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

1. **CHEMICAL:** JAU6476-desthio PC Code No.: 113961

2. **TEST MATERIAL:** JAU 6476 - Desthio (p. 14) Purity: 96.4%

Common name: JAU6476-desthio
Chemical:
IUPAC name: 2-(1-chlorcyclopropyl)-1-(2-chlophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol
CAS name: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS No.: 120983-64-4
Synonyms: SXX0665

3. **CITATION:**

Author: Drottar, K.R., T.Z. Kendall, and H.O. Drueger

Title: Desthio JAU 6476: A Flow-Through Life-Cycle Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

Study Completion Date: March 5, 2004

Laboratories: Wildlife International, Ltd.
8598 Commerce Drive
Easton, MD 21601

Sponsor: Bayer CropScience
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Research Triangle Park, NC 27709

Laboratory Report ID: 149A-126A

MRID No.: 46246033

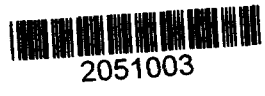
DP Barcode: D303488

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

Signature: **Date:** 8/25/04

APPROVED BY: Gregory Hess, Staff Scientist, Dynamac Corporation

Signature: **Date:** 9/20/04



5. APPROVED BY: Kevin Costello, Geologist, OPP/EFED/ERB - IV

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6. SECONDARY REVIEW BY:

Émilie Larivière, EAD, PMRA, HC
Signature: Émilie Larivière **Date:** 9/2/05 September 8, 2005

Christopher J. Salice, OPP/EFED/ERB-IV
Signature: Chris J. Salice **Date:** 8-20-05

7. STUDY PARAMETERS:

Scientific Name of Test Organism: *Pimephales promelas*

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

Definitive Test Duration: 265 Days (approximately 9 months)

Study Method: Flow-through

Type of Concentrations: Mean-measured

8. CONCLUSIONS:

The chronic toxicity of Desthio JAU 6476-Desthio (Prothioconazole metabolite) to the full life stage of Fathead Minnow (*Pimephales promelas*) was studied under flow-through conditions for approximately 9 months. Fertilized eggs (200 embryos/treatment, <24 hours old) were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 19, 38, 75, 150, and 300 ppb. Mean-measured concentrations of the parent generation were <2.00 (<LOQ, controls), 19, 37, 74, 148, and 296 ppb a.i. Mean-measured concentrations of the second-generation were <2.00 (<LOQ, controls), 19, 37, 75, 150, and 295 ppb a.i.

Following hatching on Day 5, larvae were reduced to 100 per treatment level. On Day 61, the juveniles were reduced to 50 per treatment level. On Day 174, the adults were reduced to 24 per treatment level (including reserves), and the aquaria were equipped to accommodate spawning. The P generation was terminated on Day 245. F_1-generation exposure was initiated with 100 embryos per treatment, and larvae/fry were maintained for 4 weeks post-hatch.

P Generation: No apparent treatment-related effects on time to hatch or hatching success were observed. The majority of the embryos hatched on Day 5. The NOAEC for time to hatch and hatching success was 296 ppb a.i., the highest concentration tested.

Survival through Day 61 (8 weeks post-hatch) and then from Days 61 through 174 (initiation of reproduction) was adversely affected at the 296 ppb a.i. test level compared to the pooled control (81 versus 94%, and 94 versus 100%, respectively). The NOAEC for survival through Day 174 was 148 ppb a.i. No treatment-related effect on adult survival from Days 174 to 245 was observed.

Between hatch and study termination, the majority of all surviving organisms appeared normal. However, on Days 174 and 245, a treatment-related increase in the incidence of fish with deformed upper jaws was observed at the 296 ppb a.i. treatment level. The

NOAEC for morphological deformities was therefore 148 ppb a.i.

Reproduction of fathead minnow was assessed by spawning frequency and the mean number of eggs produced per female per reproductive day. Spawning frequency averaged 16% for the pooled control, and from 6.2 to 12% in minnows exposed at ≤148 ppb a.i. Minnows exposed at 296 ppb a.i. did not spawn. Differences were statistically-different from the pooled control at the 19, 37, 148, and 296 ppb a.i. treatment levels. The study authors and reviewer concluded that the poor concentration-response for this endpoint indicates that spawning frequency was probably not a good indicator of biological effect, but that the lack of spawning in the 296 ppb a.i. group was biologically significant. Consequently, the NOAEC for spawning frequency was 148 ppb a.i. Egg production was not significantly reduced in any treatment group compared to pooled control. The NOAEC for egg production was 148 ppb a.i., the highest treatment level at which eggs were produced.

Growth was assessed 4 and 8 weeks post-hatch (combined sexes), and again on Days 174 and 245 (separate sexes). A treatment-related reduction in wet weights was observed at 8-weeks post-hatch in larvae/juveniles from the 296 ppb a.i. treatment group. A treatment-related reduction in total length and wet weights were also observed in male fish from the 296 ppb a.i. group at 174 and 245 Days. Similar reductions were not observed in females at 174 and 245 Days. The NOAEC for first-generation (P) growth was 148 ppb a.i.

F_1 Generation: No apparent treatment-related effects on time to hatch, hatching success, or 4-week post-hatch growth parameters were observed. There were significant effects on post-hatch survival at 150 ppb a.i., however, the effects were attributed to low survival in one replicate; the second replicate showed 100% survival. Hence, the NOAEC for all second-generation (F_1) endpoints was 150 ppb a.i., the highest treatment level tested due to a lack of spawning in fish from the P generation at the 296 ppb a.i. treatment level.

Based on adverse effects on first-generation larval/juvenile survival, juvenile (8-weeks post-hatch) and male growth, spawning frequency, and morphological deformities, the NOAEC and LOAEC are 148 and 296 ppb a.i., respectively.

This study is classified as SUPPLEMENTAL. This study did not fulfill the guideline requirements for a fish life-cycle toxicity test because the F_1 generation was only maintained for 4 weeks post-hatch rather than 8 weeks as required. This study is scientifically valid, and although results do not meet guideline requirements; the information may be useful for future risk assessment purposes.

Results Synopsis:

Biological Endpoint	NOAEC (ppb a.i.)	LOAEC (ppb a.i.)
P Generation		
Time to hatch	296	>296
Hatching success	296	>296
Survival, 8-weeks post-hatch (Day 61)	148	296
Survival, 24-weeks post-hatch (Day 174)	148	296
Survival, study termination (Day 245)	296	>296
Morphological deformities	148	296
Spawning frequency	148	296
Egg production	148	>148
Total length, 4-weeks post-hatch	296	>296
Total length, 8-weeks post-hatch	296	>296
Wet weight, 8-weeks post-hatch	148	296
Total length, 24-weeks post-hatch (Day 174; males)	148	296
Total length, 24-weeks post-hatch (Day 174; females)	148	>148
Wet weight, 24-weeks post-hatch (Day 174; males)	148	296
Wet weight, 24-weeks post-hatch (Day 174; females)	148	>148
Total length, study termination (Day 245; males)	148	296

Biological Endpoint	NOAEC (ppb a.i.)	LOAEC (ppb a.i.)
Total length, study termination (Day 245; females)	296	>296
Wet weight, study termination (Day 245; males)	148	296
Wet weight, study termination (Day 245; females)	296	>296
F_1 Generation		
Time to hatch	150	>150
Hatching success	150	>150
Survival, 4-weeks post-hatch	150	>150
Total length, 4-weeks post-hatch	150	>150
Wet weight, 4-weeks post-hatch	150	>150

NOAEC: 148 ppb a.i.
LOAEC: 296 ppb a.i.
MATC: 209 ppb a.i.
Endpoint(s) affected: P Generation: larval/juvenile survival (hatching through 8-weeks post-hatch), juvenile survival (8 to 24 weeks post-hatch), morphological deformities (juvenile/adult fish), spawning frequency, larval/juvenile wet weight (8-weeks post-hatch/Day 56) and juvenile/adult male growth (length and wet weight; 24-weeks/Day 174 and Day 245). F_1 Generation: None.
Most sensitive endpoint(s): Same conclusions for all affected endpoints.

9. 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 valid, and provides supplemental data on the toxicity of JAU 6476 - Desthio (Prothioconazole) 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 at the ≤150 ppb a.i. test levels would have been maintained, had the fish been observed up to 8 weeks post-hatch.

10. GUIDELINE DEVIATIONS:

1. It was not reported if the fish were fasted prior to test termination.

2. The hardness of the dilution water ranged from 120-140 mg/L as $CaCO_3$ (both generations), which greatly exceeds recommendations (40-48 mg/L as $CaCO_3$).

