DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
§72-5

1. **CHEMICAL:** 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl), nicotinic acid (Imazapyr) PC Code No.: 128821

2. **TEST MATERIAL:** AC 243997 Technical Purity: 100%

3. **CITATION:**

Author: Drottar, K.R., *et al.*

Title: Toxicity of AC 243997 (Imazapyr) Technical During the Full Life-Cycle of the Fathead Minnow (*Pimephales promelas*) Under Flow-Through Test Conditions.

Study Completion Date: February 5, 1999

Laboratories: Wildlife International Ltd.
Easton, MD.

American Cyanamide Company
Princeton, NJ.

Sponsor: American Cyanamide Company
Princeton, NJ.

Laboratory Report ID: ECO 97-101

MRID No.: 45119712

DP Barcode: D275562

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: C.E. Padova **Date:** 4/12/02

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: T. Myers **Date:** 4/12/02

5. **APPROVED BY:** Stephen Carey, Biologist, EFED

Signature: Stephen Carey **Date:** 3/10/03

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Pimephales promelas*

Age of Test Organism: <24 hours old (F_0 generation)

Definitive Test Duration: 249 Days (approximately 8 months)

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

The 8-month chronic toxicity of AC 243997 Technical to the full life cycle of Fathead Minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs (200 embryos/treatment, <24 hours old) of fathead minnow were exposed to the test material at nominal concentrations of 0 (negative control), 7.5, 15, 30, 60, and 120 mg a.i./L. No solvent carrier was used. Mean-measured concentrations were 7.2, 15, 29, 59, and 120 mg a.i./L during the parental generation (F_0) and 7.5, 15, 30, 61, and 120 mg a.i./L during the second generation (F_1). Treatment groups were compared to a negative control group.

Following hatching on Day 5, alevins were reduced to 100 per treatment level. On Day 61 (approximately 8 weeks post-hatch), the juveniles were again reduced to 50 per treatment level. At approximately 23 weeks post-hatch (approximately Day 167), 15 adult fish per treatment level were isolated for spawning. Beginning on Day 174, embryos of the F_1-generation were isolated and exposed under identical test conditions as those described for the parental generation until 4 weeks post-hatch (Day 209).

F_0-generation: No treatment-related effects were observed on embryo survival, time to hatch, or larval, juvenile, or adult survival of the F_0 generation. No treatment-related effects were observed on percent spawning frequency, the mean number of eggs produced per female per reproductive day, the mean number of fertile embryos produced per female per reproductive day, or the mean percent fertilization success. Growth of the F_0 generation was also unaffected by treatment with AC 243997 Technical, and no treatment-related signs of toxicity were observed in surviving fish throughout the study.

F_1-generation: No treatment-related effects were observed on embryo survival, time to hatch, or larval and juvenile survival of the F_1 generation. Growth of the F_1 generation was also unaffected by treatment with AC 243997 Technical, and no treatment-related signs of toxicity were observed for surviving fry during the 4-week observation period.

This study is scientifically valid, and although results do not meet guideline requirements; the information may be useful in a risk assessment.

Results Synopsis:
NOEC: 120 mg a.i./L
LOEC: >120 mg a.i./L

Most Sensitive Endpoint: None.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Because the F_1 generation was only maintained for 4 weeks post-hatch (instead of the required 8 weeks), this study does not satisfy guideline requirements for a fish life-cycle toxicity test (§72-5). This study is scientifically sound, and provides supplemental data on the toxicity of AC 243997 Technical to the life cycle of fathead minnow.

C. Repairability: This study may be upgraded to Core status if data are provided to support that assumptions of no adverse effects on the survival, appearance, or growth of second-generation larvae would have been maintained, had the fish been observed up to 8 weeks post-hatch.

