

US EPA ARCHIVE DOCUMENT

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

1. **CHEMICAL:** Propiconazole PC Code No.: 122101

2. **TEST MATERIAL:** CGA-64250 Technical Purity: 91.7%

3. **CITATION:**

Author: Breteler, R.J.

Title: The Chronic Toxicity of CGA-64250 Technical
(Propiconazole) to Sheepshead Minnow (*Cyprinodon
variegatus*).

Study Completion Date: July 18, 1988

Laboratories: Springborn Life Sciences, Inc.
790 Main Street
Wareham, MA 02571

Sponsor: Ciba-Geigy Corporation
Agricultural Division
P.O. Box 18300
Greensboro, NC 27419

Laboratory Report ID: 88-04-2685

MRID No.: 408820-01 & 401833-10

DP Barcode: D312346

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

Signature:

Date: 5/16/05

APPROVED BY: Gregory S. Hess, Staff Scientist, Dynamac Corporation

Signature:

Date: 5/24/05

5. **APPROVED BY:** William Evans, Biologist, OPP/EFED/ERB - II

Signature:



Date:

2/6/06



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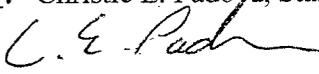
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Signature: 

Date: 7/23/05

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Cyprinodon variegatus*

Age of Test Organism: Embryos, 24 hours old (F₀ generation)

Definitive Test Duration: 100 Days

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

The 3.3-month (100 days) chronic toxicity of CGA-64250 (propiconazole) to the full life stage of sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized eggs (200 embryos/treatment, 24 hours old) were exposed to the test material at nominal concentrations of 0 (negative control), 0.019, 0.038, 0.075, 0.15, 0.30, and 0.60 ppm a.i.. Mean-measured concentrations were <0.0080 (<LOD, control), 0.016, 0.038, 0.068, 0.15, 0.29, and 0.55 ppm a.i., respectively (84-100% of nominal concentrations). Unacceptable variability (>1.5) in measured concentrations was observed in the 0.6, 0.15, and 0.075 nominal test levels due to diluter mal-function.

Following the completion of hatching on Day 4, larvae were reduced to 100 per treatment level. At 4-weeks post-hatch, the juveniles were again reduced to 50 per treatment level. Spawning was initiated approximately 7 weeks following hatching: eight groups of two male and five female per test level were assigned to spawning aquaria, and hatchability trials and early life stage studies were performed for the F₁ generation. Following hatching, the F₁ generation was maintained for 4 weeks. The F₀ portion of test was terminated on Day 95, and the F₁ portion was determined on Day 100.

F₀-generation: Significant reductions in reproduction (assessed as the number of eggs/female/day) were observed at the mean-measured 0.15, 0.29, and 0.55 ppm a.i. levels. The number of eggs/female/day averaged 13.9 for the control group, and 11.3, 11.8, 13.2, 8.9, 5.1, and 1.2 for the 0.016, 0.038, 0.068, 0.15, 0.24, and 0.55 ppm a.i. groups, respectively. No treatment-related effects were observed on hatching success, survival or growth throughout the developmental stages. However, wet weight at 4 weeks could only be assessed up to and including the 0.29 ppm a.i. level. Results based on terminal female wet weight (13 weeks) could not be accurately assessed for treatment-related reductions due to the significant effects on reproductive success (spawning) at the three highest treatment levels. The time to hatch of F₀ embryos was not statistically assessed in the study. Throughout the study, no abnormal appearance or behavior was observed in any of the treatment levels.

F₁-generation: Significant reduction in hatching success was observed at the 0.29 and 0.55 ppm a.i. treatment levels. Percent hatch averaged 75% for the control group, and 77, 75, 79, 80, 26, and 29% for the 0.016, 0.038, 0.068, 0.15, 0.29, and 0.55 ppm a.i. groups, respectively. No treatment-related effects were observed on survival or growth at 4 weeks post-hatch (termination) up to and including the 0.15 ppm a.i. treatment level. The time to hatch for F₁ embryos was also not statistically assessed in the study.

This study is classified as INVALID. This study is deemed scientifically unsound due to diluter mal-function on day 51 which resulted in unacceptable variability (>1.5) in measured concentrations in the 0.6, 0.15, and 0.075 test levels. Both the exposure levels and the toxicity levels derived from them are uncertain. In addition, this study does not fulfill the guideline requirements for a fish life-cycle toxicity test because the time to hatch was not quantitatively assessed for either generation, and the F₁ generation was only maintained for 4 weeks post-hatch. **The data obtained in this study are not considered useful for risk assessment purposes.**

Results Synopsis: Study INVALID, Results Not Reliable.

Biological Endpoint	NOEC (ppm a.i.)	LOEC (ppm a.i.)
F ₀ Generation		
Hatching success		
Time to hatch	Not assessed	
4-week survival		
4-week length		
4-week wet weight		
7-week survival		
7-week length		
13-week survival		
13-week length, male		
13-week length, female		
13-week wet weight, male		
13-week wet weight, female		

Biological Endpoint	NOEC (ppm a.i.)	LOEC (ppm a.i.)
Reproductive success (eggs/female/day)		
F ₁ Generation		
Hatching success		
Time to hatch	Not assessed	
4-week survival		
4-week length		
4-week weight		
8-week survival	Not assessed	
8-week length	Not assessed	
8-week weight	Not assessed	

NOEC:

LOEC:

Endpoint(s) Affected:

Most sensitive endpoint(s):

8. ADEQUACY OF THE STUDY:

A. Classification: INVALID

B. Rationale: Excessive analytical variability was observed on day 51 due to diluter malfunction which resulted in unacceptable variability (>1.5) in measured concentrations in the 0.6, 0.15, and 0.075 test levels. The study authors claim that a selenoid valve failure caused concentrations to drop for a brief period of time but do not adequately explain the duration of the exposure fluctuation and if immediate and appropriate actions were taken to remedy the situation. In addition, this study does not satisfy guideline requirements because the time to hatch was not quantitatively assessed for either the F₀ or F₁ generation, and F₁ generation fish were only maintained for 4 weeks post-hatch, instead of the required 8 weeks.

C. Repairability: This study may be upgraded to ACCEPTABLE status if acceptable

data are provided that demonstrate this study was a "best effort" by the laboratory (e.g., the submission of all quantitative data obtained from preliminary experiments, explanation for oxygenation of the test vessels and evidence that this had no effect on test level concentrations). In addition, data must be provided that support the assumptions that exposure to CGA-64250 (propiconazole) caused no adverse effects on the time to hatch for the F₀ and F₁ generations, or on the survival, appearance, or growth of F₁ generation larvae/fish maintained for 8 weeks post-hatch. Finally, the diluter mal-function which resulted in unacceptable variability (>1.5) in measured concentrations in the 0.6, 0.15, and 0.075 test levels must adequately explain the duration of the exposure fluctuation and if immediate and appropriate actions were taken to remedy the situation.

9. GUIDELINE DEVIATIONS:

1. According to Table 1 (page 46) of the report, unacceptable variability (>1.5 per the EPA Pesticide Registration Analysis) in measured concentrations, was only observed in the 0.6, 0.15, and 0.075 nominal test levels due to diluter mal-function.
2. The photo-period (12 hours light/12 hours dark) differed from recommendation (16 hours light/8 hours dark).
3. The flow-splitting accuracy was not reported.
4. Each tank was continuously aerated during the study using an airstone.
5. The time to hatch endpoint was not quantitatively compared for a possible treatment-related effect. It was only reported that hatching was complete (for both generations) by Day 4 of incubation.
6. Due to territorial behavior being observed, survival and growth data were collected for F₀ generation fish at 7 weeks post-hatch, instead of at 8 weeks post-hatch, and the spawning phase was subsequently initiated.
7. F₁-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 CGA-64250 Technical (Propiconazole) to the full life cycle of sheepshead minnow for the purposes of chemical re-registration.

DP Barcode: D312346

MRID No: 408820-01

11. MATERIALS AND METHODS:**A. Test Organisms**

Guideline Criteria	Reported Information
<p><u>Species</u> Prefer Sheepshead minnow (<i>Cyprinodon variegatus</i>) or Fathead minnow (<i>Pimephales promelas</i>).</p>	<p>Sheepshead minnow (<i>Cyprinodon variegatus</i>)</p>
<p><u>Source and Acclimation</u></p>	<p>Adult minnow were purchased from Aquat-tek, Pensacola, FL, and maintained for a 14-day acclimation and holding period. During acclimation the salinity range was 31-34 ‰, temp. was 27-30°C, and the pH was 7.3-7.9. The brood stock was divided into 14 groups of 7 females and 2 males each, and the eggs from the 14 groups were pooled prior to use for the chronic exposure.</p>
<p><u>Age at beginning of test</u> Embryos, 2 to 24 hours old</p>	<p>Embryos, 24 hours old</p>
<p><u>Feeding</u> Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.</p>	<p>F₀ and F₁ larvae were fed live brine shrimp nauplii three times daily (twice on weekends) during the first 28 days post-hatch. The juvenile and adult fish were fed Tetramarin[®] flakes on the same schedule.</p>

Guideline Criteria	Reported Information
<p><u>Embryo Exposure (4 to 5 Days)</u> Embryos (24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups.</p> <p>A minimum of 50 embryos (24 hrs old) per replicate cup, 4 cups per treatment should be used.</p> <p><u>Parameters measured:</u> Survival of embryos Time required to hatch Hatching success Survival of fry for 4 weeks</p> <p>Dead and fungused embryos should be counted and removed daily.</p>	<p><u>Days 0-6</u> Embryos (24 hours old), obtained from 14 groups of 7 female and 2 male minnow, were randomly assigned into embryo incubation cups.</p> <p>Each cup contained 50 embryos, with two cups per replicate and two replicate aquaria per treatment level (total of 200 embryos per treatment).</p> <p><u>Parameters measured:</u> Hatching success Survival of fry at 4 weeks post-hatch</p> <p>Mortality was determined daily. Dead embryos were removed.</p>