3. The pH of the dilution water ranged from 7.8 to 8.4 (both generations), which exceeds recommendations (7.2 to 7.6).

4. Due to low DO concentrations, mild aeration was initiated in the P generation aquaria on Day 183 (p. 22). Aeration did not impact the analytical results.

5. F_1-generation fish were maintained for only 4 weeks, rather than the required 8 weeks.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of Prothioconazole to the life cycle of fathead minnows for the purposes of chemical registration (NC).

12. 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 cultures maintained by Chesapeake Cultures, Hayes, VA. The embryos obtained were from 9 individual spawns. The culture dilution water was from the same source as the dilution water used for testing (p. 17).
Age at beginning of test Embryos, 2 to 24 hours old	Embryos, <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.	Newly hatched larvae were fed brine shrimp nauplii (*Artemia sp.*) three times/day (at 4-hour intervals) during the first 7 days post-hatch. Thereafter, fish were fed three times/day on weekdays and twice/day on weekends (6-hour intervals). Rations were adjusted each week to maintain a constant feeding rate. Starting on Day 32, parental fish were fed dry flake food and/or frozen brine shrimp at the same frequency previously described. It was not reported if the fish were fasted prior to test termination.

Guideline Criteria	Reported Information
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 Dead and fungused embryos should be counted and removed daily.	Days 0-5 Embryos (< 24 hours old) were obtained from 9 separate spawns and were randomly assigned to embryo incubation cups. Each cup contained 25 embryos, with two cups per replicate and four replicate aquaria per treatment level (total of 200 embryos per treatment) Parameters measured: · Survival of embryos · Time required to hatch · Hatching success Mortality was determined daily, and dead and fungused embryos were removed.
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 5-61 (hatch to approximately 8 weeks) When ≥90% of the negative control group embryos had hatched, larvae were reduced to 100 per treatment (25 larvae per replicate with four replicates), and the larvae were transferred from the incubation cups to the larval growth chambers in the corresponding aquarium. Parameters measured: · Survival of fry/juvenile fish (weekly). · Lengths (mm) of all surviving fish at 28 and 56 day post-hatch (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]) 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. The substrates are examined daily and embryos removed, counted, and recorded separately for each pair. 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.	Days 61 to 245 (from 8 to 34 weeks post-hatch) On Day 61, fish were reduced to 50 per treatment (25 per replicate with two replicates). On Day 174 (24 weeks post-hatch), fish were thinned to 24 per treatment (12 per replicate with two replicates). At this time, spawning substrates were added to the aquaria and two males and three females were assigned to each spawning compartment (two compartments per replicate). One additional fish/sex/replicate was maintained separately in each spawning tank. The spawning substrates are examined daily and embryos removed and counted. Individual spawning records were maintained for each spawning substrate (two per spawning compartment). Adult exposure was terminated on Day 245. Parameters measured: · Survival of adult fish · Spawning frequency · Egg production · Length (mm) and wet weight (g) of fish remaining after thinning on Day 174 · Length (mm) and wet weight (g) of all surviving fish at 245 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 0-5 In each aquarium, 50 embryos per replicate aquarium (100 per level) were incubated for the early life-stage test. The same test procedures as those employed for the parental generation were used. The embryos were observed daily.
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 2^{nd} generation fish is terminated 8 weeks after hatching. Fish are blotted, weighed, and measured before being discarded.	Days 5-78 (from hatch to 28 days post-hatch) After hatching, the larvae were reduced to 50 per treatment (25 larvae per replicate with two replicates), and the larvae were transferred from the incubation cups to the larval growth chambers in the corresponding aquarium. Each group of F_1-generation fish was terminated 4 weeks after hatching. Fish were weighed and measured for 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 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6.	N/A 1. The dilution water was prepared using moderately-hard on-site well water. The well water was sand-filtered, aerated, filtered again (0.2 µm), and UV-sterilized prior to use. 2. Hardness of 120 to 140 mg/L as $CaCO_3$ (Tables 10 and 11, pp. 38-39) and pH of 7.8 to 8.4 (Tables 8 and 9, pp. 36-37).
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 26.0°C (Tables 6 and 7, pp. 34-35).

Guideline Criteria	Reported Information
Photoperiod 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	The photo-period followed the natural photo-period times in Evansville, Indiana, with 30-minute transition periods (Appendix 3, p. 60). The photo-periods varied from 10 hours and 30 minutes to 15 hours and 45 minutes of light during the study. The light intensity ranged from 126 to 405 Lux (Tables 12 and 13, pp. 40-41).
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 serial diluters 2. Five toxicant concentrations with a dilution factor of 0.5. 3. Negative and solvent controls were 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. Flow splitting accuracy was reportedly maintained within 10% of the mean flow rate. The flow split was checked weekly throughout the test.

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).	During the juvenile/adult exposure (Days 61-245), the test chambers were 57-L glass aquaria filled with approximately 27 L of test solution (15-cm depth). During the spawning period (Days 174-245), each adult test chamber was divided into two spawning compartments and one additional compartment using stainless steel screen.
Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm.	During the first and second generation embryo/larval/juvenile exposure (Days 0-61 for the first generation and Days 0-28 for the second generation), the test chambers were 9-L glass aquaria filled with approximately 7 L of solution (15- to 16-cm depth).
Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	It was not specified if test 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.	The egg incubation cups were constructed from glass cylinders approximately 50 mm in diameter with 425 µm nylon screen mesh attached to the bottom with silicone sealant. The egg cups were placed in the growth chamber and oscillated in the test solution using a rocker arm apparatus.
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.	First generation embryo/larval/juvenile: 6.4 volume additions/day. First generation juvenile/adult: 6.7 volume additions/day. Second generation embryo/larval/juvenile: 26 volume additions/day.

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.	Initially, test aquaria were not aerated. Due to extremely low DO concentrations observed on Day 182 (as low as 42% of saturation), mild aeration was initiated in the P generation aquaria on Day 183 (p. 22). Aeration did not impact the analytical results. Aeration of the second generation aquaria was not necessary.

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 and solvent controls), 19, 38, 75, 150, and 300 ppb.
Toxicant conc. must be measured in one tank at each toxicant level every week.	Toxicant concentrations were measured weekly from alternating replicate aquaria in each test group.

Guideline Criteria	Reported Information
Other Variables 1. DO must be measured at each conc. at least once a week. 2. Test water temp. must be recorded continuously. 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.	1. DO levels were measured weekly from alternating replicate aquaria in each test group. 2. Temperature was measured weekly in each replicate aquaria, and was monitored continuously in one negative control replicate. 3. pH levels were measured weekly from alternating replicate aquaria in each test group. Hardness, alkalinity, and conductivity were measured weekly in the control and 300 ppb level concentrations.
Solvents Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Dimethylformamide (DMF), 0.050 mL/L

Comments: None.

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes

Guideline Criteria	Reported Information
Data Endpoints must include: · survival of P and F_1 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 F_1 fish at 8 weeks; · incidence of pathological or histological effects; and · observations of other effects or clinical signs.	Data Endpoints included: · survival of P and F_1 embryos, time required to hatch, and hatching success; · survival of P fish at weekly intervals following hatching; · total length of P fish at 4 and 8 weeks after hatching; · wet weights of P fish at 8 weeks after hatching · gender-specific total length and wet weight of P fish at 174 (fish remaining after thinning at the start of reproduction) and 245 Days (study termination); · spawning frequency and embryo production of P fish · total length and wet weight of F_1 fish at 4 weeks after hatching; · incidence of pathological or histological effects; · observation of other effects or clinical signs
Raw data included?	Yes

P Results:

Nominal Conc. (ppb)	Mean Measured Conc. (ppb a.i.)	% Hatch[1]			8-Week (Day 61) Post-Hatch % Survival	24-Week (Day 174) Post-Hatch % Survival	34-Week (Day 245) Post-Hatch % Survival
		Day 4	Day 5	Day 6			
Negative Control	<2.00	9.5	86	86	94	100	96
Solvent control	<2.00	1.0	92	93	93	100	96

Nominal Conc. (ppb)	Mean Measured Conc. (ppb a.i.)	% Hatch[1]			8-Week (Day 61) Post-Hatch % Survival	24-Week (Day 174) Post-Hatch % Survival	34-Week (Day 245) Post-Hatch % Survival
		Day 4	Day 5	Day 6			
19	19	4.5	88	92	95	98	96
38	37	2.5	86	90	95	100	92
75	74	4.0	88	89	97	100	100
150	148	0.5	78	82*	91	100	100
300	296	2.5	87	89	81*	94*	100

Data obtained from Tables 14 through 17, pp. 42-45.
[1] Days 4 and 5 % hatch data were reviewer-calculated. Only Day 6 data were statistically compared.
*Statistically-significant difference from pooled control ($p \leq 0.05$).

