 treatments. Negative and DMF controls. 8 Replicates.	Okay. --- *EPA requires Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.*
Number of organisms: For growth and reproduction: For survival test:	8 replicates/treatment 10 Daphnids per replicate. 80 Daphnids per treatment.	Okay. --- *22 daphnids/level;* *7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.*
Application rates nominal: measured:	Up to 4 lbs ai./A. 0, 1, 5, 10, 50 and 100 µg/L. < 0.05, 0.93, 4.1, 8.7, 44 and 87 µg/L.	Okay.
Solvent (type, percentage, if used)	Dimethylformamide – 100 µL/L.	Okay.

µg/L per adult, but the test was conducted for only 12 days, not the required 21 days.

Table 1: Effect of atrazine on growth and survival of *Daphnia pulicaria*.

Treatment (mg a.i./L) [record measured and nominal conc. used]		Mortality (Dead or Immobile)		Mean Days to First Brood	Total Number of Young (Y/Rep)	Male: Female Ratio	Mean Daphnid Length (mm) µg/L
		No. Dead	%				
DMF Control (0.1 ml/L)		3	3.8	5	2834 (354) 254?	0.0	2.1
~~Positive~~ negative control, if used		11+	14 +	5	2132* (355)	0.0	2.4
1.0	0.93	7	8.8	4	2854 (357)	0.0	2.1
5.0	4.1	4	5.0	5	3472 (434)	0.0	2.2
10	8.7	6	7.5	5	3708 (464)	0.0	2.2
50	44	21*	26	5	2475 (309)	0.0	2.1
100	87	12*	15	5	2390 (299)	0.0	2.0
NOAEC		8.7 µg/L		87 µg/L	87 µg/L	87 µg/L	44 µg/L
LOAEC		44 µg/L		> 87 µg/L	> 87 µg/L	> 87 µg/L	87 µg/L

is total # young 254 or 354?

* The count for two control replicates were uncertain and therefore they were not reported.
+ statistically different (α<0.05); therefore comparisons made to solvent control.

B. EFFECT ON REPRODUCTION:

No male daphnia were produced in any of the cultures; therefore the male:female ratio is zero

The lower number of offspring at the two highest test concentrations appear to be due to higher mortality but the differences were not significantly different from the solvent control due to high variability in the replicates The NOAEC is 87 µg ai./L and the LOAEC is > 87 µg ai./L.

The mean adult body length at 87 µg ai./L was statistically different ($p < 0.05$) from the solvent control (NOAEC, 44 µg ai./L and the LOAEC, 87 µg ai./L). Adult dry body weight was not measured as required by OPP test guideline 72-4(b). ← test is not intended to comply w/ 72-4(b). This is a special study 72-1

Based on the solvent control, the most sensitive endpoint was adult mortality (NOAEC, 8.7 µg ai./L and the LOAEC, 44 µg ai./L).

C. REPORTED STATISTICS: The following endpoints were measured and analyzed for statistical significance: adult mortality, adult body length, and the number of young. The NOAEC and LOAEC values are listed at the bottom of the columns in the above table.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: ANOVA and Williams Test

12-day LC_{50} > 87 µg ai./L 95% C.I.: N/A

Bill - on p10 William's test shows 44 and 87 µg/L treatments to have significantly lower survival compared to solvent control. Have you elected to discard this result?

NOAEC: 8.7 µg ai. /L LOAEC: 44 µg ai./L (Adult mortality)

Probit Slope: N/A 95% C.I.: N/A

I agree that TOC and TDS are not reported, but you have not made a strong argument against aged water

E. STUDY DEFICIENCIES: The use of "biologically aged water" as the dilution water is unacceptable. The dilution water is of unknown quality. TOC and particulate levels were not reported for the dilution water post-biologically aged. The test was conducted for 12 days rather than the required 21 days. Adult dry body weight was not measured as an endpoint as required. The above items are major deficiencies which render the test results incomplete and uncertain. This study is invalid.

