Data Evaluation Report on the Chronic Toxicity of JAU 6476 - Desthio (Prothioconazole Metabolite) to Freshwater invertebrates - Daphnia sp.

PMRA Submission Number 2004-0843 EPA MRID Number 46246029

Data Requirement:	PMRA DATA CODE	9.3.5
	EPA DP Barcode	D303488
	OECD Data Point	IIIA 10.2.6.1
	EPA MRID	46246029
	EPA Guideline	§72-4b

Test material: JAU 6476 - Desthio **Purity:** 96.5%
Common name: Prothioconazole metabolite
Chemical name: IUPAC: 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

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CITATION: Dorgerloh, M., and H. Sommer. 2001. Influence of JAU6476-Desthio on the Reproduction Rate of Water Fleas in a Static Renewal Laboratory Test System. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany. Laboratory ID No. E 3211987-5; Report No. DOM 21036. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated January 9, 2001 and completed September 10, 2001.

EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of JAU 6476 - Desthio (96.5% Prothioconazole - desthio) to *Daphnia magna* was studied under static renewal conditions. Nominal concentrations were 0 (negative and solvent controls), 0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 ppm, which correspond to mean-measured concentrations of 0 (controls), 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 ppm. Eight media renewals were performed. "New" test media was sampled from all test levels at 0, 9, and 19 Days, and "old" test media (after 48 or 72 hours) was sampled from the negative control, 0.025, 0.103, and 0.830 ppm levels on Days 2, 12, and 21. Recoveries for all samples ranged from 96 to 109% of nominal concentrations indicating precision and stability. Since "old" treatment solutions were not sampled for all toxicant levels and recoveries were high for those "new and old" treatment solutions sampled, mean-measured concentrations were based on all available data.

After 21 days of exposure, mortality was ≤10% for all test and control groups. The 21-day LC_{50} was >0.830 ppm, and the NOAEC for mortality was 0.830 ppm.

The mean first day of reproduction (time to first brood release) was 9.9, 10.0, 9.6, 9.8, 10.1, 10.3, 10.4, and 11.4 for the negative control, solvent control, 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 ppm test groups, respectively, and the NOAEC was 0.408 ppm. Offspring production was adversely affected by treatment at the ≥0.2 ppm test levels. At the 0.830 ppm level, dead offspring were found regularly during the study, and abnormal behavior of juvenile organisms was observed, including lack of coordination in swimming, lying at the bottom, and clinging to the water surface. The sum of offspring per parent averaged 92.7 for the solvent control, and 91.5, 96.0, 85.7, 77.1, 73.7, and 25.9 for the 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 ppm test groups, respectively. The number of offspring per parent and reproduction day averaged 7.75 for the solvent control, and 7.42, 7.90, 7.21, 6.59, 6.45, and 2.44 for the 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 ppm test groups, respectively. For both endpoints, data were significantly different from the solvent control at concentrations ≥0.206 ppm. The subsequent NOAEC was 0.103 ppm.

A statistically-significant reduction compared to the solvent control in terminal lengths was not observed. Body lengths of surviving daphnids averaged 4.08 mm for the solvent control, and 4.22, 4.13, 4.18, 4.22, 4.16, and 4.09 mm for the 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 ppm test groups, respectively.

This study fulfills the guideline requirements for an aquatic invertebrate life-cycle study using *Daphnia magna* (§72-4b). Even though not all "old" treatment solutions were analytically verified in this static-renewal design, those that were recoveries averaged 102%. Consequently, this study is classified as ACCEPTABLE. This study provides information that may be useful for risk assessment purposes. Reproduction was the most sensitive endpoint.

Results Synopsis:

Test Organism Age (eg. 1st instar): First instar, <24 hours old
Test Type (Flow through, Static, Static Renewal): Static-renewal

Mortality/immobilization:
NOAEC: **0.830 ppm**
LOAEC: >0.830 ppm
LC_{50}: >0.830 ppm

Time to First Brood Release
NOAEC: 0.408 ppm
LOAEC: 0.830 ppm

Sum of Offspring/Parent
NOAEC: 0.103 ppm
LOAEC: 0.206 ppm

Number of Offspring/Parent/Reproduction Day
NOAEC: 0.103 ppm
LOAEC: 0.206 ppm

Terminal Length
NOAEC: **0.830 ppm**
LOAEC: >0.830 ppm

Endpoint(s) Affected: Offspring production, time to first brood release
Most sensitive endpoint(s): Offspring production

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the OECD Guidelines for Testing of Chemicals, No. 211 (1998) and U.S. EPA FIFRA Guideline 72-4, *Daphnia magna* Life Cycle Chronic Toxicity Test. Deviations from U.S. EPA FIFRA Guideline §72-4b include:

1. The storage conditions of the test chemical were not reported.

2. The pretest health (including mortality) of the parental stock was not specified.

3. The hardness (196-214 mg/L as $CaCO_3$) and pH (7.1-8.7) exceeded recommended limits (160-180 mg/L as $CaCO_3$ and 7.6-8.0, respectively).