Guideline Criteria	Reported Information
<p><u>Larval-Juvenile Exposure (From Hatch to 8 Weeks)</u> 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.</p> <p><u>Parameters measured:</u> 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.</p>	<p><u>Hatch to 7 Weeks Post-Hatch</u> When hatching was complete (on Day 4), 25 larvae were impartially selected from each incubation cup and transferred to the larval growth chambers (two chambers within each replicate aquarium, 100 larvae per treatment). At 4 weeks post-hatch, juvenile fish from the two growth chambers were combined and impartially reduced to 25 per replicate (50 per treatment).</p> <p><u>Parameters measured:</u> Survival of fry/juvenile fish at 7 weeks post-hatch Total lengths (mm) of all surviving fish at 4 and 7 weeks post-hatch Wet weights (mg) of fish discontinued from exposure (at thinning) at 4 weeks post-hatch</p>

Guideline Criteria	Reported Information
<p><u>Juvenile-Adult Exposure (From 8 weeks posthatch to the end of the spawning phase [32-40 weeks])</u></p> <p>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.</p> <p>The substrates are examined daily and embryos removed, counted, and recorded separately for each pair.</p> <p>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.</p>	<p><u>7 to 13 Weeks Post-Hatch</u></p> <p>Two spawning groups (2 male and 5 female per group) were established for each replicate aquarium. The first group was established on Day 53 (49 days post-hatch), and the second 14 days following the first. Females killed by male aggression were not replaced; however, males were replaced in order to maximize egg fertilization success.</p> <p>The spawning substrates are examined daily and embryos removed, counted, and examined for fertility.</p> <p>Adult exposure was terminated on Day 95 (91 days post-hatch).</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> Survival of adult fish Reproduction (eggs/female/day) Total lengths (mm) and wet weights (g) of all surviving fish at Day 95 (gender-specific)
<p><u>Second Generation Embryo Exposure (4 to 5 days)</u></p> <p>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.</p> <p>Embryos not selected are discarded.</p>	<p><u>F₁ Embryo Exposure</u></p> <p>50 embryos from two different spawns were incubated in each incubation cup as previously described (200 embryos per treatment level).</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> Hatching success

Guideline Criteria	Reported Information
<p><u>Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 weeks)</u> After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment).</p> <p>Each group of 2nd generation fish is terminated 8 weeks after hatching.</p> <p>Fish are blotted, weighed, and measured before being discarded.</p>	<p><u>F₁ Larval-Juvenile Exposure</u> Groups of 25 newly-hatched larvae were randomly released into each larval growth chamber (100 larvae per treatment level).</p> <p>Each group of F₁-generation fish was terminated 4 weeks after hatching.</p> <p><u>Parameters measured:</u> Survival of fry/juvenile fish at 4 weeks post-hatch Total lengths (mm) and wet weights (g) at 4 weeks post-hatch</p>

Comments: At study initiation, a sub-sample of 100 F₀-embryos was microscopically examined to determine fertilization success. It was determined that 73% of eggs were fertilized.

In addition to determination of hatching success of embryo groups used to perform the F₁ early life stage exposure, hatching success was determined frequently throughout the 14-day spawning periods, usually in groups of 50 eggs (except at levels where spawning was reduced due to treatment).

B. Test System

Guideline Criteria	Reported Information
<p><u>Test Water</u> <u>Sheepshead Minnow</u> 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).</p> <p><u>Fathead Minnow</u> 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as CaCO_3 and pH of 7.2 to 7.6.</p>	<p>1. Natural filtered (20 and 5 μm) seawater collected from Cape Cod Canal, Bourne, MA. 2. Salinity of 30-32 ‰ and pH 7.9-8.2</p> <p>N/A</p>
<p><u>Test Temperature</u> <u>Sheepshead:</u> 30 C. <u>Fathead:</u> 25 C and should not remain outside the range of 24 to 26 C for more than 48 hours.</p>	<p>26-31 C N/A</p>
<p><u>Photo-period</u> 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.</p>	<p>12-hour light/12-hour dark cycle Light intensity at the surface of the water in the upper level of the test system ranged from 34-60 footcandles and from 100-200 footcandles in the lower level.</p>

Guideline Criteria	Reported Information
<p><u>Dosing Apparatus</u></p> <ol style="list-style-type: none"> 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor ≤ 0.5. 3. One control should be used. 	<ol style="list-style-type: none"> 1. Intermittent-flow proportional diluter. 2. Six toxicant concentrations with a dilution factor of 0.5. 3. A dilution water (negative) control was used.
<p><u>Toxicant Mixing</u></p> <ol style="list-style-type: none"> 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. 	<ol style="list-style-type: none"> 1. The diluter system incorporated a mixing chamber. 2. Yes 3. The flow-splitting accuracy was not reported.

Guideline Criteria	Reported Information
<p><u>Exposure System/Test Vessels</u> 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).</p> <p>Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm.</p> <p>Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.</p>	<p>Glass exposure aquaria (60 x 30 x 30 cm) were used. Each aquarium was equipped with a 15-cm high drain-end, to maintain the test solution volume at 27 L.</p> <p>The larval growth chambers were constructed of glass and 40-mesh nylon screening (ends), and measured 30 x 14.5 x 17 cm. Designated aquaria were assigned two larval growth chambers.</p> <p>Spawning baskets were constructed of 8.5-mesh nylon screening, and measured 30 x 30 cm, with a water depth of 12 cm. Each basket was placed over a removable egg collection tray. The trays were constructed with 3-cm high glass sides and 40-mesh nylon screening bottom. Designated aquaria were assigned two spawning baskets.</p>
<p><u>Embryo and Fry Chambers</u> 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.</p>	<p>The embryo incubation cups were 5 cm diameter glass jars with 40-mesh nylon screen bottoms and stainless steel wire handles. Embryo incubation cups were suspended from embryo incubation chambers with stainless steel wire hangers (refer to Reviewer's Comments section for further details). The embryo incubation chambers measured 16 x 7.5 x 7.5 cm; construction materials were not reported.</p>

Guideline Criteria	Reported Information
<p><u>Flow Rate</u> 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.</p>	<p>During the pre-spawning phase, the flow rate was 7.2 volume additions per day (1 volume replacement/3.33 hr).</p> <p>During the spawning phase, the flow rate was 5.1 volume additions per day (1 volume replacement/4.7 hr).</p>
<p><u>Aeration</u> Dilution water should be aerated to insure dissolved oxygen concentrations at or near 100% saturation. Test tanks and embryo chambers should not be aerated.</p>	<p>Continuous aeration was provided to each tank using a Hagen® aquarium pump and an airstone.</p>

C. Chemical System

Guideline Criteria	Reported Information
<p><u>Nominal Concentrations</u> Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate.</p> <p>Toxicant conc. must be measured in one tank at each toxicant level every week.</p>	<p>0 (negative control), 0.019, 0.038, 0.075, 0.15, 0.30, and 0.60 ppm a.i.</p> <p>Toxicant concentrations were measured at test initiation and approximately weekly from alternating replicate aquaria in each test group.</p>

Guideline Criteria	Reported Information
<p>Other Variables</p> <ol style="list-style-type: none"> DO must be measured at each conc. at least once a week. Test water temp. must be recorded continuously. <u>Freshwater</u>: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. <u>Natural seawater</u>: must maintain a constant salinity and not fluctuate more than 6‰ weekly; monthly pH range <0.8 pH units. 	<ol style="list-style-type: none"> DO was measured at test initiation in each aquarium, and daily thereafter in alternating replicate aquaria. Temperature was measured at test initiation in each aquarium, and daily thereafter in alternating replicate aquaria. Temperature was also continuously monitored in one aquarium from both levels of the diluter system. pH and salinity were measured at test initiation in each aquarium, and weekly thereafter in alternating replicate aquaria. The salinity and pH did not appreciably fluctuate.
<p>Solvents Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>None used.</p>

Comments: None.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes. This study was conducted in compliance with all pertinent EPA GLP regulations with the following exception: characterization and verification of the test substance identity was the responsibility of the sponsor.</p>
<p>Data Endpoints must include: survival of P and F₁ embryos, time required to hatch, and hatching success; survival and total length of P fish at 4 and 8 weeks after hatching; weights and lengths of F₁ fish at 8 weeks; incidence of pathological or histological effects; and observations of other effects or clinical signs.</p>	<p>Data Endpoints included: survival of F₀ and F₁ embryos and hatching success; survival and total lengths of F₀ fish at 4 and 7 weeks after hatching; wet weights of fish discontinued from exposure (at thinning) 4 weeks after hatching; survival of F₀ fish at 13 weeks after hatching (91 days post-hatch; test termination); total lengths and weights (gender specific) of surviving F₀ fish at 13 weeks after hatching; F₀ fecundity (eggs/female/day) total lengths and wet weights of F₁ fish at 4 weeks after hatching incidence of pathological or histological effects; observation of other effects or clinical signs</p>
<p>Raw data included?</p>	<p>Yes</p>

E₀ Results:

Nominal Conc., ppm a.i.	Mean Measured Conc., ppm a.i. (SD)	% Hatch	4-Week Post-Hatch % Survival	7-Week Post-Hatch % Survival ²	13-Week Post-Hatch (Terminal) % Survival ²
Negative Control	<0.0080	75	97	97	83
0.019	0.016 (0.0037)	80	95	95	71
0.038	0.038 (0.0077)	74	94	94	73
0.075	0.068 (0.015)	78	95	93	74
0.15	0.15 (0.030)	74	91	91	54
0.30	0.29 (0.067)	80	98	98	65
0.60	0.55 (0.13)	74	96	96	81

Data obtained from Table 1, pp. 46-47, and Table 4, p. 51.

¹ Although mean-measured values ranged from 84-100% of nominal values, analytical variability within each test level was excessive, with reviewer-calculated high-low ratios of 1.8-2.2 and coefficients of variation of 20-24%.

² Based on normalized data, adjusted for thinning of the population (refer to Reviewer's Comments section).

