Data Evaluation Report on the Chronic Toxicity of AE 0317309 (Pyrasulfotole) to Freshwater Invertebrates - Daphnia sp.

PMRA Submission Number 2006-2445 EPA MRID No. 468017-27

Data Requirement:	PMRA Data Code	9.3.3
	EPA DP Barcode	D328639
	OECD Data Point	211
	EPA MRID	468017-27
	EPA Guideline	850.1300

Test material: AE 0317309 Technical **Purity:** 95.4% ai
Common name Pyrasulfotole
Chemical name: IUPAC: (5-Hydroxy-1,3-dimethylpyrazol-4-yl)(α,α,α-trifluoro-2-mesyl-*p*-tolyl)methanone
CAS name: (5-Hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
CAS No.: 365400-11-9
Synonyms: None reported

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Reference/Submission No.: {.....}

Company Code	BCZ
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Use Site Category:	13, 14
EPA PC Code	000692

Date Evaluation Completed: 12-05-2006

CITATION: Kern, M.E., and C.V. Lam. 2004. Chronic Toxicity of AE 0317309 Technical to the *Daphnia magna* Under Static Renewal Conditions. Unpublished study performed by Bayer CropScience, Stilwell, KS. Laboratory Study No. EBAIX014. Study submitted by Bayer CropScience, Research Triangle Park, NC. Study initiated March 30, 2004 and submitted October 14, 2004.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the chronic toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a

case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of AE 0317309 Technical (pyrasulfotole) to *Daphnia magna* was studied under static renewal conditions. Daphnids were exposed to AE 0317309 at nominal concentrations of 0 (negative control), 3.13, 6.25, 12.5, 25.0, and 50.0 mg ai/L. Mean-measured concentrations were <0.29 (<LOQ, control), 3.20, 6.39, 12.8, 25.5, and 52.9 mg ai/L, respectively. The 21-day EC_{50} (with 95% C.I.) for immobility effect was >52.9 mg ai/L, the highest concentration tested. The 21-day NOAEC based on survival/immobility (accompanied by other clinical signs of toxicity), the most sensitive endpoint, was 12.8 mg ai/L.

Cumulative survival was 96, 100, 96, 100, 84, and 56% at the control, 3.20, 6.39, 12.8, 25.5, and 52.9 mg ai/L levels, respectively, with statistically significant reductions observed at the two highest test levels. In addition, at the 25.5 mg ai/L level, one organism was noted to be pale and another organism was quiescent, and at the 52.9 mg ai/L level, between 5 and 10 organisms were noted to be pale or quiescent.

The time to first brood averaged 8.4 days for the control group, compared to 8.0-10.3 days for the treatment groups, with no statistically-significant differences observed. However, the mean number of live offspring produced per parent per reproductive day was statistically-reduced at the 52.9 mg ai/L level, with an average of 4.94, compared to 14.35 for the control.

At study termination, total length was statistically-reduced compared to the control at the 52.9 mg ai/L (4.64 versus 4.38 mm). Although decreases in dry weight were observed at the ≥12.8 mg ai/L levels, no statistically-significant differences were observed when compared to the control.

This study is scientifically sound, is classified as **ACCEPTABLE**, and does satisfy guideline requirements for a chronic toxicity study with freshwater invertebrates.

Results Synopsis

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

21-day EC_{50} (immobility): >52.9 mg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 12.8 mg ai/L
LOAEC: 25.5 mg ai/L

Endpoint(s) affected: Survival, clinical effects, reproduction, and total length
Most sensitive endpoint: Survival and clinical effects

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series 72-4(b); the U.S. EPA Ecological Effects Test Guidelines, No. 850.1300; and OECD Guideline No. 211. Deviations from U.S. EPA OPPTS No. 850.1300 included:

1. The health (including mortality) of the laboratory culture and subculture were not provided.
2. Dilution water pH higher than recommended (8.0-8.5).
3. Dissolved oxygen higher than recommended (92-114%).

These deviations do not affect the scientific soundness of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material AE 0317309 Technical

Description: Light brown powder

Lot No./Batch No. : Op. 1-4

Purity: 94.5%

Stability of compound under test conditions: Verified. Freshly-prepared test solutions were sampled (all levels) on days 0, 5, 12, and 19, and aged solutions were sampled on days 5, 12, 19, and 21. Minimal variability was observed, with measured concentrations within 20% of mean values at all intervals.