9. GUIDELINE DEVIATIONS:

1. F_1-generation fish were maintained for only 4 weeks, instead of the required 8 weeks.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of AC 243997 to the life cycle of fathead minnows for the purposes of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Prefer Sheepshead minnow (*Cyprinodon variegatus*) or Fathead minnow (*Pimephales promelas*).	Fathead minnow (*Pimephales promelas*)

Guideline Criteria	Reported Information
Source and Acclimation	Embryos were obtained from brood stock cultures maintained for an unspecified period of time at Wildlife International Ltd., Easton, MD.
Age at beginning of test Embryos, 2 to 24 hours old	<24 Hours old
Feeding Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	Feeding began when ≥90% of the negative control embryos had hatched. Newly hatched larvae were fed live brine shrimp nauplii (*Artemia sp.*) three times daily, twice on weekends. Rations were adjusted based on the number of fish in each test chamber. It was not reported whether or not fish were fed at least 24 hours prior to test termination.
Embryo Exposure (4 to 5 Days) Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups. A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used. Parameters measured: · Survival of embryos · Time required to hatch · Hatching success · Survival of fry for 4 weeks Dead and fungused embryos should be counted and removed daily.	Days 0-5 Embryos (< 24 hours old) from nine separate spawns were randomly distributed to embryo cups. Each cup contained 25 embryos, with two cups per replicate and four replicates per treatment level (total of 200 embryos per treatment). Parameters measured: · Survival of embryos · Time required to hatch · Hatching success · Survival and signs of toxicity of fry/juvenile fish Mortality and clinical signs of toxicity were made daily. Dead embryos were removed.

Guideline Criteria	Reported Information
Larval-Juvenile Exposure (From Hatch to 8 Weeks) After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed. Parameters measured: · Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly). · Total lengths (mm) of all fish at 4 and 8 weeks after hatching.	Days 5-60 (hatch to approximately 8 weeks) When ≥90% of the embryos in the negative control group had hatched, larvae were impartially thinned to 25 per replicate, with four replicates per treatment (100 embryos per treatment), and the larvae were transferred from the incubation cups to the larval growth chambers. Parameters measured: · Survival and signs of toxicity of fry/juvenile fish · Total lengths (mm) of all surviving fish at 32 and 60 days (approximately 4 and 8 weeks)

Guideline Criteria	Reported Information
Juvenile-Adult Exposure (From 8 weeks posthatch to the end of the spawning phase [32-40 weeks])	Days 61 to 249 (approximately 8 to 35 weeks post-hatch) On Day 61, juvenile fish were reduced from four to two replicates per treatment (50 total fish per treatment).
At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates.	On Day 167 (approximately 23 weeks post-hatch), two males and three females were impartially assigned to each spawning compartment. There were two spawning compartments per replicate. Five additional fish were maintained in each spawning tank but were segregated from the spawning groups for a total of 15 adults per replicate (30 total fish per treatment).
The substrates are examined daily and embryos removed, counted, and recorded separately for each pair.	The spawning substrates are examined daily and embryos removed, counted, examined for fertility, and the number of fertile and non-fertile embryos were recorded for each spawning group.
For fathead minnow, adult exposure should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.	Adult exposure was terminated when no spawning occurred in any group over a 7-day period. Parameters measured: · Survival of adult fish · Spawning frequency · Number of eggs deposited · Fertilization success · Total lengths (mm) and weight (g) of all surviving fish at 249 days

Guideline Criteria	Reported Information
Second Generation Embryo Exposure (4 to 5 days) 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation. Embryos not selected are discarded.	Days 174-181 (7 days) 100 embryos from each treatment level were randomly selected and transferred to incubation cups for hatch. The same test procedures as those employed for the parental generation were used, and the same endpoints were measured. Embryos not used for the second generation exposure were maintained frozen for possible analyses of tissue residue concentration.
Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 weeks) After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment). Each group of 2nd generation fish is terminated 8 weeks after hatching. Fish are blotted, weighed, and measured before being discarded.	Days 178-209 (Hatch to 4 weeks) After hatching, 25 larvae were released in two replicate test chambers (50 total larvae per treatment). Each group of F_1-generation fish was terminated 4 weeks after hatching. Fish were blotted, weighed, and measured for total length.

Comments: None.