Mean Measured Conc. (ppb a.i.)	Mean Total Length (mm)					
	4 Weeks Post-Hatch	8 Weeks Post-Hatch	Day 174 (fish remaining after thinning for the start of reproduction)		Day 254 (test termination)	
			♂	♀	♂	♀
NegativeControl	19.8	36.3	69.4	54.4	71.3	56.7
Solvent control	20.5	35.4	64.4	54.6	73.2	56.4
19	20.4	36.7	65.0	53.6	71.9	57.6
37	20.4	36.6	63.8	53.3	72.3	55.7
74	20.7	37.8	65.9	57.7	74.4	58.9
148	20.6	35.8	63.5	54.3	72.6	59.8
296	19.8	35.6	55.3*	—	67.5*	55.1

Data obtained from Tables 20 and 22 through 25, pp. 48, and 50-53, respectively.
* Significantly different from the pooled control ($p \leq 0.05$).
— No females remaining after thinning.

Mean Measured Conc. (ppb a.i.)	Mean Wet Weight (g)				
	8 Weeks Post-Hatch	Day 174 (fish remaining after thinning for the start of reproduction)		Day 254 (test termination)	
		♂	♀	♂	♀
Negative Control	0.4610	3.0295	1.4180	4.1421	1.6331
Solvent control	0.4392	2.4633	1.4412	4.7290	1.7570
19	0.4643	2.4124	1.3111	4.1971	1.7985
37	0.4440	2.3286	1.3175	4.6389	1.6192
74	0.4905	2.6059	1.5704	4.4748	1.9684
148	0.4234	2.2913	1.3367	4.0018	2.0426
296	0.4081	1.7527*	—	3.4669*	1.6416

Data obtained from Tables 21 through 25, pp. 49-53.
* Significantly different from the pooled control ($p \leq 0.05$).
— No females remaining after thinning.

Mean Measured Conc. (ppb a.i.)	Overall % Spawning Frequency	Mean No. Eggs Per Female Per Reproductive Day
Negative Control	16.8	12.1
Solvent control	14.7	5.59
19	7.96*	3.05
37	6.16*	3.28
74	12.0	6.79
148	6.52*	3.70
296	0.00*	0.00[1]

Data obtained from Tables 18 and 19, pp. 46-47.
* Significantly different from the pooled control ($p \leq 0.05$).
[1] Excluded from statistical analysis.

Toxicity Observations: No apparent treatment-related effects on time to hatch or hatching success were observed. The majority of the embryos hatched on Day 5. Although there was a statistically-significant reduction in the percent hatch of embryos in the 148 ppb a.i. test

group compared to pooled control, this difference was slight and not concentration dependent. Consequently, the NOAEC for time to hatch and hatching success was 296 ppb a.i., the highest concentration tested.

After hatching, larvae were thinned to 100/treatment. Survival through Day 61 (8 weeks post-hatch) was adversely affected at the 296 ppb a.i. test level (Table 15, p. 43). At 8 weeks post-hatch, survival averaged 94% for the pooled control, and 95, 95, 97, 91, and 81% for the 19, 37, 74, 148, and 296 ppb a.i. treatment levels, respectively. The majority of all surviving organisms appeared normal during this period of time (Appendix 9, pp. 101-102). The NOAEC for larval/juvenile survival through Day 61 was 148 ppb a.i.

On Day 61 (8 weeks post-hatch), juvenile fish were thinned to 50/treatment. Juvenile survival through Day 174 (initiation of reproduction phase) was adversely affected at the 296 ppb a.i. test level (Table 16, p. 44). On Day 174, survival averaged 100% for the pooled control, and 98, 100, 100, 100, and 94% for the 19, 37, 74, 148, and 296 ppb a.i. treatment levels, respectively. The majority of all surviving organisms appeared normal during this period of time; however, on Day 174, five fish in the 296 ppb a.i. treatment group had deformed upper jaws (Appendix 10, pp. 103-106). Since this effect was not observed at the lower treatment levels, it was considered to be an effect of treatment. The NOAEC for juvenile/adult survival and morphological deformities was 148 ppb a.i.

On Day 174 (24 weeks post-hatch), adult fish were thinned to 24/treatment and the reproductive phase was initiated. No treatment-related effects on survival were observed from Days 174 to 245 (study termination), with average survival rates of 92 to 100% for all control and treatment levels (Table 17, p. 45). It was reported that on Day 245, six of the surviving minnows exposed at 296 ppb a.i. had the deformed jaw described above (p. 24). The NOAEC for adult survival was 296 ppb a.i., and the NOAEC for adult morphological deformities was 148 ppb a.i.

Reproduction of fathead minnow was assessed by spawning frequency and the mean number of eggs produced per female per reproductive day. Spawning frequency averaged 17 and 15% for the negative and solvent control groups, respectively (Table 18, p. 46). Minnows exposed to concentrations of $\leq$148 ppb a.i. spawned an average of 6.2 to 12% of the spawning days. Minnows exposed at 296 ppb a.i. did not spawn. Differences were statistically-different from pooled control at the 19, 37, 148, and 296 ppb a.i. treatment levels. The study authors reported that the poor concentration-response for this endpoint indicates that spawning frequency was probably not a good indicator of biological effect, but that the lack of spawning in the 296 ppb a.i. group was biologically significant (p. 24). A NOAEC for spawning frequency was not provided. The first reproductive day was Day 177, resulting in a potential of 69 reproductive days per female (p. 25). Egg production was not

significantly reduced in any treatment group (excluded the 296 ppb a.i. group, where no spawning occurred) compared to pooled control. The NOAEC for egg production was 148 ppb a.i.

No treatment-related effects on growth were observed 4 and 8 weeks post-hatch (Tables 20 and 21, pp. 48-49). Following thinning on Day 174, remaining fish were sexed and measured for wet weight and total length. Both endpoints were statistically-reduced in males from the 296 ppb a.i. group (Tables 22 and 23, pp. 50-51). At adult study termination on Day 245, wet weight and total length were also statistically-reduced in males from the 296 ppb a.i. group compared to pooled control (Tables 24 and 25, pp. 52-53). Similar reductions were not observed in females. The NOAEC for first-generation growth was 148 ppb a.i.

F_1 Results:

Mean Measured Concentration (ppb a.i.)	% Hatch[1]			4-Week Post-Hatch % Survival	4-Week Post-Hatch Length (mm)	4-Week Post-Hatch Wet Weight (g)
	Day 3	Day 4	Day 5			
Negative Control	0	2	99	96	18.6	0.0437
Solvent control	0	43	84	98	18.7	0.0439
19	0	13	80*	82	18.4	0.0417
37	0	23	87	94	19.6	0.0521
75	0	90	99*	98	18.4	0.0427
150	17	58	90	78*	16.8	0.0360
295[2]	—	—	—	—	—	—

Data obtained from Table 3, p. 31 and Tables 26 through 28, pp. 54-56.
[1] Days 3 and 4 % hatch data were reviewer-calculated. Only Day 5 data were statistically compared.
[2] No offspring were produced at this treatment level.
* Significantly different from the pooled control ($p \leq 0.05$).

Toxicity Observations: Second-generation embryos began hatching as early as Day 2 at the 150 ppb a.i. treatment level (Table 26, p. 54). Hatching was complete by Day 7, with no apparent treatment-related effects. A statistically-significant reduction in hatching success was noted at the 19 ppb a.i. treatment level compared to the pooled control; however, this was attributed to the poor hatching success in one replicate (60%) and did not appear to be an effect of treatment (p. 26). Hatching success at the 150 ppb a.i. level was significantly greater than the pooled control, and not considered to be related to treatment. The NOAEC for time to hatch and hatching success was 150 ppb a.i.

After hatching, larvae were thinned to 50/treatment. Survival and growth were assessed at 4 weeks post-hatch. Survival was statistically-reduced at the 150 ppb a.i. treatment level compared to pooled control (78 versus 97%; Table 27, p. 55). However, this difference was the result of poor post-hatch survival in one replicate, as survival in the second replicate was 100%. At test termination, five of the fish in the 150 ppb a.i. group appeared smaller than the other fish, and one of the fish had a crooked spine (Appendix 18, p. 126). No treatment-related effects on growth parameters were observed (Table 28, p. 56). Consequently, the study authors reported a NOAEC of 150 ppb a.i. for survival and growth of second-generation fish.

B. Reported Statistical Results

Endpoints that were statistically analyzed included 1) hatching success of the first and second generations, 2) survival of the first and second generation fry during the growth period, 3) total length at day 28 and 56 post-hatch, 4) wet weight of fish thinned at Day 56 post-hatch, 5) total length and wet weight of adult fish thinned on Day 174, 6) total length and wet weight of adults at test termination, 7) spawning frequency, 8) number of eggs produced, and 9) total length and wet weight of second generation fish at 28 days post-hatch.

