

US EPA ARCHIVE DOCUMENT

MRID No. 439965-03

**DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)**

1. **CHEMICAL:** Linuron PC Code No.: 035506
2. **TEST MATERIAL:** DPX-Z326-198 Purity: 98.4%
3. **CITATION:**
Authors: R.L. Boeri, J.P. Magazu, and T.J. Ward
Title: Chronic Toxicity of DPX-Z326-198
(Linuron) to the Mysid, *Mysidopsis bahia*
Study Completion Date: April, 22, 1996
Laboratory: T.R. Wilbury Laboratories, Inc.,
Marblehead, MA
Sponsor: E.I. du Pont de Nemours and Company,
Newark, DE
Laboratory Report ID: 814-DU
MRID No.: 439965-03
DP Barcode: D226341
4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Environmental
Scientist, KBN Engineering and Applied Sciences, Inc.
Signature: *[Handwritten Signature]* Date: 6/20/96
APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
KBN Engineering and Applied Sciences, Inc.
Signature: P. Kosalwat Date: 6/20/96
5. **APPROVED BY:**
Signature: *[Handwritten Signature]* Date: 2/16/99
6. **STUDY PARAMETERS:**
Age of Test Organism: <24 hours
Definitive Test Duration: 28 days
Study Method: Flow-Through
Type of Concentrations: Mean Measured
7. **CONCLUSIONS:** This study is scientifically sound but does not
fulfill the guideline requirements for an estuarine
invertebrate life cycle test using mysids. All treatment
levels tested adversely affected the mysids. The LOEC for
mysids exposed to DPX-Z326-198 was 0.297 ppm based on mean
measured concentration. An NOEC and MATC could not be
determined.



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Results Synopsis

Most sensitive endpoint: Male length
NOEC: Not determined **LOEC:** 0.297 ppm **MATC:** Not determined

LOEC's for specific effects

# Young/Female:	>1.10 ppm
Survival:	0.582 ppm
Male length:	0.297 ppm
Female length:	>1.10 ppm
Male dry weight:	1.10 ppm
Female dry weight:	0.582 ppm

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental.

B. Rationale: All treatment levels adversely affected the mysids. An NOEC and MATC could not be determined in this study.

C. Repairability: No.

9. MAJOR GUIDELINE DEVIATIONS: Since there is no EPA's SEP for a mysid life cycle toxicity test, ASTM's Standard Guide for Conducting Life-Cycle Toxicity Tests With Saltwater Mysids (E1191-90) was used as a guidance for this data evaluation. Deviations from ASTM's guidelines are noted as follows:

1. Growth of exposed mysids (male length) was reduced at all toxicant concentrations with surviving mysids. One concentration must not affect any life stage.
2. The data were not analyzed separately based on sex as recommended.

10. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably <i>Mysidopsis bahia</i>	<i>Mysidopsis bahia</i>
Duration 28 days/one generation	28 days
Source (or supplier)	In-house cultures

Guideline Criteria	Reported Information
<p>Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.</p> <p>2) Mysids should be in good health.</p>	<p>1. Mysid cultures were acclimated in the dilution water at test temperature.</p> <p>2. Yes</p>
<p>Parental Acclimation Period At least 14 days</p>	<p>≥14 days</p>
<p>Chamber Location: Treatments should be randomly assigned to test chamber locations.</p>	<p>Yes</p>
<p>Duration of the Test: A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment.</p>	<p>Test was conducted for 28 days. Sexual maturation of surviving mysids was ≥13 days.</p>
<p>Brood Stock: Test started with mysids: 1) from only one brood stock or</p> <p>2) from brood stock which has not obtain sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.</p>	<p>1. Yes</p> <p>2. N/A</p>