F. REVIEWER'S COMMENTS: Compared to the solvent control and most treatments, the blank control had unexplained high mortality (14 percent). The collection of young from two replicates of the blank controls were mixed and had to be omitted from the statistical analysis of the number of young. Dose-response curves were erratic for adult mortality and the total number of young.

This comment should be deleted in my opinion

I'm not clear as to what is the problem with biologically aged water if they show chemical analysis.

G. CONCLUSIONS: This study was not performed using good scientific practices. The "biologically aged water" was inappropriate as the dilution water. The test period was of insufficient duration (12 days rather than 21 days) and the required endpoint for adult dry body weight was not measured. This study contained unconventional methods as well as incomplete and irregular test results, which render this study of dubious value as an aquatic invertebrate life cycle test. The study shows no evidence of endocrine effects, but if atrazine has a mode of action of demasculinization, the effects would not be found in this daphnid study with all females.

The study is not intended to be an invertebrate life cycle test

it appears as though the test was intended to 'refute' the Dodson et al. 1999 study reported in SETAC.

III. REFERENCES: [Provide references that were cited in the study report: studies in the open literature, references to other study reports in the submission or other studies conducted by the proponent . Do not include references to standard guidelines or methodologies.]

Dodson, S. I., C. M. Merritt, L. Torrentera, J. Shannahan and C. M. Shults. 1999. Low exposure concentrations of atrazine increase male production in *Daphnia pulicaria*. Environmental Toxicology and Chemistry 18(7):1568-1573.

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Zar, J. H. 1984. Biostatistical Analysis. Second Ed., Prentice-Hall, Inc. Englewood Cliffs, NJ.

Conover, W. J. 1980. Pratical [sic] nonparametric statistics. 2nd Edition.

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TITLE: Atrazine – Daphnia pulicaria Partial Life Cycle – Adult Survival

Atrazine measured by Insite(TM) plate kit immunoassay (Beacon Analytical Systems) with limit of quantification 0.05 µg/L

TRANSFORM: LOG BASE 10(Y) NUMBER OF GROUPS: 7

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	DMF Control	1	10.0000	1.0000
1	DMF Control	2	10.0000	1.0000
1	DMF Control	3	10.0000	1.0000
1	DMF Control	4	10.0000	1.0000
1	DMF Control	5	9.0000	0.9542
1	DMF Control	6	8.0000	0.9031
1	DMF Control	7	10.0000	1.0000
1	DMF Control	8	10.0000	1.0000
2	Control	1	6.0000	0.7782
2	Control	2	10.0000	1.0000

According to the report, the decision to pool the control groups was made of each variable by considering both whether the amount of difference between the control groups was biologically important and whether the amount of difference was statistically significant

2	Control	3	8.0000	0.9031
2	Control	4	10.0000	1.0000
2	Control	5	8.0000	0.9031
2	Control	6	8.0000	0.9031
2	Control	7	10.0000	1.0000
2	Control	8	9.0000	0.9542
3	0.93	1	10.0000	1.0000
3	0.93	2	10.0000	1.0000
3	0.93	3	10.0000	1.0000
3	0.93	4	9.0000	0.9542
3	0.93	5	10.0000	1.0000
3	0.93	6	10.0000	1.0000
3	0.93	7	6.0000	0.7782
3	0.93	8	8.0000	0.9031
4	4.1	1	10.0000	1.0000
4	4.1	2	9.0000	0.9542
4	4.1	3	9.0000	0.9542
4	4.1	4	9.0000	0.9542
4	4.1	5	10.0000	1.0000
4	4.1	6	10.0000	1.0000
4	4.1	7	9.0000	0.9542
4	4.1	8	10.0000	1.0000
5	8.7	1	10.0000	1.0000
5	8.7	2	10.0000	1.0000
5	8.7	3	9.0000	0.9542
5	8.7	4	8.0000	0.9031
5	8.7	5	10.0000	1.0000
5	8.7	6	9.0000	0.9542
5	8.7	7	9.0000	0.9542
5	8.7	8	9.0000	0.9542
6	44	1	7.0000	0.8451
6	44	2	5.0000	0.6990
6	44	3	8.0000	0.9031
6	44	4	9.0000	0.9542
6	44	5	7.0000	0.8451
6	44	6	9.0000	0.9542
6	44	7	4.0000	0.6021
6	44	8	10.0000	1.0000
7	87	1	9.0000	0.9542
7	87	2	10.0000	1.0000
7	87	3	10.0000	1.0000
7	87	4	9.0000	0.9542
7	87	5	9.0000	0.9542
7	87	6	3.0000	0.4771
7	87	7	9.0000	0.9542
7	87	8	9.0000	0.9542