4. Dissolved oxygen was generally not provided in terms of percent saturation.

5. The study design differed from EPA guidance: in this static renewal study, 1 daphnid per test chamber was maintained, with ten replicate chambers per concentration (total of 10 daphnids/concentration). EPA guidance recommends 22 daphnids/level for static renewal studies, where seven test chambers should contain one daphnid each (to collect data on survival, growth, and reproduction), and three test chambers should contain five daphnids each (to collect data on survival only).

6. The time to first brood release was not statistically analyzed.

7. Not all terminal growth measurements of surviving parental daphnid were measured. Terminal length is currently a required endpoint, and dry weight is a recommended endpoint. Terminal length was measured.

8. Not all "old" treatment solutions were analytically verified during the exposure period.

The fact that not all "old" treatment solutions were analytically verified (so nominal rather than mean-measured treatment concentrations were used to determine toxicity values) affected the acceptability of this study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance

statements were provided. This study was conducted in accordance with the current version of Annex 1 of the Chemicals Law (Chem G; 1994) and the current OECD Principles of GLP.

A. MATERIALS:

1. Test Material JAU 6476 - Desthio (Prothioconazole metabolite)

Description: Light beige powder

Lot No./Batch No.: RUX76-105/8A

Purity: 96.5%

Stability of Compound Under Test Conditions: The stability of JAU 6476 - Desthio in "old" test medium was verified on Days 2 (after 48 hours), 12 (after 72 hours), and 21 (after 72 hours). Samples were collected from the negative control, 0.025, 0.10, and 0.80 ppm test levels. Recoveries were 96 to 109% of nominal concentrations (Table 9, p. 31).

Storage conditions of test chemicals: Not reported.

OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound). The following OECD requirements were reported:

Water solubility: 51 mg/L

2. Test organism:

Species: *Daphnia magna*

Age of the parental stock: Parental stock were 21- to 28-day old; first instars were <24 hours old

Source: In-house laboratory cultures (> 10 years).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: None reported. Nominal concentrations for the definitive study were reportedly based on the results of historical acute testing with daphnids (p. 12).

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 Continuous in-house cultures were maintained. Same as test *Daphnia* cultures were fed three times/week with single cell green algea *Scenedesmus subspicatus* and occasionally with commercial ornamental fish food (TetraMin®) in aqueous suspension. Not reported	The organisms used for the definitive study were <24 hours old and were from the fourth brood or later. The parental stock were 21- to 28-days old at instar collection.
Test condition: Static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal	 Static-renewal N/A Every 48 hours during the week, and at 72 hours over weekends.	 *For flow-through study: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.*
Aeration, if any	Not aerated during the exposure period.	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.*
Duration of the test	21 days	*EPA requires 21 days for static renewal*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: growth/reproduction test: survival test: Fill volume: growth/reproduction test: survival test:	Glass beakers 250 mL Same 100 mL (4-cm depth) Same	The growth/reproduction and survival portions of the test were performed simultaneously in the same replicate test chambers/beakers. The beakers were covered with plexi glass plates. *1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in each vessel.*
Source of dilution water	M7 Medium prepared using deionized water, mineral salts, and vitamins (components listed on p. 11). The water was aerated prior to use.	Results of analysis of the dilution water (February 2001) for contaminants are provided in Appendix E, pp. 66-69. *Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness	11-12 °dH (196-214 mg/L $CaCO_3$)	The hardness and pH exceeded the recommended limits of the EPA, but are acceptable according to OECD Guideline 211.
pH	7.1-8.7	
Dissolved oxygen	7.9-9.2 mg/L (≥88% saturation) in freshly prepared media; ≥5.0 mg/L in 48- or 72-hour old media (Tables 1.1 and 1.2, pp. 16-17)	Dissolved oxygen was generally not provided in terms of percent saturation.
Temperature	19.0-20.1 °C	
Total Organic Carbon Particulate matter Metals Pesticides Chlorine	<2 mg/L (Feb. 2001) Not specified <LOD <0.05 µg/L <0.01 mg/L (Feb. 2001)	***hardness*** *160 to 180 mg/L as $CaCO_3$; OECD requires > 140 mg/L as $CaCO_3$* ***pH*** *7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH rang 6 - 9 and should not vary more than 1.5 units in any one test.* ***Dissolved Oxygen*** *Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥ 60% throughout test.* ***Temperature*** *20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C* *OECD requires total organic carbon < 2 mg/L*
Interval of water quality measurements	DO and pH were measured at all test levels from "new" and "old" test media. Alkalinity, hardness, and conductivity were measured prior to each transfer of the parent daphnia. Temperature was measured continuously in one vessel (not specified) and in one control vessel at the "old" media samplings.	
Number of organisms:	10 daphnids/level	Did not follow recommended EPA test design, but followed OECD Guideline 211.
growth/reproduction test:	1 daphnid per replicate with 10 replicate chambers	
survival test:	(Not differentiated; same test chambers as above)	*EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.*