(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of Test chemicals: Ambient conditions

Physiochemical properties of AE 0317309.

Parameter	Value	Comment
Molecular weight	362.3 g/mol	
Water Solubility (g/L) at 20°C	4.2 at pH 4 69.1 at pH 7 49.0 at pH 9	Very soluble
Vapor Pressure/Volatility	2.7×10^{-7} Pa at 20°C 6.8×10^{-7} Pa at 25°C	Non-volatile
UV Absorption	water $\lambda_{max} = 264$ 0.1M HCl $\lambda_{max} = 241$ 0.1M NaOH $\lambda_{max} = 216$	Not likely to undergo photolysis.
Pka	4.2 ± 0.15	
log K_{ow} at 23°C	0.276 at pH 4 -1.362 at pH 7 -1.58 at pH 9	Not likely to bioaccumulate
Stability of compound at room temperature, if provided		No significant degradation over 12 months at ambient temperatures.

Data obtained from pyrasulfatole chemistry review of Submission 2006-2445.

2. Test Organism:

Species: *Daphnia magna*, first instar, <24 hours old
EPA and OECD recommend Daphnia magna

Age of the parental stock: 15 days old
EPA recommends that young daphnids #24 hours old from a separate parental culture be used

Source: Laboratory cultures were maintained in hard blended water under static renewal conditions (renewed three times/week). The subculture for the test was initiated on 03/16/04 under the same conditions as the culture. Two neonates (<24 hours old) were impartially assigned to each of ten 400-ml beakers for a total of 20 neonates. The glass beakers were filled to a volume of 300 ml with culture water. First instar daphnids (<24 hours old) produced by 15-day old adults from the subculture were used to begin the study. The daphnids used in the definitive study were from the third brood or older.

EPA requires all test organisms must be produced from laboratory reared culture that has been maintained for at least 21 days at test conditions in dilution water with renewal of the culture medium at least three times per week.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A preliminary study was conducted at nominal concentrations of 0, 0.08, 0.8, 8.0, and 80 mg/L. A significant effect on immobility was observed at the 80 mg/L level. No apparent effect was observed at the lower test levels. Additional details regarding methods and results were not reported.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	15 days (age of parents)	*EPA recommends that prior to testing, daphnids that are at least 10-12 days old (those that have had at least one brood) should be separated from the culture, put in separate container and maintained for at least 21 days to insure that good health conditions are present*
Conditions: (same as test or not)	Same as test	
Feeding:	A combination of green algae (*Pseudokirchneriella subcapitata*) and blended Tetrafin® flaked fish food.	
Health (any mortality observed):	Not reported	
Test condition: static renewal/flow-through:	Static renewal	*(EPA requires consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Type of dilution system- for flow through method.	N/A	
Renewal rate for static renewal	3 times/week	
Aeration, if any	The dilution water was intensely aerated prior to use, and no further aeration was used during the study.	*EPA recommends test chambers should not be aerated*
Duration of the test	21 days	*Recommended duration is 21 days.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size (for growth and reproduction/survival test): Fill volume:	Glass 1 L with 900-ml fill volume for survival and sublethal effects 250 ml with 200-ml fill volume for survival, sublethal effects, growth, and reproduction	*1. Recommended Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Recommended Size: 250 ml with 200 ml fill volume; 100 ml with 80 ml fill volume* *OECD guideline recommends that parent animals be maintained individually; one per vessel, with 50 - 100 ml of medium in each vessel.*
Source of dilution water	Hard blended water was prepared by combining spring water that had been filtered and UV-sterilized with municipal water that had been de-chlorinated with sodium metabisulfite, filtered, demineralized by conventional softeners, and treated by double pass reverse osmosis. All batch dilution water was checked to ensure that residual chlorine levels were <3 µg/L. The dilution water was stored in polypropylene or PVC holding tanks and intensely aerated before use.	Results of the spring water and reverse-osmosis water for analysis of metals, inorganics, pesticides, and PCBs were provided (water analyzed on February 18, 2004). In addition, weekly monitoring of the dilution water for total suspended solids, unionized ammonia, and residual chlorine were provided. *Recommended source of dilution water includes unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*
Water parameters: Hardness pH	 172-174 mg/L as $CaCO_3$ 8.0-8.5	Dilution water pH higher than recommended (8.0-8.5). Dissolved oxygen higher than recommended (supersaturated).

