

Data Evaluation Report on the Chronic Toxicity of AE F130060 Technical to Freshwater Invertebrates - *Daphnia* sp.
PMRA Submission Number {.....} EPA MRID Number 45386305

Data Requirement: PMRA DATA CODE
EPA DP Barcode D284719
OECD Data Point
EPA MRID 45386305
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

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: *Rebecca Bryan*
Date: 9/8/03

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{EPA/OECD/PMRA}

Date: *7/1/04* *Tim Bargar*

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 122009

Date Evaluation Completed:

CITATION: Sowig, P., et al. 2000. First Amendment to Report CE97/098-1: C008780: 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 Unpublished study performed by Aventis CropScience GmbH, Frankfurt am Main, Germany.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 CE97/098-2. Study submitted by Aventis CropScience, Research Triangle Park, NC. Study initiated June 5, 1997 and completed October 18, 2000.



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EXECUTIVE SUMMARY:

Further investigations on the 21-day chronic toxicity of AE F130060 Technical (Mesosulfuron-methyl) to *Daphnia magna* was studied under static-renewal conditions. This study was conducted at lower concentrations levels in attempts to further define a NOEC for terminal growth measurements. Results from the original life-cycle study was provided in MRID 45386304 (Laboratory Study Identification CE97/098-1). The original study was conducted a nominal concentrations of 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. This study was conducted at nominal concentrations of 0 (negative control), 1.0, 1.8, 3.2, 5.6, 10, and 18 ppm; mean-measured concentrations were <0.03 (LOD, control), 0.99, 1.7, 3.0, 5.1, 8.8, and 16.0 ppm a.i.

In CE97/098-1 (MRID 45386304), 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. Results from the current study supported these findings.

In CE97/098-1, a treatment-related reduction in terminal length was observed at all test levels. The resultant NOEC for length was <9.3 ppm a.i. In the present study, no treatment-related effect on terminal length was observed in any test level in animals maintained under both conditions. The NOEC for length was 16.0 ppm a.i. As these results were contradictory, a conservative NOEC was concluded to be 5.1 ppm a.i.

In CE97/098-1, 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. In the present study, the weight of adult daphnids was statistically-reduced in animals maintained under both conditions, although at different levels. In daphnids maintained individually, the NOEC for terminal weight was 1.7 ppm a.i. and in daphnids maintained in groups of five, the NOEC was 8.8 ppm a.i.. The resultant NOEC was concluded to be 1.7 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 dry weight.

This study, in conjunction with MRID 45386304, is scientifically sound, fulfills the guideline requirements for an aquatic invertebrate life cycle test with *Daphnia magna* (§ 72-4b), and is classified CORE.

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 (from CE97/098-1; MRID 45386304)

LC/EC₅₀: >90.0 ppm a.i.

NOEC: 90.0 ppm a.i.

LOEC: >90.0 ppm a.i.

Time to First Brood Release (from CE97/098-1; MRID 45386304)

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

Reproduction (Offspring/adult) (from CE97/098-1; MRID 45386304)

NOEC: 28.91 mg a.i./L
LOEC: 50.10 mg a.i./L

Length

NOEC: 5.1 ppm a.i. (next lowest concentration tested; from CE97/098-2)
LOEC: 9.3 ppm a.i. (from CE97/098-1; MRID 45386304)

Dry Weight

NOEC: 1.7 ppm a.i. *
LOEC: 3.0 ppm a.i.

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

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 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, 3, 10, 17, and 19. Old test medium was sampled on Days 3, 5, 12, 19, and 21. Recovery rates ranged from 92.4-105.3% of nominal concentrations, with no pattern of decline (Tables 6.2.2 and 6.2.4, pp. 29-30).

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 3, 5, 7, 10, 12, 14, 17, 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.
Aeration, if any	No aeration during the study.	Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.
Duration of the test	21 days	EPA requires 21 days for static renewal

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Parameter	Details	Remarks
		Criteria
<p><u>Test vessel</u> Material: (glass/stainless steel)</p> <p>Size:</p> <p style="padding-left: 40px;">growth/reproduction test: 175 mL (reviewer-calculated)</p> <p style="padding-left: 40px;">survival test: 600 mL</p> <p>Fill volume:</p> <p style="padding-left: 40px;">growth/reproduction test: 100 mL (5.8- to 6.1-cm depth)</p> <p style="padding-left: 40px;">survival test: 400 mL (7.8- to 8.1-cm depth)</p>		<p>1. <u>Material:</u> Glass, No. 316 stainless steel, or perfluorocarbon plastics</p> <p>2. <u>Size:</u> 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.</p> <p>OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in each vessel.</p>
<p>Source of dilution water</p>	<p>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.</p>	<p>Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</p>