Parameter	Details	Remarks *Criteria*
Application rates nominal: measured:	0 (negative and solvent controls), 0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 ppm "New" test media (without food or test animals) was sampled from all test levels at 0, 9, and 19 Days, and "old" test media (after 48 or 72 hours) was sampled from the negative control and 0.025, 0.10, and 0.80 ppm levels on Days 2, 12, and 21 (Tables 8 and 9, p. 31). Recoveries for all samples ranged from 96 to 109% of nominal concentrations indicating precision and stability; therefore, nominal concentrations are reported.	Fresh stock solutions were analyzed on Days 0, 9, and 19; recoveries were 97-100% of nominal concentrations (Table 7, p. 30). *EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.*
Solvent (type, percentage, if used)	Dimethylformamide (DMF), 0.1 mL/L (200 µL/2000 mL)	*EPA requires: solvent not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethyl formamide, triethylene glycol, methanol, acetone and ethanol. OECD requires ≤ 0.1 ml/L*
Lighting	16:8 hour light/dark cycle.	Light intensity was approximately 1500 lux (p. 12). *EPA/OECD requires: 16 hours light, 8 hours dark.*
Feeding	Daphnids were fed daily with living single cell green algae (*Scenedesmus subspicatus*) at 0.2 mg TOC/100 mL test solution (1 x 10^8 cells/L).	On the first Friday (only), a three-fold amount was fed for the weekend. Thereafter, the daphnids were fed daily.

Parameter	Details	Remarks *Criteria*
Stability of chemical in the test system	Verified. The stability of JAU 6476 - Desthio in "old" test medium was verified on Days 2 (after 48 hours), 12 (after 72 hours), and 21 (after 72 hours). Samples were collected from the negative control, 0.025, 0.10, and 0.80 ppm test levels. Recoveries were 96 to 109% of nominal concentrations (Table 9, p. 31).	Not all "old" treatment solutions were analytically verified during the exposure period.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	96-108% of nominal "New" media sampled on Days 0, 9, and 19 Not reported 0.0046 ppm (p. 30)	
Positive control {if used, indicate the chemical and concentrations}	see Reviewer's Comments section of this DER.	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Data end points measured (list)	- Survival of first-generation daphnids - Number of young produced per adult per reproduction day - Terminal length - Observations of other effects	Dry weights should have been determined according to the EPA, but are not a requirement of OECD Guideline 211. *EPA requires:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (recommended) and length (required)* of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.* **current requirement until the Agency provides specific guidance indicating otherwise (Pesticide Rejection Rate Analysis, p. 132).*
Observation intervals	Daphnids were observed daily. Offspring production was recorded daily beginning on Day 8 (first day of offspring production). Body length was determined at test termination.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 21 days of exposure, mortality was ≤10% for all treatment and control groups (Tables 2.1 and 2.2, p. 19). At the negative control and 0.05 ppm levels, the single death was caused by a mechanical accident, and was not considered to be treatment-related. The 21-day LC_{50} was >0.8 ppm, and the NOAEC for mortality was 0.8 ppm.

Table 1: Effect of JAU 6476 - Desthio (Prothioconazole Metabolite) on Growth and Survival of Daphnia sp.

Treatment, ppm Nominal Concn.	Mortality[1] (Dead or Immobile)		Sum of Offspring per Parent	Offspring per Parent and Reproduction Day	Length (mm)
	No. Dead	%			
Negative control	1	10	89.3	7.37	4.28
Solvent control	0	0	92.7	7.74	4.08
0.025	0	0	91.5	7.42	4.22
0.05	1	10	96.0	7.90	4.13
0.10	0	0	85.7	7.21	4.19*
0.20	0	0	77.1*	6.59*	4.22
0.40	1	10	73.7*	6.45*	4.16*
0.80	0	0	25.9*	2.44*	4.09*
NOAEC, ppm (nominal)	0.8		0.10	0.10	0.20
LOAEC, ppm (nominal)	>0.8		0.20	0.20	0.40
MATC, ppm (nominal)	ND		ND	ND	ND
LC_{50}/EC_{50} (95% C.I.), ppm (nominal)	>0.8				

* Significantly different from pooled control ($\alpha = 0.05$).
[1] At the negative control and 0.05 ppm levels, the observed single death was caused by a mechanical accident, and not by treatment.
ND - Not determined.

B. EFFECT ON REPRODUCTION AND GROWTH:

The first appearance of neonates occurred on Day 8 (Tables 1 through 9 of Section A of Appendix D, pp. 61-64). The mean first day of reproduction was 9.9, 10.0, 9.6, 9.8, 10.1, 10.3, 10.4, and 11.4 for the negative control, solvent control, nominal 0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 ppm treatment groups, respectively. This endpoint was not statistically assessed by the study authors.

Offspring production was adversely affected by treatment at the ≥0.2 ppm test levels. In the solvent control and 0.025 through 0.2 ppm treatment levels, aborted eggs were found temporarily (p. 20). No dead offspring were found at the 0.40 ppm level. At the 0.80 ppm level, dead offspring were found regularly during the study, and abnormal behavior of juvenile organisms was observed, including lack of coordination in swimming, lying at the bottom, and clinging to the water surface. The sum of offspring per parent averaged 91.1 for the pooled control, and 91.5, 96.0, 85.7, 77.1, 73.7, and 25.9 for the nominal 0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 ppm treatment groups, respectively (Table 4, p. 24). The number of offspring per parent and reproduction day averaged 7.60 for the pooled control, and 7.42, 7.90, 7.21, 6.59, 6.45, and 2.44 for the 0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 ppm treatment groups, respectively (Table 5, p. 26). For both endpoints, data were significantly different from pooled control at concentrations ≥0.20 ppm. The subsequent NOAEC was 0.10 ppm.