Guideline Criteria	Reported Information
<p>Distribution: No. of mysids before pairing: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/level.</p> <p>No. of mysids after pairing: ≥ 20 randomly selected pairs/treatment (excess males should be held in separate compartment to replace paired males).</p>	<p>Before pairing: 15 mysids/retention chamber; 2 chambers/test vessel; 2 replicate vessels/ treatment (60 mysids/level).</p> <p>After pairing: Up to 10 pairs/replicate vessel. Excess mysids were held in a two extra test chambers.</p>
<p>Pairing: 1) Should be conducted when most of the mysids are sexually mature (usually 10-14 days after test initiation).</p> <p>2) Should be paired on the same day.</p>	<p>1. Day 13 for the controls and two lowest test concentrations, and day 15 for the third lowest test level. There was no pairing in the two highest test concentrations (dead or not reach sexual maturation).</p> <p>2. Not on the same day.</p>
<p>Feeding: 1) Mysids should be fed live brine shrimp nauplii at least once daily.</p> <p>2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.</p>	<p>1. Newly hatched <i>Artemia Salina</i> nauplii 2-3 times a day</p> <p>2. 150 nauplii/mysid/day</p>
<p>Counts: Live adult mysids should be counted at test initiation, at pairing, and daily after pairing.</p> <p>Live young must be counted and removed daily.</p> <p>Missing or impinged animals should be recorded.</p>	<p>Live adults and young were counted according to the schedule at left.</p> <p>Occurrence of sublethal effects on behavior or appearance were recorded at 24-hour intervals. Dead animals were removed when observed.</p>

Guideline Criteria	Reported Information
<p>Controls: Survival in any control chamber (between pairing and test termination) must not be less than 70%.</p>	<p>85% survival in the dilution water control and 87% survival in the solvent control based on total number of mysids exposed. Survival of paired adults was 100% in both controls.</p>
<p>Controls: Negative control and carrier control (when applicable) are required.</p>	<p>A dilution water control and a solvent control were included.</p>

Comments: None

B. Physical System:

Guideline Criteria	Reported Information
<p>Test Water:</p> <p>1) May be natural (sterilized and filtered) or a commercial mixture with a salinity between 15 and 30 g/kg, and free of pollutants.</p> <p>2) During the test, salinity should be measured daily and the difference between highest and lowest must be less than 10 g/kg.</p> <p>3) pH should be measured at the beginning, end of test and weekly. Measured values should be between 7.6 and 8.2, and not deviate by more than one unit for more than 48 hours.</p> <p>6) DO must be measured at each conc. at least once a week. (see details in ASTM)</p>	<p>1. Natural seawater (aerated, filtered, and U.V. sterilized) with salinity adjusted with deionized water.</p> <p>2. Salinity measured daily was between 15 and 16 ‰ during the test.</p> <p>3. pH measured daily in each test vessel was between 7.8 and 8.4 during the test.</p> <p>6. DO measured daily in each test vessel was maintained at $\geq 75\%$ of saturation throughout the test.</p>

Guideline Criteria	Reported Information
<p>Test Temperature:</p> <p>1) Mean measured temperature for each chamber at test termination should be within 1°C of selected test temperature. For mysid shrimp, 27°C is recommended.</p> <p>2) Each individual measured temperature must be within 3°C of the mean of the time-weighted averages.</p> <p>4) Whenever temperature is measured concurrently in more than one test chamber the highest & lowest temp. must not differ by more than 2°C.</p>	<p>Target temperature was 25 ±2°C. The highest temperature and lowest temperature did not differ more than 2°C on any given day.</p>
<p>Photoperiod: Recommend 16L/8D.</p>	<p>16-hour light/8-hour dark</p>
<p>Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters with a dilution factor not greater than 0.5 (a minimum of 5 toxicant concentrations and a control).</p>	<p>Intermittent-flow proportional diluter with a dilution factor of approximately 0.5.</p>
<p>Toxicant Mixing:</p> <p>1) Mixing chamber is recommended but not required;</p> <p>2) Aeration should not be used for mixing;</p> <p>3) It must be demonstrated that the test solution is completely mixed before intro. into the test system;</p> <p>4) Flow splitting accuracy must be within 10%.</p>	<p>1. Mixing chambers were used.</p> <p>2. The diluter toxicant solution was mixed by a high shear pump.</p> <p>3. Chemical analysis showed consistent levels of toxicant in solution.</p> <p>4. Not reported.</p>

