

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	355006
OECD Data Point	{.....}
EPA MRID	47471204
EPA Guideline	OPPTS 850.4400 (123-2)

Test material: Dimethyl Disulfide **Purity:** 99.6%

Common name

Chemical name: IUPAC: Not reported
 CAS name: Not reported
 CAS No. 624-92-0
 Synonyms: Not reported

Primary Reviewer: Moncie Wright
 Staff Scientist, Cambridge Environmental

Moncie V Wright
Signature:
Date: 09/09/08

Secondary Reviewer: Teri S. Myers
 Senior Scientist, Cambridge Environmental

Teri S Myers
Signature:
Date: 11/03/08

Primary Reviewer: Edward Odenkirchen
 {EPA/OECD/PMRA}

Edward Odenkirchen
Date: {.....}

Secondary Reviewer(s): Valerie Woodard
 {EPA/OECD/PMRA}

Valerie Woodard
Date: 3-20-09

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

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
Use Site Category: {.....} [For PMRA]
EPA PC Code 029088

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Minderhout, T., Kendall, T.Z., and H.O. Krueger. 2008. Dimethyl Disulfide: A 7-Day Static-Renewal Toxicity Test with Duckweed (*Lemna gibba* G3). Unpublished study performed by Wildlife International, Easton, MD. Laboratory Project No.: 524A-122. Study sponsored by Arkema, Inc., Philadelphia, Pennsylvania. Study completed June 3, 2008.

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 acute toxicity of a pesticide to aquatic vascular plants. 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



Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

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.

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants Duckweed (*Lemna gibba*) were exposed to dimethyl disulfide at nominal concentrations of 0 (negative control), 3.1, 6.3, 13, 25, 50, and 100 mg a.i./L under static renewal conditions. Time-weighted average concentrations were <2.00 (<LOQ, control), 3.2, 5.6, 12, 24, 48, and 95 mg a.i./L.

The 7-Day NOAEC and EC₅₀ values for frond density, the most sensitive endpoint, were 3.2 and 28 mg a.i./L, respectively. The % growth inhibition in frond density, in the treated algal culture as compared to the negative control, ranged from 2.6 to 86%.

Chlorosis was observed at all treatment levels and was more widespread in the highest two treatment levels. Necrosis was observed in all treatment levels except the lowest test concentration, and was more prevalent in the three highest treatment levels. Mortality was mostly observed at the highest treatment level.

This toxicity study is classified as scientifically sound and satisfies the guideline requirement for a Tier II vascular plant toxicity study with the freshwater species, *Lemna gibba*.

Results Synopsis

Test Organism: *Lemna gibba*

Test Type (Flow-through, Static, Static Renewal): Static renewal

Frond density

EC₀₅: 6.0 mg a.i./L 95% C.I.: 3.3 to 11 mg a.i./L

EC₅₀: 28 mg a.i./L 95% C.I.: 22 to 36 mg a.i./L

NOAEC: 3.2 mg a.i./L

Probit Slope: 2.45 ± 0.302

Growth rate

EC₀₅: 9.7 mg a.i./L 95% C.I.: 5.8 to 16 mg a.i./L

EC₅₀: 34 mg a.i./L 95% C.I.: 28 to 42 mg a.i./L

NOAEC: 12 mg a.i./L

Probit Slope: 2.98 ± 0.383

Biomass (Dry weight)

EC₀₅: 17 mg a.i./L 95% C.I.: 14 to 21 mg a.i./L

EC₅₀: 48 mg a.i./L 95% C.I.: 44 to 52 mg a.i./L

NOAEC: 12 mg a.i./L

Probit Slope: 3.69 ± 0.258

Endpoint(s) Affected: frond density, growth rate, and biomass

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

This study was conducted following US EPA Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.4400, ASTM Standard Guide 1415-91 E (1991), and the OECD Guideline 221: *Lemna sp. Growth Inhibition Test*. The following deviations from OPPTS 850.4400 were noted:

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

1. The physicochemical properties of the test material were not reported.
2. Pretest health of the test species was not reported.
3. At test initiation and termination, the pH of the solutions ranged from 7.8 to 8.0 and 8.3 to 9.0, respectively, well above the pH suggested by OPPTS guidelines of 7.5.

These deviations do not affect the acceptability of this study.

COMPLIANCE:

Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. A certificate of analysis was also provided. This study was conducted in compliance with U.S. EPA GLP standards (40 CFR Parts 160 and 792), OECD Principles of GLP and JMAFF GLP (1999), with the following exception: Periodic water screening analysis for potential contaminants was performed using a certified laboratory and standard US EPA analytical methods.

A. MATERIALS:

1. Test material Dimethyl Disulfide

Description: Liquid.

Lot No./Batch No. : 05.03.06 (Batch no.)

Purity: 99.6%

Stability of compound under test conditions:

The day 0-7 reviewer-calculated mean-measured concentrations yielded recoveries of 89-105% of the nominal test concentrations, indicating that dimethyl disulfide was relatively stable under the test conditions.