A statistically-significant reduction compared to the pooled control in terminal lengths was observed at the 0.40 and 0.80 ppm treatment levels (p. 21 and Table 3, p. 22). Body lengths of surviving daphnids averaged 4.18 mm for the pooled control, and 4.22, 4.13, 4.18, 4.22, 4.16, and 4.09 mm for the nominal 0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 ppm treatment groups, respectively. The NOAEC for total length was 0.20 ppm.

C. REPORTED STATISTICS:

The sum of offspring per parent, the number of offspring per parent per reproduction day, and terminal lengths of surviving adult daphnia were statistically analyzed. For all endpoints, the negative control and solvent control groups were analyzed using the Kolmogorrof-Smirnov test for normality and the Bartlett's test for homogeneity of variances. The data were then subjected to ANOVA followed by the Student t-test. Negative and solvent control data were pooled for all statistical analyses due to a lack of significant differences for all endpoints.

Except for total length data, the data sets were determined to be normal (Kolmogoroff-Smirnoff test) and homogenous (Bartlett's test), and were analyzed by ANOVA followed the Student's t-test . Total length data were normal, but non-homogeneous, and were analyzed using the Student t-test for non-homogeneous variances. Statistical analyses were performed via "Easy Assay Multiple Testing" statistical software for MS-DOS using nominal concentrations.

Mortality/immobilization:
NOAEC: 0.80 ppm
LOAEC: >0.80 ppm
LC_{50}: >0.80 ppm

Time to First Brood Release
NOAEC: Not determined
LOAEC: Not determined

Sum of Offspring/Parent
NOAEC: 0.10 ppm
LOAEC: 0.20 ppm

Number of Offspring/Parent/Reproduction Day
NOAEC: 0.10 ppm
LOAEC: 0.20 ppm

Terminal Length
NOAEC: 0.20 ppm
LOAEC: 0.40 ppm

Endpoint(s) Affected: Offspring production and terminal length
Most sensitive endpoint(s): Offspring production

D. VERIFICATION OF STATISTICAL RESULTS:

The 21-day LC_{50} and NOAEC and LOAEC values were visually determined because mortality did not exceed 10% in any control or treatment level by test termination. Reproduction (sum of offspring per parent, the number of offspring per parent per reproduction day, and the first day of reproduction) and terminal length data were determined to be normally distributed and the variances were homogeneous. NOAEC and LOAEC values for reproduction and terminal length were determined using ANOVA followed by William's multiple comparison test. For all endpoints, the solvent control data was used for statistical analyses. The above statistical analyses were performed via TOXSTAT statistical software. All calculations were performed using the mean-measured treatment concentrations although not all treatment levels were analytically verified throughout the exposure period.

Mortality/immobilization:
NOAEC: 0.830 ppm
LOAEC: >0.830 ppm
LC_{50}: >0.830 ppm

Time to First Brood Release
NOAEC: 0.408 ppm
LOAEC: 0.830 ppm

Sum of Offspring/Parent
NOAEC: 0.103 ppm
LOAEC: 0.206 ppm

Number of Offspring/Parent/Reproduction Day
NOAEC: 0.103 ppm
LOAEC: 0.206 ppm

Terminal Length
NOAEC: 0.830 ppm
LOAEC: >0.830 ppm

Endpoint(s) Affected: Offspring production, time to first brood release

Most sensitive endpoint(s): Offspring production

E. STUDY DEFICIENCIES:

This study does not fulfill the guideline requirements for an aquatic invertebrate life-cycle study using *Daphnia magna* (§72-4b) because not all "old" treatment solutions were analytically verified in this static-renewal design during the exposure period, including the treatment level determined to be the NOAEC and LOAEC values (nominal 0.10 and 0.20 ppm) for the most sensitive endpoints (offspring production; total number and number/parent/reproduction day). Consequently, this study is classified as SUPPLEMENTAL although it may be useful for risk assessment purposes.

All other deficiencies were considered minor and did not affect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to those of the study authors although the review used mean-measured concentrations instead of nominal concentrations. The reviewer-determined LC_{50} value was identical to that of the study authors and the NOAEC and LOAEC values based on mortality and reproduction (sum of offspring and number of offspring per parent per reproductive day) were also similar. The reviewer also statistically verified the reported results for the first day of reproduction (time to first brood release) because toxicity values for this endpoint were not determined by the study authors. The reviewer-determined NOAEC and LOAEC values (0.830 and >0.830 ppm, respectively) based on terminal length were two treatment levels higher than those of the study authors (0.20 and 0.40 ppm, respectively), presumably due to the different statistical methods used. The reviewer determined statistics are presented in the conclusions.