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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	1.63-1.72 mmol/L (Ca ²⁺ + Mg ²⁺) 7.6-8.0 8.1-9.1 mg/L 19.2-20.0°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. <hr/> 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 range 6 - 9 and should not vary more than 1.5 units in any one test. Dissolved Oxygen <u>Renewal</u> : 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	<hr/> 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: measured:</p>	<p>0 (negative control), 1.0, 1.8, 3.2, 5.6, 10, and 18 ppm <0.03 (LOD, control), 0.99, 1.7, 3.0, 5.1, 8.8, and 16.0 ppm a.i.</p>	<p>Fresh and/or aged water samples were collected and analyzed on Days 0, 3, 5, 10, 12, 17, 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. 29-30.</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	<p>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.</p>	

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Parameter	Details	Remarks
		Criteria
Stability of chemical in the test system	Verified. New test medium was sampled on Days 0, 3, 10, 17, and 19. Old test medium was sampled on Days 3, 5, 12, 19, and 21. Recovery rates ranged from 92.4-105.3% of nominal concentrations, with no pattern of decline (Tables 6.2.2 and 6.2.4, pp. 29-30).	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	94.2-101.5% of nominal Days 0, 3, 5, 10, 12, 17, 19, and 21 0.03 ppm 0.04 ppm	Based on matrix spikes analyzed concurrently with the samples at each sampling interval (Tables 6.2.2 and 6.2.4, pp. 29-30).
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	<p>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.</p>	
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 >18 ppm (nominal).

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	77.5	14.1	10
0.99 (1.0)	0	0	59.4	2.8	10
1.7 (1.8)	0	0	76.5	17.0	10
3.0 (3.2)	0	0	72.2	16.6	10
5.1 (5.6)	0	0	68.3	16.9	10
8.8 (10.0)	0	0	79.6	19.4	10
16.0 (18.0)	0	0	65.8	14.2	10
NOEC, ppm	18		18	18	18
LOEC, ppm	>18		>18	>18	>18
MATC, ppm	ND		ND	ND	ND
LC ₅₀ /EC ₅₀ , ppm	>16		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 10 for all test and control groups (p. 24). No immobilization of neonates was observed. In addition, no treatment-related effects on the total mean number of young produced or the number of offspring/adult individually assessed on Days 7, 10, 12, 14, 17, 19, and 21 (Tables 6.6 and 6.7, pp. 32-33, and Figures 7.3 and 7.4, pp. 38-39). The overall mean number of offspring/adult was not assessed. The subsequent NOEC for time to first brood release, mortality of neonates, and reproduction was 18 ppm. These data supported the results of the previous study.

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 7.5-7.8, pp. 40-43). The study authors reported that the data were difficult to interpret.

In this study, no treatment-related effect on terminal length was observed in any test level in animals maintained under both conditions. The NOEC for length was 18 ppm. This was contradictory to the previous study, in

which a statistically-significant reduction in terminal length was observed in animals (maintained either singly or in groups) at all test levels, including the 10 and 18 ppm levels (nominal). The study authors reported that in order to harmonize findings from both studies, the NOEC was considered to be 5.6 ppm, (which is the test level below 10 ppm).

The weight of adult daphnids was statistically-reduced in animals maintained under both conditions, although at different levels. In daphnids maintained individually, the NOEC for terminal weight was 1.8 ppm and in daphnids maintained in groups of five, the NOEC was 10 ppm. The study authors reported that due to lack of a clear dose-response in singly-maintained adults (Table 6.7, p. 33), the NOEC for terminal weight was concluded to be 10 ppm (p. 25).

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	4.50	4.26	1.23	1.16
0.99 (1.0)	4.37	4.28	1.28	1.20
1.7 (1.8)	4.34	4.40	1.19	1.19
3.0 (3.2)	4.45	4.33	1.06*	1.16
5.1 (5.6)	4.35	4.31	0.99*	1.10
8.8 (10.0)	4.60	4.43	1.13	1.10
16.0 (18.0)	4.46	4.30	1.07*	1.02*
NOEC, ppm	18	18	1.8	10
LOEC, ppm	>18	>18	3.2	18
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.