Guideline Criteria	Reported Information
<p>Test Vessels:</p> <p>1) Material: all glass, No. 316 stainless steel, or perfluorocarbon plastic.</p> <p>2) Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume acceptable.</p> <p>3) Test compartments: 90 or 140 mm inside diameter glass Petri dish bottoms with collars made of 200-250 μm mesh screen.</p>	<p>1. Glass</p> <p>2. 20-liter aquaria (20 X 40 X 26 cm) with a fill volume up to 8 liters.</p> <p>3. Before paring, two retention chambers or compartments (10-cm dia. glass petri dishes with 15-cm high Nitex screen collars) were placed in each test vessel. Upon pairing, ≤ 12 retention chambers (6-cm dia. petri dishes with 12-cm high Nitex screen collars) were used in each test vessel (10 for mysid pairs; 2 for extra mysids).</p>
<p>Covers</p> <p>1) Renewal: Test vessels should be covered with a glass plate.</p> <p>2) Flow-through: Openings in the test compartments should be covered with nylon mesh or stainless steel screen.</p>	<p>1. N/A</p> <p>2. Test vessels were loosely covered.</p>
<p>Flow Rate:</p> <p>1) Flow rates should provide 5 to 10 volume additions per 24 hr.</p> <p>2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level.</p> <p>3) Meter systems calibrated before study and checked twice daily during test period.</p>	<p>1. 9.6 volume additions/24 hours</p> <p>2. DO maintained $\geq 75\%$ of saturation.</p> <p>3. Yes</p>

Guideline Criteria	Reported Information
<p>Aeration:</p> <p>1) Dilution water should be aerated to insure DO concentration at or near 100% saturation.</p> <p>2) Test tanks may be aerated.</p>	<p>1. Dilution water was aerated prior to use.</p> <p>2. No aeration.</p>

Comments: None

C. Chemical System:

Guideline Criteria	Reported Information
<p>Concentrations:</p> <p>1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <p>2) Toxicant conc. must be measured in one tank at each toxicant level every week.</p> <p>3) One concentration must adversely affect a life stage and one concentration must not affect any life stage.</p> <p>4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured concentration for >10% of the duration of the test.</p> <p>5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.</p>	<p>1. A dilution water control; solvent control (0.1 ml/L DMF); 0.30, 0.65, 1.3, 2.5, and 5.0 mg/L.</p> <p>2. Toxicant concentrations were measured in each test vessel at least weekly.</p> <p>3. NOEC was not obtained.</p> <p>4. Measured concentrations were consistent over the test period.</p> <p>5. See above.</p>

Guideline Criteria	Reported Information
<p>Solvents: 1) Should not exceed 0.1 ml/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.</p>	<p>1. Maximum solvent concentration: 0.1 ml DMF/L. 2. DMF</p>

Comments: None

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes</p>
<p>1) At least 75% of the paired 1st generation females in the control produced young or 2) the average number of young produced by the 1st generation females in the control(s) was more than 3.</p>	<p>1. Yes 2. Yes</p>
<p>Data Endpoints must include: · Survival of first-generation mysids (female and male), · Number of live young produced per female, · Dry weight and length of each first-generation mysid alive at the end of the test (female and male), · Incidence of pathological or histological effects, and observations of other effects or clinical signs.</p>	<p>All data endpoints listed at left were reported.</p>

Guideline Criteria	Reported Information
Raw data included? (Y/N)	Yes

Effects Data:

Concentration (mg/L)		Mean # Young/fem./repro. day	Survival (28 days)			Mean Total Length (mm)			Mean Dry Weight (mg)		
Nom.	Meas.		♂ ¹	♀ ¹	♂ & ♀ ²	♂	♀	♂ & ♀	♂	♀	♂ & ♀
Cont.	<0.10	7.2	100	100	85.0	8.74	8.75	8.8	0.76	1.00	0.87
Solv Cont.	<0.10	8.3	100	100	86.7	8.78	8.73	8.8	0.77	0.93	0.85
0.30	0.297	11.5	100	100	85.0	8.68	8.73	8.5	0.72	1.01	0.82
0.65	0.582	9.7	72	78	60.0	8.6	8.68	8.6	0.65	0.79	0.72
1.3	1.10	4.6	91	100	43.3	8.64	8.68	8.7	0.59	0.73	0.68
2.5	2.53	0.0	-	-	0	-	-	-	-	-	-
5.0	4.29	0.0	-	-	0	-	-	-	-	-	-

¹ Represents survival of those adults paired in spawning compartments.

² Represents survival of all first generation mysids (i.e., those paired for spawning and those maintained as extras).

Toxicity Observations: During the first six days of the study, erratic swimming and lethargy were exhibited by mysids in the two highest test concentrations. All mysids in the two highest concentrations were dead by Day 7. No other sublethal effects were observed.

Statistical Results:

Endpoint	Method	NOEC	LOEC
Survival	ANOVA and Bonferroni's t test	0.297 ppm	0.582 ppm
# Young/female	ANOVA and Bonferroni's t test	1.10 ppm	2.53 ppm

Length	ANOVA and Bonferroni's t test	1.10 ppm	2.53 ppm
Wet Weight	ANOVA and Bonferroni's t test	0.297 ppm	0.582 ppm
Dry Weight	ANOVA and Bonferroni's t test	0.297 ppm	0.582 ppm

Comments: Treatment data were compared to the pooled control data. Data were not analyzed by sex, but rather male and female data were analyzed collectively.