Parameter	Details	Remarks *Criteria*
Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	92-114% saturation at 20°C 19.9-20.7°C 2.1 mg/L for the spring water and <0.50 mg/L for the reverse-osmosis water (typical characteristics) 5 mg/L (total suspended solids; typical dilution water) Mg – ≤9900 µg/L; K – ≤1900 µg/L; Na – 1400-120000 µg/L; (typical characteristics) <LOD (typical characteristics) <0.003 mg/L (typical dilution water)	***Recommended hardness:*** *160 to 180 mg/L as $CaCO_3$; OECD recommends > 140 mg/L as $CaCO_3$* ***Recommended pH:*** *7.6 to 8.0 pH should not deviate by more than 1.0 unit for more than 48 hours. OECD recommends that pH range be 6 - 9 and does not vary more than 1.5 units in any one test.* ***Recommended dissolved oxygen:*** *renewal should not drop below 50% for more than 48 hours.* *Recommended flow-through: ∃ 60% throughout test.* ***Recommended temperature:*** *20EC ∀ 2EC.; should not deviate from 20EC by more than 5EC for more than 48 hours. OECD recommends a range of 18 - 22°C; temperature should not vary more than ∀ 2°C* *OECD guideline recommends that total organic carbon < 2 mg/L*
Number of replicates: For survival and sublethal effects: For survival, sublethal effects, growth, and reproduction:	3 10	*Number of replicates should include a control(s) and at least 5 test concentrations; dilution factor should not be greater than 50%..* *OECD recommends that at least 5 test concentrations be used in a geometric series with a separation factor not exceeding 3.2.*
Number of organisms: For survival and sublethal effects: For survival, sublethal effects, growth, and reproduction:	5 per replicate 1 per replicate	*Recommended number of organisms include 22 daphnids/test concentration;* *7 test chambers should contain 1 daphnid each, and 3 test chambers contain 5 daphnids each.* *OECD recommends holding a minimum of 10 daphnids individually for static tests.* *For flow-through tests, 40 animals should be divided into 4 groups of 10 animals at each test concentration.*

Parameter	Details	Remarks *Criteria*
Treatment Concentrations: nominal: measured:	0 (negative control), 3.13, 6.25, 12.5, 25.0, and 50.0 mg ai/L <0.29 (<LOQ, control), 3.20, 6.39, 12.8, 25.5, and 52.9 mg ai/L	Mean-measured concentrations were determined from freshly-prepared test solutions sampled (all levels) on days 0, 5, 12, and 19, and aged solutions sampled on days 5, 12, 19, and 21.
Solvent (type, percentage, if used)	N/A	*Solvent concentration should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD recommends # 0.1 ml/L of solvent.*
Lighting	16 hours light/8 hours dark, with 30-minute dawn/dusk transition periods	Light intensity ranged from 43 to 64 foot candles (mean of 563 lux). *Recommended photoperiod is 16 hours light and 8 hours of dark.*
Recovery of chemical: Frequency of measurement: LOD: LOQ:	100 ± 6% 9 samples analyzed on a single day Not reported 0.29 mg ai/L	Based on method validation recoveries. During sample analysis, concurrently-analyzed laboratory spikes yielded recoveries of 94-109% of nominal concentrations.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any Feeding:	The daphnia were fed once daily with algae (*Pseudokirchneriella subcapitata*) and on Monday, Wednesday, and Friday with a slurry of Tetrafin®. The alga was dispensed equally to all vessels at a minimum rate of 2 x 10^8 algal cells/L of test solution.	

Parameter	Details	Remarks *Criteria*
Water quality monitoring:	Dissolved oxygen, pH, and hardness were measured in freshly-prepared and aged solutions (as applicable) from all levels on days 0, 5, 12, 19, and 21. Hourly temperature readings were collected from a centrally located vessel, and manual temperatures were also recorded daily.	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
		Criteria
Data endpoints measured (list)	- Survival (including immobility) of first-generation daphnids - Sublethal effects - Time to first brood - Number of offspring/adult/-reproductive day - Total length and dry weight of surviving first generation daphnids	*Recommended endpoints measured:* *- 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.*
Observation intervals	Survival was recorded daily. On the day of first release, neonates were counted and removed, and then were subsequently counted and removed on a Monday-Wednesday-Friday schedule. Growth was determined at study termination.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Statistically-significant decreases in survival (immobility) were observed at the two highest test levels. Following 21 days of exposure, cumulative survival was 96, 100, 96, 100, 84, and 56% at the control, 3.20, 6.39, 12.8, 25.5, and 52.9 mg ai/L levels, respectively. The NOAEC for immobilization was 12.8 mg ai/L.