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	DMF Control	8	0.903	1.000	0.982
2	Control	8	0.778	1.000	0.930
3	0.93	8	0.778	1.000	0.954
4	4.1	8	0.954	1.000	0.977
5	8.7	8	0.903	1.000	0.965
6	44	8	0.602	1.000	0.850
7	87	8	0.477	1.000	0.906

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	DMF Control	0.001	0.036	0.013
2	Control	0.006	0.076	0.027
3	0.93	0.006	0.079	0.028
4	4.1	0.001	0.024	0.009
5	8.7	0.001	0.034	0.012
6	44	0.019	0.137	0.048
7	87	0.030	0.175	0.062

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.106	0.018	2.000
Within (Error)	49	0.450	0.009	
Total	55	0.556		

Critical F value = 2.34 (0.05,6,40); Since F < Critical F FAIL TO REJECT Ho: All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	DMF Control	0.982	9.625		
2	Control	0.930	8.625	1.095	
3	0.93	0.954	9.125	0.585	
4	4.1	0.977	9.500	0.106	
5	8.7	0.965	9.250	0.362	
6	44	0.850	7.375	2.779	*
7	87	0.906	8.500	1.605	

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

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	DMF Control	8			
2	Control	8	0.112	1.2	1.000
3	0.93	8	0.112	1.2	0.500
4	4.1	8	0.112	1.2	0.125
5	8.7	8	0.112	1.2	0.375
6	44	8	0.112	1.2	2.250
7	87	8	0.112	1.2	1.125

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	DMF Control	8	9.625	0.982	0.982
2	Control	8	8.625	0.930	0.957
3	0.93	8	9.125	0.954	0.957
4	4.1	8	9.500	0.977	0.957
5	8.7	8	9.250	0.965	0.957
6	44	8	7.375	0.850	0.878
7	87	8	8.500	0.906	0.878

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
DMF Control	0.982				
Control	0.957	0.531		1.68	k= 1, v=49
0.93	0.957	0.531		1.76	k= 2, v=49

4.1	0.957	0.531		1.79	k= 3, v=49
8.7	0.957	0.531		1.80	k= 4, v=49
44	0.878	2.169	*	1.80	k= 5, v=49
87	0.878	2.169	*	1.81	k= 6, v=49

s = 0.096; Note: df used for table values are approximate when v > 20.

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle -- Number of Young

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.618	13.068	20.628	13.068	3.618
OBSERVED	4	12	26	8	4

Calculated Chi-Square goodness of fit test statistic = 3.5324
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Shapiro-Wilks test for normality
******** Shapiro-Wilks Test is aborted ********
This test can not be performed because total number of replicates is greater than 50.
Total number of replicates = 54

Hartley test for homogeneity of variance
Calculated H statistic (max Var/min Var) = 19.31
Closest, conservative, Table H statistic = 20.0 (alpha = 0.01)
Used for Table H ==> R (# groups) = 7, df (# reps-1) = 7
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 6.71 (average df used)
Data PASS homogeneity test. Continue analysis.
NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Bartletts test for homogeneity of variance
Calculated B statistic = 13.57
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 6.71
Used for Chi-square table value ==> df (#groups-1) = 6
Data PASS homogeneity test at 0.01 level. Continue analysis.
NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

GROUP1 (SOLVENT CONTROL) MEAN = 354.2500 CALCULATED t VALUE = 2.5236
GROUP2 (BLANK CONTROL) MEAN = 252.8333 DEGREES OF FREEDOM = 12
DIFFERENCE IN MEANS = 101.4167
TABLE t VALUE (0.05 (2),12) = 2.179** SIGNIFICANT DIFFERENCE at alpha = 0.05
TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha = 0.01

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle – Number of Young (cont.)

