

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the Chronic Toxicity of AE F130060 Technical to Freshwater Invertebrates - *Daphnia* sp.
PMRA Submission Number {.....} EPA MRID Number 45386304

Data Requirement: PMRA DATA CODE
EPA DP Barcode D284719
OECD Data Point
EPA MRID 45386304
EPA Guideline §72-4b

Test material: AE F 130060 Technical Purity: 94.6%
Common name: Mesosulfuron-methyl
Chemical name: IUPAC: Methyl 2-[3-(4,6-dimethoxyprimidin-2-yl)ureidosulfonyl]-4-methanesulfonamidomethylbenzoate
CAS name: Not reported
CAS No.: Not reported
Synonyms: Code: AE F130060 00 1C95 0001

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Date: 9/8/03

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{EPA/OECD/PMRA}

Date: 01/09/04

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 122009

Date Evaluation Completed:

CITATION: Sowig, P., et al. 2000. Effects on growth and reproduction of *Daphnia magna* (Waterflea), AE F130060; substance, technical. Unpublished study performed by Aventis CropScience GmbH, Frankfurt am Main, Germany. Laboratory Study Identification CE97/098. Study submitted by Aventis CropScience, Research Triangle Park, NC. Study initiated June 5, 1997 and completed October 18, 2000.

EXECUTIVE SUMMARY:

The 21-day chronic toxicity of AE F130060 Technical (Mesosulfuron-methyl) to *Daphnia magna* was studied under static-renewal conditions. Nominal concentrations were 0 (negative control), 10, 18, 32, 56, and 100 ppm; mean-measured concentrations were <0.26 (LOD, control), 9.3, 16, 28.7, 49.4, and 90.0 ppm a.i.

No mortalities/immobilizations were observed in any test vessel during the 21-day study. The 21-day LC/EC₅₀ was >90.0 ppm a.i. In addition, no immobilization of neonates was observed. The first brood release occurred on Day 9 for the ≤49.4 ppm a.i. groups, and on Day 12 for the 90.0 ppm a.i. group, indicating a treatment-related effects at this level. The NOEC for time to first brood release was 49.4 ppm a.i. Instead of providing the overall mean number of offspring/adult, the number of offspring/adult were individually assessed on Days 9, 12, 14, 16, 19, and 21. A treatment-related reduction in the number of offspring/test vessel was observed on Days 9, 12, and 16. The NOEC for offspring/adult was determined to be 28.7 ppm a.i., based on reduced reproduction on Days 9 and 12. **A treatment-related reduction in terminal length was observed at all test levels. The resultant NOEC for length was <9.3 ppm a.i.** A treatment-related reduction in terminal dry weight was observed at the ≥49.4 ppm a.i. levels. The resultant NOEC for terminal weight was 28.7 ppm a.i. Overall the endpoints affected by treatment with AE F130060 Technical included the time for first brood release, the number of offspring/adult, and terminal growth measurements. The most sensitive endpoint was terminal length, where all test levels were affected.

This study is scientifically sound, but does not fulfill the guideline requirements for an aquatic invertebrate life cycle test with *Daphnia magna* (§ 72-4b). An NOEC for growth was not established as effects were observed at the lowest tested dosage.

Results Synopsis:

Test Organism Age (eg. 1st instar): 1st instar, ≤24 hours old
Test Type (Flowthrough, Static, Static Renewal): Static Renewal

Adult and Juvenile immobility

LC/EC₅₀: >90.0 ppm a.i.
NOEC: 90.0 ppm a.i.
LOEC: >90.0 ppm a.i.

Time to First Brood Release

NOEC: 49.4 ppm a.i.
LOEC: 90.0 ppm a.i.

Reproduction (Offspring/adult)

NOEC: 28.7 ppm a.i.
LOEC: 49.4 ppm a.i.

Length

NOEC: <9.3 ppm a.i.
LOEC: 9.3 ppm a.i.