Mean Measured Conc. (ppm a.i.)	Mean Total Length (mm)				Wet Weight (g)		
	4 Weeks Post-hatch	7 Weeks Post-hatch	Test Termination		4 Weeks Post-hatch	Test Termination	
Control	25	34	39	34	0.22	1.11	0.73
0.016	25	34	38	35	0.23	1.08	0.74
0.038	26	35	40	36	0.26	1.26	0.76
0.068	25	35	39	35	0.21	1.09	0.72
0.15	25	35	41	35	0.24	1.37	0.81
0.29	25	35	40	34	0.23	1.24	0.73
0.55	24	34	39	39	0.22	1.15	1.16 ²

Data obtained from Table 5, p. 52.

¹ Although mean-measured values ranged from 84-100% of nominal values, analytical variability within each test level was excessive, with reviewer-calculated high-low ratios of 1.8-2.2 and coefficients of variation of 20-24%.

² Significantly higher compared to the control group, but attributed to the large number of unspawned eggs present in the females, and not a result of exposure.

DP Barcode: D312346

MRID No: 408820-01

Mean Measured Conc. (ppm a.i.) ¹	Total Number of Eggs ²	Number of Eggs/ Female/Day (± SD) ³
Control	7740	13.9 (± 10.3)
0.016	5262	11.3 (± 8.5)
0.038	6393	11.8 (± 8.8)
0.068	6074	13.2 (± 10.5)
0.15	4515	8.6 (± 1.6)*
0.29	2135 ⁴	5.1 (± 7.6)*
0.55	620	1.2 (± 4.4)*

Data obtained from Table 6, p. 53.

¹ Although mean-measured values ranged from 84-100% of nominal values, analytical variability within each test level was excessive, with reviewer-calculated high-low ratios of 1.8-2.2 and coefficients of variation of 20-24%.

² Based on eight spawnings of 14 days each.

³ Based on eight spawnings of 14 days each and corrected for the number of females alive on each day of spawning.

⁴ Second digit of value was illegible; the reviewer best-guessed the value to be "1".

* Significantly reduced compared to the control, based on Williams' Test.

Toxicity Observations: Hatching was completed within 4 days of egg release. However, the time to hatch was not quantitatively assessed. No treatment-related effect on embryo hatchability or survival of F₀ fish at any stage was observed. By exposure Day 52 (48 days post-hatch), nearly all exposed fish in all treatment levels and control completed their sexual development, as evidenced by clear sexual dimorphism and aggressive behavior of the male fish. The concentrations of CGA-64250 did not appear to significantly alter the time required for the fish to reach this developmental stage.

Reproduction was affected by exposure to CGA-64250, as evidenced by a statistically-significant reduction in the number of eggs/female/day at the mean-measured 0.15, 0.29, and 0.55 ppm a.i. levels. Based on this effect, the NOEC, LOEC, and geometric MATC were 0.068, 0.15, and 0.10 ppm a.i., respectively.

No treatment-related effect on growth was observed at any interval. Although the terminal weights of surviving females from the 0.55 ppm a.i. level (highest test level) were notably higher than controls (by 59%), the study author attributed this difference to the large number of unspawned eggs present in the females at this test concentration which significantly reduced spawning.

Throughout the study, no abnormal appearance or behavior was observed in any of the treatment levels. It was reported that during spawning trials, females were occasionally lost due to persistent male territorial behavior (chasing and biting). Female mortalities could

clearly be ascribed to this behavioral pattern, rather than to any toxicant-mediated stress, as evidenced by the random occurrence of such mortalities in both concentration and control spawn groups.

F₁ Results:

Mean Measured Concentration (ppm a.i.) ¹	% Hatch	Supplemental % Hatch ²		4 Week Post-Hatch % Survival	4 Week Post-Hatch Length (mm)	4 Week Post-Hatch Wet Weight (g)
		A	B			
Control	75	74	75	95	23	0.17
0.016	77	76	80	98	22	0.18
0.038	75	77	77	93	23	0.18
0.068	79	82	73	92	22	0.16
0.15	80	76	84	100	23	0.17
0.29	26*	13*	34*	100 ³	21 ³	0.16 ³
0.55	29*	12*	27*	---	---	---

Data obtained from Tables 8 through 10, pp. 55-57.

¹ Although mean-measured values ranged from 84-100% of nominal values, analytical variability within each test level was excessive, with reviewer-calculated high-low ratios of 1.8-2.2 and coefficients of variation of 20-24%.

² Supplemental data; based on frequent determinations throughout the 14-day spawning period, including one set from fish which had been used once previously for spawning.

³ Based on survival (of 100%) in one replicate, since the number of available fish for F₁ exposure was limited due to the reduced spawning in the F₀ generation. This value should not be considered reliable since it is based on the results from one replicate rather than four replicates for all lower treatment levels and the control group.

* Significantly reduced compared to the control, based on Williams' Test.

— = Fish not available due to treatment-related effect on spawning in the F₀ generation.

Toxicity Observations: Hatching was completed within 4 days of egg release. However, the time to hatch was not quantitatively assessed. In addition to determination of hatching success of embryo groups used to perform the F₁ early life-stage exposure, hatching success was determined frequently throughout the 14-day spawning periods. In all cases, hatching success was statistically-reduced at the 0.29 and 0.55 ppm a.i. levels. Based on hatching success, the NOEC, LOEC, and geometric MATC were 0.15, 0.29, and 0.21 ppm a.i., respectively.

No treatment-related effects on survival or growth were observed in F₁-generation fish 4 weeks following hatch at concentrations of 0.15 ppm a.i.. Reduced spawning at the highest two exposure concentrations prevented performance of an early life-stage exposure at these concentrations (one replicate was tested at the mean-measured 0.29 ppm a.i. level as noted

above in the F₁ Results Table).

B. Reported Statistical Results

Statistical Method (s): Data endpoints statistically assessed included F₀ and F₁ embryo hatching success, survival, total length, and wet weight; and F₀ reproductive success. Mean-measured concentrations were used in the calculations.

A two-factor analysis of variance (ANOVAs) was conducted with F₀ reproductive success (spawning) and F₁ hatching data to determine whether data obtained from four separate spawning groups per treatment and control group (which were not conducted simultaneously) should be treated as a single replicated data set in subsequent statistical analyses. Data points used in these analyses were entered as mean values for each of the A and B replicates (maximum of four A and four B replicates per treatment and control group). The independent factor in these 2-way ANOVAs was concentration, while the reproductive success and the percent hatching success were considered dependent factors. No statistical differences were found among the four spawning groups for either endpoint. Therefore the data were pooled for subsequent analyses.

Percent survival and percent hatch data were arcsine square-root transformed prior to analysis. Bartlett's test (99% level of certainty) indicated a homogeneity of variance in all data endpoints, which were subsequently compared using Williams' method (95% level of certainty; Williams, 1971, 1972). Reproductive success and hatching success data points were entered as individual values by replicate (i.e., four values per replicate), while growth and survival data were entered by replicate (i.e., one mean value per replicate).

The no observed effect concentration (NOEC) is the highest test concentration causing no adverse effects. The lowest observed effect concentration (LOEC) is the lowest test concentration causing adverse effects. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and the LOEC.

Biological Endpoint	NOEC (ppm a.i.)	LOEC (ppm a.i.)	MATC (ppm a.i.)
F ₀ Generation			
Hatching success	0.55	>0.55	>0.55
Time to hatch	Not assessed		
4-week survival	0.55	>0.55	>0.55
4-week length	0.55	>0.55	>0.55
4-week wet weight	0.55	>0.55	>0.55
7-week survival	0.55	>0.55	>0.55
7-week length	0.55	>0.55	>0.55
13-week survival	0.55	>0.55	>0.55
13-week length, male	0.55	>0.55	>0.55
13-week length, female	0.55	>0.55	>0.55
13-week wet weight, male	0.55	>0.55	>0.55
13-week wet weight, female	0.55	>0.55	>0.55
Reproductive success (eggs/female/day)	0.068	0.15	0.10
F ₁ Generation			
Hatching success	0.15	0.29	0.21
Time to hatch	Not assessed		
4-week survival	0.55	>0.55	>0.55
4-week length	0.55	>0.55	>0.55
4-week weight	0.55	>0.55	>0.55
8-week survival	Not assessed		
8-week length	Not assessed		
8-week weight	Not assessed		

NOEC: 0.068 ppm a.i.

LOEC: 0.15 ppm a.i.

MATC: 0.10 ppm a.i.

Endpoint(s) Affected: F₀ reproductive success and F₁ hatching success

Most sensitive endpoint(s): F₀ reproductive success

13. REVIEWER'S STATISTICAL RESULTS:

Statistical Method(s): After confirming normality and homogeneity of variances, F₀ generation hatching success, survival at 13 weeks, male and female wet-weights (week 13) and the mean number of eggs per female per day were assessed for significant ($p < 0.05$) treatment-related reductions compared to the control group using ANOVA and William's multiple comparison test. F₀ fish length and wet-weight at 4 weeks, length at 7 weeks, and male lengths at 13 weeks did not meet the assumptions of ANOVA and were assessed for treatment-related reductions relative to the control non-parametrically using Kruskal-Wallis ANOVA by ranks and Dunn's multiple comparison test. Only enough extra fish used to assess F₀ wet-weights at 4 weeks were available to fill one of two possible replicates for the mean-measured 0.55 ppm a.i. treatment group. Consequently, the reviewer was only able to determine a NOEC for this endpoint up to and including the mean-measured 0.29 ppm a.i. treatment level. The above statistical analyses were performed via TOXSTAT statistical software. The NOEC and LOEC values for F₀ survival at 4 and 7 weeks and female length at 13 weeks were visually determined since survival was 91% in all treatment groups and the control and female terminal lengths (13 weeks) in all treatment groups were greater than or equal to those of the control group.

F₁ generation hatching success treatment response data met the assumptions of ANOVA and were assessed for treatment-related reductions relative to the control data using William's test. NOEC and LOEC values for F₁ survival, lengths and wet-weights at 4 weeks were determined visually since treatment results were comparable to those of the control up to and including the mean-measured 0.15 ppm a.i. treatment group. The 0.29 ppm a.i. treatment group results were not considered reliable by the reviewer since the number of embryos was limited due to treatment-related effects on F₀ spawning. Consequently, only one replicate was tested and could not be statistically compared to the control results. Due to a nearly complete treatment-related reduction in F₀ reproductive success (number of eggs/female/day) and F₁ hatching success at the mean-measured 0.55 ppm a.i. treatment level, no groups of embryos were available for testing at this level during the early-life stage portion of the F₁ exposure.