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	DMF Control	1	319.0000	319.0000
1	DMF Control	2	301.0000	301.0000
1	DMF Control	3	493.0000	493.0000
1	DMF Control	4	354.0000	354.0000
1	DMF Control	5	185.0000	185.0000
1	DMF Control	6	341.0000	341.0000
1	DMF Control	7	453.0000	453.0000
1	DMF Control	8	388.0000	388.0000
2	Control	1	241.0000	241.0000
2	Control	2	245.0000	245.0000
2	Control	3	220.0000	220.0000
2	Control	4	262.0000	262.0000
2	Control	5	298.0000	298.0000
2	Control	6	251.0000	251.0000
3	0.93	1	370.0000	370.0000
3	0.93	2	341.0000	341.0000
3	0.93	3	512.0000	512.0000
3	0.93	4	373.0000	373.0000
3	0.93	5	304.0000	304.0000
3	0.93	6	374.0000	374.0000
3	0.93	7	241.0000	241.0000
3	0.93	8	339.0000	339.0000
4	4.1	1	375.0000	375.0000
4	4.1	2	463.0000	463.0000
4	4.1	3	403.0000	403.0000
4	4.1	4	406.0000	406.0000
4	4.1	5	561.0000	561.0000
4	4.1	6	413.0000	413.0000
4	4.1	7	393.0000	393.0000
4	4.1	8	458.0000	458.0000
5	8.7	1	492.0000	492.0000
5	8.7	2	558.0000	558.0000
5	8.7	3	503.0000	503.0000
5	8.7	4	442.0000	442.0000
5	8.7	5	570.0000	570.0000
5	8.7	6	405.0000	405.0000
5	8.7	7	381.0000	381.0000
5	8.7	8	357.0000	357.0000
6	44	1	240.0000	240.0000
6	44	2	116.0000	116.0000
6	44	3	373.0000	373.0000
6	44	4	414.0000	414.0000
6	44	5	334.0000	334.0000
6	44	6	396.0000	396.0000
6	44	7	189.0000	189.0000
6	44	8	413.0000	413.0000
7	87	1	164.0000	164.0000
7	87	2	283.0000	283.0000

7	87	3	483.0000	483.0000
7	87	4	343.0000	343.0000
7	87	5	343.0000	343.0000
7	87	6	118.0000	118.0000
7	87	7	301.0000	301.0000
7	87	8	355.0000	355.0000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	DMF Control	8	185.000	493.000	354.250
2	Control	6	220.000	298.000	252.833
3	0.93	8	241.000	512.000	356.750
4	4.1	8	375.000	561.000	434.000
5	8.7	8	357.000	570.000	463.500
6	44	8	116.000	414.000	309.375
7	87	8	118.000	483.000	298.750

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	DMF Control	9005.929	94.900	33.552
2	Control	681.367	26.103	10.657
3	0.93	5940.500	77.075	27.250
4	4.1	3556.286	59.635	21.084
5	8.7	6382.571	79.891	28.246
6	44	12934.268	113.729	40.209
7	87	13158.500	114.711	40.556

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	248610.273	41435.046	5.406
Within (Error)	47	360253.208	7664.962	
Total	53	608863.481		

Critical F value = 2.34 (0.05,6,40); Since F > Critical F REJECT Ho: All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	DMF Control	354.250	354.250		
2	Control	252.833	252.833	2.145	
3	0.93	356.750	356.750	-0.057	
4	4.1	434.000	434.000	-1.822	
5	8.7	463.500	463.500	-2.496	
6	44	309.375	309.375	1.025	
7	87	298.750	298.750	1.268	

Bonferroni T table value = 2.50 (1 Tailed Value, P=0.05, df=40,6)

BONFERRONI T-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	DMF Control	8			
2	Control	6	118.158	33.4	101.417