12. Reviewer's Statistical Results:

Endpoint	Method	NOEC	LOEC
Survival	Williams'	0.297 ppm	0.582 ppm
# Young/female	Williams'	1.10 ppm	>1.10 ppm
Male Length	Bonferroni's	ND	0.297 ppm
Female Length	Bonferroni's	1.10 ppm	>1.10 ppm
Male Dry Weight	Bonferroni's	0.582 ppm	1.10 ppm
Female Dry Weight	Bonferroni's	0.297 ppm	0.582 ppm

ND = Not determined.

Comments: Wet weight data were not analyzed by the reviewer. According to the ASTM, "wet weight is not acceptable due to large variations resulting from retention of water in female brood sacs." Male and female growth data were combined and statistically analyzed by the authors. These data should have been analyzed separately based on sex. The reviewer's results are more conservative than the authors' results.

13. Reviewer's Conclusions: The length of the first-generation male mysids were significantly reduced at all treatment levels when compared to that of the solvent control. Therefore, the NOEC could not be determined. This study is scientifically sound but does not fulfill the guideline requirements. The study is classified as **Supplemental**.

N's, means and standard deviations based on dependent variable: Male Length (mm)

* Indicates statistics are collapsed over this factor

Factors: T R	N	Mean	S.D.
**	110	8.7018	0.1375
1 * Control	26	8.7423	0.1554
2 * Solvent Control	24	8.7833	0.1373
3 * 0.297 mg/L	32	8.6844	0.1019
4 * 0.582 mg/L	18	8.6000	0.1029
5 * 1.10 mg/L	10	8.6400	0.0966
* 1	50	8.7120	0.1560
* 2	60	8.6933	0.1206
1 1	14	8.7429	0.1828
1 2	12	8.7417	0.1240
2 1	11	8.8273	0.1421
2 2	13	8.7462	0.1266
3 1	12	8.6917	0.0996
3 2	20	8.6800	0.1056
4 1	6	8.6000	0.1265
4 2	12	8.6000	0.0953
5 1	7	8.6000	0.0816
5 2	3	8.7333	0.0577

A total of 18 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

Fmax for testing homogeneity of between subjects variances: 10.02
 Number of variances= 10 df per variance=

Analysis of Variance		Dependent variable: Male Length			
Source	df	SS (H)	MSS	F	P
Between Subjects	109	2.0596			
T (TRT)	4	0.4367	0.1092	7.064	0.0000
R (REP)	1	0.0034	0.0034	0.222	0.6388
TR	4	0.0741	0.0185	1.199	0.3135
Subj w Groups	100	1.5454	0.0155		

Post-hoc tests for factor T (TRT)

Level	Mean
1	8.742
2	8.783
3	8.684
4	8.600
5	8.640

Comparison	Tukey-A*	Bonferroni
1 < 2		
1 > 3		
1 > 4	0.0100	0.0033
1 > 5		
2 > 3		0.0399
2 > 4	0.0100	0.0000
2 > 5	0.0100	0.0282
3 > 4		
3 > 5		
4 < 5		

Male length was significantly lower than that of the control at all test levels.

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
 A blank means the P-value is greater than 0.0500.

Analysis of Variance

File: Linuron/Mysid

Date: 06-18-1996

N's, means and standard deviations based on dependent variable: Female Length (mm)

* Indicates statistics are collapsed over this factor

Factors: T R	N	Mean	S.D.
**	106	8.7170	0.1175
1 * Control	25	8.7480	0.1418
2 * Solvent Control	28	8.7250	0.1076
3 * 0.297 mg/L	19	8.7316	0.1376
4 * 0.582 mg/L	18	8.6833	0.0925
5 * 1/10 mg/L	16	8.6750	0.0775
* 1	58	8.7155	0.1182
* 2	48	8.7187	0.1179
1 1	11	8.7182	0.1722
1 2	14	8.7714	0.1139
2 1	15	8.7400	0.0910
2 2	13	8.7077	0.1256
3 1	13	8.7308	0.1377
3 2	6	8.7333	0.1506
4 1	11	8.6818	0.0982
4 2	7	8.6857	0.0900
5 1	8	8.6875	0.0641
5 2	8	8.6625	0.0916

A total of 22 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

Fmax for testing homogeneity of between subjects variances: 7.22
 Number of variances= 10 df per variance= 9.