B. Test System

Guideline Criteria	Reported Information
Test Water Sheepshead Minnow 1. Natural seawater (sterilized and filtered) or a commercial mixture. 2. Natural seawater with a salinity of ≥15 parts per thousand (weekly range of salinity <6% and monthly pH range <0.8 pH units). Fathead Minnow 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 160 to 180 mg/L as $CaCO_3$ and pH of 7.6 to 8.0.	N/A 1. Well water (40-meters deep) located on-site at the laboratory. The water was filtered, aerated, and UV-sterilized prior to use. 2. Hardness of 124 to 140 mg/L as $CaCO_3$ and pH of 7.5 to 8.4.
Test Temperature Sheepshead: 30°C. Fathead: 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours.	N/A Range of 24.0 to 25.6°C.

Guideline Criteria	Reported Information
Photoperiod 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	F_0-generation: Ranged from 10.5 to 15.75 hours per day and were adjusted every 2 weeks to approximate the natural sunlight of Evansville, IN. Light intensity, ranged from 414 to 995 Lux (lumen/m^2) F_1-generation: 16-hours light/8-hours dark, 362 to 545 Lux.
Dosing Apparatus 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor $\leq$0.5. 3. One control should be used.	1. Continuous-flow diluter. 2. Five toxicant concentrations with a dilution factor of 0.5. 3. One negative control was used.
Toxicant Mixing 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked.	1. A mixing chamber was used for each toxicant level. 2. Yes 3. Maximum deviation reportedly less than 10%.

Guideline Criteria	Reported Information
Exposure System/Test Vessels Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheepshead). Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm. Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	During the F_0-generation embryo/larval/juvenile exposure (Days 0-60), 9-L glass aquaria were used, with a fill volume of 7 L and depth of 16 cm. During the F_0-generation juvenile/adult exposure (Days 61-249), 54-L glass aquaria were used, with a fill volume of 27 L and depth of 15 cm. During the F_1-generation embryo/larval/juvenile exposure (Days 174-209), 9-L glass aquaria were used, with a fill volume of 7 L and depth of 17 cm. It was not specified if larval chambers had drains to allow for water level reduction.
Embryo and Fry Chambers 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.	Glass cylinders, 50-mm diameter (depth not specified) and sealed on one end with 425-µm nylon mesh screen. The embryo cups were suspended in the water column of each chamber and slowly reciprocated (2 rpm) using a rocking arm.

Guideline Criteria	Reported Information
Flow Rate Flow rates to adult tanks or larval chambers should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	During the F_0-generation embryo/larval/juvenile exposure (Days 0-60), the flow rate was adjusted to provide approximately 6.4 volume additions per day. During the F_0-generation juvenile/adult exposure (Days 61-249), the flow rate was adjusted to provide approximately 6 volume additions per day. During the F_1-generation embryo/larval/juvenile exposure (Days 174-209), the flow rate was adjusted to provide approximately 10 volume additions per day. On Day 112 of the parental generation exposure, the DO dropped to 4.7 mg/L (57% of saturation). Mild aeration was initiated in all parental generation test chambers and thereafter, DO levels remained ≥6.3 mg/L (76% of saturation). DO levels in the second generation chambers remained ≥7.6 mg/L (93% of saturation) and aeration was not necessary. The toxicant level never fell below 20% of nominal values. The mild aeration required from Days 112 to study termination did not adversely affect toxicant levels.

Guideline Criteria	Reported Information
Aeration Dilution water should be aerated to insure dissolved oxygen concentrations at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	Dilution water was aerated prior to entering the mixing chamber. In addition, F_0-generation test tanks were mildly aerated from Days 112 to 249.

C. Chemical System

Guideline Criteria	Reported Information
Nominal Concentrations Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate.	0 (negative control), 7.5, 15, 30, 60, and 120 mg a.i./L. Concentrations were adjusted for the purity of the test material.
Toxicant conc. must be measured in one tank at each toxicant level every week.	Toxicant concentrations were measured from alternating replicate aquaria in each test group at test initiation, at approximately weekly intervals during the test, and at test termination. Samples were collected at mid-depth and were analyzed immediately.