Dry Weight

NOEC: 28.7 ppm a.i.
LOEC: 49.4 ppm a.i.

Most Sensitive Endpoint: Length

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the OECD Guideline No. 202 (1984, and proposed update 1996), the U.S. EPA Pesticide Assessment Guidelines, Series §72-4 (1982), and the EU Directive 92/69/EEG Annex Part C:C.2. Deviations from U.S. EPA FIFRA Guideline §72-4b include:

1. The storage conditions of the test material were not reported.
2. The pretest health (including mortality) of the parental stock was not specified. In addition, a 21-day isolated sub-culture was not performed.
3. The water hardness in terms of mg/L as CaCO₃ was not provided.
4. The dissolved oxygen content in terms of percent saturation was not reported.
5. The pH range (7.1-7.8) was slightly lower than the recommended range (7.6-8.0).
6. The total organic carbon and particulate matter contents, and levels of metals, pesticides, and chlorine in the dilution water were not reported.

These deviations did not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in accordance with OECD principles of GLP (p. 3).

A. MATERIALS:

1. Test Material AE F 130060 Technical (Mesosulfuron-methyl)

Description: Light beige powder

Lot No./Batch No. : Code: AE F130060 00 1C95 0001

Purity: 94.6%

Stability of Compound Under Test Conditions: New test medium was sampled on Days 0, 7, 9, 14, and 19. Old test medium was sampled on Days 2, 9, 12, 16, and 21. Recovery rates ranged from 91.8-101.1% of nominal concentrations, with no pattern of decline (Tables 6.2.2 and 6.2.4, pp. 28-29).

Storage conditions of test chemicals: Not reported.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. Test organism:

Species: *Daphnia magna*
Age of the parental stock: Not specified
Source: In-house laboratory cultures.

B. STUDY DESIGN:

1. Experimental Conditions

- a. Range-finding Study: A range-finding study was not reported.
- b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
<u>Parental acclimation:</u> Period: Conditions (same as test or not): Feeding: Health: (any mortality observed)	Continuous culture Same as test Unicellular green algae, <i>Selenastrum subspicatus</i> , provided twice a week. Not reported	
<u>Test condition:</u> static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal	Static renewal N/A Days 2, 5, 7, 9, 12, 14, 16, and 19	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.

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Parameter	Details	Remarks
		Criteria
Aeration, if any	No aeration during the study.	<i>Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.</i>
Duration of the test	21 days	<i>EPA requires 21 days for static renewal</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: growth/reproduction test: survival test: Fill volume: growth/reproduction test: survival test:	Glass beakers with glass lids 175 mL (reviewer-calculated) 600 mL 100 mL (6.8- to 7.0-cm depth) 400 mL (7.9- to 8.2-cm depth)	1. <i>Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics</i> 2. <i>Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.</i> <i>OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in each vessel.</i>
Source of dilution water	Deionized water and artificial mineral medium M4 (Elendt 1990) were used to prepare eight different stock solutions, each containing different chemical components (pp. 14-16). Varying volumes of each of the prepared solutions were combined to make 1 L of the dilution water.	<i>Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</i>

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Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	1.66-1.70 mmol/L (Ca ²⁺ + Mg ²⁺) 7.1-7.8 5.5-9.5 mg/L 19.7-20.4°C Not reported Not reported Not reported Not reported Not reported	The water hardness in terms of mg/L as CaCO ₃ was not provided. The dissolved oxygen content in terms of percent saturation was not reported. The pH range (7.1-7.8) was slightly lower than the recommended range (7.6-8.0). EPA requires: 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
<u>Number of organisms:</u> growth/reproduction test: survival test:	25 daphnids/level for the control and test groups. 10 daphnids, divided into 10 chambers, with 1 daphnid/chamber 15 daphnids, divided into three chambers, with 5 daphnids/chamber	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.

