

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

1. **CHEMICAL:** XDE-105 PC Code No.: 11003

2. **TEST MATERIAL:** Spinosad  
Purity: 88%

3. **CITATION**

Authors: Jirk, H.D., A.M. Landre, J.A. Miller, J.M. Hugo and M.D. Martin

Title: Evaluation of the Chronic Toxicity of XDE-105 Insecticide to the Daphnid, Daphnia magna Straus Following Flow-through Exposure

Study Completion Date: 1995

Laboratory: Environmental Toxicology and Chemistry Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI.

Sponsor: DowElanco

Laboratory Report ID: DECO-ES-3018

MRID No.: 43848801

DP Barcode: D221275

4. **REVIEWED BY:** Joanne S. Edwards, Entomologist, EEB, EFED

Signature: *Joanne S. Edwards*

Date: 4/27/96

5. **APPROVED BY:** Leslie Touart, Head of Section 1, EEB, EFED

Signature: *LGTD*

Date: 3/25/96

6. **STUDY PARAMETERS**

<b>Scientific Name of Test Organism:</b>	<u>Daphnia magna</u>
<b>Age of Test Organism:</b>	Instars <24-hr old
<b>Definitive Test Duration:</b>	21 days
<b>Study Method:</b>	Flow-through
<b>Type of Concentrations:</b>	Mean measured

7. **CONCLUSIONS:**

**Results Synopsis**

NOEC: 0.616 ppb

LOEC: 1.15 ppb

MATC: 0.842 ppb

LOEC's for specific effects

Egg Production: 2.19 ppb

Growth (length): 1.15 ppb

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

1. Dry weight measurements of each first generation daphnid should have been made; only length measurements were taken.

10 SUBMISSION PURPOSE:

11. MATERIALS AND METHODS

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
<u>Species</u> <i>Daphnia magna</i>	<i>Daphnia magna</i>
<u>Source</u>	laboratory-reared culture
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Yes
<u>Parental Acclimation Period</u> At least 21 days.	Yes
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	>14 days (and which had at least four broods)
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Algal diet ( <u>Ankistrodesmus convolutus</u> )
<u>Food Concentration</u> 5 mg/L (dry wt.) of synthetic food or 10 <sup>8</sup> cells/L of algae is recommended.	During test all replicates were fed 20 mL of an algal diet/525 mL of test solution at least twice daily.

Guideline Criteria	Reported Information
Were daphnids in good health during acclimation period?	Not reported

### B. Test System

Guideline Criteria	Reported Information
<p><b>Test Water</b> Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</p>	Lake Huron water- before use the water was sand-filtered, pH-adjusted with gaseous CO <sub>2</sub> , carbonfiltered and UV-irradiated. Most recent analyses for contaminants made on 4/7/95
<p><b>Water Temperature</b> 20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.</p>	Target: 20°C Range: 19.3 to 20.5°C
<p><b>pH</b> 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.</p>	7.4 to 7.7
<p><b>Total Hardness</b> 160 to 180 mg/L as CaCO<sub>3</sub> is recommended.</p>	66-73 mg/L as CaCO <sub>3</sub>
<p><b>Dissolved Oxygen</b> <u>Renewal</u>: must not drop below 50% for more than 48 hours. <u>Flow-through</u>: ≥ 60% throughout test.</p>	>82%
<p><b>Test Vessels or Compartments</b> 1. <u>Material</u>: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <u>Size</u>: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.</p>	600 mL glass beakers with 7.9 X 14 cm insets; beakers contained approx. 525 mL test solution