Guideline Criteria	Reported Information
Other Variables 1. DO must be measured at each conc. at least once a week.	1. DO was measured in each replicates at initiation, once weekly, and at test termination.
2. Test water temp. must be recorded continuously.	2. Temperature measured in each replicate aquaria at initiation, once weekly, and at test termination. Also, continuously monitored in one negative control replicate.
3. Freshwater: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. Natural seawater: must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.	3. pH was measured at test initiation, once weekly, and at test termination. Hardness, alkalinity, and specific conductance were measured during the F_0-generation exposure in the control and one treatment group at test initiation, once weekly, and at test termination.
Solvents Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	N/A

Comments: None.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Data Endpoints must include: · survival of F_0 and F_1 embryos, time required to hatch, and hatching success; · survival and total length of F_0 fish at 4 and 8 weeks after hatching; · weights and lengths of F_1 fish at 8 weeks; · incidence of pathological or histological effects; and · observations of other effects or clinical signs.	Data Endpoints included: · daily monitoring of survival of F_0 and F_1 embryos, time required to hatch, and hatching success; · daily monitoring of survival and signs of toxicity of fry/juvenile/adult F_0 fish · total lengths of surviving F_0 fish at approximately 4 and 8 weeks and total lengths and wet weights of surviving fish at approximately 36 weeks · spawning frequency, number of eggs deposited, and fertilization success of F_0 spawning groups · total lengths and wet weights of F_1 fish at approximately 4 weeks instead of 8 weeks..
Raw data included?	Yes

F_0 Results:

Nominal Conc. (mg a.i./L)	Mean Measured Conc. (mg a.i./L) (SD)	% Hatch[1]	4-Week Post-Hatch % Survival[2]	8-Week Post-Hatch % Survival[2]	Test Termination (Day 249) % Survival[3]
Negative Control	<LOD[4]	96	92	88	97
7.2	7.2 ± 0.49	97	91	84	90
15	15 ± 1.1	95	91	87	100
30	29 ± 2.0	94	92	89	100
60	59 ± 3.2	94	89	83	93
120	120 ± 6.3	95	91	88	97

[1] Percent hatching success = (number hatched ÷ number exposed) x 100
[2] Relative to 100 initial larvae/treatment.
[3] Relative to 30 fish/treatment, thinned on Day 167.
[4] LOD = 1.0 mg a.i./L.

Mean Measured Conc. (mg a.i./L)	Mean Total Length (mm)				Wet Weight (g)		
	Day 32 (4 Weeks)	Day 60 (8 Weeks)	Day 249 (Test Termination)		Day 60 (8 Weeks)	Day 249 (Test Termination)	
			♂	♀		♂	♀
Control	18.3	33	72	58	0.3284	4.3117	2.1631
7.2	18.2	32	74	61	0.3372	4.8279	2.4345
15	17.9	32	72	61	0.3695	4.0690	2.3257
29	18.9	33	72	60	0.2920	4.5711	2.2701
59	18.9	33	73	61	0.3053	4.4532	2.2854
120	18.2	32	73	60	0.3019	4.4234	2.1941

Mean Measured Conc. (mg a.i./L)	Number of Spawns[1]	Total Number of Eggs[1]	Number of Eggs/ Spawn	Number of Spawns/Female[2]	Number of Eggs/ Female
Control	42	4467	106	3.5	372
7.2	36	4460	124	3.0	372
15	36	3763	105	3.0	314
29	34	4576	135	2.8	381
59	51	5766	113	4.2	480
120	35	3800	109	2.9	317

[1] Values represent sum of two replicates.
[2] Six females per replicate, with 12 per treatment.

Toxicity Observations: No treatment-related effects were observed on embryo survival, time to hatch, or larval, juvenile, growth or adult survival of the F_0 generation. No treatment-related effects were observed on percent spawning frequency, the mean number of eggs produced per female per reproductive day, the mean number of fertile embryos produced per female per reproductive day, or the mean percent fertilization success.

No treatment-related signs of toxicity were observed in surviving fish throughout the study.

F_1 Results:

Mean Measured Concentration (mg a.i./L)	% Hatch	28-Day Post-Hatch % Survival	28-Day Post-Hatch Length (mm)	28-Day Post-Hatch Wet Weight (g)
Control	83	88	20	0.0878
7.5	83	88	19	0.0875
15	81	84	20	0.0946
30	86	82	20	0.0865
61	82	88	19	0.0885
120	86	82	20	0.0848

Toxicity Observations: No treatment-related effects were observed on embryo survival, time to hatch, or larval, juvenile survival and growth of the F_1 generation.