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Parameter	Details	Remarks
		Criteria
<p><u>Application rates:</u> nominal:</p> <p>measured:</p>	<p>0 (negative control), 10, 18, 32, 56, and 100 ppm</p> <p><0.26 (LOD, control), 9.3, 16, 28.7, 49.4, and 90.0 ppm a.i.</p>	<p>Fresh and/or aged water samples were collected and analyzed on Days 0, 2, 7, 9, 12, 14, 16, 19, and 21. Mean-measured concentrations were reviewer-calculated from corrected (for purity) fresh and aged analytical data in Tables 6.2.2 and 6.2.4, pp. 28-29.</p> <hr/> <p><i>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.</i></p>
Solvent (type, percentage, if used)	N/A	<hr/> <p><i>EPA requires: solvent 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. OECD requires ≤ 0.1 ml/L</i></p>
Lighting	16:8 hour light/dark cycle	<hr/> <p><i>EPA/OECD requires: 16 hours light, 8 hours dark.</i></p>
Feeding	During the test, daphnids were fed unicellular green algae, <i>Selenastrum subspicatus</i> , daily during the week and provided a three fold amount on Fridays for the weekend. The amount of food for Days 0-8 was 10 million cells/adult/day and for Days 9-21 was 15 million cells/adult/day.	

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Parameter	Details	Remarks
		Criteria
Stability of chemical in the test system	Verified. New test medium was sampled on Days 0, 7, 9, 14, and 19. Old test medium was sampled on Days 2, 9, 12, 16, and 21. Recovery rates ranged from 91.8-101.1% of nominal concentrations, with no pattern of decline (Tables 6.2.2 and 6.2.4, pp. 28-29).	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	97.0-104.0% of nominal Days 0, 2, 7, 9, 12, 14, 16, 19, and 21 0.26 ppm 0.44 ppm	Based on matrix spikes analyzed concurrently with the samples at each sampling interval (Tables 6.2.2 and 6.2.4, pp. 28-29).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
		Criteria
Data end points measured (list)	<ul style="list-style-type: none"> - Survival of first-generation daphnids and neonates - # young produced per test vessel - 1st appearance of juveniles - Total length - Dry weight 	<p><i>EPA requires:</i></p> <ul style="list-style-type: none"> - Survival of first-generation daphnids, - Number of young produced per female, - Wet or 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	Mortality and juvenile production and condition of parent daphnia (abnormalities) were observed at every renewal (three times weekly). Total length and body weight were determined at the end of the test.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No mortalities/immobilizations were observed in any test vessel (those maintained for survival as well as reproduction/growth) during the 21-day study (p. 24). The 21-day LC/EC₅₀ was >100 ppm (nominal).

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Table 1: Effect of AE F130060 Technical on Growth, Reproduction, and Survival of *Daphnia* sp.

Treatment, ppm a.i. Measured and (Nominal) Concentrations	Mortality (dead or immobile)		Total Mean No. of Young	Mean No. of Young per Test Vessel (Day 21) ¹	Mean Day of 1 st Brood
	No. Dead	%			
Negative control	0	0	134.9	20.1	9
9.3 (10)	0	0	119.5	11.4	9
16 (18)	0	0	123.3	15.0	9
28.7 (32)	0	0	121.1	14.3	9
49.4 (56)	0	0	100.7*	10.1	9
90.0 (100)	0	0	80.1*	13.8	12*
NOEC, ppm	100		32	32	56
LOEC, ppm	>100		56	56	100
MATC, ppm	ND		ND	42.3	ND
LC ₅₀ /EC ₅₀ , ppm	>100		ND	ND	ND

¹ Data for Day 21 only. An overall mean was not determined.

* Values with differences versus control, as determined by the study authors.

ND = Not determined.

B. EFFECT ON REPRODUCTION AND GROWTH:

The first brood release occurred on Day 9 for the ≤56 ppm groups, and on Day 12 for the 100 ppm group, indicating a treatment-related effects at this level. The NOEC for time to first brood release was 56 ppm.

No immobilization of neonates was observed.