Guideline Criteria	Reported Information
<p><b><u>Covers</u></b>  <b><u>Renewal:</u></b> Test vessels should be covered with a glass plate.  <b><u>Flow-through:</u></b> openings in test compartments should be covered with mesh nylon or stainless steel screen.</p>	<p>Not indicated if test vessels were covered</p>
<p><b><u>Type of Dilution System</u></b>  Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	<p>Intermittent-flow proportional diluter system</p>
<p><b><u>Flow Rate</u></b>  Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.</p>	<p>At least 6 turnovers per 24 hours</p>
<p><b><u>Aeration</u></b>  Dilution water should be vigorously aerated, but the test tanks should not be aerated.</p>	<p>Test vessels were not aerated during the study</p>
<p><b><u>Photoperiod</u></b>  16 hours light, 8 hours dark.</p>	<p>16 hours light, 8 hours dark  light readings (lux) 1133 ± 202</p>
<p><b><u>Solvents</u></b>  Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.</p>	<p>Solvent: DMF  Maximum conc.: not reported</p>

**C. Test Design**

Guideline Criteria	Reported Information
<p><b><u>Duration</u></b>  21 days</p>	<p>21 days</p>

Guideline Criteria	Reported Information
<p><b><u>Nominal Concentrations</u></b> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.</p>	<p>Six nominal concentrations of 7.7, 4.6, 2.8, 1.7, 1.1, and 0.6 ppb; solvent control and dilution water control</p>
<p><b><u>Number of Test Organisms</u></b> 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.</p>	<p>four replicates/level; 5 organisms/replicate</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>First instars were impartially placed into the test solutions</p>
<p><b><u>Renewal</u></b> Parent daphnids in all beakers must be transferred to containers with fresh test solution (&lt; 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).</p>	<p>N/A</p>
<p><b><u>Water Parameter Measurements</u></b> 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.</p>	<p>All criteria were met</p>
<p><b><u>Chemical Analysis</u></b> Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.</p>	<p>From each individual test vessel, the concentration of XDE-105 was determined on days 0, 8, 14, and 21</p>

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes
<b><u>Control Mortality</u> ≤ 30%</b>	0%
<b>Did daphnids in each control produce at least 40 young after 21 days?</b>	Yes
<b>Were no ehippia produced in any of the controls?</b>	Yes
<b><u>Data Endpoints</u></b> - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs.	mortality of 1st generation daphnids  average # young produced per female  length of each first generation daphnid  observations of other effects or clinical signs.
<b>Raw data included?</b>	Excerpted

Effects Data

Toxicant Concentration ( $\mu\text{g/L}$ )		% Dead or Immobile (21 Days)	Young per Female per Repro. Day	Total Length (mm)
Nominal	Measured			
Control	-	0	76.9	4.68
Solvent Control	-	0	83.3	4.66
0.6	0.392	0	91.8	4.67
1.1	0.617	0	85.3	4.51
1.7	1.15	10	79.5	4.27
2.8	2.19	15	61.5	3.99
4.6	3.96	25	43.6	3.88
7.7	5.81	35	39.9	3.73

Toxicity Observations:

**B. Statistical Results:** Dunnett's Test

Most sensitive endpoint:

Endpoint	Method	NOEC (ppb)	LOEC (ppb)	MATC (ppb)
Mortality	Due to the low mortality and sublethal effects, it was not possible to calculate LC/EC <sub>50</sub> values.	1.15	2.19	1.59
Reproduction (average # young/adult)	Dunnett's	1.15	2.19	1.59
Length (mm)	Dunnett's	0.617	1.15	0.842

The statistically derived LOEC for growth (length) was 1.15 ppb. This was a 9% reduction in length. At this level there was no corresponding reduction in reproductive capacity. The study authors do not believe the 9% difference is predictive of an adverse impact on aquatic invertebrate populations, and that the combined LOECs for reproduction and growth are 2.19 ppb.

**13. VERIFICATION OF STATISTICAL RESULTS**

Most sensitive endpoint:

Endpoint	Method	NOEC (ppb)	LOEC (ppb)	MATC (ppb)
Survival	TOXANOL-could not be determined due to low mortality	-	-	-
Reproduction (total # of progeny produced per replicate)	Dunnett's	1.15	2.19	1.59
Length	Dunnett's	0.617	1.15	0.842

**14. REVIEWER'S COMMENTS:**

The following study deviations were noted:

- o The recommended hardness of water is 160 to 180 mg/L as CaCO<sub>3</sub>. The hardness of the water in this test was lower (66-73 mg/L as CaCO<sub>3</sub>).
- o Dry weight measurements of each first generation daphnid should have been made; only length measurements were taken.