No treatment-related signs of toxicity were observed in surviving fry during the 4-week observation period.

B. Reported Statistical Results

Data obtained for the F_0 generation that were statistically analyzed included (1) hatching success, (2) survival, (3) total length at 4 and 8 weeks post-hatch, (4) total wet weight at 8 weeks post-hatch, (5) total length and wet weight on Days 167 and 249 (study termination), (6) spawning frequency, and (7) the number of fertile eggs produced. Data obtained for the F_1 generation that were statistically analyzed included (1) hatching success, (2) survival, (3) and total length and wet weight at 4 weeks post-hatch.

Data were analyzed by standard statistical techniques using a computer program (TOXSTAT, Ver. 3.5 or SPSS/PC, Ver. 2.0). Discrete-variable data (mortality proportions) were analyzed using 2X2 contingency tables to identify treatment groups that showed a statistically-significant difference ($p \leq 0.05$) from the negative control group. Continuous-variable data (weight and length) were evaluated for normality using Shapiro-Wilkes test and for homogeneity of variance using Bartlett's test ($p=0.01$). Dunnett's test was used to evaluate difference between treatment and the control means ($p \leq 0.05$).

Biological Endpoint	NOEC (mg a.i./L)	LOEC (mg a.i./L)
F_0 hatching success	120	>120
F_0 4-week survival	120	>120
F_0 4-week length	120	>120
F_0 8-week survival	120	>120
F_0 8-week length	120	>120
F_0 8-week weight	120	>120
F_0 test termination survival	120	>120
F_0 test termination length (Males)	120	>120
F_0 test termination length (Females)	120	>120
F_0 test termination weight (Males)	120	>120
F_0 test termination weight (Females)	120	>120
F_0 # of spawns/female	120	>120

Biological Endpoint	NOEC (mg a.i./L)	LOEC (mg a.i./L)
F_0 # of eggs/female	120	>120
F_1 hatching success	120	>120
F_1 4-week survival	120	>120
F_1 4-week length	120	>120
F_1 4-week weight	120	>120
F_1 8-week survival	Not determined	Not determined
F_1 8-week length	Not determined	Not determined
F_1 8-week weight	Not determined	Not determined

NOEC: 120 mg a.i./L **LOEC:** >120 mg a.i./L

13. REVIEWER'S STATISTICAL RESULTS:

The reviewer analyzed data to verify conclusions made for the following parental endpoints: hatching success, 4-week, 8-week, and terminal survival, # of spawning days, total eggs produced, # eggs/female/reproductive day, # of fertile embryos, # fertile embryos/female/reproductive day, male and female length and wet weight from days 32, 60, 167, and test termination. Data analyzed from the second generation included: hatching success, 4-week survival, total wet weight and length.

There was no significant effect for any endpoint. This could be determined via visual inspection of the data for many parameters. In cases where the reviewer could not determine the LOEC and NOEC visually, data were analyzed using TOXSTAT. Data were tested to determine if their distribution was normal and variances were homogeneous. For those parameters that satisfied these assumptions, the LOEC and NOEC were determined using Dunnett's test (or William's test, for dose-dependent responses). For parameters that did not satisfy these assumptions, the LOEC and NOEC were determined using the non-parametric Kruskall-Wallis test.

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'. This study is scientifically valid. However, because the F_1-generation fish were only maintained for 4 weeks post-hatch, although results do not meet guideline requirements; the information may be useful in a risk assessment. The NOEC and LOEC were 120 mg a.i./L and >120 mg a.i./L, respectively, under the conditions tested in this study.

The study authors reported that nominal test concentrations were chosen based on results from the study entitled "Toxicity of AC 243997 During the Early Life-Stages of the Fathead Minnow (*Pimephales promelas*)", Cyanamid Study Number 954-97-102. No adverse effects on any life-stage were observed at concentrations of up to 118 mg a.i./L.

Since initiation of the F_1-generation exposure was based on the availability of eggs in the parental generation spawning compartments, not all treatment levels or replicates were initiated on the same day. The second-generation exposures were thus initiated over a 3-day period, with the majority of exposures initiated on Day 174 of the test.