A method validation was conducted concurrently with test solution analysis. However, results from these injections were only provided in terms of peak area (Table B3, p. 41).

The study authors reported (p. 32) that an acute toxicity test was performed on 2001-04-02 under the same conditions using the reference substance $K_2Cr_2O_7$ (potassium dichromate), reagent grade (test concentrations: 0.75,1.00, 1.33, 1.78, 2.37 and 3.16 mg/L). The 24 hour EC_{50} (with 95% C.I.) was 1.45 (0.87-2.91) ppm, which was within the required range of 0.9-1.9 ppm. The gradient of the regression line (after Litchfield & Wilcoxon) was s = 1.46.

G. CONCLUSIONS:

This study does not fulfill the guideline requirements for an aquatic invertebrate life-cycle study using *Daphnia magna* (§72-4b) because not all "old" treatment solutions were analytically verified in this static-renewal design during the exposure period, including the treatment level determined to be the LOAEC values (nominal 0.103 and 0.206 ppm) for the most sensitive endpoints (offspring production; total number and number/parent/reproduction day). Consequently, this study is classified as SUPPLEMENTAL. This study provides information that may be useful for risk assessment purposes. Reproduction and growth were affected by treatment. Offspring production was the most sensitive endpoint.

Mortality/immobilization:
NOAEC: 0.830 ppm
LOAEC: >0.830 ppm

LC_{50}: >0.830 ppm

Time to First Brood Release
NOAEC: 0.408 ppm
LOAEC: 0.830 ppm

Sum of Offspring/Parent
NOAEC: 0.103 ppm
LOAEC: 0.206 ppm

Number of Offspring/Parent/Reproduction Day
NOAEC: 0.103 ppm
LOAEC: 0.206 ppm

Terminal Length
NOAEC: 0.830 ppm
LOAEC: >0.830 ppm

Endpoint(s) Affected: Offspring production, time to first brood release
Most sensitive endpoint(s): Offspring production

III. REFERENCES:

No references were cited.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Daphnia adult length - desthio
File: jdapleng.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.206	0.034	0.603
Within (Error)	61	3.468	0.057	
Total	67	3.674		

Critical F value = 2.25 (0.05,6,60)
Since F < Critical F FAIL TO REJECT Ho: All equal

Daphnia sum of offspring per adult - desthio
File: jpapsum.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	34779.618	5796.603	38.117
Within (Error)	61	9276.500	152.074	
Total	67	44056.118		

Critical F value = 2.25 (0.05,6,60)
Since F > Critical F REJECT Ho: All equal

Daphnia sum of offspring per adult - desthio
File: jpapsum.dat 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	solv control	92.700	92.700		
2	.025	91.500	91.500	0.218	
3	.052	96.000	96.000	-0.582	
4	.103	85.700	85.700	1.269	
5	.206	77.100	77.100	2.829	*
6	.408	73.667	73.667	3.359	*
7	.830	25.900	25.900	12.113	*

Bonferroni t table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)

Daphnia sum of offspring per adult - desthio
File: jpapsum.dat 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	solv control	10			
2	.025	10	13.583	14.7	1.200
3	.052	9	13.956	15.1	-3.300
4	.103	10	13.583	14.7	7.000
5	.206	10	13.583	14.7	15.600
6	.408	9	13.956	15.1	19.033
7	.830	10	13.583	14.7	66.800

Daphnia sum of offspring per adult - desthio
File: jpapsum.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solv control	10	92.700	92.700	93.310
2	.025	10	91.500	91.500	93.310
3	.052	9	96.000	96.000	93.310
4	.103	10	85.700	85.700	85.700
5	.206	10	77.100	77.100	77.100
6	.408	9	73.667	73.667	73.667
7	.830	10	25.900	25.900	25.900

Daphnia sum of offspring per adult - desthio
File: jpapsum.dat 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
solv control	93.310				
.025	93.310	0.111		1.67	k= 1, v=61
.052	93.310	0.108		1.75	k= 2, v=61
.103	85.700	1.269		1.77	k= 3, v=61
.206	77.100	2.829	*	1.78	k= 4, v=61
.408	73.667	3.359	*	1.79	k= 5, v=61
.830	25.900	12.113	*	1.79	k= 6, v=61

s = 12.332
Note: df used for table values are approximate when v > 20.

Daphnia offspring/parent/reproduction day- desthio
File: jdaprep.dat Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	211.616	35.269	28.518
Within (Error)	61	75.441	1.237	
Total	67	287.056		

Critical F value = 2.25 (0.05,6,60)
Since F > Critical F REJECT Ho: All equal

Daphnia offspring/parent/reproduction day - desthio
File: jdaprep.dat 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	solv control	7.745	7.745		
2	.025	7.418	7.418	0.657	
3	.052	7.887	7.887	-0.277	
4	.103	7.211	7.211	1.074	
5	.206	6.586	6.586	2.330	
6	.408	6.448	6.448	2.539	*
7	.830	2.435	2.435	10.677	*

Bonferroni t table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)