Biological Endpoint	NOEC (ppm a.i.)	LOEC (ppm a.i.)	Statistical Method (s)
F₀ Generation			
Hatching success	0.55	>0.55	William's
Time to hatch	Not assessed in study		
4-week survival	0.55	>0.55	Visually
4-week length	0.55	>0.55	Dunn's
4-week wet weight	0.29	>0.29	Dunn's
7-week survival	0.55	>0.55	Visually
7-week length	0.55	>0.55	Dunn's
13-week survival	0.55	>0.55	William's
13-week length, male	0.55	>0.55	Dunn's
13-week length, female	0.55	>0.55	Visually
13-week wet weight, male	0.55	>0.55	William's
13-week wet weight, female	0.55	>0.55	William's*
Reproductive success (eggs/female/day)	0.15	0.29	William's*
F₁ Generation			
Hatching success	0.15	0.29	William's*
Time to hatch	Not assessed in study		
4-week survival	0.15	>0.15	Visually
4-week length	0.15	>0.15	Visually
4-week weight	0.15	>0.15	Visually
8-week survival	Not assessed in study		
8-week length	Not assessed in study		
8-week weight	Not assessed in study		

* See Reviewer's Comments section of this DER for further details.

14. REVIEWER'S COMMENTS:

The reviewer's conclusions (NOEC and LOEC values) were identical to those of the study author for F_0 generation survival at 4, 7, and 13 weeks, and length at 4 weeks, male and female length at 13 weeks, male wet weight at 13 weeks, reproductive success (mean number of eggs/female/day), and F_1 hatching success. The reviewer-determined NOEC and LOEC values for F_0 wet weight at 4 weeks (0.29 and >0.29 ppm a.i., respectively) were one treatment level lower than those of the study author (0.55 and >0.55 ppm a.i., respectively). This difference was due to the fact that only enough extra fish used to assess F_0 wet-weights at 4 weeks were available to fill one of two possible replicates for the mean-measured 0.55 ppm a.i. treatment group. Consequently, the reviewer was only able to determine a NOEC for this endpoint up to and including the mean-measured 0.29 ppm a.i. treatment level. Similarly, due to the significant ($p < 0.05$) reduction in F_0 reproductive success at the three highest treatment levels tested and a lack of available F_1 fish post-hatch at the two highest treatment levels, the reviewer was only able to verify the NOEC and LOEC values for F_1 survival, length and wet weight by 4 weeks up to and including the 0.15 ppm a.i. treatment level. Consequently, the reviewer concluded the NOEC and LOEC values to be 0.15 and >0.15 ppm a.i. for these three endpoints and not 0.55 and >0.55 ppm a.i. as reported by the study author.

The study author noted that there were no treatment-related effects on growth, although, terminal weights (13 weeks) of surviving females from the 0.55 ppm a.i. level (highest test level) were notably higher than controls (by 59%). The study author attributed this difference to the large number of unspawned eggs present in the females at this test concentration which significantly reduced spawning. Although statistically verified, the reviewer did not consider the NOEC and LOEC values for female wet weight at 13 weeks reliable given the study author-provided explanation and the statistically verified treatment-related reduction in reproductive success at the three highest treatment levels tested.

It was reported that a total of eight spawn groups were evaluated for F_0 fish (four per replicate aquarium). Spawn group 2 was initiated within a few days of spawn group 1 using females which were in spawning condition. Spawn group 3 was started several weeks later, while spawn group 4 used females which previously had been placed in a spawn group. The study author reported that this practice was considered acceptable as sheepshead minnow spawn continuously. It was also reported that a two-factor ANOVA indicated no significant differences between spawning groups, so the results were pooled for statistical analysis of F_0 reproductive success. Reproductive success and hatching success data points were entered as individual values by replicate (i.e., four values per replicate), while growth and survival data were entered as mean values by replicate (i.e., one mean value per replicate). The pooling of the A and B replicate values, as committed by the study author, to boost treatment replicate size from four to eight was regarded by the reviewer as pseudo-replication. While

the study author demonstrated that spawning groups did not differ, a similar comparison was not made for replicates A and B. As a result, the reviewer statistically verified the reported toxicity values for F_0 reproductive success and F_1 hatching success using the mean replicate data (two replicates per level, as mandated by the overall study design). For comparative purposes, the reviewer also statistically verified the results for both endpoints using the pseudo-replication method of combining replicates A and B to total eight replicates per treatment and control group. The results of this pseudo-replication statistical analysis were identical to those of the study author. Results of the appropriate mean replicate analysis revealed that the reviewer-determined NOEC and LOEC values for F_0 reproductive success (0.15 and 0.29 ppm a.i.) were higher than those of the study author (0.068 and 0.15 ppm a.i.). The reviewer agrees with the study author's results, that the NOEC and LOEC for reproductive success should in fact be 0.068 and 0.15 ppm a.i.. However, the study design (two replicates per level) did not allow for enough statistical power to detect the apparent biologically-significant reduction in reproductive success at the three highest treatment levels tested (0.15, 0.29, and 0.55 ppm a.i.).

In summary, the most conservative toxicity values are reported in the Conclusion section of this DER.

The study author reported that prior to initiating the chronic study, the stability of CGA-64250 in seawater was established; however, data were not submitted to support this conclusion. Furthermore, the study author noted that since the measured concentrations averaged 84 to 100% of nominal values, and since the coefficients of variation (CV's) were consistent for each tested concentration and were between 20 and 24% of the mean measurements, that CGA-64250 was soluble and stable at the concentrations selected for testing. However, the reviewer considered these CV values to be excessive (although there is no guidance pertaining to aquatic concentrations and CV), which was supported by calculation of the high-low ratios for the mean-measured data. For all test concentrations, the analytical high-low ratios exceeded the 1.5 limit, and ranged from 1.8 to 2.2 (excluding the obvious outliers on Day 51). The extraction and analysis procedures were not a factor, as concurrently-run QC samples averaged $104 \pm 4.21\%$ (see comment below), nor was solubility a factor. However, the use of continuous oxygenation of the test vessels may have contributed to the highly variable concentrations. As the study author did not acknowledge excessive analytical variability, no explanation was provided. As a result, this study is considered scientifically invalid, and data obtained are not useful for risk assessment purposes. This study may be upgraded to ACCEPTABLE status if acceptable data are provided that demonstrate this study was a "best effort" by the laboratory (e.g., the submission of all quantitative data obtained from preliminary experiments, explanation for oxygenation of the test vessels and evidence that this had no effect on test level concentrations). In addition, data must be provided that support the assumptions that exposure to CGA-64250 (propiconazole) caused no adverse effects on the time to hatch for the F_0 and F_1 generations, or on the survival, appearance, or growth of F_1 generation

larvae/fish maintained for 8 weeks post-hatch.

Two additional factors that were noted under protocol deviations contributed to the observed analytical variability. One deviation included initiation of the toxicant delivery system on Day 0, instead of a minimum of 48 hours prior to study initiation. Measured concentrations averaged 64% of nominal on Day 0. In addition, six toxicant delivery system malfunctions occurred, three of which were on regular sampling days, and only one of which was reported to have affected the measured test concentration: on Day 51, a solenoid valve failure was discovered in the test system, which caused concentrations to drop for a brief period of time (not further specified). Therefore, all Day 51 values were excluded from the mean measurements. For the remaining three malfunctions, judgements on the probability of the concentrations being affected were made and no samplings were conducted.

An early life stage range-finding study conducted in January 1987 was concurrently-submitted [MRID 401833-10: Foster, R.B. 1987. Fish Full Life-Cycle Test with Sheepshead Minnow, *Cyprinodon Variegatus* and CGA-64250 (Propiconazole). Unpublished study performed by Springborn Bionomics, Inc., Wareham, MA. Laboratory Study No. 1781.6132. Final report submitted April 21, 1987]. In this experiment, sheepshead embryos (number of embryos and replicates not reported) were exposed under flow-through (presumed) conditions to CGA-64250 (propiconazole) at nominal concentrations of 0 (negative control), 0 (acetone control), 0.038, 0.075, 0.15, 0.30, and 0.60 ppm a.i. for 27 days (5-day hatching period and 22-day post-hatch period). It was reported that concentrations of the test substance were stable under the conditions employed, with measured amounts at approximately 85% of nominal concentrations (detailed analytical results not provided). After 27 days of exposure, no treatment-related effects were observed on egg hatchability, fry survival, or growth (length and weight) up to and including the highest concentration tested of 0.603 ppm a.i. (mean-measured). Summarized quantitative data were not provided. Upon consultation with the sponsor, a conservative approach was elected to establish the definitive test concentrations. Although a LOEC was not established in this study, it was reported that the likelihood of establishing an unequivocal NOEC is asserted with a higher degree of certainty at somewhat lower exposure concentrations, and therefore nominal concentrations selected for use in the definitive study were 0.018, 0.038, 0.075, 0.15, 0.30, and 0.60 ppm a.i.. It was also determined in the preliminary study that there was a potential solvent control effect. Lengths of solvent control fish were significantly longer than those of the negative control fish (averaging 27 versus 24 mm, respectively) and of the fish at all treatment levels (23-24 mm). Therefore, additional trials were performed to introduce CGA-64250 into seawater without the aid of a co-solvent. It was concluded from the additional work that following a 48-hour mixing period, the test article was soluble up to 25 ppm a.i. in seawater, and that seawater stock solutions were proven to be homogeneous and stable for at least 7 days (the maximum period of stock usage in the toxicant delivery apparatus). Detailed methods and quantitative results for these solubility and stability trials were not provided.