On Day 169, one male and one female from a spawning compartment of the 7.5-mg a.i./L treatment group were found dead. Since the spawning period had just begun (on Day 167), the fish were replaced from the pool of five additional fish in the test chamber.

Guidelines specify that the photoperiod provide 10-100 lumens at the water surface. In this study, light intensity was reported 414 - 995 Lux in the F_0 generation and 362-545 Lux (lumens/m^2) in the F_1 generation.

At test initiation, fish from different treatment groups were exposed to Imazapyr at different pH levels. The pH was lower at higher treatment levels and tend to increase with decreasing treatment levels.

It was not specified if larval chambers had drains to allow for water level reduction.

15. REFERENCES:

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16. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

parental hatching success
File: 9712ph Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	8.500	1.700	1.302
Within (Error)	18	23.500	1.306	
Total	23	32.000		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

parental hatching success
File: 9712ph 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	48.000	48.000		
2	7.2	48.500	48.500	-0.619	
3	15	47.500	47.500	0.619	
4	29	46.750	46.750	1.547	
5	59	47.000	47.000	1.237	
6	120	47.250	47.250	0.928	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

parental hatching success
File: 9712ph 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	4			
2	7.2	4	1.947	4.1	-0.500
3	15	4	1.947	4.1	0.500
4	29	4	1.947	4.1	1.250
5	59	4	1.947	4.1	1.000
6	120	4	1.947	4.1	0.750

parental hatching success
File: 9712ph Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	48.000	48.000	48.250

2	7.2	4	48.500	48.500	48.250
3	15	4	47.500	47.500	47.500
4	29	4	46.750	46.750	47.000
5	59	4	47.000	47.000	47.000
6	120	4	47.250	47.250	47.000

parental hatching success
File: 9712ph 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	48.250				
7.2	48.250	0.309		1.73	k= 1, v=18
15	47.500	0.619		1.82	k= 2, v=18
29	47.000	1.238		1.85	k= 3, v=18
59	47.000	1.238		1.86	k= 4, v=18
120	47.000	1.238		1.87	k= 5, v=18

s = 1.143
Note: df used for table values are approximate when v > 20.

4-week parental survival
File: 9712p4s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.500	0.300	0.318
Within (Error)	18	17.000	0.944	
Total	23	18.500		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

4-week parental survival
File: 9712p4s 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	23.000	23.000		
2	7.2	22.750	22.750	0.364	
3	15	22.750	22.750	0.364	
4	29	23.000	23.000	0.000	
5	59	22.250	22.250	1.092	
6	120	22.750	22.750	0.364	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

4-week parental survival
File: 9712p4s 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	4			
2	7.2	4	1.656	7.2	0.250
3	15	4	1.656	7.2	0.250
4	29	4	1.656	7.2	0.000
5	59	4	1.656	7.2	0.750
6	120	4	1.656	7.2	0.250

4-week parental survival
File: 9712p4s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	23.000	23.000	23.000
2	7.2	4	22.750	22.750	22.833
3	15	4	22.750	22.750	22.833
4	29	4	23.000	23.000	22.833
5	59	4	22.250	22.250	22.500
6	120	4	22.750	22.750	22.500

4-week parental survival
File: 9712p4s 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	23.000				
7.2	22.833	0.243		1.73	k= 1, v=18
15	22.833	0.243		1.82	k= 2, v=18
29	22.833	0.243		1.85	k= 3, v=18
59	22.500	0.728		1.86	k= 4, v=18
120	22.500	0.728		1.87	k= 5, v=18

s = 0.972
Note: df used for table values are approximate when v > 20.

8-week parental survival
File: 9712p8s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	7.375	1.475	1.095
Within (Error)	18	24.250	1.347	
Total	23	31.625		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

8-week parental survival
File: 9712p8s 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	22.000	22.000		
2	7.2	21.000	21.000	1.219	
3	15	21.750	21.750	0.305	
4	29	22.250	22.250	-0.305	
5	59	20.750	20.750	1.523	
6	120	22.000	22.000	0.000	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

8-week parental survival
File: 9712p8s 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	4			
2	7.2	4	1.978	9.0	1.000
3	15	4	1.978	9.0	0.250
4	29	4	1.978	9.0	-0.250
5	59	4	1.978	9.0	1.250
6	120	4	1.978	9.0	0.000