The total mean number of young produced was statistically-reduced at the ≥56 ppm test levels, with a NOEC of 32 ppm. Instead of providing the overall mean number of offspring/adult, the number of offspring/adult were individually assessed on Days 9, 12, 14, 16, 19, and 21 (Table 6.6, p. 32 and Figure 7.5, p. 36). A treatment-related reduction in the number of offspring/test vessel was observed on Days 9, 12, and 16, although the study authors noted that the Day-16 data were non-homogeneous (even with log-transformation, p.24). The NOEC for offspring/adult was determined to be 32 ppm, based on reduced reproduction on Days 9 and 12. The study authors noted that the lower NOEC on these days may indicate a slight retardation of the start of reproduction.

The terminal growth of daphnids maintained in groups (for survival) were assessed in addition to those maintained individually (Table 6.7, p. 33 and Figures 7.7-7.10, pp. 38-41). Statistical comparisons revealed a treatment-related effect on the length of adult daphnids maintained under both conditions at all treatment levels. The NOEC for terminal length was <10 ppm. The weight of adult daphnids also was statistically-reduced in animals maintained under both conditions, although at different levels. In daphnids maintained individually, the NOEC for terminal weight was 56 ppm and in daphnids maintained in groups of five, the NOEC was <10 ppm.

The NOEC for terminal weight was concluded to be <10 ppm.

Table 2. Effect of AE F130060 Technical on Terminal Growth of *Daphnia* sp.

Measured and (nominal) concentrations (ppm a.i.) ¹	Length (cm)		Weight (g)	
	Single	Group	Single	Group
Negative control	4.37	4.36	0.95	0.91
9.3 (10)	4.05*	3.96*	0.91	0.73*
16 (18)	4.09*	3.92*	0.84	0.67*
28.7 (32)	4.03*	4.12*	0.86	0.72*
49.4 (56)	4.14*	4.02*	0.82	0.81*
90.0 (100)	4.11*	4.14*	0.70*	0.67*
NOEC, ppm	<10	<10	56	<10
LOEC, ppm	ND	ND	100	ND
MATC, ppm	ND	ND	ND	ND

* Values with differences versus control, as determined by the study authors.
 ND - Not determined.

C. REPORTED STATISTICS:

The study authors reported that the NOEC and LOEC was determined using General Linear Models using DUNCAN's Multiple Range Test Procedures (SAS, 1989). The ANOVA assumption of homogeneity of variance was conducted, followed by Bartlett's Test. In cases where the assumption of homogeneity could not be verified, data were transformed logarithmically. The MATC was the geometric mean of the NOEC and LOEC values. The LC/EC₅₀ values were estimated based on the reproduction and survival data.

D. VERIFICATION OF STATISTICAL RESULTS:

Length, weight, and reproductive data (from singled adult females) were determined to satisfy the assumptions of ANOVA (i.e., normality and homogeneity of variances). The NOEC and LOEC for these endpoints were determined using ANOVA and, if necessary, Dunnett's (length) or William's (weight and reproduction) multiple comparison test via TOXSTAT statistical software. There was no immobility for adults or juveniles in this study, so the NOEC and LOEC for these endpoints were determined visually. The time to first brood release was also determined visually. Results are based on the average of measured (and corrected for purity) fresh and aged test solutions.

Adult and Juvenile immobility

LC/EC₅₀: >90.0 ppm a.i.

NOEC: 90.0 ppm a.i.

LOEC: >90.0 ppm a.i.

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Time to First Brood Release

NOEC: 49.4 ppm a.i.

LOEC: 90.0 ppm a.i.

Reproduction (Offspring/adult)

NOEC: 28.7 ppm a.i.

LOEC: 49.4 ppm a.i.

Length

NOEC: <9.3 ppm a.i.

LOEC: 9.3 ppm a.i.

Dry Weight

NOEC: 28.7 ppm a.i.

LOEC: 49.4 ppm a.i.