In a supplemental short-term experiment (reported within the definitive study), two groups of control F₁ embryos (25 per group or 50 per level; 24 hours old) were transferred and incubated at the mean-measured 0.29 and 0.55 ppm a.i. levels (highest two concentrations). The hatching success of these embryos was 80% for the 0.29 ppm a.i. level and 76% for the 0.55 ppm a.i. level, correlating positively with data obtained from the F₀ embryos exposed at these levels. After hatching, the fry were exposed at these concentration levels for 14 days, when they were transferred to a recirculating seawater-only system. The total exposure time was 18 days. Thirty-four fish (68% of initial embryos) and 32 fish (64%) temporarily exposed to 0.29 and 0.55 ppm a.i., respectively, reached maturity. Following maturation, two spawning groups (two male and five female each) were established for each level. Reproduction was evaluated for 14-day spawning periods as previously described in the definitive study. Similarly, hatching success was evaluated in several embryo groups during the 14-day spawning periods. No treatment-related effect on reproduction or hatching success of the progeny were observed. The number of eggs/female/day averaged 16.2 and 14.5 for the 0.29 and 0.55 ppm a.i. levels, respectively (compared to 13.9 eggs/female/day in controls). Hatching success averaged 63 and 67% for the 0.29 and 0.55 ppm a.i. levels, respectively (compared to 75% in controls). The study author noted that any adverse effects caused by the test substance did not occur during the earliest, generally most sensitive life stages.

At the request of the sponsor, the study report was submitted to two independent outside aquatic toxicologists to review the report and determine the MATC, the NOEC, and provide a rationale for selection of the statistical method used. The reports sent to the reviewers did not reveal the compound name, the name of the study sponsor, nor any indication of the laboratory's statistical interpretation of the data. One outside reviewer corroborated the laboratory's conclusion, while the other reviewer indicated that, based on Dunnett's test, the NOEC is 0.15 ppm a.i.. The study author noted that the second reviewer failed to apply Williams' test, which was considered to be more appropriate. Both statistical reviews were included as appendices in the definitive study report.

In the definitive study, fish survival after thinning of the population was calculated based on the number present on day 1 post-hatch, after normalizing the data in the following example for Replicate A of the control group: On day 1 post-hatch, 50 fish were alive. Only 49 fish were alive on day 32 (or 98%). After thinning, 25 fish remained. On day 52, all 25 fish were still alive. The "adjusted survival" was calculated by multiplying the number of fish alive by the thinning ratio: $(49/25) \times 25 = 49$ (or 98%). Similarly, day 95 survival was 23 fish. Adjusted survival on that day was therefore $(49/25) \times 23 = 45$ (or 90%).

The embryo incubation chambers that were attached at the head of each applicable aquarium were originally designed to hold two embryo incubation cups. However, it became evident that the water temperature in the incubation chambers could not be maintained at the required range of 28-32 C due to the position of the chambers above the surface of the

aquaria and the temperature regulation provided by the heated water bath. Instead, the embryo incubation cups were suspended from the embryo incubation chambers with stainless steel wire hangers into the main area of the exposure aquaria where they were aerated from below to circulate water around the embryos.

At 46 days post-hatch (nearly 7 weeks), territorial behavior was observed in several males at two treatment levels of the study. Growth and survival data that were originally scheduled for 56 days post-hatch (8 weeks) were thus collected, and the spawning phase of the study was initiated at 49 days post-hatch (Day 53 of exposure). This deviation was not considered to be significant by the reviewer, as required endpoints were collected at the change of the developmental stage (i.e., beginning of spawning).

In addition to the preliminary range-finding study with sheepshead minnow, MRID 401833-10 report compared the known acute and chronic toxicity of propiconazole to various aquatic species, and calculated applicable acute:chronic ratios (ACRs) based on this known data. The following table summarizes the results provided for the acute and chronic comparisons:

Species	Acute test type	Acute LC ₅₀ , ppm a.i.	Chronic test type	Chronic MATC, ppm a.i.	ACR
Freshwater					
<i>Daphnia magna</i>	48-hr static	1.34	21-day flow-through	0.46	2.9
Bluegill sunfish	96-hr static	3.76	---	---	---
Rainbow trout	96-hr static	1.04	---	---	---
Fathead minnow	96-hr static	7.64		0.132	58
Saltwater					
Mysid shrimp	96-hr static	0.59	---	---	---
	96-hr flow-through	0.51	28-day flow-through	0.32	1.6
Oyster	48-hr embryo larval	3.4	---	---	---
	96-hr shell deposition	1.7	---	---	---
Spot	96-hr static	2.2	---	---	---
Sheepshead minnow	96-hr static	2.1	27-day flow-through*	>0.60	<3.5

*Obtained from current preliminary testing.

In the comparison discussion, it was reported that propiconazole is acutely toxic generally in the range of 0.5-5.0 ppm a.i. to aquatic organisms, including the freshwater species *daphnia magna*, bluegill sunfish, and rainbow trout, and saltwater species mysid shrimp (most sensitive), oyster, spot, and sheepshead minnow. Fathead minnow were slightly less sensitive, with an LC₅₀ of 7.64 ppm a.i. It was further noted that since the LC₅₀ values varied minimally (<10X) across a wide range of standard fish and invertebrate organisms, that there was a greater confidence that chronic data from a few species would represent protective levels for most organisms. The ACR values obtained for daphnids and mysids were not indicative of significant risk from cumulative or chronic toxic action in these aquatic species. The study author reported that although the ACR obtained for fathead minnow (58) indicated a higher potential risk to fish under conditions of continuous chronic exposure, examination of the time-dependent response of fathead minnows in the fish early life-stage study places considerable doubt on the validity of an ACR of this magnitude.

Propiconazole is the active ingredient in the formulated product TILT 3.6E. The chemical

name provided for propiconazole was 1-[2-(2',4'-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole (MRID 401833-10).

In the definitive study (MRID 408820-01), quality control samples were prepared at each sampling interval and remained with the set of exposure solution samples throughout the analytical process; samples were prepared in saltwater at a nominal concentrations of 0.0300, 0.0350, 0.0400, 0.0500, 0.0600, 0.0900, 0.200, 0.250, 0.300, 0.400, 0.450, 0.500, 20.0, 28.0, and/or 34.0 ppm a.i.. Recoveries ranged from 93.6 to 113%, and averaged $104 \pm 4.21\%$. The mean recovery value was the same as that obtained a method validation study conducted prior to study initiation ($104 \pm 4.0\%$).

The test system for the definitive study included two tiers, consisting of an upper and lower level water bath. Each water bath contained 14 aquaria. The minimum-maximum temperature readings from both baths ranged from 26-31 C, with two exceptions: on Day 85, the heaters in the lower level water bath went off due to a tripped electrical breaker, and the lowest temperature recorded in the monitoring tank was 24 C. The following day (Day 86), a heater failed completely and had to be replaced. During this repair, a system malfunction, which affected only the monitoring tank and its replicate, caused the temperature to briefly drop to 20 C, but returned to 26 C within 5.5 hours.

The total organic carbon content of the seawater used for the definitive study, determined once, was 22.36 mg/L.

15. REFERENCES:

- Macek, K.J. and B.H. Sleight, III. 1977. Utility of toxicity tests with embryo and fry of fish in evaluating hazards associated with chronic toxicity of chemicals to fishes. Symposium Proceedings, ASTM, Memphis, Tennessee, October, 1976: 137-146.
- McKim, J.M. 1977. Evaluation of tests with early-life-stage of fish for predicting long-term toxicity. *J. Fish. Res. Board Can.* 34: 1148-1154.
- Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. *Water Res.* 1: 20-29. 196 pp.
- Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Res.* 3: 793-821.
- Williams, D.A. 1971. A test for the difference between treatment means when several dose levels are compared with a zero dose control. *Biometrics.* 27: 103-117.
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519-531.

16. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

F0 Percent Hatchability (mean-meas. ppm a.i.), NOEC:
 File: 2001h1d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	96.429	16.071	0.389
Within (Error)	7	289.000	41.286	
Total	13	385.429		

Critical F value = 3.87 (0.05,6,7)
 Since F < Critical F FAIL TO REJECT Ho:All groups equal

F0 Percent Hatchability (mean-meas. ppm a.i.)
 File: 2001h1d Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	75.000	75.000		
2	0.016	80.500	80.500	-0.856	
3	0.038	74.000	74.000	0.156	
4	0.068	78.000	78.000	-0.467	
5	0.15	74.000	74.000	0.156	
6	0.29	79.500	79.500	-0.700	
7	0.55	74.000	74.000	0.156	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

F0 Percent Hatchability (mean-meas. ppm a.i.)
 File: 2001h1d Transform: NO TRANSFORMATION

DUNNETT'S 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	neg control	2			
2	0.016	2	18.120	24.2	-5.500
3	0.038	2	18.120	24.2	1.000
4	0.068	2	18.120	24.2	-3.000
5	0.15	2	18.120	24.2	1.000
6	0.29	2	18.120	24.2	-4.500
7	0.55	2	18.120	24.2	1.000

F0 Percent Hatchability (mean-meas. ppm a.i.)
 File: 2001h1d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	75.000	75.000	77.750
2	0.016	2	80.500	80.500	77.750
3	0.038	2	74.000	74.000	76.375
4	0.068	2	78.000	78.000	76.375
5	0.15	2	74.000	74.000	76.375
6	0.29	2	79.500	79.500	76.375
7	0.55	2	74.000	74.000	74.000

FO Percent Hatchability (mean-meas. ppm a.i.)
 File: 2001hid 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
neg control	77.750				
0.016	77.750	0.428		1.89	k= 1, v= 7
0.038	76.375	0.214		2.00	k= 2, v= 7
0.068	76.375	0.214		2.04	k= 3, v= 7
0.15	76.375	0.214		2.06	k= 4, v= 7
0.29	76.375	0.214		2.07	k= 5, v= 7
0.55	74.000	0.156		2.08	k= 6, v= 7

s = 6.425

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

FO % Survival @ Day 91 (13 weeks, mean-meas. ppm a.i.), NOEC:
 File: 2001s2d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1171.857	195.310	0.518
Within (Error)	7	2638.500	376.929	
Total	13	3810.357		

Critical F value = 3.87 (0.05,6,7)