3	0.93	8	109.393	30.9	-2.500
4	4.1	8	109.393	30.9	-79.750
5	8.7	8	109.393	30.9	-109.250
6	44	8	109.393	30.9	44.875
7	87	8	109.393	30.9	55.500

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	DMF Control	8	354.250	354.250	378.553
2	Control	6	252.833	252.833	378.553
3	0.93	8	356.750	356.750	378.553
4	4.1	8	434.000	434.000	378.553
5	8.7	8	463.500	463.500	378.553
6	44	8	309.375	309.375	309.375
7	87	8	298.750	298.750	298.750

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
DMF Control	378.553				
Control	378.553	0.514		1.68	k= 1, v=47
0.93	378.553	0.555		1.76	k= 2, v=47
4.1	378.553	0.555		1.79	k= 3, v=47
8.7	378.553	0.555		1.80	k= 4, v=47
44	309.375	1.025		1.80	k= 5, v=47
87	298.750	1.268		1.81	k= 6, v=47

s = 87.550; Note: df used for table values are approximate when v > 20.

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle – Adult Body Length

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.752	13.552	21.392	13.552	3.752
OBSERVED	5	15	16	16	4

Calculated Chi-Square goodness of fit test statistic = 2.3875
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Shapiro-Wilks test for normality

******** Shapiro-Wilks Test is aborted ********

This test can not be performed because total number of replicates is greater than 50.
Total number of replicates = 56

Hartley test for homogeneity of variance
Calculated H statistic (max Var/min Var) = 14.71
Closest, conservative, Table H statistic = 20.0 (alpha = 0.01)
Used for Table H ==> R (# groups) = 7, df (# reps-1) = 7
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 7.00
Data PASS homogeneity test. Continue analysis.
NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Bartletts test for homogeneity of variance
Calculated B statistic = 15.52
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 6
Data PASS homogeneity test at 0.01 level. Continue analysis.
NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN
GROUP1 (SOLVENT CRTL) MEAN = 2.1125 CALCULATED t VALUE = -8.0643
GROUP2 (BLANK CRTL) MEAN = 2.4125 DEGREES OF FREEDOM = 14
DIFFERENCE IN MEANS = -0.3000
TABLE t VALUE (0.05 (2),14) = 2.145** SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2),14) = 2.977** SIGNIFICANT DIFFERENCE at alpha=0.01

TITLE: Atrazine -- Daphnia pulicaria Partial Life Cycle – Adult Body Length (cont.)

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	DMF Control	1	2.1000	2.1000
1	DMF Control	2	2.1000	2.1000
1	DMF Control	3	2.1000	2.1000

1	DMF Control	4	2.2000	2.2000
1	DMF Control	5	1.9000	1.9000
1	DMF Control	6	2.2000	2.2000
1	DMF Control	7	2.2000	2.2000
1	DMF Control	8	2.1000	2.1000
2	Control	1	2.4000	2.4000
2	Control	2	2.4000	2.4000
2	Control	3	2.4000	2.4000
2	Control	4	2.4000	2.4000
2	Control	5	2.4000	2.4000
2	Control	6	2.5000	2.5000
2	Control	7	2.4000	2.4000
2	Control	8	2.4000	2.4000
3	0.93	1	2.1000	2.1000
3	0.93	2	2.2000	2.2000
3	0.93	3	2.2000	2.2000
3	0.93	4	2.2000	2.2000
3	0.93	5	2.1000	2.1000
3	0.93	6	2.2000	2.2000
3	0.93	7	2.0000	2.0000
3	0.93	8	2.1000	2.1000
4	4.1	1	2.1000	2.1000
4	4.1	2	2.3000	2.3000
4	4.1	3	2.0000	2.0000
4	4.1	4	2.2000	2.2000
4	4.1	5	2.2000	2.2000
4	4.1	6	2.1000	2.1000
4	4.1	7	2.2000	2.2000
4	4.1	8	2.1000	2.1000
5	8.7	1	2.2000	2.2000
5	8.7	2	2.3000	2.3000
5	8.7	3	2.3000	2.3000
5	8.7	4	2.2000	2.2000
5	8.7	5	2.2000	2.2000
5	8.7	6	2.2000	2.2000
5	8.7	7	2.2000	2.2000
5	8.7	8	2.2000	2.2000
6	44	1	1.9000	1.9000
6	44	2	2.0000	2.0000
6	44	3	2.2000	2.2000
6	44	4	2.2000	2.2000
6	44	5	2.1000	2.1000
6	44	6	2.2000	2.2000
6	44	7	2.0000	2.0000
6	44	8	2.2000	2.2000
7	87	1	1.8000	1.8000
7	87	2	2.0000	2.0000
7	87	3	2.2000	2.2000
7	87	4	2.0000	2.0000
7	87	5	2.0000	2.0000
7	87	6	1.9000	1.9000