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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 and weight) 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: >16.0 ppm a.i.
NOEC: 16.0 ppm a.i.
LOEC: >16.0 ppm a.i.

Time to First Brood Release

NOEC: 16.0 ppm a.i.
LOEC: 16.0 ppm a.i.

Reproduction (Offspring/adult)

NOEC: 16.0 ppm a.i.
LOEC: >16.0 ppm a.i.

Length

NOEC: 16.0 ppm a.i.
LOEC: >16.0 ppm a.i.

Dry Weight

NOEC: 1.7 ppm a.i.
LOEC: 3.0 ppm a.i.

Endpoints Affected: Dry weight

E. STUDY DEFICIENCIES:

This study was not conducted to stand alone. It was performed to provide supplemental data on growth endpoints. Results from the original study were provided in the concurrently-submitted MRID 45386304 (Laboratory Identification No. CE97/098-1). When considered in conjunction with MRID 45386304, 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 results were similar to the study authors'. Length was not significantly affected at any treatment level in this study; however, because it was adversely affected at all levels in CE97/098-1 (MRID 45386304), the NOEC was determined to be the concentration lower than the highest concentration tested in CE97/098-1. Both the reviewer's and study authors' analysis revealed significant reductions in weight at the nominal 3.2 ppm (3.0 ppm a.i.) treatment level; however, the study authors dismissed this effect as a basis for a NOEC because the response was not dose-dependent. The reviewer's conclusion regarding the weight NOEC is reported in the Executive Summary and Conclusions sections. Reproduction was not affected in this study, so the NOEC was

based on adverse effects detected in CE97/098-1; MRID 45386304. The study authors based toxicity values on the nominal concentrations, while the reviewer based them on the mean-measured concentrations. Results provided in the Executive Summary and Conclusions sections represent harmonization of the conclusions from both studies.

No ehippia were produced by any test individual.

G. CONCLUSIONS:

The study is scientifically sound and in conjunction with MRID 45386304, fulfills the guideline requirements for an aquatic invertebrate life cycle test with the *Daphnia magna* (§72-4b). In combination, these studies are classified as CORE. The most sensitive endpoint was terminal dry weight.

Adult and Juvenile immobility (from CE97/098-1; MRID 45386304)

LC/EC₅₀: >90.0 ppm a.i.

NOEC: 90.0 ppm a.i.

LOEC: >90.0 ppm a.i.

Time to First Brood Release (from CE97/098-1; MRID 45386304)

NOEC: 49.4 ppm a.i.

LOEC: 90.0 ppm a.i.

Reproduction (Offspring/adult) (from CE97/098-1; MRID 45386304)

NOEC: 28.91 mg a.i./L

LOEC: 50.10 mg a.i./L

Length

NOEC: 5.1 ppm a.i. (next lowest concentration tested; from CE97/098-2)

LOEC: 9.3 ppm a.i. (from CE97/098-1; MRID 45386304)

Dry Weight

NOEC: 1.7 ppm a.i.

LOEC: 3.0 ppm a.i.

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

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.
- Bradley, M.C. 1988. Report of the results and conclusions of the EEC *Daphnia magna* genetic typing exercise. University of Sheffield, Department of Zoology, February 3rd 1988.
- Deutsches Institut für Normung (DIN). 1989. German standard methods for the examination of water, waste water, and sludge. Normenausschuß Wasserwesen (NAW) im DIN Deutsches Institut für Normung e.V. Berlin.
- 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: 63051 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.530	0.088	2.444
Within (Error)	63	2.299	0.036	
Total	69	2.828		

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

length

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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	4.502	4.502		
2	0.99	4.373	4.373	1.520	
3	1.68	4.340	4.340	1.909	
4	2.99	4.456	4.456	0.542	
5	5.11	4.353	4.353	1.756	
6	8.78	4.602	4.602	-1.179	
7	16.02	4.464	4.464	0.448	

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

Ho: Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	10			
2	0.99	10	0.199	4.4	0.129