In all cases, negative and solvent control data were compared using Fisher's Exact test , and pooled for subsequent comparisons. The negative and solvent control groups were found to be significantly different only for hatching success of the second generation; however, for consistency, the controls were pooled as for all other endpoints (p. 26).

Discrete-variable data (e.g. mortality proportions) were analyzed using Fischer's Exact test. Continuous-variable data (e.g., weight and length) were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. The Bonferroni t-test was then used to evaluated differences between treatment and pooled control.

All statistical tests were performed using TOXSTAT 3.5 or SAS Version 8.0 statistical software using mean-measured concentrations.

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

Biological Endpoint	NOAEC (ppb a.i.)	LOAEC (ppb a.i.)
P Generation		
Time to hatch	296	>296
Hatching success	296	>296
Survival, 8-weeks post-hatch (Day 61)	148	296
Survival, 24-weeks post-hatch (Day 174)	148	296
Survival, study termination (Day 245)	296	>296
Morphological deformities	148	296
Spawning frequency	148	296
Egg production	148	>148
Total length, 4-weeks post-hatch	296	>296
Total length, 8-weeks post-hatch	296	>296
Wet weight, 8-weeks post-hatch	296	>296
Total length, 24-weeks post-hatch (Day 174; males)	148	296
Total length, 24-weeks post-hatch (Day 174; females)	148	>148
Wet weight, 24-weeks post-hatch (Day 174; males)	148	296
Wet weight, 24-weeks post-hatch (Day 174; females)	148	>148
Total length, study termination (Day 245; males)	148	296
Total length, study termination (Day 245; females)	296	>296

Biological Endpoint	NOAEC (ppb a.i.)	LOAEC (ppb a.i.)
Wet weight, study termination (Day 245; males)	148	296
Wet weight, study termination (Day 245; females)	296	>296
F_1 Generation		
Time to hatch	150	>150
Hatching success	150	>150
Survival, 4-weeks post-hatch	150	>150
Total length, 4-weeks post-hatch	150	>150
Wet weight, 4-weeks post-hatch	150	>150

NOAEC: 148 ppb a.i.
LOAEC: 296 ppb a.i.
MATC: 209 ppb a.i.
Endpoint(s) affected: P Generation: larval/juvenile survival (hatching through 8-weeks post-hatch), juvenile survival (8 to 24 weeks post-hatch), morphological deformities (juvenile/adult fish), spawning frequency, and juvenile/adult male growth (length and wet weight). F_1 Generation: None.
Most sensitive endpoint(s): Same conclusions for all affected endpoints.

14. REVIEWER'S STATISTICAL RESULTS:

Endpoints that were statistically analyzed included 1) hatching success of the first and second generations (P and F1, respectively), 2) percent survival of the first and second generation fry during the growth period, 3) total length at Day 28 and 56 post-hatch, 4) wet weight of fish thinned at Day 56 post-hatch, 5) total length and wet weight of adult fish thinned on Day 174, 6) total length and wet weight of adults at test termination (Day 245), 7) spawning frequency (P), 8) number of eggs produced (P), and 9) total length and wet weight of second generation fish at 28 days post-hatch.

With the exception of time to hatch (P and F1), adult survival (Days 174 and 245), and adult morphological deformities, data for all analyzed endpoints satisfied the assumptions of

ANOVA (i.e., normal distribution and variance homogeneity), so the NOAEC and LOAEC for these endpoints were determined using ANOVA and William's multiple comparison test (if necessary). For all endpoints, the solvent control was compared to the negative control using a Student's t-test and the two were pooled for comparison to the treatment groups. The above analyses were conducted via TOXSTAT statistical software using mean-measured concentrations. Adult survival (Days 174 and 245) data were visually assessed due to the lack of a >10% effect in the control or treatment groups. Time to hatch (P and F1) and adult morphological deformities data were also assessed visually.

The mean-measured 296 ppb a.i. treatment group was excluded from the statistical analysis of P spawning frequency and egg production due to the obvious treatment-related effect (no spawning). Raw rather than replicate data for wet weight and total length of P fish thinned on Day 174 was used for statistical analysis due to the lack of females in all replicates (treatment level 37 ppb a.i. replicate "B" and 296 ppb a.i. replicates "A" and "B", Table 23, p. 51 and Appendix 14-15, pp. 110-117).

Biological Endpoint	NOAEC (ppb a.i.)	LOAEC (ppb a.i.)
P Generation		
Time to hatch	296	>296
Hatching success	296	>296
Survival, 8-weeks post-hatch (Day 61)	148	296
Survival, 24-weeks post-hatch (Day 174)	148	296
Survival, study termination (Day 245)	296	>296
Morphological deformities	148	296
Spawning frequency	148	296
Egg production	148	>148
Total length, 4-weeks post-hatch	296	>296
Total length, 8-weeks post-hatch	296	>296
Wet weight, 8-weeks post-hatch	148	296

Biological Endpoint	NOAEC (ppb a.i.)	LOAEC (ppb a.i.)
Total length, 24-weeks post-hatch (Day 174; males)	148	296
Total length, 24-weeks post-hatch (Day 174; females)	148	>148
Wet weight, 24-weeks post-hatch (Day 174; males)	148	296
Wet weight, 24-weeks post-hatch (Day 174; females)	148	>148
Total length, study termination (Day 245; males)	148	296
Total length, study termination (Day 245; females)	296	>296
Wet weight, study termination (Day 245; males)	148	296
Wet weight, study termination (Day 245; females)	296	>296
F_1 Generation		
Time to hatch	150	>150
Hatching success	150	>150
Survival, 4-weeks post-hatch	150	>150
Total length, 4-weeks post-hatch	150	>150
Wet weight, 4-weeks post-hatch	150	>150

NOAEC: 148 ppb a.i.
LOAEC: 296 ppb a.i.
Endpoint(s) affected: P Generation: larval/juvenile survival (hatching through 8-weeks post-hatch), juvenile survival (8 to 24 weeks post-hatch), morphological deformities (juvenile/adult fish), spawning frequency, larval/juvenile wet weight (8-weeks post-hatch/Day 56) and juvenile/adult male growth (length and wet weight; 24-weeks/Day 174

and Day 245). F_1 Generation: None.
Most sensitive endpoint(s): Same conclusions for all affected endpoints.

15. REVIEWER'S COMMENTS:

With the exception of parent generation wet weight at 8-weeks post-hatch, the reviewer's conclusions were identical to those of the study authors. The reviewer determined a statistically significant treatment-related effect in parent generation wet weight at 8-weeks post-hatch at the 296 ppb a.i. treatment level while the study authors reported no significant effect, presumably due to the different statistical methods used. Consequently, the NOAEC and LOAEC values (148 and 296 ppb a.i., respectively) are reported in the CONCLUSION section of this DER because they are a more conservative estimate of the chronic toxicity of JAU 6476 - Desthio (Prothioconazole metabolite) to the growth of the Fathead Minnow (*Pimephales promelas*).

The study authors reported that spawning frequency averaged 16% for the pooled control, and from 6.2 to 12% in minnows exposed at ≤148 ppb a.i. Minnows exposed at 296 ppb a.i. did not spawn. Differences were statistically-different from the pooled control at the 19, 37, 148, and 296 ppb a.i. treatment levels, but not the 74 ppb a.i. level. The reviewer determined that the spawning frequency was significantly reduced (William's test) at the 19 and 148 ppb a.i. treatment levels only. Given the obvious non-linear response (Table 18, p. 46) the reviewer agreed with the study authors' conclusion that the poor concentration-response for this endpoint indicates that spawning frequency was probably not a good indicator of biological effect, but that the lack of spawning in the 296 ppb a.i. group was biologically significant.

On Day 85, one of two test substance syringe pumps stopped delivering stock to the nominal 38, 150, and 300 ppb treatment groups (p. 21). Analytical samples were collected at that time, and determined that recoveries were 48-52% of nominal concentrations (reviewer-calculated from data provided in Table 2, p. 30). The pump was promptly restarted, and the values obtained on Day 85 were included in the mean-measured concentration calculations.

On Day 105, the results of the analysis (for concentration verification) for the nominal 19 ppb group was unusually low (23% of nominal) without any obvious signs of diluter malfunction (p. 21). An additional sample was collected and analyzed on Day 106, resulting in a 96% recovery. Therefore, it was concluded that the difference was due to a error in analysis of the Day 105 sample, and the data was therefore excluded from the mean-measured concentration calculation.