Table 3: Effect of AE 0317309 on Growth and Survival of Daphnia sp.

Nominal Treatment mg ai/L Mean-measured (and nominal)	Mortality (dead or immobile)		Time to First Brood	Mean No. Offspring per Adult per Reproductive Day	Length, mm	Dry Weight, mg
	No. Dead	%				
Control (dilution water only)	1	4	8.4	14.35	4.64	1.189
3.20 (3.13)	0	0	8.0	13.70	4.67	1.255
6.39 (6.25)	1	4	9.5	16.65	4.66	1.188
12.8 (12.5)	0	0	8.4	14.87	4.68	1.069
25.5 (25.0)	4	16*	9.0	11.25	4.62	1.028
52.9 (50.0)	11	44*	10.3	4.94*	4.38*	1.007
NOAEC, mg ai/L	12.8		52.9	25.5	25.5	52.9
LOAEC, mg ai/L	22.5		>52.9	52.9	52.9	>52.9

* Statistically-significant difference from the control ($p<0.05$).

B. SUB-LETHAL EFFECTS AND EFFECT ON REPRODUCTION:

No sub-lethal effects were observed at the control, 3.20, or 12.8 mg ai/L levels. One daphnid from the 6.39 mg ai/L was pale on days 4 and 5, but appeared normal by day 6 through the remainder of the study. As no effects were observed at the next higher level, this brief observation was not considered to be dose-related. However, at the 25.5 mg ai/L level, one organism was noted to be pale and another organism was quiescent, and at the 52.9 mg ai/L level, between 5 and 10 organisms were noted to be pale or quiescent (durations were not reported).

The time to first brood averaged 8.4 days for the control group, compared to 8.0-10.3 days for the treatment groups, with no statistically-significant differences observed. The mean number of live offspring produced per parent per reproductive day was statistically-reduced at the 52.9 mg ai/L level, with an average of 4.94, compared to 14.35 for the controls. The NOAEC for reproductive effects was 25.5 mg ai/L.

At study termination, total length was statistically-reduced compared to the control at the 52.9 mg ai/L (4.64 versus 4.38 mm). Although decreases in dry weight were observed at the ≥12.8 mg ai/L levels, no statistically-significant differences were observed. The subsequent NOAEC, based on total weight findings, was 25.5 mg ai/L.

C. REPORTED STATISTICS:

Endpoints that were statistically assessed included survivorship (including immobilization), time to first brood release, number of offspring produced per adult per reproductive day, and total lengths and dry weights of surviving adults. For all endpoints, the experimental unit was each replicate test chamber.

The data were first assessed for normality using the chi-square test and for homogeneity of variances using Bartlett's test. All endpoints were then analyzed by a one-way analysis of variance (ANOVA) followed by the Dunnett's test and William's test, or a nonparametric analysis if the assumptions of normality and/or homogeneity of variance were not met.

Statistical analyses were conducted using mean-measured concentrations and TOXSTAT (1994) statistical software at a 95% significant level. The NOAEC and LOAEC values for all endpoints were based on significance data.

The 21-day EC_{50} for survivorship/immobilization could not be calculated because mortality did not exceed 50% at any treatment level.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Reproduction, length, and weight data satisfied the assumptions of normality and homogeneity of variances, while survival data did not. The NOAEC for survival was determined using the non-parametric Steele's many-one rank test, while the NOAEC for reproduction and growth endpoints were determined using ANOVA, followed by William's test (reproduction). These analyses were conducted using Toxstat statistical software. The LC_{50} value could be determined visually, as could the EC_{50} value, because reductions did not exceed 50% for any endpoint.

21-day EC_{50} (immobility): >52.9 mg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 12.8 mg ai/L
LOAEC: 25.5 mg ai/L

Endpoint(s) affected: Survival, clinical effects, and reproduction
Most sensitive endpoint: Clinical effects

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWERS' COMMENTS:

The reviewers' conclusions differed from the study authors' for survival and length endpoints. For both endpoints, the reviewer's analysis revealed a higher NOAEC. Differences are attributed to the different statistical methods used (nonparametric Steele's many-one rank test and ANOVA without multiple comparison tests for survival and length data, respectively). Because the study authors' toxicity estimates are more conservative, they are reported in the Executive Summary and Conclusions sections.