The reviewer disagrees with the study authors' interpretation of the data that the combined LOECs for reproduction and growth should be 2.19 ppb and corresponding NOECs for both, 1.15 ppb. The most sensitive endpoint in this study was growth, and the overall NOEC, LOEC and MATC values are based on this parameter.

In conclusion, based on a statistically significant reduction (p < 0.05) in reproduction capacity as measured by progeny produced at the 2.19 ppb treatment level and a statistically significant reduction in growth as measured by length data at the 1.15 ppb treatment level, the NOEC, LOEC, and MATC for daphnids

exposed to XDE-105 for 21 days is 0.616 ppb, 1.15 ppb, and 0.842 ppb, respectively.

1	control	4.662	4.662	
2	.392	4.677	4.677	-0.239
3	.617	4.567	4.567	1.450
4	1.15	4.268	4.268	5.905 *
5	2.19	3.994	3.994	9.880 *
6	3.96	3.880	3.880	11.164 *
7	5.81	3.728	3.728	12.779 *

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 Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=110,6)

length

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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	control	20			
2	.392	20	0.158	3.4	-0.015
3	.617	20	0.158	3.4	0.094
4	1.15	18	0.162	3.5	0.393
5	2.19	17	0.164	3.5	0.668
6	3.96	15	0.170	3.7	0.782
7	5.81	13	0.178	3.8	0.933

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NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	4.7000	4.7000
1	control	2	4.8500	4.8500
1	control	3	4.8500	4.8500
1	control	4	4.7000	4.7000
1	control	5	4.8500	4.8500
1	control	6	4.5500	4.5500
1	control	7	4.7000	4.7000
1	control	8	4.5500	4.5500
1	control	9	4.8500	4.8500
1	control	10	4.7000	4.7000
1	control	11	4.3900	4.3900
1	control	12	4.2400	4.2400
1	control	13	4.7000	4.7000
1	control	14	4.5500	4.5500
1	control	15	4.7000	4.7000
1	control	16	4.5500	4.5500
1	control	17	4.7000	4.7000
1	control	18	4.7000	4.7000
1	control	19	4.8500	4.8500

1	control	20	4.5500	4.5500
2	.392	1	4.5500	4.5500
2	.392	2	4.5500	4.5500
2	.392	3	4.7000	4.7000
2	.392	4	4.8500	4.8500
2	.392	5	4.8500	4.8500
2	.392	6	4.7000	4.7000
2	.392	7	4.7000	4.7000
2	.392	8	4.8500	4.8500
2	.392	9	4.7000	4.7000
2	.392	10	4.5500	4.5500
2	.392	11	4.7000	4.7000
2	.392	12	4.3900	4.3900
2	.392	13	4.7000	4.7000
2	.392	14	4.8500	4.8500
2	.392	15	4.7000	4.7000
2	.392	16	4.5500	4.5500
2	.392	17	4.5500	4.5500
2	.392	18	4.7000	4.7000
2	.392	19	4.7000	4.7000
2	.392	20	4.7000	4.7000
3	.617	1	4.5500	4.5500
3	.617	2	4.7000	4.7000
3	.617	3	4.3900	4.3900
3	.617	4	4.9400	4.9400
3	.617	5	4.3900	4.3900
3	.617	6	4.5500	4.5500
3	.617	7	4.5500	4.5500
3	.617	8	4.5500	4.5500
3	.617	9	4.5500	4.5500
3	.617	10	4.3900	4.3900
3	.617	11	4.5500	4.5500
3	.617	12	4.5500	4.5500
3	.617	13	4.8500	4.8500
3	.617	14	4.5500	4.5500
3	.617	15	4.7000	4.7000
3	.617	16	4.5500	4.5500
3	.617	17	4.7000	4.7000
3	.617	18	4.8500	4.8500
3	.617	19	3.7900	3.7900
3	.617	20	4.7000	4.7000
4	1.15	1	4.2400	4.2400
4	1.15	2	4.3900	4.3900
4	1.15	3	4.2400	4.2400
4	1.15	4	4.5500	4.5500
4	1.15	5	3.9400	3.9400
4	1.15	6	3.9400	3.9400
4	1.15	7	3.9400	3.9400
4	1.15	8	4.2400	4.2400
4	1.15	9	3.9400	3.9400
4	1.15	10	3.9400	3.9400
4	1.15	11	4.2400	4.2400
4	1.15	12	4.2400	4.2400
4	1.15	13	4.2400	4.2400
4	1.15	14	4.5500	4.5500
4	1.15	15	4.5500	4.5500
4	1.15	16	4.5500	4.5500
4	1.15	17	4.5500	4.5500
4	1.15	18	4.5500	4.5500
5	2.19	1	4.2400	4.2400