Analysis of Variance Dependent variable: Female Length

Source	df	SS (H)	MSS	F	P
Between Subjects	105	1.4494			
T (TRT)	4	0.0785	0.0196	1.402	0.2375
R (REP)	1	0.0000	0.0000	0.003	0.9598
TR	4	0.0273	0.0068	0.487	0.7467
Subj w Groups	96	1.3436	0.0140		

Post-hoc tests for factor T (TRT)

Level	Mean
1	8.748
2	8.725
3	8.732
4	8.683
5	8.675

Comparison Tukey-A* Bonferroni

- 1 > 2
- 1 > 3
- 1 > 4
- 1 > 5
- 2 < 3
- 2 > 4
- 2 > 5
- 3 > 4
- 3 > 5
- 4 > 5

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
 A blank means the P-value is greater than 0.0500.

N's, means and standard deviations based on dependent variable: Male Dry Weight (mg)

* Indicates statistics are collapsed over this factor

Factors: T R	N	Mean	S.D.
**	110	0.7158	0.1483
1 * Control	26	0.7577	0.1081
2 * Solvent Control	24	0.7692	0.1723
3 * 0.297 mg/L	32	0.7178	0.1282
4 * 0.582 mg/L	18	0.6489	0.1194
5 * 1.10 mg/L	10	0.5930	0.1900
* 1	50	0.7206	0.1511
* 2	60	0.7118	0.1471
1 1	14	0.7914	0.1017
1 2	12	0.7183	0.1057
2 1	11	0.7773	0.1588
2 2	13	0.7623	0.1892
3 1	12	0.6983	0.1157
3 2	20	0.7295	0.1366
4 1	6	0.5983	0.0987
4 2	12	0.6742	0.1245
5 1	7	0.6329	0.2162
5 2	3	0.5000	0.0600

A total of 18 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

Fmax for testing homogeneity of between subjects variances: 12.99
Number of variances= 10 df per variance= 7.

Analysis of Variance Dependent variable: Male Dry Weight

Source	df	SS (H)	MSS	F	P
Between Subjects	109	2.3965			
T (TRT)	4	0.3455	0.0864	4.435	0.0024
R (REP)	1	0.0035	0.0035	0.182	0.6709
TR	4	0.0997	0.0249	1.279	0.2812
Subj w Groups	100	1.9478	0.0195		

Post-hoc tests for factor T (TRT)

Level	Mean
1	0.758
2	0.769
3	0.718
4	0.649
5	0.593

Comparison	Tukey-A*	Bonferroni
1 < 2		
1 > 3		
1 > 4		
1 > 5	0.0100	0.0203
2 > 3		
2 > 4		
2 > 5	0.0100	0.0114
3 > 4		
3 > 5		
4 > 5		

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

N's, means and standard deviations based on dependent variable: Female Dry Weight (mg)

* Indicates statistics are collapsed over this factor

Factors: T R	N	Mean	S.D.
**	106	0.9051	0.1710
1 * Control	25	0.9968	0.1594
2 * Solvent Control	28	0.9268	0.1229
3 * 0.297 mg/L	19	1.0047	0.1439
4 * 0.582 mg/L	18	0.7939	0.1218
5 * 1.10 mg/L	16	0.7306	0.1431
* 1	58	0.9221	0.1849
* 2	48	0.8846	0.1520
1 1	11	1.0518	0.1936
1 2	14	0.9536	0.1163
2 1	15	0.9680	0.1183
2 2	13	0.8792	0.1143
3 1	13	1.0062	0.1584
3 2	6	1.0017	0.1196
4 1	11	0.7473	0.1192
4 2	7	0.8671	0.0901
5 1	8	0.7612	0.1089
5 2	8	0.7000	0.1729

A total of 22 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

Fmax for testing homogeneity of between subjects variances: 4.61
 Number of variances= 10 df per variance= 9.