Daphnia offspring/parent/reproduction day - desthio
File: jdaprep.dat 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	solv control	10			
2	.025	10	1.225	15.8	0.327
3	.052	9	1.259	16.2	-0.142
4	.103	10	1.225	15.8	0.534
5	.206	10	1.225	15.8	1.159
6	.408	9	1.259	16.2	1.297
7	.830	10	1.225	15.8	5.310

Daphnia offspring/parent/reproduction day - desthio
File: jdaprep.dat Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solv control	10	7.745	7.745	7.745

2	.025	10	7.418	7.418	7.640
3	.052	9	7.887	7.887	7.640
4	.103	10	7.211	7.211	7.211
5	.206	10	6.586	6.586	6.586
6	.408	9	6.448	6.448	6.448
7	.830	10	2.435	2.435	2.435

Daphnia offspring/parent/reproduction day - desthio
File: jdaprep.dat 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
solv control	7.745				
.025	7.640	0.211		1.67	k= 1, v=61
.052	7.640	0.205		1.75	k= 2, v=61
.103	7.211	1.074		1.77	k= 3, v=61
.206	6.586	2.330	*	1.78	k= 4, v=61
.408	6.448	2.539	*	1.79	k= 5, v=61
.830	2.435	10.677	*	1.79	k= 6, v=61

s = 1.112
Note: df used for table values are approximate when v > 20.

Daphnia first day of reproduction - desthio
File: jdapfday.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	20.658	3.443	5.306
Within (Error)	61	39.578	0.649	
Total	67	60.235		

Critical F value = 2.25 (0.05,6,60)
Since F > Critical F REJECT Ho: All equal

Daphnia first day of reproduction - desthio
File: jdapfday.dat 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	solv control	10.000	10.000		
2	.025	9.600	9.600	1.110	
3	.052	9.778	9.778	0.600	
4	.103	10.100	10.100	-0.278	
5	.206	10.300	10.300	-0.833	
6	.408	10.444	10.444	-1.201	
7	.830	11.400	11.400	-3.886	

Bonferroni t table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)

Daphnia first day of reproduction - desthio
File: jdapfday.dat 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	solv control	10			
2	.025	10	0.887	8.9	0.400
3	.052	9	0.912	9.1	0.222
4	.103	10	0.887	8.9	-0.100
5	.206	10	0.887	8.9	-0.300
6	.408	9	0.912	9.1	-0.444
7	.830	10	0.887	8.9	-1.400

Daphnia first day of reproduction - desthio
File: jdapfday.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solv control	10	10.000	10.000	9.793
2	.025	10	9.600	9.600	9.793
3	.052	9	9.778	9.778	9.793
4	.103	10	10.100	10.100	10.100
5	.206	10	10.300	10.300	10.300
6	.408	9	10.444	10.444	10.444
7	.830	10	11.400	11.400	11.400

Daphnia first day of reproduction - desthio
File: jdapfday.dat 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
solv control	9.793				
.025	9.793	0.574		1.67	k= 1, v=61
.052	9.793	0.559		1.75	k= 2, v=61
.103	10.100	0.278		1.77	k= 3, v=61
.206	10.300	0.833		1.78	k= 4, v=61
.408	10.444	1.201		1.79	k= 5, v=61
.830	11.400	3.886	*	1.79	k= 6, v=61

s = 0.805
Note: df used for table values are approximate when v > 20.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 24, 2005

PMRA Submission Number: 2004-0843

Study Type: Chronic (Life-Cycle) Toxicity to *Daphnia* sp.

Dorgerloh, M., and H. Sommer. 2001. Influence of JAU6476-Desthio on the Reproduction Rate of Water Fleas in a Static Renewal Laboratory Test System. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany. Laboratory ID No. E 3211987-5; Report No. DOM 21036. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated January 9, 2001 and completed September 10, 2001.

PMRA DATA CODE	9.3.5
EPA DP Barcode	D303488
OECD Data Point	IIIA 10.2.6.1
EPA MRID	46246029
EPA Guideline	§72-4b

Reviewing Agency: U.S. EPA

EAD Executive Summary:

The 21-day-chronic toxicity of the transformation product JAU 6476-desthio (96.5% Prothioconazole - desthio) to *Daphnia magna* was studied under static renewal conditions. The study was conducted following OECD Guideline 211 and EPA FIFRA Guideline 72-4, and was in compliance with German and OECD Principles of GLP. Nominal concentrations were 0 (negative and solvent controls), 0.025, 0.05, 0.10, 0.20, 0.40, and 0.80 mg JAU6476-desthio/L, which correspond to mean measured concentrations of 0 (controls), 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 mg JAU6476-desthio/L. Eight media renewals were performed. "New" test media was sampled from all test levels at 0, 9, and 19 Days, and "old" test media (after 48 or 72 hours) was sampled from the negative control, 0.025, 0.103, and 0.830 mg JAU6476-desthio/L levels on Days 2, 12, and 21. Recoveries for all samples ranged from 96 to 109% of nominal concentrations indicating precision and stability. Since "old" treatment solutions were not sampled for all toxicant levels and recoveries were high for those "new and old" treatment solutions sampled, mean measured concentrations were based on all available data.