On Day 182 of the P generation exposure, DO concentrations dropped as low as 3.5 mg/L

(42% of saturation; p. 22). Mild aeration was therefore instituted for the remainder of the test. Thereafter (beginning on Day 183), DO levels remained ≥5.7 mg/L (70% of saturation). DO concentrations in the F_1 generation exposure remained ≥7.3 mg/L (89% of saturation) and mild aeration was not necessary. Aeration did not impact the analytical results, since measured concentrations of Desthio JAU 6476 were comparable before and after aeration was instituted.

It was not specified if test chambers had drains to allow for water level reduction, although a flow-through exposure system was used.

This study was performed in compliance with Good Laboratory Practice Standards as published by the U.S. EPA 40 CFR Part 160 (1989), OECD Principles of GLP (1998), and Japan MAFF (1984). A Quality Assurance Statement was provided.

16. REFERENCES:

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Benoit, D.A. 1981. *User's Guide for Conducting Life-Cycle Chronic Toxicity Tests with Fathead Minnows (Pimephales promelas).* U.S. Environmental Protection Agency, Office of Research and Development. EPA-600/8-81-011.

APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. Water Polluction Control Federation, New York.

Martin, J.W. 1967. *A Method for Measuring Lengths of Juvenile Salmon from Photographs.* Prgr. Fish-Cult. 29:238-240.

West, Inc., and D.D. Gulley. 1996. TOXSTAT® 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

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17. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

P GENERATION:

Hatching success P (Day6; %)
File: 6033hpd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	260.357	52.071	0.659
Within (Error)	22	1738.500	79.023	
Total	27	1998.857		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Hatching success P (Day6; %)
File: 6033hpd 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	GRPS 1&2 POOLED	89.250	89.250		
2	19	92.000	92.000	-0.505	
3	37	90.000	90.000	-0.138	
4	74	89.000	89.000	0.046	
5	148	81.500	81.500	1.424	
6	296	89.000	89.000	0.046	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Survival P at Day 61
File: 6033s1d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	675.714	135.143	4.073
Within (Error)	22	730.000	33.182	
Total	27	1405.714		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F **REJECT Ho:All groups equal**

Survival P at Day 61
File: 6033s1d 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	GRPS 1&2 POOLED	93.500	93.500		
2	19	95.000	95.000	-0.425	
3	37	95.000	95.000	-0.425	
4	74	97.000	97.000	-0.992	
5	148	91.000	91.000	0.709	
6	296	81.000	81.000	3.544	*

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Survival P at Day 61
File: 6033s1d 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	GRPS 1&2 POOLED	8			
2	19	4	8.850	9.5	-1.500
3	37	4	8.850	9.5	-1.500
4	74	4	8.850	9.5	-3.500
5	148	4	8.850	9.5	2.500
6	296	4	8.850	9.5	12.500

Survival P at Day 61
File: 6033s1d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	93.500	93.500	94.800
2	19	4	95.000	95.000	94.800
3	37	4	95.000	95.000	94.800
4	74	4	97.000	97.000	94.800
5	**148**	**4**	**91.000**	**91.000**	**91.000**
6	296	4	81.000	81.000	81.000

Survival P at Day 61
File: 6033s1d 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
GRPS 1&2 POOLED	94.800				
19	94.800	0.369		1.72	k= 1, v=22
37	94.800	0.369		1.80	k= 2, v=22
74	94.800	0.369		1.83	k= 3, v=22
148	**91.000**	**0.709**		**1.84**	**k= 4, v=22**
296	81.000	3.544	*	1.85	k= 5, v=22

s = 5.760
Note: df used for table values are approximate when v > 20.

Spawning frequency (%)
File: 6033sfd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	197.384	49.346	2.898
Within (Error)	7	119.199	17.028	
Total	11	316.583		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Spawning frequency (%)
File: 6033sfd 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	GRPS 1&2 POOLED	15.775	15.775		
2	19	7.955	7.955	2.188	
3	37	6.160	6.160	2.691	
4	74	11.950	11.950	1.070	
5	148	6.520	6.520	2.590	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

Egg Production P (# eggs/female/repro. day)
File: 6033epd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	75.708	18.927	1.894
Within (Error)	7	69.955	9.994	
Total	11	145.663		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Egg Production P (# eggs/female/repro. day)
File: 6033epd 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	GRPS 1&2 POOLED	8.842	8.842		
2	19	3.045	3.045	2.118	
3	37	3.275	3.275	2.034	
4	74	6.790	6.790	0.750	
5	148	3.700	3.700	1.878	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

Egg Production P (# eggs/female/repro. day)
File: 6033epd 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	GRPS 1&2 POOLED	4			
2	19	2	7.781	88.0	5.798
3	37	2	7.781	88.0	5.567
4	74	2	7.781	88.0	2.052
5	148	2	7.781	88.0	5.142

Length at Day 28 (4 weeks; mm)
File: 60331p4d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.289	0.458	1.055
Within (Error)	22	9.551	0.434	
Total	27	11.840		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Length at Day 28 (4 weeks; mm)
File: 60331p4d 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	GRPS 1&2 POOLED	20.138	20.138		
2	19	20.425	20.425	-0.713	
3	37	20.375	20.375	-0.589	
4	74	20.700	20.700	-1.394	
5	148	20.550	20.550	-1.023	
6	296	19.775	19.775	0.899	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Length P at Day 56 (8 weeks; mm)
File: 60331p8d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	14.644	2.929	4.233
Within (Error)	22	15.223	0.692	
Total	27	29.867		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F **REJECT Ho:All groups equal**

Length P at Day 56 (8 weeks; mm)
File: 60331p8d 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	GRPS 1&2 POOLED	35.825	35.825		
2	19	36.650	36.650	-1.620	
3	37	36.550	36.550	-1.423	
4	74	37.800	37.800	-3.877	
5	148	35.800	35.800	0.049	
6	296	35.575	35.575	0.491	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Length P at Day 56 (8 weeks; mm)
File: 60331p8d 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	GRPS 1&2 POOLED	8			
2	19	4	1.278	3.6	-0.825
3	37	4	1.278	3.6	-0.725
4	74	4	1.278	3.6	-1.975
5	148	4	1.278	3.6	0.025
6	296	4	1.278	3.6	0.250

Length P at Day 56 (8 weeks; mm)
File: 60331p8d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	35.825	35.825	36.530
2	19	4	36.650	36.650	36.530
3	37	4	36.550	36.550	36.530
4	74	4	37.800	37.800	36.530
5	148	4	35.800	35.800	35.800
6	**296**	**4**	**35.575**	**35.575**	**35.575**

Length P at Day 56 (8 weeks; mm)
File: 60331p8d 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

GRPS 1&2 POOLED	36.530			
19	36.530	1.384	1.72	k= 1, v=22
37	36.530	1.384	1.80	k= 2, v=22
74	36.530	1.384	1.83	k= 3, v=22
148	35.800	0.049	1.84	k= 4, v=22
296	**35.575**	**0.491**	**1.85**	**k= 5, v=22**

s = 0.832
Note: df used for table values are approximate when v > 20.

Wet weight of P at Day 56 (8 weeks; g)
File: 6033wp8d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.017	0.003	3.000
Within (Error)	22	0.029	0.001	
Total	27	0.046		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F **REJECT Ho:All groups equal**

Wet weight of P at Day 56 (8 weeks; g)
File: 6033wp8d 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	GRPS 1&2 POOLED	0.450	0.450		
2	19	0.464	0.464	-0.732	
3	37	0.444	0.444	0.318	
4	74	0.491	0.491	-2.089	
5	148	0.423	0.423	1.381	
6	296	0.408	0.408	2.169	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Wet weight of P at Day 56 (8 weeks; g)
File: 6033wp8d 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	GRPS 1&2 POOLED	8			
2	19	4	0.049	10.8	-0.014
3	37	4	0.049	10.8	0.006

4	74	4	0.049	10.8	-0.040
5	148	4	0.049	10.8	0.027
6	296	4	0.049	10.8	0.042

Wet weight of P at Day 56 (8 weeks; g)
File: 6033wp8d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	0.450	0.450	0.460
2	19	4	0.464	0.464	0.460
3	37	4	0.444	0.444	0.460
4	74	4	0.491	0.491	0.460
5	**148**	**4**	**0.423**	**0.423**	**0.423**
6	296	4	0.408	0.408	0.408

Wet weight of P at Day 56 (8 weeks; g)
File: 6033wp8d 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
GRPS 1&2 POOLED	0.460				
19	0.460	0.437		1.72	k= 1, v=22
37	0.460	0.437		1.80	k= 2, v=22
74	0.460	0.437		1.83	k= 3, v=22
148	**0.423**	**1.207**		**1.84**	**k= 4, v=22**
296	0.408	1.895	*	1.85	k= 5, v=22

s = 0.036
Note: df used for table values are approximate when v > 20.