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

FO % Survival @ Day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001s2d 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	neg control	83.500	83.500		
2	0.016	71.500	71.500	0.618	
3	0.038	73.500	73.500	0.515	

DP Barcode: D312346

MRID No: 408820-01

4	0.068	74.500	74.500	0.464
5	0.15	54.000	54.000	1.519
6	0.29	65.000	65.000	0.953
7	0.55	80.500	80.500	0.155

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Fo % Survival @ Day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001s2d 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	neg control	2			
2	0.016	2	54.749	65.6	12.000
3	0.038	2	54.749	65.6	10.000
4	0.068	2	54.749	65.6	9.000
5	0.15	2	54.749	65.6	29.500
6	0.29	2	54.749	65.6	18.500
7	0.55	2	54.749	65.6	3.000

Fo % Survival @ Day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001s2d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	83.500	83.500	83.500
2	0.016	2	71.500	71.500	73.167
3	0.038	2	73.500	73.500	73.167
4	0.068	2	74.500	74.500	73.167
5	0.15	2	54.000	54.000	66.500
6	0.29	2	65.000	65.000	66.500
7	0.55	2	80.500	80.500	66.500

Fo % Survival @ Day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001s2d 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
neg control	83.500				
0.016	73.167	0.532		1.89	k= 1, v= 7
0.038	73.167	0.532		2.00	k= 2, v= 7
0.068	73.167	0.532		2.04	k= 3, v= 7
0.15	66.500	0.876		2.06	k= 4, v= 7
0.29	66.500	0.876		2.07	k= 5, v= 7
0.55	66.500	0.876		2.08	k= 6, v= 7

s = 19.415

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

Fo Length @ Day 28 (4 weeks, mean-meas. ppm a.i.), NOEC:
 File: 200111d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	2.714	0.452	1.266
Within (Error)	7	2.500	0.357	
Total	13	5.214		

Critical F value = 3.87 (0.05,6,7)
 Since F < Critical F FAIL TO REJECT Ho:All groups equal

Fo Length @ Day 28 (4 weeks, mean-meas. ppm a.i.)
 File: 200111d 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	neg control	24.500	24.500		
2	0.016	25.000	25.000	-0.837	
3	0.038	25.500	25.500	-1.674	
4	0.068	24.500	24.500	0.000	
5	0.15	24.500	24.500	0.000	
6	0.29	24.500	24.500	0.000	
7	0.55	24.000	24.000	0.837	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Fo Length @ Day 28 (4 weeks, mean-meas. ppm a.i.)
 File: 200111d 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	neg control	2			
2	0.016	2	1.685	6.9	-0.500
3	0.038	2	1.685	6.9	-1.000
4	0.068	2	1.685	6.9	0.000
5	0.15	2	1.685	6.9	0.000
6	0.29	2	1.685	6.9	0.000
7	0.55	2	1.685	6.9	0.500

Fo Length @ Day 28 (4 weeks, mean-meas. ppm a.i.)
 File: 200111d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
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1	neg control	2	24.500	24.500	25.000
2	0.016	2	25.000	25.000	25.000
3	0.038	2	25.500	25.500	25.000
4	0.068	2	24.500	24.500	24.500
5	0.15	2	24.500	24.500	24.500
6	0.29	2	24.500	24.500	24.500
7	0.55	2	24.000	24.000	24.000

Fo Length @ Day 28 (4 weeks, mean-meas. ppm a.i.)
 File: 200111d 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
neg control	25.000				
0.016	25.000	0.837		1.89	k= 1, v= 7
0.038	25.000	0.837		2.00	k= 2, v= 7
0.068	24.500	0.000		2.04	k= 3, v= 7
0.15	24.500	0.000		2.06	k= 4, v= 7
0.29	24.500	0.000		2.07	k= 5, v= 7
0.55	24.000	0.837		2.08	k= 6, v= 7

s = 0.598

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

Fo Length @ Day 28 (4 weeks, mean-meas. ppm a.i.)
 File: 200111d Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	24.500	24.500	13.500
2	0.016	25.000	25.000	20.000
3	0.038	25.500	25.500	24.000
4	0.068	24.500	24.500	13.500
5	0.15	24.500	24.500	13.500
6	0.29	24.500	24.500	13.500
7	0.55	24.000	24.000	7.000

Calculated H Value = 6.393 Critical H Value Table = 12.590
 Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

Fo Length @ Day 28 (4 weeks, mean-meas. ppm a.i.)
 File: 200111d Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP
7	0.55	24.000	24.000	0 0 0 0 0 0 0 7 4 5 6 1 2 3

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4	0.068	24.500	24.500	. \
5	0.15	24.500	24.500	. . \
6	0.29	24.500	24.500	. . . \
1	neg control	24.500	24.500 \
2	0.016	25.000	25.000 \
3	0.038	25.500	25.500 \

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 3.742

Fo Length @ Day 48 (7 weeks, mean-meas. ppm a.i.), NOEC:
 File: 200112d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	5.714	0.952	4.449
Within (Error)	7	1.500	0.214	
Total	13	7.214		

Critical F value = 3.87 (0.05,6,7)
 Since F > Critical F REJECT Ho:All groups equal

Fo Length @ Day 48 (7 weeks, mean-meas. ppm a.i.)
 File: 200112d 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	neg control	33.500	33.500		
2	0.016	33.500	33.500	0.000	
3	0.038	35.000	35.000	-3.243	
4	0.068	34.500	34.500	-2.162	
5	0.15	35.000	35.000	-3.243	
6	0.29	35.000	35.000	-3.243	
7	0.55	34.000	34.000	-1.081	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Fo Length @ Day 48 (7 weeks, mean-meas. ppm a.i.)
 File: 200112d 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	neg control	2			
2	0.016	2	1.305	3.9	0.000
3	0.038	2	1.305	3.9	-1.500
4	0.068	2	1.305	3.9	-1.000
5	0.15	2	1.305	3.9	-1.500
6	0.29	2	1.305	3.9	-1.500

Fo Length @ Day 48 (7 weeks, mean-meas. ppm a.i.)
 File: 200112d 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	0		
2		0.016	33.500	33.500	\							
1	neg control		33.500	33.500	.	\						
7		0.55	34.000	34.000	.	.	\					
4		0.068	34.500	34.500	.	.	.	\				
5		0.15	35.000	35.000	\			
6		0.29	35.000	35.000	\		
3		0.038	35.000	35.000	\	

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 3.813

Fo Male Length @ day 91 (13 weeks, mean-meas. ppm a.i.), NOEC:
 File: 20011md Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	8.857	1.476	0.590
Within (Error)	7	17.500	2.500	
Total	13	26.357		

Critical F value = 3.87 (0.05,6,7)
 Since F < Critical F FAIL TO REJECT Ho:All groups equal

Fo Male Length @ day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 20011md 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	neg control	38.500	38.500		
2		0.016	38.000	38.000	0.316
3		0.038	40.000	40.000	-0.949
4		0.068	39.000	39.000	-0.316
5		0.15	40.500	40.500	-1.265
6		0.29	39.500	39.500	-0.632
7		0.55	39.000	39.000	-0.316

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Fo Male Length @ day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 20011md 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	neg control	2			
2	0.016	2	4.459	11.6	0.500
3	0.038	2	4.459	11.6	-1.500
4	0.068	2	4.459	11.6	-0.500
5	0.15	2	4.459	11.6	-2.000
6	0.29	2	4.459	11.6	-1.000
7	0.55	2	4.459	11.6	-0.500

Fo Male Length @ day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001lmd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	38.500	38.500	38.250
2	0.016	2	38.000	38.000	38.250
3	0.038	2	40.000	40.000	39.500
4	0.068	2	39.000	39.000	39.500
5	0.15	2	40.500	40.500	39.667
6	0.29	2	39.500	39.500	39.667
7	0.55	2	39.000	39.000	39.667

Fo Male Length @ day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001lmd 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
neg control	38.250				
0.016	38.250	0.158		1.89	k= 1, v= 7
0.038	39.500	0.632		2.00	k= 2, v= 7
0.068	39.500	0.632		2.04	k= 3, v= 7
0.15	39.667	0.738		2.06	k= 4, v= 7
0.29	39.667	0.738		2.07	k= 5, v= 7
0.55	39.667	0.738		2.08	k= 6, v= 7

s = 1.581
 Note: df used for table values are approximate when v > 20.

Fo Male Length @ day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001lmd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM

DP Barcode: D312346

MRID No: 408820-01

1	neg control	38.500	38.500	11.000
2	0.016	38.000	38.000	7.000
3	0.038	40.000	40.000	20.000
4	0.068	39.000	39.000	14.000
5	0.15	40.500	40.500	21.500
6	0.29	39.500	39.500	18.000
7	0.55	39.000	39.000	13.500

Calculated H Value = 4.789 Critical H Value Table = 12.590
 Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

Fo Male Length @ day 91 (13 weeks, mean-meas. ppm a.i.)
 File: 2001lmd 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	
2	0.016	38.000	38.000	2	1	4	7	6	3	5
1	neg control	38.500	38.500
4	0.068	39.000	39.000
7	0.55	39.000	39.000
6	0.29	39.500	39.500
3	0.038	40.000	40.000
5	0.15	40.500	40.500

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 4.081

Fo Weight @ Day 28 (4 weeks, mean-meas. ppm a.i.), NOEC:
 File: 2001wld Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0033	0.0007	1.400
Within (Error)	6	0.0029	0.0005	
Total	11	0.0062		

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

Fo Weight @ Day 28 (4 weeks, mean-meas. ppm a.i.)
 File: 2001wld Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.215	0.215		
2	0.016	0.225	0.225	-0.447	

DP Barcode: D312346

MRID No: 408820-01

3	0.038	0.260	0.260	-2.012
4	0.068	0.210	0.210	0.224
5	0.15	0.240	0.240	-1.118
6	0.29	0.230	0.230	-0.671

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

Fo Weight @ Day 28 (4 weeks, mean-meas. ppm a.i.)
File: 2001wld Transform: NO TRANSFORM