After 21 days of exposure, mortality was ≤10% for all test and control groups. The 21-day LC_{50} was >0.830 mg JAU6476-desthio/Ln, and the NOEC for mortality was 0.830 mg JAU6476-

desthio/L.

The mean first day of reproduction (time to first brood release) was 9.9, 10.0, 9.6, 9.8, 10.1, 10.3, 10.4, and 11.4 for the negative control, solvent control, 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 mg JAU6476-desthio/L test groups, respectively, and the NOEC was 0.408 mg JAU6476-desthio/L. At the 0.830 mg JAU6476-desthio/L level, dead offspring were found regularly during the study, and abnormal behaviour of juvenile organisms was observed, including lack of coordination in swimming, lying at the bottom, and clinging to the water surface. The sum of offspring per parent averaged 89.3, 92.7, 91.5, 96.0, 85.7, 77.1, 73.7, and 25.9 for the negative control, solvent control, 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 mg JAU6476-desthio/L test groups, respectively. The number of offspring per parent and reproduction day averaged 7.37, 7.74, 7.42, 7.89, 7.21, 6.59, 6.45, and 2.44 for the negative control, solvent control, 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 mg JAU6476-desthio/L test groups, respectively. For both endpoints, data were significantly different from the solvent control at concentrations ≥0.206 mg JAU6476-desthio/L. The subsequent NOEC was 0.103 mg JAU6476-desthio/L for the sum of offspring per parent and the number of offspring per parent and reproduction day.

A statistically-significant reduction compared to the solvent control in terminal lengths was not observed. Body lengths of surviving daphnids averaged 4.08 mm for the solvent control, and 4.22, 4.13, 4.18, 4.22, 4.16, and 4.09 mm for the 0.025, 0.052, 0.103, 0.206, 0.408, and 0.83 mg JAU6476-desthio/L test groups, respectively. The NOEC based on length was 0.830 mg JAU6476-desthio/L.

Results Synopsis:

Test Organism Age (eg. 1st instar): First instar, <24 hours old
Test Type (Flow through, Static, Static Renewal): Static-renewal

Mortality/immobilization:
NOEC: 0.830 mg JAU6476-desthio/L
LOEC: >0.830 mg JAU6476-desthio/L
LC_{50}: >0.830 mg JAU6476-desthio/L

Time to First Brood Release
NOEC: 0.408 mg JAU6476-desthio/L
LOEC: 0.830 mg JAU6476-desthio/L

Sum of Offspring/Parent
NOEC: 0.103 mg JAU6476-desthio/L
LOEC: 0.206 mg JAU6476-desthio/L

Number of Offspring/Parent/Reproduction Day
NOEC: 0.103 mg JAU6476-desthio/L
LOEC: 0.206 mg JAU6476-desthio/L

Terminal Length
NOEC: 0.830 mg JAU6476-desthio/L
LOEC: >0.830 mg JAU6476-desthio/L

Endpoint(s) Affected: Offspring production, time to first brood release
Most sensitive endpoint(s): Offspring production

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, name of PMRA secondary reviewer) was added to the EPA-DER as well as information on the chemical name (IUPAC name, CAS name and synonym) available from the PMRA Chemistry review.

2. The water hardness and the pH were within the limits recommended by the OECD guideline. A comment was added in the Comments section in Table 1, under Water parameters for clarification purposes.

3. The OECD guideline requires that at least 10 animals, held individually, be used in semi-static tests. The Remark on test design in Table 1 for the Number of Organisms was modified to clarify that even though the design did not follow EPA guidance, it was conducted according to OECD Guideline 211.

4. Measuring old solutions in all treatment vessels is not required according to OECD guideline 211 if the concentration is expected to remain within ±20 percent of the nominal concentration. As a minimum, the highest and lowest concentrations are to be analyzed when freshly prepared and at the time of renewal on one occasion during the first week of the test and repeated at weekly intervals thereafter.

5. Measurements of dry weight are not required under OECD Guideline 211. A clarification has been added to the Remarks column in Table 2.

6. The EAD reviewer verified the statistical analyses with SigmaStat sofware. Total length was analyzed using a Kruskal-Wallis One Way ANOVA on Ranks, as the assumptions of normality and homogeneity of variances were not met. No significant differences were found between any treatment and the solvent control. A t-test showed that the control and solvent control differed in

mean total length. In all tests, the same conclusions were reached when combining controls (when appropriate), or when using the solvent control only.

The EAD reviewer obtained identical conclusions as the EPA reviewer for mortality, sum of offspring, and time to first brood. A NOEC of 0.408 mg JAU6476-desthio/L was obtained for the number of offspring/parent/reproduction day, which is 2 treatment levels higher than the the NOEC obtained by the EPA reviewer and the study author. The differences may be a result of different statistical packages. The results of the EPA reviewer are acceptable.

Study Acceptability: The study is scientifically sound, and fulfills the guideline requirements for an aquatic invertebrate life cycle test with the *Daphnia magna*.