Endpoints Affected: Time to first brood release, number offspring/adult, length, dry weight
Most Sensitive Endpoint: Length

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-4b that affected the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study authors'. Length was significantly affected at all treatment levels. Another concurrently-submitted study (CE97/098-2; MRID 45386305) was conducted at lower levels to determine a NOEC for the chronic effects of AE F130060 on daphnid growth. The study author also assessed growth in vessels containing individual as well as group-held daphnia, whereas the reviewer assessed the data only from the individually-maintained daphnia. The study authors based toxicity values on the nominal concentrations, while the reviewer based them on the mean-measured concentrations.

No ephippia were produced by any test individual.

G. CONCLUSIONS:

The study is scientifically sound and fulfills the guideline requirements for an aquatic invertebrate life cycle test with the *Daphnia magna* (§72-4b). This study is classified as Core. The most sensitive endpoint was terminal length.

Adult and Juvenile immobility

LC/EC₅₀: >90.0 ppm a.i.

NOEC: 90.0 ppm a.i.

LOEC: >90.0 ppm a.i.

Time to First Brood Release

NOEC: 49.4 ppm a.i.
LOEC: 90.0 ppm a.i.

Reproduction (Offspring/adult)

NOEC: 28.7 ppm a.i.
LOEC: 49.4 ppm a.i.

Length

NOEC: <9.3 ppm a.i.
LOEC: 9.3 ppm a.i.

Dry Weight

NOEC: 28.7 ppm a.i.
LOEC: 49.4 ppm a.i.

Endpoints Affected: Time to first brood release, number offspring/adult, length, dry weight
Most Sensitive Endpoint: Length

III. REFERENCES:

- Organization for Economic Co-operation and Development. 1984. OECD Guideline for Testing of Chemicals; Guideline No. 202: *Daphnia* sp., Acute Immobilization Test and Reproduction Test, 04 April 1984.
- Organization for Economic Co-operation and Development. 1996. Proposal for Updating Guideline No. 202, Part II: *Daphnia magna* Reproduction Test.
- U.S. Environmental Protection Agency (EPA). 1975. Committee on Methods for Toxicity Tests with Aquatic Organisms, Method for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-660/3-75-009.
- U.S. Environmental Protection Agency (EPA). 1982. Pesticide Assessment Guidelines. Subdivision E, §72-4, Fish early life stage and aquatic invertebrate life-cycle studies.
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- Elendt, B.P. 1990. Selenium deficiency in Crustacea. An ultrastructural approach to antennal damage in *Daphnia magna* Straus., *Protoplasma* 154: 25-33.
- Stephan, C.E. 1982. A Computer Program for Calculating an LC₅₀, U.S. Environmental Protection Agency, Duluth, Mn. Letter to Dr. Lowell Bahner, Chairman of the ASTM Task group on Calculating LC50s; September 10, 1982.
- SAS. 1989, SAS Institute Inc., Cary, NC USA, Release 6.12 TS407.
- Stephenson, R.R. & S.A. Watts. 1984. Chronic toxicity tests with *Daphnia magna*: the effects of different food and temperature regimes on survival, reproduction and growth. *Environmental Pollution (Series A)* 95:95-107.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

length

File: 63041 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.732	0.146	3.561
Within (Error)	54	2.222	0.041	
Total	59	2.954		

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

length

File: 63041 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	4.367	4.367		
2	9.3	4.055	4.055	3.445	*
3	16.0	4.088	4.088	3.081	*
4	28.7	4.034	4.034	3.677	*
5	49.4	4.144	4.144	2.463	*
6	90.0	4.112	4.112	2.816	*

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

length

File: 63041 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	9.3	10	0.209	4.8	0.312
3	16.0	10	0.209	4.8	0.279
4	28.7	10	0.209	4.8	0.333