Analysis of Variance Dependent variable: Female Dry Weight

Source	df	SS (H)	MSS	F	P
Between Subjects	105	3.0716			
T (TRT)	4	1.1217	0.2804	15.304	0.0000
R (REP)	1	0.0372	0.0372	2.030	0.1575
TR	4	0.1537	0.0384	2.097	0.0865
Subj w Groups	96	1.7591	0.0183		

Post-hoc tests for factor T (TRT)

Level	Mean
1	0.997
2	0.927
3	1.005
4	0.794
5	0.731

Comparison	Tukey-A*	Bonferroni
1 > 2		
1 < 3		
1 > 4	0.0100	0.0000
1 > 5	0.0100	0.0000
2 < 3		
2 > 4	0.0500	0.0161
2 > 5	0.0100	0.0000
3 > 4	0.0100	0.0000
3 > 5	0.0100	0.0000
4 > 5		

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
 A blank means the P-value is greater than 0.0500.

ANALYSIS OF GROWTH DATA

Data listing

File: Linuron/Mysid

Date: 06-18-1996

Obs.	TRT	REP	MLEN	FLEN	MDWT	FDWT
1	1	1	8.5	8.5	0.95	1.44
2	1	1	8.7	8.8	0.67	1.16
3	1	1	8.4	8.4	0.81	1.09
4	1	1	8.6	8.6	0.70	0.78
5	1	1	8.7	9.0	0.90	1.20
6	1	1	8.7	8.7	0.85	0.86
7	1	1	9.0	8.7	0.81	1.18
8	1	1	8.8	8.8	0.91	1.10
9	1	1	8.9	8.9	0.62	0.88
10	1	1	9.1	8.7	0.88	0.89
11	1	1	8.7	8.8	0.81	0.99
12	1	1	8.7	.	0.70	.
13	1	1	8.8	.	0.78	.
14	1	1	8.8	.	0.69	.
15	1	2	8.6	8.8	0.78	0.94
16	1	2	8.6	8.7	0.72	1.09
17	1	2	8.7	8.7	0.76	1.00
18	1	2	8.7	8.9	0.79	1.01
19	1	2	8.6	8.6	0.65	0.61
20	1	2	8.8	9.0	0.81	1.04
21	1	2	9.0	8.8	0.70	0.98
22	1	2	8.9	8.7	0.73	0.94
23	1	2	8.7	8.6	0.91	0.99
24	1	2	8.8	8.8	0.68	1.01
25	1	2	8.7	8.8	0.53	0.98
26	1	2	8.8	8.9	0.56	0.94
27	1	2	.	8.7	.	0.82
28	1	2	.	8.8	.	1.00
29	2	1	8.9	8.8	1.14	1.10
30	2	1	8.8	8.7	0.80	1.08
31	2	1	8.7	8.9	0.90	0.91
32	2	1	8.8	8.7	0.60	1.03
33	2	1	8.9	8.8	0.78	1.00
34	2	1	9.1	8.7	0.56	0.95
35	2	1	8.7	8.9	0.88	0.93
36	2	1	8.8	8.6	0.74	0.85
37	2	1	8.6	8.7	0.71	0.88
38	2	1	9.0	8.8	0.76	1.21
39	2	1	8.8	8.7	0.68	1.08
40	2	1	.	8.8	.	0.99
41	2	1	.	8.7	.	0.78
42	2	1	.	8.6	.	0.81
43	2	1	.	8.7	.	0.92
44	2	2	8.7	8.6	0.62	0.93
45	2	2	8.8	8.8	0.92	0.76