No undissolved test material was noted in any test concentration.

In-life dates for the definitive study were March 31 – April 21, 2004.

G. CONCLUSIONS:

This study is scientifically sound and is classified as **ACCEPTABLE**. AE 0317309 Technical adversely affected the survival, reproduction, and growth of *Daphnia magna*. Clinical effects were also observed at the two highest treatment levels. The most sensitive endpoint was survival, accompanied by other clinical signs of toxicity.

21-day EC_{50} (immobility): >52.9 mg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 12.8 mg ai/L
LOAEC: 25.5 mg ai/L

Endpoint(s) affected: Survival, clinical effects, reproduction, and total length
Most sensitive endpoint: Survival and clinical effects

III. REFERENCES:

American Public Health Association. 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition, Washington DC.

American Society for Testing and Materials. 1997. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. ASTM Standard E1193, Philadelphia, PA.

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U.S. Environmental Protection Agency. 1993. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, 4th Edition. EPA 600/4-90/027F. Office of Research and Development, Cincinnati, OH.

U.S. Environmental Protection Agency. 1996. *Draft* OPPTS 850.1300: Daphnid Chronic Toxicity Test.

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APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

percent survival
File: 1727s Transform: NO TRANSFORM

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	control	92.308				
2	3.20	100.000	182.00	132.00	13.00	
3	6.39	92.308	175.50	132.00	13.00	
4	12.8	100.000	182.00	132.00	13.00	
5	25.5	81.538	157.00	132.00	13.00	
6	52.9	46.154	125.00	132.00	13.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

reproduction
File: 1727r Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	863.658	172.732	10.417
Within (Error)	54	895.384	16.581	
Total	59	1759.042		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

reproduction
File: 1727r Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	14.349	14.349		
2	3.20	13.694	13.694	0.360	
3	6.39	16.653	16.653	-1.265	
4	12.8	14.870	14.870	-0.286	
5	25.5	11.248	11.248	1.703	
6	52.9	4.938	4.938	5.168	*

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

reproduction
File: 1727r Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	10			
2	3.20	10	4.207	29.3	0.655
3	6.39	10	4.207	29.3	-2.304
4	12.8	10	4.207	29.3	-0.521
5	25.5	10	4.207	29.3	3.101
6	52.9	10	4.207	29.3	9.411

reproduction
File: 1727r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	14.349	14.349	14.899
2	3.20	10	13.694	13.694	14.899
3	6.39	10	16.653	16.653	14.899
4	12.8	10	14.870	14.870	14.870
5	25.5	10	11.248	11.248	11.248
6	52.9	10	4.938	4.938	4.938

reproduction
File: 1727r 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
control	14.899				
3.20	14.899	0.302		1.68	k= 1, v=54
6.39	14.899	0.302		1.76	k= 2, v=54
12.8	14.870	0.286		1.79	k= 3, v=54
25.5	**11.248**	**1.703**		**1.80**	**k= 4, v=54**
52.9	4.938	5.168	*	1.80	k= 5, v=54

s = 4.072
Note: df used for table values are approximate when v > 20.

length
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.299	0.060	2.308
Within (Error)	44	1.159	0.026	
Total	49	1.459		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	4.641	4.641		
2	3.20	4.672	4.672	-0.417	
3	6.39	4.656	4.656	-0.190	
4	12.8	4.680	4.680	-0.525	
5	25.5	4.623	4.623	0.238	
6	52.9	4.380	4.380	2.695	*

Bonferroni T table value = 2.42 (1 Tailed Value, P=0.05, df=40,5)

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	9			
2	3.20	10	0.180	3.9	-0.031
3	6.39	9	0.184	4.0	-0.014
4	12.8	10	0.180	3.9	-0.039
5	25.5	8	0.190	4.1	0.019
6	52.9	4	0.235	5.1	0.261

length
File: 17271 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	9	4.641	4.641	4.663
2	3.20	10	4.672	4.672	4.663
3	6.39	9	4.656	4.656	4.663
4	12.8	10	4.680	4.680	4.663
5	25.5	8	4.623	4.623	4.623
6	52.9	4	4.380	4.380	4.380

length
File: 17271 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
control	4.663				
3.20	4.663	0.292		1.68	k= 1, v=44
6.39	4.663	0.285		1.76	k= 2, v=44
12.8	4.663	0.292		1.79	k= 3, v=44
25.5	4.623	0.236		1.80	k= 4, v=44
52.9	4.380	2.677	*	1.80	k= 5, v=44

s = 0.162
Note: df used for table values are approximate when v > 20.