5	2.19	2	3.7900	3.7900
5	2.19	3	3.7900	3.7900
5	2.19	4	4.5500	4.5500
5	2.19	5	4.3900	4.3900
5	2.19	6	3.9400	3.9400
5	2.19	7	3.9400	3.9400
5	2.19	8	3.9400	3.9400
5	2.19	9	4.0900	4.0900
5	2.19	10	3.9400	3.9400
5	2.19	11	3.9400	3.9400
5	2.19	12	3.9400	3.9400
5	2.19	13	3.9400	3.9400
5	2.19	14	3.7900	3.7900
5	2.19	15	3.7900	3.7900
5	2.19	16	3.9400	3.9400
5	2.19	17	3.9400	3.9400
6	3.96	1	3.9400	3.9400
6	3.96	2	4.0900	4.0900
6	3.96	3	3.9400	3.9400
6	3.96	4	3.7900	3.7900
6	3.96	5	3.9400	3.9400
6	3.96	6	3.9400	3.9400
6	3.96	7	3.7900	3.7900
6	3.96	8	3.7900	3.7900
6	3.96	9	3.9400	3.9400
6	3.96	10	3.7900	3.7900
6	3.96	11	3.7900	3.7900
6	3.96	12	3.9400	3.9400
6	3.96	13	3.7900	3.7900
6	3.96	14	3.7900	3.7900
6	3.96	15	3.9400	3.9400
7	5.81	1	3.3300	3.3300
7	5.81	2	3.3300	3.3300
7	5.81	3	3.7900	3.7900
7	5.81	4	3.4800	3.4800
7	5.81	5	3.6400	3.6400
7	5.81	6	3.4800	3.4800
7	5.81	7	3.7900	3.7900
7	5.81	8	3.9400	3.9400
7	5.81	9	3.7900	3.7900
7	5.81	10	3.4800	3.4800
7	5.81	11	4.2400	4.2400
7	5.81	12	3.9400	3.9400
7	5.81	13	4.2400	4.2400

length

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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	20	4.240	4.850	4.662
2	.392	20	4.390	4.850	4.677
3	.617	20	3.790	4.940	4.567
4	1.15	18	3.940	4.550	4.268
5	2.19	17	3.790	4.550	3.994

Since  $F > \text{Critical } F$  REJECT  $H_0$ : All groups equal

length

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DUNNETTS TEST

\*\*\*\*\* WARNING \*\*\*\*\*

This data set has unequal replicates. The Bonferroni T-test should be used instead of the Dunnetts test.

length

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DUNNETTS TEST - TABLE 1 OF 2

$H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	4.662	4.662		
2	.392	4.677	4.677	-0.239	
3	.617	4.567	4.567	1.450	
4	1.15	4.268	4.268	5.905	*
5	2.19	3.994	3.994	9.880	*
6	3.96	3.880	3.880	11.164	*
7	5.81	3.728	3.728	12.779	*

Dunnett table value = 2.35 (1 Tailed Value,  $P=0.05$ ,  $df=60,6$ )

length

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DUNNETTS TEST - TABLE 2 OF 2