Obs.	TRT	REP	MLEN	FLEN	MDWT	FDWT
46	2	2	8.7	8.8	0.70	0.70
47	2	2	8.6	8.7	0.74	0.83
48	2	2	9.0	8.7	0.86	0.92
49	2	2	8.8	8.4	0.78	0.86
50	2	2	8.9	8.6	0.66	1.01
51	2	2	8.7	8.8	0.72	0.80
52	2	2	8.8	8.7	1.31	0.82
53	2	2	8.8	8.9	0.64	0.94
54	2	2	8.7	8.7	0.59	0.77
55	2	2	8.5	8.7	0.71	1.10
56	2	2	8.7	8.8	0.66	0.99
57	3	1	8.5	8.7	0.77	0.88
58	3	1	8.8	8.6	0.69	0.99
59	3	1	8.6	8.6	0.74	1.01
60	3	1	8.7	8.6	0.87	1.00
61	3	1	8.7	9.0	0.71	1.34
62	3	1	8.6	8.7	0.61	0.89
63	3	1	8.8	8.8	0.68	1.01
64	3	1	8.6	8.7	0.56	0.96
65	3	1	8.7	8.7	0.78	1.12
66	3	1	8.8	8.8	0.74	1.16
67	3	1	8.7	8.6	0.79	1.02
68	3	1	8.8	8.7	0.44	1.04
69	3	1	.	9.0	.	0.66
70	3	2	8.5	8.8	0.74	1.05
71	3	2	8.8	8.6	0.69	1.20
72	3	2	8.6	8.6	0.67	0.85
73	3	2	8.7	8.7	0.65	0.99
74	3	2	8.7	8.7	0.74	0.92
75	3	2	8.6	9.0	0.78	1.00
76	3	2	8.8	.	0.84	.
77	3	2	8.6	.	1.02	.
78	3	2	8.7	.	1.03	.
79	3	2	8.6	.	0.65	.
80	3	2	8.6	.	0.84	.
81	3	2	8.8	.	0.86	.
82	3	2	8.7	.	0.61	.
83	3	2	8.5	.	0.75	.
84	3	2	8.8	.	0.71	.
85	3	2	8.6	.	0.49	.
86	3	2	8.8	.	0.61	.
87	3	2	8.8	.	0.58	.
88	3	2	8.6	.	0.65	.
89	3	2	8.8	.	0.68	.
90	4	1	8.5	8.6	0.61	0.83
91	4	1	8.4	8.7	0.54	0.87
92	4	1	8.7	8.7	0.57	0.71
93	4	1	8.7	8.8	0.52	0.63
94	4	1	8.6	8.7	0.79	0.72
95	4	1	8.7	8.8	0.56	0.56
96	4	1	.	8.5	.	0.70
97	4	1	.	8.7	.	0.88
98	4	1	.	8.8	.	0.70

Obs.	TRT	REP	MLEN	FLEN	MDWT	FDWT
99	4	1	.	8.6	.	0.67
100	4	1	.	8.6	.	0.95
101	4	2	8.5	8.8	0.74	0.84
102	4	2	8.4	8.7	0.66	0.93
103	4	2	8.7	8.7	0.83	0.85
104	4	2	8.7	8.6	0.62	0.73
105	4	2	8.6	8.6	0.88	0.80
106	4	2	8.6	8.6	0.60	0.92
107	4	2	8.7	8.8	0.69	1.00
108	4	2	8.6	.	0.67	.
109	4	2	8.7	.	0.72	.
110	4	2	8.6	.	0.70	.
111	4	2	8.6	.	0.59	.
112	4	2	8.5	.	0.39	.
113	5	1	8.6	8.7	0.46	0.78
114	5	1	8.7	8.8	0.49	0.91
115	5	1	8.6	8.6	0.43	0.94
116	5	1	8.6	8.7	0.99	0.71
117	5	1	8.5	8.6	0.63	0.73
118	5	1	8.5	8.7	0.87	0.64
119	5	1	8.7	8.7	0.56	0.68
120	5	1	.	8.7	.	0.70
121	5	2	8.7	8.7	0.44	0.86
122	5	2	8.8	8.6	0.50	0.63
123	5	2	8.7	8.7	0.56	0.90
124	5	2	.	8.7	.	0.65
125	5	2	.	8.5	.	0.71
126	5	2	.	8.6	.	0.86
127	5	2	.	8.8	.	0.38
128	5	2	.	8.7	.	0.61

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	4	7	3	0

Calculated Chi-Square goodness of fit test statistic = 2.5413
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.012

W = 0.944

Critical W (P = 0.05) (n = 14) = 0.874

Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 FILE: 43996503.sur

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	0.8330	0.8330
1	GRPS 1&2 POOLED	2	0.8670	0.8670
1	GRPS 1&2 POOLED	3	0.8670	0.8670
1	GRPS 1&2 POOLED	4	0.8670	0.8670
2	0.297	1	0.8330	0.8330
2	0.297	2	0.8670	0.8670
3	0.582	1	0.5670	0.5670
3	0.582	2	0.6330	0.6330
4	1.10	1	0.5000	0.5000
4	1.10	2	0.3670	0.3670
5	2.53	1	0.0000	0.0000
5	2.53	2	0.0000	0.0000
6	4.29	1	0.0000	0.0000
6	4.29	2	0.0000	0.0000

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	GRPS 1&2 POOLED	4	0.833	0.867	0.859
2	0.297	2	0.833	0.867	0.850
3	0.582	2	0.567	0.633	0.600
4	1.10	2	0.367	0.500	0.434
5	2.53	2	0.000	0.000	0.000
6	4.29	2	0.000	0.000	0.000