8-week parental survival
File: 9712p8s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	22.000	22.000	21.500
2	7.2	4	21.000	21.000	21.500
3	15	4	21.750	21.750	21.583
4	29	4	22.250	22.250	21.583
5	59	4	20.750	20.750	21.583
6	120	4	22.000	22.000	22.000

8-week parental survival
File: 9712p8s 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	21.500				

7.2	21.500	0.609	1.73	k= 1, v=18
15	21.583	0.508	1.82	k= 2, v=18
29	21.583	0.508	1.85	k= 3, v=18
59	21.583	0.508	1.86	k= 4, v=18
120	22.000	0.000	1.87	k= 5, v=18

s = 1.161
Note: df used for table values are approximate when v > 20.

 # spawning days
File: 9712sf Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	21.000	21.000	12.500
2	7.2	18.000	18.000	13.000
3	15	18.000	18.000	12.500
4	29	17.000	17.000	8.000
5	59	25.500	25.500	22.000
6	120	17.500	17.500	10.000

Calculated H Value = 4.554 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

spawning days
File: 9712sf Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 4 6 2 3 1 5
4	29	17.000	17.000	\
6	120	17.500	17.500	. \
2	7.2	18.000	18.000	. . \
3	15	18.000	18.000	. . . \
1	control	21.000	21.000	. . . . \
5	59	25.500	25.500	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.561

total eggs produced
File: 9712e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1319843.000	263968.600	1.065
Within (Error)	6	1487119.000	247853.167	
Total	11	2806962.000		

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

total eggs produced
File: 9712e 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	2233.500	2233.500		
2	7.2	2230.000	2230.000	0.007	
3	15	1881.500	1881.500	0.707	
4	29	2288.000	2288.000	-0.109	
5	59	2883.000	2883.000	-1.305	
6	120	1900.000	1900.000	0.670	

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

total eggs produced
File: 9712e 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	7.2	2	1408.911	63.1	3.500
3	15	2	1408.911	63.1	352.000
4	29	2	1408.911	63.1	-54.500
5	59	2	1408.911	63.1	-649.500
6	120	2	1408.911	63.1	333.500

total eggs produced
File: 9712e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	2233.500	2233.500	2303.200
2	7.2	2	2230.000	2230.000	2303.200
3	15	2	1881.500	1881.500	2303.200
4	29	2	2288.000	2288.000	2303.200
5	59	2	2883.000	2883.000	2303.200
6	120	2	1900.000	1900.000	1900.000

total eggs produced
File: 9712e 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	2303.200				

7.2	2303.200	0.140	1.94	k= 1, v= 6
15	2303.200	0.140	2.06	k= 2, v= 6
29	2303.200	0.140	2.10	k= 3, v= 6
59	2303.200	0.140	2.12	k= 4, v= 6
120	1900.000	0.670	2.13	k= 5, v= 6

s = 497.849
Note: df used for table values are approximate when v > 20.

 # eggs/female/reproductive day
File: 9712erd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	5.336	1.067	1.065
Within (Error)	6	6.011	1.002	
Total	11	11.347		

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

eggs/female/reproductive day
File: 9712erd 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	4.485	4.485		
2	7.2	4.480	4.480	0.005	
3	15	3.775	3.775	0.709	
4	29	4.595	4.595	-0.110	
5	59	5.790	5.790	-1.304	
6	120	3.815	3.815	0.669	

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

eggs/female/reproductive day
File: 9712erd 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	7.2	2	2.833	63.2	0.005
3	15	2	2.833	63.2	0.710
4	29	2	2.833	63.2	-0.110
5	59	2	2.833	63.2	-1.305
6	120	2	2.833	63.2	0.670

eggs/female/reproductive day
File: 9712erd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	4.485	4.485	4.625
2	7.2	2	4.480	4.480	4.625
3	15	2	3.775	3.775	4.625
4	29	2	4.595	4.595	4.625
5	59	2	5.790	5.790	4.625
6	120	2	3.815	3.815	3.815

eggs/female/reproductive day
File: 9712erd 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	4.625				
7.2	4.625	0.140		1.94	k= 1, v= 6
15	4.625	0.140		2.06	k= 2, v= 6
29	4.625	0.140		2.10	k= 3, v= 6
59	4.625	0.140		2.12	k= 4, v= 6
120	3.815	0.669		2.13	k= 5, v= 6

s = 1.001
Note: df used for table values are approximate when v > 20.