7	87	7	2.0000	2.0000
7	87	8	2.2000	2.2000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	DMF Control	8	1.900	2.200	2.112
2	Control	8	2.400	2.500	2.413
3	0.93	8	2.000	2.200	2.138
4	4.1	8	2.000	2.300	2.150
5	8.7	8	2.200	2.300	2.225
6	44	8	1.900	2.200	2.100
7	87	8	1.800	2.200	2.013

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	DMF Control	0.010	0.099	0.035
2	Control	0.001	0.035	0.012
3	0.93	0.006	0.074	0.026
4	4.1	0.009	0.093	0.033
5	8.7	0.002	0.046	0.016
6	44	0.014	0.120	0.042
7	87	0.018	0.136	0.048

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.769	0.128	14.222
Within (Error)	49	0.420	0.009	
Total	55	1.189		

Critical F value = 2.34 (0.05,6,40); Since F > Critical F REJECT Ho: All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	DMF Control	2.112	2.112		
2	Control	2.413	2.413	-6.325	
3	0.93	2.138	2.138	-0.527	
4	4.1	2.150	2.150	-0.791	
5	8.7	2.225	2.225	-2.372	
6	44	2.100	2.100	0.264	
7	87	2.013	2.013	2.108	

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

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	DMF Control	8			
2	Control	8	0.112	5.3	-0.300
3	0.93	8	0.112	5.3	-0.025
4	4.1	8	0.112	5.3	-0.038
5	8.7	8	0.112	5.3	-0.112
6	44	8	0.112	5.3	0.012

7	87	8	0.112	5.3	0.100

BONFERRONI T-TEST - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	DMF Control	2.112	2.112		
2	Control	2.413	2.413	-6.325	
3	0.93	2.138	2.138	-0.527	
4	4.1	2.150	2.150	-0.791	
5	8.7	2.225	2.225	-2.372	
6	44	2.100	2.100	0.264	
7	87	2.013	2.013	2.108	

Bonferroni T table value = 2.50 (1 Tailed Value, P=0.05, df=40,6)

BONFERRONI T-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	DMF Control	8			
2	Control	8	0.119	5.6	-0.300
3	0.93	8	0.119	5.6	-0.025
4	4.1	8	0.119	5.6	-0.038
5	8.7	8	0.119	5.6	-0.112
6	44	8	0.119	5.6	0.012
7	87	8	0.119	5.6	0.100

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	DMF Control	8	2.112	2.112	2.263
2	Control	8	2.413	2.413	2.263
3	0.93	8	2.138	2.138	2.171
4	4.1	8	2.150	2.150	2.171
5	8.7	8	2.225	2.225	2.171
6	44	8	2.100	2.100	2.100
7	87	8	2.013	2.013	2.013

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
DMF Control	2.263				
Control	2.263	3.241	*	1.68	k= 1, v=49
0.93	2.171	1.260		1.76	k= 2, v=49
4.1	2.171	1.260		1.79	k= 3, v=49
8.7	2.171	1.260		1.80	k= 4, v=49
44	2.100	0.270		1.80	k= 5, v=49
87	2.013	2.160	*	1.81	k= 6, v=49

s = 0.093; Note: df used for table values are approximate when v > 20.