$H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	20			
2	.392	20	0.152	3.3	-0.015
3	.617	20	0.152	3.3	0.094
4	1.15	18	0.156	3.4	0.393
5	2.19	17	0.159	3.4	0.668
6	3.96	15	0.164	3.5	0.782
7	5.81	13	0.172	3.7	0.933

length

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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	control	4.662	4.662		
2	.392	4.677	4.677	-0.239	
3	.617	4.567	4.567	1.450	
4	1.15	4.268	4.268	5.905	*
5	2.19	3.994	3.994	9.880	*
6	3.96	3.880	3.880	11.164	*
7	5.81	3.728	3.728	12.779	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=110,6)

length

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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	control	20			
2	.392	20	0.158	3.4	-0.015
3	.617	20	0.158	3.4	0.094
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5	2.19	17	0.164	3.5	0.668
6	3.96	15	0.170	3.7	0.782
7	5.81	13	0.178	3.8	0.933

TITLE: reproduction

FILE: repro

TRANSFORM: NO TRANSFORM

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	485.0000	485.0000
1	control	2	489.0000	489.0000
1	control	3	415.0000	415.0000
1	control	4	277.0000	277.0000
2	.392	1	471.0000	471.0000
2	.392	2	455.0000	455.0000
2	.392	3	438.0000	438.0000
2	.392	4	472.0000	472.0000
3	.617	1	387.0000	387.0000
3	.617	2	408.0000	408.0000

3	.617	3	416.0000	416.0000
3	.617	4	494.0000	494.0000
4	1.15	1	419.0000	419.0000
4	1.15	2	477.0000	477.0000
4	1.15	3	346.0000	346.0000
4	1.15	4	347.0000	347.0000
5	2.19	1	321.0000	321.0000
5	2.19	2	310.0000	310.0000
5	2.19	3	266.0000	266.0000
5	2.19	4	332.0000	332.0000
6	3.96	1	200.0000	200.0000
6	3.96	2	143.0000	143.0000
6	3.96	3	274.0000	274.0000
6	3.96	4	255.0000	255.0000
7	5.81	1	176.0000	176.0000
7	5.81	2	169.0000	169.0000
7	5.81	3	193.0000	193.0000
7	5.81	4	260.0000	260.0000

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reproduction

File: repro

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

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GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	277.000	489.000	416.500
2	.392	4	438.000	472.000	459.000
3	.617	4	387.000	494.000	426.250
4	1.15	4	346.000	477.000	397.250
5	2.19	4	266.000	332.000	307.250
6	3.96	4	143.000	274.000	218.000
7	5.81	4	169.000	260.000	199.500

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reproduction

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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

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GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	9803.667	99.013	49.507
2	.392	256.667	16.021	8.010
3	.617	2189.583	46.793	23.396
4	1.15	3994.917	63.205	31.603
5	2.19	836.917	28.930	14.465
6	3.96	3484.667	59.031	29.516
7	5.81	1728.333	41.573	20.787

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reproduction

File: repro

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	264613.000	44102.167	13.847
Within (Error)	21	66884.250	3184.964	
Total	27	331497.250		

Critical F value = 2.57 (0.05,6,21)  
 Since F > Critical F REJECT Ho:All groups equal

reproduction

File: repro

Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	416.500	416.500		
2	.392	459.000	459.000	-1.065	
3	.617	426.250	426.250	-0.244	
4	1.15	397.250	397.250	0.482	
5	2.19	307.250	307.250	2.738	*
6	3.96	218.000	218.000	4.974	*
7	5.81	199.500	199.500	5.438	*

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

reproduction

File: repro

Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	.392	4	98.169	23.6	-42.500
3	.617	4	98.169	23.6	-9.750
4	1.15	4	98.169	23.6	19.250
5	2.19	4	98.169	23.6	109.250
6	3.96	4	98.169	23.6	198.500
7	5.81	4	98.169	23.6	217.000

reproduction

File: repro

Transform: NO TRANSFORM