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	neg control	2			
2	0.016	2	0.063	29.4	-0.010
3	0.038	2	0.063	29.4	-0.045
4	0.068	2	0.063	29.4	0.005
5	0.15	2	0.063	29.4	-0.025
6	0.29	2	0.063	29.4	-0.015

Fo Weight @ Day 28 (4 weeks, mean-meas. ppm a.i.)
File: 2001wld Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	0.215	0.215	0.215
2	0.016	2	0.225	0.225	0.225
3	0.038	2	0.260	0.260	0.235
4	0.068	2	0.210	0.210	0.235
5	0.15	2	0.240	0.240	0.235
6	0.29	2	0.230	0.230	0.235

Fo Weight @ Day 28 (4 weeks, mean-meas. ppm a.i.)
File: 2001wld Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.215				
0.016	0.225	0.456		1.94	k= 1, v= 6
0.038	0.235	0.913		2.06	k= 2, v= 6
0.068	0.235	0.913		2.10	k= 3, v= 6
0.15	0.235	0.913		2.12	k= 4, v= 6
0.29	0.235	0.913		2.13	k= 5, v= 6

s = 0.022

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

DP Barcode: D312346

MRID No: 408820-01

Fo Weight @ Day 28 (4 weeks, mean-meas. ppm a.i.)
File: 2001wld Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	0.215	0.215	6.500
2	0.016	0.225	0.225	13.000
3	0.038	0.260	0.260	20.500
4	0.068	0.210	0.210	5.500
5	0.15	0.240	0.240	15.500
6	0.29	0.230	0.230	17.000

Calculated H Value = 7.320 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Fo Weight @ Day 28 (4 weeks, mean-meas. ppm a.i.)
File: 2001wld Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP
4	0.068	0.210	0.210	\
1	neg control	0.215	0.215	. \
2	0.016	0.225	0.225	. . \
6	0.29	0.230	0.230	. . . \
5	0.15	0.240	0.240 \
3	0.038	0.260	0.260 \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.477

Fo Male Weight @ Day 91 (13 weeks, ppm a.i.), NOEC:
File: 2001wmd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.144	0.024	1.043
Within (Error)	7	0.163	0.023	
Total	13	0.307		

Critical F value = 3.87 (0.05,6,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Fo Male Weight @ Day 91 (13 weeks, ppm a.i.)
File: 2001wmd 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	neg control	1.110	1.110		
2	0.016	1.080	1.080	0.198	
3	0.038	1.265	1.265	-1.022	
4	0.068	1.090	1.090	0.132	
5	0.15	1.375	1.375	-1.747	
6	0.29	1.235	1.235	-0.824	
7	0.55	1.150	1.150	-0.264	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Fo Male Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wmd 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	neg control	2			
2	0.016	2	0.428	38.5	0.030
3	0.038	2	0.428	38.5	-0.155
4	0.068	2	0.428	38.5	0.020
5	0.15	2	0.428	38.5	-0.265
6	0.29	2	0.428	38.5	-0.125
7	0.55	2	0.428	38.5	-0.040

Fo Male Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wmd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	1.110	1.110	1.095
2	0.016	2	1.080	1.080	1.095
3	0.038	2	1.265	1.265	1.178
4	0.068	2	1.090	1.090	1.178
5	0.15	2	1.375	1.375	1.253
6	0.29	2	1.235	1.235	1.253
7	0.55	2	1.150	1.150	1.253

Fo Male Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wmd 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
neg control	1.095				
0.016	1.095	0.098		1.89	k= 1, v= 7
0.038	1.178	0.442		2.00	k= 2, v= 7
0.068	1.178	0.442		2.04	k= 3, v= 7

DP Barcode: D312346

MRID No: 408820-01

0.15	1.253	0.939	2.06	k= 4, v= 7
0.29	1.253	0.939	2.07	k= 5, v= 7
0.55	1.253	0.939	2.08	k= 6, v= 7

s = 0.153

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

Po Male Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wmd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	1.110	1.110	11.000
2	0.016	1.080	1.080	8.000
3	0.038	1.265	1.265	22.000
4	0.068	1.090	1.090	9.000
5	0.15	1.375	1.375	21.000
6	0.29	1.235	1.235	21.000
7	0.55	1.150	1.150	13.000

Calculated H Value = 6.514 Critical H Value Table = 12.590
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

Po Male Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wmd 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	0
				2	4	1	7	6	3	5
2	0.016	1.080	1.080	\						
4	0.068	1.090	1.090	- \						
1	neg control	1.110	1.110	. . \						
7	0.55	1.150	1.150	. . . \						
6	0.29	1.235	1.235 \						
3	0.038	1.265	1.265 \						
5	0.15	1.375	1.375 \						

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 4.165

Po Female Weight @ Day 91 (13 weeks, ppm a.i.), NOEC:
 File: 2001wfd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.294	0.049	3.063
Within (Error)	7	0.115	0.016	

Total 13 0.409

Critical F value = 3.87 (0.05,6,7)
 Since F < Critical F FAIL TO REJECT Ho:All groups equal

Fo Female Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wfd 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	neg control	0.730	0.730		
2	0.016	0.745	0.745	-0.119	
3	0.038	0.765	0.765	-0.277	
4	0.068	0.720	0.720	0.079	
5	0.15	0.810	0.810	-0.632	
6	0.29	0.725	0.725	0.040	
7	0.55	1.155	1.155	-3.360	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Fo Female Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wfd 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	neg control	2			
2	0.016	2	0.357	48.9	-0.015
3	0.038	2	0.357	48.9	-0.035
4	0.068	2	0.357	48.9	0.010
5	0.15	2	0.357	48.9	-0.080
6	0.29	2	0.357	48.9	0.005
7	0.55	2	0.357	48.9	-0.425

Fo Female Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wfd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	0.730	0.730	0.730
2	0.016	2	0.745	0.745	0.743
3	0.038	2	0.765	0.765	0.743
4	0.068	2	0.720	0.720	0.743
5	0.15	2	0.810	0.810	0.768
6	0.29	2	0.725	0.725	0.768
7	0.55	2	1.155	1.155	1.155

DP Barcode: D312346

MRID No: 408820-01

Fo Female Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wfd 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
neg control	0.730				
0.016	0.743	0.104		1.89	k= 1, v= 7
0.038	0.743	0.104		2.00	k= 2, v= 7
0.068	0.743	0.104		2.04	k= 3, v= 7
0.15	0.768	0.292		2.06	k= 4, v= 7
0.29	0.768	0.292		2.07	k= 5, v= 7
0.55	1.155	3.313	*	2.08	k= 6, v= 7

s = 0.128

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

Fo Female Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wfd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	0.730	0.730	8.500
2	0.016	0.745	0.745	13.000
3	0.038	0.765	0.765	14.000
4	0.068	0.720	0.720	11.000
5	0.15	0.810	0.810	18.500
6	0.29	0.725	0.725	13.000
7	0.55	1.155	1.155	27.000

Calculated H Value = 6.414 Critical H Value Table = 12.590
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

Fo Female Weight @ Day 91 (13 weeks, ppm a.i.)
 File: 2001wfd 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	0		
4	0.068	0.720	0.720	\								
6	0.29	0.725	0.725	. \								
1	neg control	0.730	0.730	. . \								
2	0.016	0.745	0.745	. . . \								
3	0.038	0.765	0.765 \								
5	0.15	0.810	0.810 \								
7	0.55	1.155	1.155 \								

* = significant difference (p=0.05)
 Table q value (0.05,7) = 3.038

. = no significant difference
 SE = 4.174

Fo Mean # Eggs/Female/Day (mean-meas. ppm a.i.), NOEC: Note, only two replicates per treatment level were used for statistical analysis, see Reviewer's Statistical Results for further details.

File: 2001rd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	257.429	42.905	5.723
Within (Error)	7	52.480	7.497	
Total	13	309.909		

Critical F value = 3.87 (0.05,6,7)
 Since F > Critical F REJECT Ho:All groups equal

Fo Mean # Eggs/Female/Day (mean-meas. ppm a.i.)
 File: 2001rd 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	neg control	13.900	13.900		
2	0.016	11.900	11.900	0.730	
3	0.038	11.850	11.850	0.749	
4	0.068	13.200	13.200	0.256	
5	0.15	8.650	8.650	1.917	
6	0.29	5.550	5.550	3.050	*
7	0.55	1.250	1.250	4.620	*

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Fo Mean # Eggs/Female/Day (mean-meas. ppm a.i.)
 File: 2001rd 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	neg control	2			
2	0.016	2	7.721	55.5	2.000
3	0.038	2	7.721	55.5	2.050
4	0.068	2	7.721	55.5	0.700
5	0.15	2	7.721	55.5	5.250
6	0.29	2	7.721	55.5	8.350
7	0.55	2	7.721	55.5	12.650

Fo Mean # Eggs/Female/Day (mean-meas. ppm a.i.)
 File: 2001rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	13.900	13.900	13.900
2	0.016	2	11.900	11.900	12.317
3	0.038	2	11.850	11.850	12.317
4	0.068	2	13.200	13.200	12.317
5	0.15	2	8.650	8.650	8.650
6	0.29	2	5.550	5.550	5.550
7	0.55	2	1.250	1.250	1.250

Fo Mean # Eggs/Female/Day (mean-meas. ppm a.i.)
 File: 2001rd 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
neg control	13.900				
0.016	12.317	0.578		1.89	k= 1, v= 7
0.038	12.317	0.578		2.00	k= 2, v= 7
0.068	12.317	0.578		2.04	k= 3, v= 7
0.15	8.650	1.917		2.06	k= 4, v= 7
0.29	5.550	3.050	*	2.07	k= 5, v= 7
0.55	1.250	4.620	*	2.08	k= 6, v= 7

s = 2.738

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

Fo Mean # Eggs/Female/Day (mean-meas. ppm a.i.)
 File: 2001rd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	13.900	13.900	23.000
2	0.016	11.900	11.900	20.000
3	0.038	11.850	11.850	20.000
4	0.068	13.200	13.200	21.000
5	0.15	8.650	8.650	11.000
6	0.29	5.550	5.550	7.000
7	0.55	1.250	1.250	3.000

Calculated H Value = 10.686 Critical H Value Table = 12.590
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