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	GRPS 1&2 POOLED	0.000	0.017	0.009	1.98
2	0.297	0.001	0.024	0.017	2.83
3	0.582	0.002	0.047	0.033	7.78
4	1.10	0.009	0.094	0.066	21.69
5	2.53	0.000	0.000	0.000	N/A
6	4.29	0.000	0.000	0.000	N/A

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.785	0.357	229.082
Within (Error)	8	0.012	0.002	
Total	13	1.798		

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

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur 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	0.859	0.859		
2	0.297	0.850	0.850	0.249	
3	0.582	0.600	0.600	7.561	*
4	1.10	0.434	0.434	12.431	*
5	2.53	0.000	0.000	25.111	*
6	4.29	0.000	0.000	25.111	*

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

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur 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	0.297	2	0.099	11.5	0.009
3	0.582	2	0.099	11.5	0.259
4	1.10	2	0.099	11.5	0.425
5	2.53	2	0.099	11.5	0.859
6	4.29	2	0.099	11.5	0.859

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur 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	0.859	0.859	0.859
2	0.297	2	0.850	0.850	0.850
3	0.582	2	0.600	0.600	0.600
4	1.10	2	0.434	0.434	0.434
5	2.53	2	0.000	0.000	0.000
6	4.29	2	0.000	0.000	0.000

DPX-Z326-198: 28-Day Survival of Exposed Adult Mysids
 File: 43996503.sur 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	0.859				
0.297	0.850	0.248		1.86	k= 1, v= 8
0.582	0.600	7.557	*	1.96	k= 2, v= 8
1.10	0.434	12.425	*	2.00	k= 3, v= 8
2.53	0.000	25.098	*	2.01	k= 4, v= 8
4.29	0.000	25.098	*	2.02	k= 5, v= 8

s = 0.039

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

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	7.2000	CALCULATED t VALUE =	-0.8087
GRP2 (BLANK CRTL) MEAN =	8.3000	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	-1.1000		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.670	2.420	3.820	2.420	0.670
OBSERVED	0	4	2	4	0

Calculated Chi-Square goodness of fit test statistic = 4.2703
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 20.820

W = 0.955

Critical W (P = 0.05) (n = 10) = 0.842

Critical W (P = 0.01) (n = 10) = 0.781

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: DPX-Z326-198: Reproduction of Exposed Adult Mysids
 FILE: 43996503.rep
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	8.0000	8.0000
1	GRPS 1&2 POOLED	2	6.4000	6.4000
1	GRPS 1&2 POOLED	3	7.2000	7.2000
1	GRPS 1&2 POOLED	4	9.4000	9.4000
2	0.297	1	9.9000	9.9000
2	0.297	2	13.0000	13.0000
3	0.582	1	9.0000	9.0000
3	0.582	2	10.4000	10.4000
4	1.10	1	2.3000	2.3000
4	1.10	2	6.8000	6.8000

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	GRPS 1&2 POOLED	4	6.400	9.400	7.750
2	0.297	2	9.900	13.000	11.450
3	0.582	2	9.000	10.400	9.700
4	1.10	2	2.300	6.800	4.550

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	GRPS 1&2 POOLED	1.637	1.279	0.640	16.51
2	0.297	4.805	2.192	1.550	19.14
3	0.582	0.980	0.990	0.700	10.21
4	1.10	10.125	3.182	2.250	69.93

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	53.064	17.688	5.097
Within (Error)	6	20.820	3.470	
Total	9	73.884		

Critical F value = 4.76 (0.05,3,6)
 Since F > Critical F REJECT Ho: All equal

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep 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	7.750	7.750		
2	0.297	11.450	11.450	-2.294	
3	0.582	9.700	9.700	-1.209	
4	1.10	4.550	4.550	1.984	

Bonferroni t table value = 2.75 (1 Tailed Value, P=0.05, df=6,3)

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep 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	0.297	2	4.435	57.2	-3.700
3	0.582	2	4.435	57.2	-1.950
4	1.10	2	4.435	57.2	3.200

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep 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	7.750	7.750	9.163
2	0.297	2	11.450	11.450	9.163
3	0.582	2	9.700	9.700	9.163
4	1.10	2	4.550	4.550	4.550

DPX-Z326-198: Reproduction of Exposed Adult Mysids
 File: 43996503.rep 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	9.163				
0.297	9.163	0.876		1.94	k= 1, v= 6
0.582	9.163	0.876		2.06	k= 2, v= 6
1.10	4.550	1.984		2.10	k= 3, v= 6

s = 1.863

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