Male length at Day 174 (24 weeks; mm)
File: 6033ml24d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	195.209	39.042	6.444
Within (Error)	8	48.475	6.059	
Total	13	243.684		

Critical F value = 3.69 (0.05,5,8)

Since F > Critical F **REJECT Ho:All groups equal**

Male length at Day 174 (24 weeks; mm)
File: 6033ml24d 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	GRPS 1&2 POOLED	66.850	66.850		
2	19	65.000	65.000	0.868	
3	37	63.800	63.800	1.431	
4	74	65.900	65.900	0.446	
5	148	63.500	63.500	1.571	
6	296	55.250	55.250	5.442	*

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Male length at Day 174 (24 weeks; mm)
File: 6033ml24d 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	GRPS 1&2 POOLED	4			
2	19	2	6.176	9.2	1.850
3	37	2	6.176	9.2	3.050
4	74	2	6.176	9.2	0.950
5	148	2	6.176	9.2	3.350
6	296	2	6.176	9.2	11.600

Male length at Day 174 (24 weeks; mm)
File: 6033ml24d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	66.850	66.850	66.850
2	19	2	65.000	65.000	65.000
3	37	2	63.800	63.800	64.850
4	74	2	65.900	65.900	64.850
5	**148**	**2**	**63.500**	**63.500**	**63.500**
6	296	2	55.250	55.250	55.250

Male length at Day 174 (24 weeks; mm)

File: 6033ml24d 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
GRPS 1&2 POOLED	66.850				
19	65.000	0.868		1.86	k= 1, v= 8
37	64.850	0.938		1.96	k= 2, v= 8
74	64.850	0.938		2.00	k= 3, v= 8
148	**63.500**	**1.571**		**2.01**	**k= 4, v= 8**
296	55.250	5.441	*	2.02	k= 5, v= 8

s = 2.462
Note: df used for table values are approximate when v > 20.

Female length at Day 174 (week 24; mm)
File: 6033fl2d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	156.875	39.219	3.173
Within (Error)	56	692.207	12.361	
Total	60	849.082		

Critical F value = 2.61 (0.05,4,40)
Since F > Critical F **REJECT Ho:All groups equal**

Female length at Day 174 (week 24; mm)
File: 6033fl2d 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	GRPS 1&2 POOLED	54.136	54.136		
2	19	53.273	53.273	0.665	
3	37	53.250	53.250	0.611	
4	74	57.615	57.615	-2.829	
5	148	54.857	54.857	-0.472	

Bonferroni T table value = 2.31 (1 Tailed Value, P=0.05, df=50,4)

Female length at Day 174 (week 24; mm)
File: 6033fl2d 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	GRPS 1&2 POOLED	22			
2	19	11	3.000	5.5	0.864
3	37	8	3.355	6.2	0.886
4	74	13	2.842	5.3	-3.479
5	148	7	3.526	6.5	-0.721

Female length at Day 174 (week 24; mm)
File: 6033fl2d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	22	54.136	54.136	53.732
2	19	11	53.273	53.273	53.732
3	37	8	53.250	53.250	53.732
4	74	13	57.615	57.615	56.650
5	**148**	**7**	**54.857**	**54.857**	**56.650**

Female length at Day 174 (week 24; mm)
File: 6033fl2d 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
GRPS 1&2 POOLED	53.732				
19	53.732	0.312		1.68	k= 1, v=56
37	53.732	0.279		1.76	k= 2, v=56
74	56.650	2.044	*	1.79	k= 3, v=56
148	**56.650**	**1.648**		**1.80**	**k= 4, v=56**

s = 3.516
Note: df used for table values are approximate when v > 20.

Male wet weight at Day 174 (24 weeks; g)
File: 6033mw2d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.435	0.287	3.932
Within (Error)	8	0.584	0.073	
Total	13	2.019		

Critical F value = 3.69 (0.05,5,8)
Since F > Critical F **REJECT Ho:All groups equal**

Male wet weight at Day 174 (24 weeks; g)
File: 6033mw2d 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	GRPS 1&2 POOLED	2.746	2.746		
2	19	2.412	2.412	1.427	
3	37	2.329	2.329	1.785	
4	74	2.606	2.606	0.600	
5	148	2.291	2.291	1.945	
6	296	1.753	1.753	4.247	*

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Male wet weight at Day 174 (24 weeks; g)
File: 6033mw2d 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	GRPS 1&2 POOLED	4			
2	19	2	0.678	24.7	0.334
3	37	2	0.678	24.7	0.418
4	74	2	0.678	24.7	0.141
5	148	2	0.678	24.7	0.455
6	296	2	0.678	24.7	0.994

Male wet weight at Day 174 (24 weeks; g)
File: 6033mw2d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	2.746	2.746	2.746
2	19	2	2.412	2.412	2.449
3	37	2	2.329	2.329	2.449
4	74	2	2.606	2.606	2.449
5	**148**	**2**	**2.291**	**2.291**	**2.291**
6	296	2	1.753	1.753	1.753

Male wet weight at Day 174 (24 weeks; g)
File: 6033mw2d 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
GRPS 1&2 POOLED	2.746				
19	2.449	1.271		1.86	k= 1, v= 8
37	2.449	1.271		1.96	k= 2, v= 8
74	2.449	1.271		2.00	k= 3, v= 8
148	**2.291**	**1.945**		**2.01**	**k= 4, v= 8**
296	1.753	4.246	*	2.02	k= 5, v= 8

s = 0.270
Note: df used for table values are approximate when v > 20.

Female wet weight at Day 174 (24 weeks; g)
File: 6033fw2d Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.515	0.129	1.344
Within (Error)	56	5.377	0.096	
Total	60	5.892		

Critical F value = 2.61 (0.05,4,40)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Female wet weight at Day 174 (24 weeks; g)
File: 6033fw2d Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.406	1.406		
2	19	1.314	1.314	0.798	
3	37	1.317	1.317	0.689	
4	74	1.572	1.572	-1.535	
5	148	1.385	1.385	0.157	

Bonferroni T table value = 2.31 (1 Tailed Value, P=0.05, df=50,4)

Female wet weight at Day 174 (24 weeks; g)

File: 6033fw2d Transform: NO TRANSFORM

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	GRPS 1&2 POOLED	22			
2	19	11	0.264	18.8	0.091
3	37	8	0.296	21.0	0.088
4	74	13	0.250	17.8	-0.166
5	148	7	0.311	22.1	0.021

Female wet weight at Day 174 (24 weeks; g)
File: 6033fw2d Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	22	1.406	1.406	1.414
2	19	11	1.314	1.314	1.414
3	37	8	1.317	1.317	1.414
4	74	13	1.572	1.572	1.414
5	**148**	**7**	**1.385**	**1.385**	**1.385**

Female wet weight at Day 174 (24 weeks; g)
File: 6033fw2d 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
GRPS 1&2 POOLED	1.414				
19	1.414	0.073		1.68	k= 1, v=56
37	1.414	0.065		1.76	k= 2, v=56
74	1.414	0.077		1.79	k= 3, v=56
148	**1.385**	**0.157**		**1.80**	**k= 4, v=56**

s = 0.310
Note: df used for table values are approximate when v > 20.

Male length at termination (Day 245; mm)
File: 6033ml3d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	60.124	12.025	6.542

Within (Error)	8	14.705	1.838
Total	13	74.829	

Critical F value = 3.69 (0.05,5,8)
Since F > Critical F **REJECT Ho:All groups equal**

Male length at termination (Day 245; mm)
File: 6033ml3d 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	GRPS 1&2 POOLED	72.200	72.200		
2	19	71.850	71.850	0.298	
3	37	72.300	72.300	-0.085	
4	74	74.400	74.400	-1.874	
5	148	72.600	72.600	-0.341	
6	296	67.100	67.100	4.344	*

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Male length at termination (Day 245; mm)
File: 6033ml3d 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	GRPS 1&2 POOLED	4			
2	19	2	3.401	4.7	0.350
3	37	2	3.401	4.7	-0.100
4	74	2	3.401	4.7	-2.200
5	148	2	3.401	4.7	-0.400
6	296	2	3.401	4.7	5.100

Male length at termination (Day 245; mm)
File: 6033ml3d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	72.200	72.200	72.592
2	19	2	71.850	71.850	72.592
3	37	2	72.300	72.300	72.592
4	74	2	74.400	74.400	72.592
5	**148**	**2**	**72.600**	**72.600**	**72.592**

6	296	2	67.100	67.100	67.100

Male length at termination (Day 245; mm)
File: 6033ml3d 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
GRPS 1&2 POOLED	72.592				
19	72.592	0.334		1.86	k= 1, v= 8
37	72.592	0.334		1.96	k= 2, v= 8
74	72.592	0.334		2.00	k= 3, v= 8
148	**72.592**	**0.334**		**2.01**	**k= 4, v= 8**
296	67.100	4.344	*	2.02	k= 5, v= 8

s = 1.356
Note: df used for table values are approximate when v > 20.