Total length

t-test Wednesday, August 24, 2005, 11:28:13

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.059)

Equal Variance Test: Passed (P = 0.078)

Group Name	N	Missing	Mean	Std Dev	SEM
control	10	1	4.283	0.141	0.0471
solvent	10	0	4.080	0.101	0.0318

Difference 0.203

t = 3.642 with 17 degrees of freedom. (P = 0.002)

95 percent confidence interval for difference of means: 0.0855 to 0.321

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.002).

Power of performed test with alpha = 0.050: 0.924

One Way Analysis of Variance Wednesday, August 24, 2005, 11:44:04

Data source: Data 1 in Notebook

Normality Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, August 24, 2005, 11:44:04

Data source: Data 1 in Notebook

Group	N	Missing	Median	25%	75%
solvent	10	0	0.607	0.602	0.613
0.025 mg/L	10	0	0.633	0.613	0.653
0.052 mg/L	10	1	0.628	0.588	0.635
0.103 mg/L	10	0	0.626	0.613	0.628
0.206 mg/L	10	0	0.633	0.623	0.633
0.408 mg/L	10	1	0.618	0.610	0.630
0.83 mg/L	10	0	0.613	0.602	0.628

H = 11.178 with 6 degrees of freedom. (P = 0.083)

The differences in the median 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 = 0.083)

Number of offspring/parent/reproduction day

One Way Analysis of Variance Wednesday, August 24, 2005, 13:24:49

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.130)

Group Name	N	Missing	Mean	Std Dev	SEM
solvent	10	0	7.745	0.888	0.281
0.025 mg/L	10	0	7.418	1.466	0.463
0.052 mg/L	10	1	7.887	1.362	0.454
0.103 mg/L	10	0	7.211	0.593	0.188
0.206 mg/L	10	0	6.586	1.195	0.378
0.408 mg/L	10	1	6.448	1.322	0.441
0.83 mg/L	10	0	2.435	0.681	0.215

Source of Variation	DF	SS	MS	F	P
Between Groups	6	211.616	35.269	28.518	<0.001
Residual	61	75.441	1.237		
Total	67	287.056			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: treatment

Comparison	Diff of Means	q'	P	P<0.050
solvent vs. 0.83 mg/L	5.310	10.677	--	Yes
solvent vs. 0.408 mg/L	1.297	2.539	--	No
solvent vs. 0.206 mg/L	1.159	2.330	--	Do Not Test
solvent vs. 0.103 mg/L	0.534	1.074	--	Do Not Test
solvent vs. 0.025 mg/L	0.327	0.657	--	Do Not Test
solvent vs. 0.052 mg/L	0.142	0.277	--	Do Not Test

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are

greater or less than the critical values of .05 and .01.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Brood time

One Way Analysis of Variance Wednesday, August 24, 2005, 13:34:33

Data source: Data 1 in Notebook

Normality Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, August 24, 2005, 13:34:33

Data source: Data 1 in Notebook

Group	N	Missing	Median	25%	75%
solvent	10	0	10.000	10.000	10.000
0.025 mg/L	10	0	10.000	10.000	10.000
0.052 mg/L	10	0	10.000	10.000	10.000
0.103 mg/L	10	0	10.000	10.000	11.000
0.206 mg/L	10	0	10.000	10.000	11.000
0.408 mg/L	10	0	10.000	10.000	11.000
0.83 mg/L	10	0	11.000	11.000	12.000

H = 25.931 with 6 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunn's Method) :

Comparison	Diff of Ranks	Q	P<0.05
0.83 mg/L vs solvent	32.500	3.571	Yes
0.408 mg/L vs solvent	10.900	1.198	No
0.206 mg/L vs solvent	9.050	0.994	Do Not Test
0.103 mg/L vs solvent	5.100	0.560	Do Not Test

0.052 mg/L vs solvent	1.900	0.209	Do Not Test
0.025 mg/L vs solvent	1.050	0.115	Do Not Test

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Sum of offspring

One Way Analysis of Variance Wednesday, August 24, 2005, 12:01:57

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.341)

Group Name	N	Missing	Mean	Std Dev	SEM
solvent	10	0	92.700	10.446	3.303
0.025 mg/L	10	0	91.500	15.792	4.994
0.052 mg/L	10	1	96.000	14.748	4.916
0.103 mg/L	10	0	85.700	9.696	3.066
0.206 mg/L	10	0	77.100	14.843	4.694
0.408 mg/L	10	1	73.667	11.113	3.704
0.83 mg/L	10	0	25.900	7.400	2.340

Source of Variation	DF	SS	MS	F	P
Between Groups	6	34779.618	5796.603	38.117	<0.001
Residual	61	9276.500	152.074		
Total	67	44056.118			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: treatment

Comparison	Diff of Means	q'	P	P<0.050
solvent vs. 0.83 mg/L	66.800	12.113	--	Yes
solvent vs. 0.408 mg/L	19.033	3.359	--	Yes
solvent vs. 0.206 mg/L	15.600	2.829	--	Yes
solvent vs. 0.103 mg/L	7.000	1.269	--	No
solvent vs. 0.052 mg/L	3.300	0.582	--	Do Not Test
solvent vs. 0.025 mg/L	1.200	0.218	--	Do Not Test

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.