weight
File: 1727w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.397	0.079	1.837
Within (Error)	44	1.899	0.043	
Total	49	2.295		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

weight
File: 1727w 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	1.189	1.189		
2	3.20	1.255	1.255	-0.691	
3	6.39	1.188	1.188	0.011	
4	12.8	1.069	1.069	1.262	
5	25.5	1.028	1.028	1.603	
6	52.9	1.007	1.007	1.463	

Bonferroni T table value = 2.42 (1 Tailed Value, P=0.05, df=40,5)

weight
File: 1727w 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	control	9			
2	3.20	10	0.231	19.4	-0.066
3	6.39	9	0.237	19.9	0.001
4	12.8	10	0.231	19.4	0.120
5	25.5	8	0.244	20.5	0.162
6	52.9	4	0.302	25.4	0.182

weight
File: 1727w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	9	1.189	1.189	1.224
2	3.20	10	1.255	1.255	1.224
3	6.39	9	1.188	1.188	1.188
4	12.8	10	1.069	1.069	1.069
5	25.5	8	1.028	1.028	1.028
6	52.9	4	1.007	1.007	1.007

weight

File: 1727w 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
control	1.224				
3.20	1.224	0.363		1.68	k= 1, v=44
6.39	1.188	0.011		1.76	k= 2, v=44
12.8	1.069	1.259		1.79	k= 3, v=44
25.5	1.028	1.600		1.80	k= 4, v=44
52.9	1.007	1.460		1.80	k= 5, v=44

s = 0.208
Note: df used for table values are approximate when v > 20.

length
6
9
10
9
10
8
4
control
4.79
4.42
4.52
4.44
4.51
4.71
4.93
4.56
4.89
3.20
4.71
4.49
4.97
4.6
4.78
4.46
4.62
4.79
4.55
4.75
6.39
4.93
4.57
4.56
4.63
4.57
4.65
4.76
4.63
4.6
12.8
4.5
4.71
4.68
4.51
4.57
4.66
4.72
4.76
4.91
4.78
25.5
4.72
4.67
4.77
4.59
4.7
4.22

4.68
4.63
52.9
4.35
4.72
4.17
4.28

reproduction
6
10
10
10
10
10
10
control
9.38
15.29
10.67
12.13
17.69
12.69
21.69
18.45
11.93
13.57
3.20
13.43
18.58
11.8
11.67
7.92
14.53
12.13
15
15.03
16.85
6.39
18.15
15.08
12.27
15.71
16.62
17
12.07
19.64
24.45
15.54
12.8
16.38
14.69
14.46
12.87
10.67
18.46
17.75
15.93
11.87
15.62
25.5
0
16.5
15.23
12.55

17.73
14.85
11.85
11.13
12.64
0
52.9
2.5
0
6.31
0
5.5
10
6
0
9.4
9.67

percent survival
6
13
13
13
13
13
13
control
100
100
100
0
100
100
100
100
100
100
100
100
100
3.20
100
100
100
100
100
100
100
100
100
100
100
100
6.39
100
100
100
100
0
100
100
100
100
100
100
100
100
100
12.8
100
100
100
100
100
100

100
100
100
100
100
100
100
25.5
80
80
100
0
100
100
100
100
100
100
100
100
0
52.9
60
80
60
0
0
100
0
0
100
0
0
100
100

weight
6
9
10
9
10
8
4
control
1.154
1.268
1.091
0.866
1.557
1.149
1.169
1.406
1.041
3.20
1.488
1.168
1.496
1.036
0.826
0.94
1.296
1.594
1.539
1.165
6.39
1.384
1.521
1.258
1.136
1.159
1.092
1.139
1.183
0.819
12.8
1.071
1
1.269
1.095
0.763
1.214
0.953
1.271
1.003
1.049
25.5
1.025
1.008
1.044
1.199
0.962
0.842

0.757
1.383
52.9
0.734
1.051
1.024
1.218