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5	49.4	10	0.209	4.8	0.223
6	90.0	10	0.209	4.8	0.255

length

File: 63041 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	4.367	4.367	4.367
2	9.3	10	4.055	4.055	4.087
3	16.0	10	4.088	4.088	4.087
4	28.7	10	4.034	4.034	4.087
5	49.4	10	4.144	4.144	4.087
6	90.0	10	4.112	4.112	4.087

length

File: 63041 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	4.367				
9.3	4.087	3.091	*	1.68	k= 1, v=54
16.0	4.087	3.091	*	1.76	k= 2, v=54
28.7	4.087	3.091	*	1.79	k= 3, v=54
49.4	4.087	3.091	*	1.80	k= 4, v=54
90.0	4.087	3.091	*	1.80	k= 5, v=54

s = 0.203

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

weight

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.379	0.076	4.222
Within (Error)	54	0.970	0.018	

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 Total 59 1.349

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

weight
 File: 6304w 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	0.945	0.945		
2	9.3	0.913	0.913	0.533	
3	16.0	0.836	0.836	1.817	
4	28.7	0.858	0.858	1.450	
5	49.4	0.816	0.816	2.150	
6	90.0	0.696	0.696	4.150	*

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

weight
 File: 6304w 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	9.3	10	0.139	14.7	0.032
3	16.0	10	0.139	14.7	0.109
4	28.7	10	0.139	14.7	0.087
5	49.4	10	0.139	14.7	0.129
6	90.0	10	0.139	14.7	0.249

weight
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED
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	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	control	10	0.945	0.945	0.945
2	9.3	10	0.913	0.913	0.913
3	16.0	10	0.836	0.836	0.847
4	28.7	10	0.858	0.858	0.847
5	49.4	10	0.816	0.816	0.816
6	90.0	10	0.696	0.696	0.696

weight
 File: 6304w 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	0.945				
9.3	0.913	0.534		1.68	k= 1, v=54
16.0	0.847	1.635		1.76	k= 2, v=54
28.7	0.847	1.635		1.79	k= 3, v=54
49.4	0.816	2.152	*	1.80	k= 4, v=54
90.0	0.696	4.153	*	1.80	k= 5, v=54

s = 0.134

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

reproduction

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	19268.333	3853.667	7.561
Within (Error)	54	27523.400	509.693	
Total	59	46791.733		

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

reproduction

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Data Evaluation Report on the Chronic Toxicity of AE F130060 Technical to Freshwater Invertebrates - Daphnia sp.
 PMRA Submission Number {.....} EPA MRID Number 45386304

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	134.900	134.900		
2	9.3	119.500	119.500	1.525	
3	16.0	123.300	123.300	1.149	
4	28.7	121.100	121.100	1.367	
5	49.4	100.700	100.700	3.387	*
6	90.0	80.100	80.100	5.428	*

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

reproduction
 File: 6304r 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	9.3	10	23.323	17.3	15.400
3	16.0	10	23.323	17.3	11.600
4	28.7	10	23.323	17.3	13.800
5	49.4	10	23.323	17.3	34.200
6	90.0	10	23.323	17.3	54.800

reproduction
 File: 6304r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	134.900	134.900	134.900
2	9.3	10	119.500	119.500	121.400
3	16.0	10	123.300	123.300	121.400
4	28.7	10	121.100	121.100	121.100
5	49.4	10	100.700	100.700	100.700
6	90.0	10	80.100	80.100	80.100

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Data Evaluation Report on the Chronic Toxicity of AE F130060 Technical to Freshwater Invertebrates - Daphnia sp.
 PMRA Submission Number {.....} EPA MRID Number 45386304

reproduction
 File: 6304r 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	134.900				
9.3	121.400	1.337		1.68	k= 1, v=54
16.0	121.400	1.337		1.76	k= 2, v=54
28.7	121.100	1.367		1.79	k= 3, v=54
49.4	100.700	3.387	*	1.80	k= 4, v=54
90.0	80.100	5.428	*	1.80	k= 5, v=54

s = 22.576

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

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APPENDIX

Page ___ is not included in this copy.

Pages 34 through 42 are not included in this copy.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
- FIFRA registration data.
- The document is a duplicate of page(s) _____.
- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