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3	1.68	10	0.199	4.4	0.162
4	2.99	10	0.199	4.4	0.046
5	5.11	10	0.199	4.4	0.149
6	8.78	10	0.199	4.4	-0.100
7	16.02	10	0.199	4.4	0.038

length

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	4.502	4.502	4.502
2	0.99	10	4.373	4.373	4.431
3	1.68	10	4.340	4.340	4.431
4	2.99	10	4.456	4.456	4.431
5	5.11	10	4.353	4.353	4.431
6	8.78	10	4.602	4.602	4.431
7	16.02	10	4.464	4.464	4.431

length

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	4.502				
0.99	4.431	0.827		1.67	k= 1, v=63
1.68	4.431	0.827		1.75	k= 2, v=63
2.99	4.431	0.827		1.77	k= 3, v=63
5.11	4.431	0.827		1.78	k= 4, v=63
8.78	4.431	0.827		1.79	k= 5, v=63
16.02	4.431	0.827		1.79	k= 6, v=63

s = 0.191

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

weight

File: 6305w

Transform: NO TRANSFORMATION

ANOVA TABLE

Data Evaluation Report on the Chronic Toxicity of AE F130060 Technical to Freshwater Invertebrates - Daphnia sp.
 PMRA Submission Number {.....} EPA MRID Number 45386305

SOURCE	DF	SS	MS	F
Between	6	0.647	0.108	8.308
Within (Error)	63	0.800	0.013	
Total	69	1.448		

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

weight
 File: 6305w 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	1.232	1.232		
2	0.99	1.275	1.275	-0.843	
3	1.68	1.192	1.192	0.784	
4	2.99	1.057	1.057	3.432	*
5	5.11	0.985	0.985	4.844	*
6	8.78	1.130	1.130	2.000	
7	16.02	1.073	1.073	3.118	*

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

weight
 File: 6305w 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	0.99	10	0.120	9.7	-0.043
3	1.68	10	0.120	9.7	0.040
4	2.99	10	0.120	9.7	0.175
5	5.11	10	0.120	9.7	0.247
6	8.78	10	0.120	9.7	0.102
7	16.02	10	0.120	9.7	0.159

Data Evaluation Report on the Chronic Toxicity of AE F130060 Technical to Freshwater Invertebrates - Daphnia sp.
 PMRA Submission Number [.....] EPA MRID Number 45386305

weight
 File: 6305w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	1.232	1.232	1.254
2	0.99	10	1.275	1.275	1.254
3	1.68	10	1.192	1.192	1.192
4	2.99	10	1.057	1.057	1.061
5	5.11	10	0.985	0.985	1.061
6	8.78	10	1.130	1.130	1.061
7	16.02	10	1.073	1.073	1.061

weight
 File: 6305w 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	1.254				
0.99	1.254	0.426		1.67	k= 1, v=63
1.68	1.192	0.793		1.75	k= 2, v=63
2.99	1.061	3.387	*	1.77	k= 3, v=63
5.11	1.061	3.387	*	1.78	k= 4, v=63
8.78	1.061	3.387	*	1.79	k= 5, v=63
16.02	1.061	3.387	*	1.79	k= 6, v=63

s = 0.113

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

reproduction
 File: 6305r Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	3160.343	526.724	2.197
Within (Error)	63	15101.100	239.700	
Total	69	18261.443		

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

reproduction
 File: 6305r 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	77.500	77.500		
2	0.99	59.400	59.400	2.614	*
3	1.68	76.500	76.500	0.144	
4	2.99	72.200	72.200	0.765	
5	5.11	68.300	68.300	1.329	
6	8.78	79.600	79.600	-0.303	
7	16.02	65.800	65.800	1.690	

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

reproduction
 File: 6305r 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	0.99	10	16.271	21.0	18.100
3	1.68	10	16.271	21.0	1.000
4	2.99	10	16.271	21.0	5.300
5	5.11	10	16.271	21.0	9.200
6	8.78	10	16.271	21.0	-2.100
7	16.02	10	16.271	21.0	11.700

reproduction
 File: 6305r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
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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 45386305

1	control	10	77.500	77.500	77.500
2	0.99	10	59.400	59.400	71.200
3	1.68	10	76.500	76.500	71.200
4	2.99	10	72.200	72.200	71.200
5	5.11	10	68.300	68.300	71.200
6	8.78	10	79.600	79.600	71.200
7	16.02	10	65.800	65.800	65.800

reproduction

File: 6305r

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	77.500				
0.99	71.200	0.910		1.67	k= 1, v=63
1.68	71.200	0.910		1.75	k= 2, v=63
2.99	71.200	0.910		1.77	k= 3, v=63
5.11	71.200	0.910		1.78	k= 4, v=63
8.78	71.200	0.910		1.79	k= 5, v=63
16.02	65.800	1.690		1.79	k= 6, v=63

s = 15.482

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

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