Female length at termination (Day 245; mm)
File: 6033fl3d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	34.317	6.863	7.668
Within (Error)	8	7.158	0.895	
Total	13	41.475		

Critical F value = 3.69 (0.05,5,8)
Since F > Critical F **REJECT Ho:All groups equal**

Female length at termination (Day 245; mm)
File: 6033fl3d 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	GRPS 1&2 POOLED	56.525	56.525		
2	19	57.550	57.550	-1.251	
3	37	55.700	55.700	1.007	
4	74	58.850	58.850	-2.838	
5	148	59.800	59.800	-3.997	
6	296	55.100	55.100	1.739	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Female length at termination (Day 245; mm)
File: 6033f13d 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	GRPS 1&2 POOLED	4			
2	19	2	2.374	4.2	-1.025
3	37	2	2.374	4.2	0.825
4	74	2	2.374	4.2	-2.325
5	148	2	2.374	4.2	-3.275
6	296	2	2.374	4.2	1.425

Female length at termination (Day 245; mm)
File: 6033f13d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	56.525	56.525	57.492
2	19	2	57.550	57.550	57.492
3	37	2	55.700	55.700	57.492
4	74	2	58.850	58.850	57.492
5	148	2	59.800	59.800	57.492
6	**296**	**2**	**55.100**	**55.100**	**55.100**

Female length at termination (Day 245; mm)
File: 6033f13d 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
GRPS 1&2 POOLED	57.492				
19	57.492	1.180		1.86	k= 1, v= 8
37	57.492	1.180		1.96	k= 2, v= 8
74	57.492	1.180		2.00	k= 3, v= 8
148	57.492	1.180		2.01	k= 4, v= 8
296	**55.100**	**1.740**		**2.02**	**k= 5, v= 8**

s = 0.946
Note: df used for table values are approximate when v > 20.

Male wet weight at termination (Day 245; g)
File: 6033mw3d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.105	0.421	4.576
Within (Error)	8	0.739	0.092	
Total	13	2.844		

Critical F value = 3.69 (0.05,5,8)
Since F > Critical F **REJECT Ho:All groups equal**

Male wet weight at termination (Day 245; g)
File: 6033mw3d 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	GRPS 1&2 POOLED	4.436	4.436		
2	19	4.197	4.197	0.908	
3	37	4.639	4.639	-0.774	
4	74	4.475	4.475	-0.150	
5	148	4.002	4.002	1.651	
6	296	3.401	3.401	3.940	*

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Male wet weight at termination (Day 245; g)
File: 6033mw3d 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	GRPS 1&2 POOLED	4			
2	19	2	0.761	17.2	0.238
3	37	2	0.761	17.2	-0.203
4	74	2	0.761	17.2	-0.039
5	148	2	0.761	17.2	0.434
6	296	2	0.761	17.2	1.035

Male wet weight at termination (Day 245; g)
File: 6033mw3d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED

	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	GRPS 1&2 POOLED	4	4.436	4.436	4.436
2	19	2	4.197	4.197	4.436
3	37	2	4.639	4.639	4.436
4	74	2	4.475	4.475	4.436
5	**148**	**2**	**4.002**	**4.002**	**4.002**
6	296	2	3.401	3.401	3.401

Male wet weight at termination (Day 245; g)
File: 6033mw3d 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
GRPS 1&2 POOLED	4.436				
19	4.436	0.003		1.86	k= 1, v= 8
37	4.436	0.003		1.96	k= 2, v= 8
74	4.436	0.003		2.00	k= 3, v= 8
148	**4.002**	**1.648**		**2.01**	**k= 4, v= 8**
296	3.401	3.932	*	2.02	k= 5, v= 8

s = 0.304
Note: df used for table values are approximate when v > 20.

Female wet weight at termination (Day 245; g)
File: 6033fw3d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.329	0.066	4.714
Within (Error)	8	0.111	0.014	
Total	13	0.439		

Critical F value = 3.69 (0.05,5,8)
Since F > Critical F **REJECT Ho:All groups equal**

Female wet weight at termination (Day 245; g)
File: 6033fw3d 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	GRPS 1&2 POOLED	1.695	1.695		

2	19	1.799	1.799	-1.010
3	37	1.619	1.619	0.740
4	74	1.968	1.968	-2.667
5	148	2.043	2.043	-3.392
6	296	1.642	1.642	0.521

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Female wet weight at termination (Day 245; g)
File: 6033fw3d 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	GRPS 1&2 POOLED	4			
2	19	2	0.297	17.5	-0.103
3	37	2	0.297	17.5	0.076
4	74	2	0.297	17.5	-0.273
5	148	2	0.297	17.5	-0.348
6	296	2	0.297	17.5	0.053

Female wet weight at termination (Day 245; g)
File: 6033fw3d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	1.695	1.695	1.803
2	19	2	1.799	1.799	1.803
3	37	2	1.619	1.619	1.803
4	74	2	1.968	1.968	1.803
5	148	2	2.043	2.043	1.803
6	**296**	**2**	**1.642**	**1.642**	**1.642**

Female wet weight at termination (Day 245; g)
File: 6033fw3d 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
GRPS 1&2 POOLED	1.803				
19	1.803	1.061		1.86	k= 1, v= 8
37	1.803	1.061		1.96	k= 2, v= 8
74	1.803	1.061		2.00	k= 3, v= 8
148	1.803	1.061		2.01	k= 4, v= 8
296	**1.642**	**0.524**		**2.02**	**k= 5, v= 8**

s = 0.118
Note: df used for table values are approximate when v > 20.

F1 GENERATION:
Hatching success of F1 gen. (%)
File: 6033hfd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	388.667	97.167	0.365
Within (Error)	7	1863.000	266.143	
Total	11	2251.667		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Hatching success of F1 gen. (%)
File: 6033hfd 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	GRPS 1&2 POOLED	91.500	91.500		
2	19	80.000	80.000	0.814	
3	37	87.000	87.000	0.319	
4	75	99.000	99.000	-0.531	
5	150	90.000	90.000	0.106	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

Survival of F1 generation Day 0 to Day 28 (4 weeks; %)
File: 6033s3d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	760.000	190.000	0.794
Within (Error)	7	1676.000	239.429	
Total	11	2436.000		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Survival of F1 generation Day 0 to Day 28 (4 weeks; %)
File: 6033s3d 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	GRPS 1&2 POOLED	97.000	97.000		
2	19	82.000	82.000	1.119	
3	37	94.000	94.000	0.224	
4	75	98.000	98.000	-0.075	
5	150	78.000	78.000	1.418	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

F1 gen length at Day 28, termination (week 4; mm)
File: 6033fld Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	8.254	2.064	3.040
Within (Error)	7	4.755	0.679	
Total	11	13.009		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

F1 gen length at Day 28, termination (week 4; mm)
File: 6033fld 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	GRPS 1&2 POOLED	18.650	18.650		
2	19	18.400	18.400	0.350	
3	37	19.600	19.600	-1.331	-
4	75	18.350	18.350	0.420	
5	150	16.800	16.800	2.592	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

F1 generation wet weight at Day 28 (week 4; g)
File: 6033fwd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.027	0.007	2.333
Within (Error)	7	0.023	0.003	
Total	11	0.050		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

F1 generation wet weight at Day 28 (week 4; g)
File: 6033fwd 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	GRPS 1&2 POOLED	0.438	0.438		
2	19	0.417	0.417	0.443	
3	37	0.521	0.521	-1.739	
4	75	0.427	0.427	0.232	
5	150	0.360	0.360	1.655	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

DP Barcode: D303488 MRID No: 46246033
Submission No: 2004-0843

Data Evaluation Report on the Toxicity of the transformation product JAU6476-desthio to Freshwater Fish - Life Cycle Toxicity Test

PMRA Submission Number 2004-0843 EPA MRID Number 46246033

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: September 8, 2005

PMRA Submission Number: 2004-0843

Study Type: Chronic Toxicity to Freshwater Fish - Life Cycle Test

Drottar, K.R., T.Z. Kendall and H.O. Krueger. 2004. Desthio JAU 6476: a flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*). Performed by: Wildlife International, Ltd., Easton, MD. Performing Laboratory ID 149A-126A. Submitted by: Bayer CropScience, RTP, NC. Bayer Study Number J6851201. Bayer Report No. 200108. March 5, 2004.