 # eggs/female/reproductive day
File: 9712fe Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1300206.000	260041.200	1.120
Within (Error)	6	1393617.000	232269.500	
Total	11	2693823.000		

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

eggs/female/reproductive day
File: 9712fe 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	2164.000	2164.000		
2	7.2	2135.500	2135.500	0.059	
3	15	1790.000	1790.000	0.776	

4	29	2209.000	2209.000	-0.093
5	59	2783.000	2783.000	-1.284
6	120	1809.500	1809.500	0.736

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

eggs/female/reproductive day
File: 9712fe 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	7.2	2	1363.900	63.0	28.500
3	15	2	1363.900	63.0	374.000
4	29	2	1363.900	63.0	-45.000
5	59	2	1363.900	63.0	-619.000
6	120	2	1363.900	63.0	354.500

eggs/female/reproductive day
File: 9712fe Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	2164.000	2164.000	2216.300
2	7.2	2	2135.500	2135.500	2216.300
3	15	2	1790.000	1790.000	2216.300
4	29	2	2209.000	2209.000	2216.300
5	59	2	2783.000	2783.000	2216.300
6	120	2	1809.500	1809.500	1809.500

eggs/female/reproductive day
File: 9712fe 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	2216.300				
7.2	2216.300	0.109		1.94	k= 1, v= 6
15	2216.300	0.109		2.06	k= 2, v= 6
29	2216.300	0.109		2.10	k= 3, v= 6
59	2216.300	0.109		2.12	k= 4, v= 6
120	1809.500	0.736		2.13	k= 5, v= 6

s = 481.943
Note: df used for table values are approximate when v > 20.

 # fertile embryos/female/reproductive day
File: 9712ferd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	5.245	1.049	1.121
Within (Error)	6	5.617	0.936	
Total	11	10.863		

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

fertile embryos/female/reproductive day
File: 9712ferd 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	4.345	4.345		
2	7.2	4.285	4.285	0.062	
3	15	3.595	3.595	0.775	
4	29	4.435	4.435	-0.093	
5	59	5.590	5.590	-1.287	
6	120	3.635	3.635	0.734	

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

fertile embryos/female/reproductive day
File: 9712ferd 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	7.2	2	2.738	63.0	0.060
3	15	2	2.738	63.0	0.750
4	29	2	2.738	63.0	-0.090
5	59	2	2.738	63.0	-1.245
6	120	2	2.738	63.0	0.710

fertile embryos/female/reproductive day
File: 9712ferd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	4.345	4.345	4.450
2	7.2	2	4.285	4.285	4.450
3	15	2	3.595	3.595	4.450
4	29	2	4.435	4.435	4.450
5	59	2	5.590	5.590	4.450
6	120	2	3.635	3.635	3.635

fertile embryos/female/reproductive day
File: 9712ferd 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	4.450				
7.2	4.450	0.109		1.94	k= 1, v= 6
15	4.450	0.109		2.06	k= 2, v= 6
29	4.450	0.109		2.10	k= 3, v= 6
59	4.450	0.109		2.12	k= 4, v= 6
120	3.635	0.734		2.13	k= 5, v= 6

s = 0.968
Note: df used for table values are approximate when v > 20.

2nd generation 4 week survival
File: 97122s4 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	22.000	22.000	18.000
2	7.5	22.000	22.000	17.000
3	15	21.000	21.000	11.000
4	30	20.500	20.500	7.500
5	61	22.000	22.000	17.000
6	120	20.500	20.500	7.500

Calculated H Value = 5.006 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

2nd generation 4 week survival
File: 97122s4 Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 4 6 3 2 5 1
4	30	20.500	20.500	\
6	120	20.500	20.500	. \
3	15	21.000	21.000	. . \
2	7.5	22.000	22.000	. . . \
5	61	22.000	22.000	. . . . \
1	control	22.000	22.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.484