Fo Mean # Eggs/Female/Day (mean-meas. ppm a.i.)
 File: 2001rd 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 0
				7 6 5 3 2 4 1

7	0.55	1.250	1.250	\
6	0.29	5.550	5.550	. \
5	0.15	8.650	8.650	. . \
3	0.038	11.850	11.850	. . . \
2	0.016	11.900	11.900 \
4	0.068	13.200	13.200 \
1	neg control	13.900	13.900 \

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 4.183

Fo Mean # of Eggs/Female/Day (8 reps/level as reported), NOEC: Note, eight replicates per treatment level were used for statistical analysis (verification purposes only), see Reviewer's Statistical Results for further details.
 File: 2001r2d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1028.554	171.426	6.538
Within (Error)	49	1284.780	26.220	
Total	55	2313.334		

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

Fo Mean # of Eggs/Female/Day (8 reps/level as reported)
 File: 2001r2d 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	neg control	13.875	13.875		
2	0.016	11.913	11.913	0.767	
3	0.038	11.800	11.800	0.810	
4	0.068	13.187	13.187	0.269	
5	0.15	8.625	8.625	2.051	
6	0.29	5.538	5.538	3.256	*
7	0.55	1.238	1.238	4.936	*

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

Fo Mean # of Eggs/Female/Day (8 reps/level as reported)
 File: 2001r2d 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	neg control	8			
2	0.016	8	6.068	43.7	1.962

3	0.038	8	6.068	43.7	2.075
4	0.068	8	6.068	43.7	0.688
5	0.15	8	6.068	43.7	5.250
6	0.29	8	6.068	43.7	8.337
7	0.55	8	6.068	43.7	12.637

Fo Mean # of Eggs/Female/Day (8 reps/level as reported)
 File: 2001r2d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	8	13.875	13.875	13.875
2	0.016	8	11.913	11.913	12.300
3	0.038	8	11.800	11.800	12.300
4	0.068	8	13.187	13.187	12.300
5	0.15	8	8.625	8.625	8.625
6	0.29	8	5.538	5.538	5.538
7	0.55	8	1.238	1.238	1.238

Fo Mean # of Eggs/Female/Day (8 reps/level as reported)
 File: 2001r2d 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
neg control	13.875				
0.016	12.300	0.615		1.68	k= 1, v=49
0.038	12.300	0.615		1.76	k= 2, v=49
0.068	12.300	0.615		1.79	k= 3, v=49
0.15	8.625	2.051	*	1.80	k= 4, v=49
0.29	5.538	3.256	*	1.80	k= 5, v=49
0.55	1.238	4.936	*	1.81	k= 6, v=49

s = 5.121

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

Fo Mean # of Eggs/Female/Day (8 reps/level as reported)
 File: 2001r2d Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	13.875	13.875	310.000
2	0.016	11.913	11.913	271.000
3	0.038	11.800	11.800	297.000
4	0.068	13.187	13.187	297.500
5	0.15	8.625	8.625	216.000
6	0.29	5.538	5.538	153.000
7	0.55	1.238	1.238	51.500

DP Barcode: D312346

MRID No: 408820-01

Calculated H Value = 25.864 Critical H Value Table = 12.590
 Since Calc H > Crit H REJECT Ho: All groups are equal.

F0 Mean # of Eggs/Female/Day (8 reps/level as reported)
 File: 2001r2d 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	0	
7	0.55	1.238	1.238	\							
6	0.29	5.538	5.538	.	\						
5	0.15	8.625	8.625	.	.	\					
3	0.038	11.800	11.800	*	.	.	\				
2	0.016	11.913	11.913	*	.	.	.	\			
4	0.068	13.187	13.187	*	\		
1	neg control	13.875	13.875	*	\	

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 8.154

F1 Mean % Embryos Hatched following 4 days of incubat., NOEC: Note, only two replicates per treatment level were used for statistical analysis, see Reviewer's Statistical Results for further details.

File: 2001h2d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	8975.429	1495.905	25.293
Within (Error)	7	414.000	59.143	
Total	13	9389.429		

Critical F value = 3.87 (0.05,6,7)
 Since F > Critical F REJECT Ho: All groups equal

F1 Mean % Embryos Hatched following 4 days of incubat.
 File: 2001h2d 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	neg control	74.500	74.500		
2	0.016	78.000	78.000	-0.455	
3	0.038	77.000	77.000	-0.325	
4	0.068	77.500	77.500	-0.390	
5	0.15	80.000	80.000	-0.715	
6	0.29	23.500	23.500	6.632	*
7	0.55	19.500	19.500	7.152	*

Dunnnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

F1 Mean % Embryos Hatched following 4 days of incubat.
 File: 2001h2d 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	neg control	2			
2	0.016	2	21.687	29.1	-3.500
3	0.038	2	21.687	29.1	-2.500
4	0.068	2	21.687	29.1	-3.000
5	0.15	2	21.687	29.1	-5.500
6	0.29	2	21.687	29.1	51.000
7	0.55	2	21.687	29.1	55.000

F1 Mean % Embryos Hatched following 4 days of incubat.
 File: 2001h2d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	74.500	74.500	77.400
2	0.016	2	78.000	78.000	77.400
3	0.038	2	77.000	77.000	77.400
4	0.068	2	77.500	77.500	77.400
5	0.15	2	80.000	80.000	77.400
6	0.29	2	23.500	23.500	23.500
7	0.55	2	19.500	19.500	19.500

F1 Mean % Embryos Hatched following 4 days of incubat.
 File: 2001h2d 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
neg control	77.400				
0.016	77.400	0.377		1.89	k= 1, v= 7
0.038	77.400	0.377		2.00	k= 2, v= 7
0.068	77.400	0.377		2.04	k= 3, v= 7
0.15	77.400	0.377		2.06	k= 4, v= 7
0.29	23.500	6.632	*	2.07	k= 5, v= 7
0.55	19.500	7.152	*	2.08	k= 6, v= 7

s = 7.690

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

F1 Mean % Embryos Hatched following 4 days of incubat.
 File: 2001h2d Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	74.500	74.500	13.000
2	0.016	78.000	78.000	20.500
3	0.038	77.000	77.000	21.000
4	0.068	77.500	77.500	18.000
5	0.15	80.000	80.000	22.500
6	0.29	23.500	23.500	6.000
7	0.55	19.500	19.500	4.000

Calculated H Value = 9.685 Critical H Value Table = 12.590
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

F1 Mean % Embryos Hatched following 4 days of incubat.
 File: 2001h2d 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	0		
7	0.55	19.500	19.500	\								
6	0.29	23.500	23.500	. \								
1	neg control	74.500	74.500	. . \								
3	0.038	77.000	77.000	. . . \								
4	0.068	77.500	77.500 \								
2	0.016	78.000	78.000 \								
5	0.15	80.000	80.000 \								

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 4.174

F1 Mean % Embryos Hatched (<=8 reps/level as reported), NOEC: Note, less than or equal to eight replicates per treatment level were used for statistical analysis (verification purposes only), see Reviewer's Statistical Results for further details.
 File: 2001h3d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	22001.157	3666.860	22.780
Within (Error)	40	6438.843	160.971	
Total	46	28440.000		

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

F1 Mean % Embryos Hatched (<=8 reps/level as reported)
 File: 2001h3d Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	74.429	74.429		
2	0.016	77.875	77.875	-0.525	
3	0.038	76.875	76.875	-0.373	
4	0.068	77.500	77.500	-0.468	
5	0.15	80.714	80.714	-0.927	
6	0.29	25.600	25.600	6.573	*
7	0.55	19.250	19.250	6.939	*

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

F1 Mean % Embryos Hatched (<=8 reps/level as reported)
 File: 2001h3d Transform: NO TRANSFORMATION

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	neg control	7			
2	0.016	8	16.409	22.0	-3.446
3	0.038	8	16.409	22.0	-2.446
4	0.068	8	16.409	22.0	-3.071
5	0.15	7	16.948	22.8	-6.286
6	0.29	5	18.565	24.9	48.829
7	0.55	4	19.873	26.7	55.179

F1 Mean % Embryos Hatched (<=8 reps/level as reported)
 File: 2001h3d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	7	74.429	74.429	77.474
2	0.016	8	77.875	77.875	77.474
3	0.038	8	76.875	76.875	77.474
4	0.068	8	77.500	77.500	77.474
5	0.15	7	80.714	80.714	77.474
6	0.29	5	25.600	25.600	25.600
7	0.55	4	19.250	19.250	19.250

F1 Mean % Embryos Hatched (<=8 reps/level as reported)
 File: 2001h3d 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
neg control	77.474				
0.016	77.474	0.464		1.68	k= 1, v=40
0.038	77.474	0.464		1.76	k= 2, v=40

DP Barcode: D312346

MRID No: 408820-01

0.068	77.474	0.464		1.79	k= 3, v=40
0.15	77.474	0.449		1.80	k= 4, v=40
0.29	25.600	6.573	*	1.80	k= 5, v=40
0.55	19.250	6.939	*	1.81	k= 6, v=40

s = 12.687

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

F1 Mean % Embryos Hatched (<=8 reps/level as reported)
 File: 2001h3d Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	74.429	74.429	169.500
2	0.016	77.875	77.875	234.500
3	0.038	76.875	76.875	220.500
4	0.068	77.500	77.500	214.500
5	0.15	80.714	80.714	240.500
6	0.29	25.600	25.600	31.500
7	0.55	19.250	19.250	17.000

Calculated H Value = 22.448 Critical H Value Table = 12.590
 Since Calc H > Crit H REJECT Ho: All groups are equal.

F1 Mean % Embryos Hatched (<=8 reps/level as reported)
 File: 2001h3d Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP
7	0.55	19.250	19.250	\
6	0.29	25.600	25.600	. \
1	neg control	74.429	74.429	. . \
3	0.038	76.875	76.875	. . . \
4	0.068	77.500	77.500 \
2	0.016	77.875	77.875 \
5	0.15	80.714	80.714	* * \

* = significant difference (p=0.05)
 Table q value (0.05,7) = 3.038

. = no significant difference
 Unequal reps - multiple SE values