PMRA DATA CODE: 9.5.3.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.2.5
EPA MRID: 46246033
EPA Guideline: §72-5; 850.1500

Company Code: BCZ
Active Code: PRB
Use Site Category: 7, 13, 14
EPA PC Code: 113961

EAD Executive Summary:

The chronic toxicity of JAU 6476-desthio (Prothioconazole transformation product; purity 96.4%) to the full life stage of Fathead Minnow (*Pimephales promelas*) was studied under flow-through conditions for approximately 9 months. This study followed the U.S. EPA OPPTS 850.1500 Guideline and was performed in compliance with Good Laboratory Practice Standards as published by the U.S. EPA 40 CFR Part 160 (1989), OECD Principles of GLP (1998), and Japan MAFF (1984). Fertilized eggs (200 embryos/treatment, <24 hours old) were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 19, 38, 75, 150, and 300 µg a.i./L. Mean measured concentrations of the parent generation were <2.00 (<LOQ,

controls), 19, 37, 74, 148, and 296 µg a.i./L. Mean measured concentrations of the second-generation were <2.00 (<LOQ, controls), 19, 37, 75, 150, and 295 µg a.i./L.

Following hatching on Day 5, larvae were reduced to 100 per treatment level. On Day 61, the juveniles were reduced to 50 per treatment level. On Day 174, the adults were reduced to 24 per treatment level (including reserves), and the aquaria were equipped to accommodate spawning. The P generation was terminated on Day 245. F_1-generation exposure was initiated with 100 embryos per treatment, and larvae/fry were maintained for 4 weeks post-hatch.

P Generation: No apparent treatment-related effects on time to hatch or hatching success were observed. The majority of the embryos hatched on Day 5. The NOEC for time to hatch and hatching success was 296 µg a.i./L, the highest concentration tested.

Survival through Day 61 (8 weeks post-hatch) and then from Days 61 through 174 (initiation of reproduction) was adversely affected at the 296 µg a.i./L test level compared to the pooled control (81 versus 94%, and 94 versus 100%, respectively). The NOEC for survival through Day 174 was 148 µg a.i./L. No treatment-related effect on adult survival from Days 174 to 245 was observed.

Between hatch and study termination, the majority of all surviving organisms appeared normal. However, on Days 174 and 245, a treatment-related increase in the incidence of fish with deformed upper jaws was observed at the 296 µg a.i./L treatment level. The NOEC for morphological deformities was therefore 148 µg a.i./L.

Reproduction of fathead minnow was assessed by spawning frequency and the mean number of eggs produced per female per reproductive day. Spawning frequency averaged 16% for the pooled control, and from 6.2 to 12% in minnows exposed at ≤148 µg a.i./L. Minnows exposed at 296 µg a.i./L did not spawn. Differences were statistically-different from the pooled control at the 19, 37, 148, and 296 µg a.i./L treatment levels. The study authors and reviewers concluded that the poor concentration-response for this endpoint indicates that spawning frequency was probably not a good indicator of biological effect, but that the lack of spawning in the 296 µg a.i./L group was biologically significant. Consequently, the NOEC for spawning frequency was 148 µg a.i./L. Egg production was not significantly reduced in any treatment group compared to pooled control. The NOEC for egg production was 148 µg a.i./L, the highest treatment level at which eggs were produced.

Growth was assessed 4 and 8 weeks post-hatch (combined sexes), and again on Days 174 and 245 (separate sexes). A treatment-related reduction in wet weights was observed at 8-weeks post-hatch in larvae/juveniles from the 296 µg a.i./L treatment group. A treatment-related reduction in total length and wet weights were also observed in male fish from the 296 µg a.i./L group at 174 and 245 Days. Similar reductions were not observed in females at 174 and 245

Days. The NOEC for first-generation (P) growth was 148 µg a.i./L.

F_1 Generation: No apparent treatment-related effects on time to hatch, hatching success, or 4-week post-hatch growth parameters were observed. There were significant effects on post-hatch survival at 150 µg a.i./L, however, the effects were attributed to low survival in one replicate; the second replicate showed 100% survival. Hence, the NOEC for all second-generation (F_1) endpoints was 150 µg a.i./L, the highest treatment level tested due to a lack of spawning in fish from the P generation at the 296 µg a.i./L treatment level.

Based on adverse effects on first-generation larval/juvenile survival, juvenile (8-weeks post-hatch) and male growth, spawning frequency, and morphological deformities, the NOEC and LOEC are 148 and 296 µg a.i./L, respectively.

Results Synopsis:

Biological Endpoint	NOEC (µg a.i./L)	LOAEC (µg a.i./L)
P Generation		
Time to hatch	296	>296
Hatching success	296	>296
Survival, 8-weeks post-hatch (Day 61)	148	296
Survival, 24-weeks post-hatch (Day 174)	148	296
Survival, study termination (Day 245)	296	>296
Morphological deformities	148	296
Spawning frequency	148	296
Egg production	148	>148
Total length, 4-weeks post-hatch	296	>296
Total length, 8-weeks post-hatch	296	>296
Wet weight, 8-weeks post-hatch	148	296

Biological Endpoint	NOEC (µg a.i./L)	LOAEC (µg a.i./L)
Total length, 24-weeks post-hatch (Day 174; males)	148	296
Total length, 24-weeks post-hatch (Day 174; females)	148	>148
Wet weight, 24-weeks post-hatch (Day 174; males)	148	296
Wet weight, 24-weeks post-hatch (Day 174; females)	148	>148
Total length, study termination (Day 245; males)	148	296
Total length, study termination (Day 245; females)	296	>296
Wet weight, study termination (Day 245; males)	148	296
Wet weight, study termination (Day 245; females)	296	>296
F_1 Generation		
Time to hatch	150	>150
Hatching success	150	>150
Survival, 4-weeks post-hatch	150	>150
Total length, 4-weeks post-hatch	150	>150
Wet weight, 4-weeks post-hatch	150	>150

NOEC: 148 µg a.i./L
LOEC: 296 µg a.i./L

Endpoint(s) affected: P Generation: larval/juvenile survival (hatching through 8-weeks post-hatch), juvenile survival (8 to 24 weeks post-hatch), morphological deformities (juvenile/adult fish), spawning frequency, larval/juvenile wet weight (8-weeks post-hatch/Day 56) and juvenile/adult male growth (length and wet weight; 24-weeks/Day 174

and Day 245). F_1 Generation: None.
Most sensitive endpoint(s): Same conclusions for all affected endpoints.

EAD Evaluator Comments:

1. The appropriate PMRA information (PMRA Submission Number, PMRA Data Code, PMRA company code, PMRA active ingredient code, PMRA use site category, OECD data point) was added to the PMRA review portion of the DER. The PMRA Submission Number was added to the Header of the DER. Information on the chemical name (IUPAC name, CAS name and synonym) available from the study report, the PMRA Chemistry review and other sources of information was added at the beginning of the DER. The name of the EAD secondary reviewer was added to the front portion of the DER.

2. The EAD reviewer verified the statistical analyses for wet weight of the parental generation at day 56 and did not observe any significant differences between treatments (p=0.052). However, as the p value was very close to the rejection level, the EAD reviewer will agree with the EPA reviewer and the NOEC for this endpoint will be set at 148 µg a.i./L. Other endpoints also have a NOEC of 148 µg a.i./L, therefore there effect of setting a NOEC of 148 instead of 296 µg a.i./L will not have an impact on the risk assessment.

3. The EAD reviewer carefully reviewed the data and the statistical analyses of both the study authors and the EPA reviewer, and found that the results and conclusions are acceptable. The EAD reviewer feels that an additional verification of statistical analyses would not have generated different results.

Study Acceptability: This is study is acceptable to the PMRA. This study is scientifically valid and the information may be useful for future risk assessment purposes even if the F_1 generation was only maintained for 4 weeks post-hatch rather than 8 weeks as required .

DP Barcode: D303488 MRID No: 46246033
Submission No: 2004-0843

Statistical verification of the EAD reviewer:

Wet Weight of Parental-Generation fish at day 56

One Way Analysis of Variance Thursday, September 08, 2005, 11:17:46

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.662)

Group Name	N	Missing	Mean	Std Dev	SEM
pooled controls	8	0	0.450	0.0267	0.00945
19 ug a.i/L	4	0	0.464	0.0420	0.0210
37 ug a.i./L	4	0	0.444	0.0358	0.0179
74 ug a.i./L	4	0	0.491	0.0111	0.00556
148 ug a.i/L	4	0	0.423	0.0427	0.0214
296 ug a.i./L	4	0	0.408	0.0541	0.0270

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.0172	0.00344	2.630	0.052
Residual	22	0.0288	0.00131		
Total	27	0.0459			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.052).

Power of performed test with alpha = 0.050: 0.456

The power of the performed test (0.456) is below the desired power of 0.800.
You should interpret the negative findings cautiously.