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

Storage conditions of test chemicals:

Test material was stored under ambient conditions.

Physicochemical properties of Dimethyl Disulfide.

Parameter	Values	Comments
Water solubility at 20EC	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

2. Test organism:

Name: Duckweed (*Lemna gibba*) EPA requires a vascular species: *Lemna gibba*.

Strain, if provided: G3

Source: In-house cultures originally obtained from the USDA.

Age of inoculum: At least 2 weeks

Method of cultivation: Grown under test conditions (20X-AAP)

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
Acclimation period:	Continuous.	
Culturing media and conditions: (same as test or not)	Temperature and photoperiod appeared to be the same as test conditions.	
Health: (any mortality observed)	Not reported.	
<u>Test system</u> Static/static renewal	Static renewal	
Renewal rate for static renewal	Test solutions were renewed on days 3 and 5.	EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).
Incubation facility	Test vessels were placed in a temperature-controlled environmental chamber.	
Duration of the test	7 days	EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Parameter	Details	Remarks
Criteria		
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass 300 mL 200 mL	
<u>Details of growth medium name</u> pH at test initiation: pH at test termination: Chelator used: Carbon source:	7.8-8.0 8.3-9.0 Yes NaHCO ₃	----- <i>EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
<u>Dilution water</u> source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Purified well water Adjusted to 7.5 ± 0.1. Filter-sterilized. Not reported. Not reported. ND-34.9 mg/L ND Not reported.	----- <i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Test material was added directly into the medium.	

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Parameter	Details	Remarks
		<i>Criteria</i>
Aeration or agitation	Neither.	
<u>Sediment used (for rooted aquatic vascular plants)</u> Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	N/A	
<u>Number of replicates</u> Control: Solvent control: Treatments:	3 N/A 3	
Number of plants/replicate	4 plants	<i>EPA requires 5 plants.</i>
Number of fronds/plant	3 fronds per plant	<i>EPA requires 3 fronds per plant.</i>
<u>Test concentrations</u> Nominal: Measured:	0 (negative control), 3.1, 6.3, 13, 25, 50, and 100 mg a.i./L <2.00 (<LOQ, control), 3.2, 5.6, 12, 24, 48, and 95 mg a.i./L	Time-weighted average concentrations were nearly identical to the mean-measured concentrations, which were calculated as the arithmetic average of all old and new samples for a given concentration. <i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A	

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Parameter	Details	Remarks
		Criteria
Method and interval of analytical verification	All exposure solution, calibration standards, and matrix blank samples were analyzed using HPLC with UV detection (200 nm). Test solutions were analyzed at time 0, before renewal on days 3 and 5, after renewal on days 3 and 5, and at test termination.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	23.5-25.2°C Continuous. 4310 to 5410 lux Warm-white fluorescent lighting	
<u>Reference chemical (if used)</u> name: concentrations:	N/A	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, growth rate, and biomass	
Measurement technique for frond number and other end points	Visual counts were used for frond density. Dry weight (biomass) was determined by drying fronds for 2 days and then weighing. Growth rate was determined based on cell density and biomass.	
Observation intervals	Days 0, 3, 5, and 7.	
Other observations, if any	See Inhibitory Effects.	
Indicate whether there was an exponential growth in the control	Yes. Frond density was 116 fronds/replicate in the negative	

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Parameters	Details	Remarks/Criteria
	control at test termination.	
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By test termination, frond density averaged 116 fronds/rep in the negative control, yielding inhibitions of 2.6, 8.9, 11, 37, 84, and 86% when compared to the negative control in the mean-measured 3.2, 5.5, 12, 24, 48, and 95 mg a.i./L treatment groups, respectively. Based on frond density, the study author's NOAEC and EC₅₀ values were 5.5 and 31 mg a.i./L, respectively.

Growth rate based on frond density averaged 0.324 days⁻¹ in the negative control, yielding inhibitions of 1.2, 4.3, 5.3, 20, 80, and 87% when compared to the negative control. Based on growth rate, the NOAEC and EC₅₀ values were 5.5 and 36 mg a.i./L, respectively.

Biomass (dry weight) averaged 9.5 mg in the negative control, yielding inhibitions of 4.6, 7.7, 8.4, 18, 53, and 87% when compared to the negative control. Based on biomass, the NOAEC and EC₅₀ values were 5.5 and 46 mg a.i./L, respectively.

The study authors also analyzed growth rate based on biomass, which resulted in an average growth rate of 0.336 days⁻¹ in the negative control, yielding inhibitions of 1.8, 3.3, 3.4, 7.4, 28, and 67% when compared to the negative control. Based on growth rate due to biomass, the NOAEC and EC₅₀ values were 5.5 and 75 mg a.i./L, respectively.

The study authors used mean-measured concentrations for calculations of endpoints, and compared frond density, growth rate, and biomass treatment groups to the negative control.

Chlorosis was observed at all treatment levels and was more widespread in the highest two treatment levels. Necrosis was observed in all treatment levels except the lowest test concentration, and was more prevalent in the three highest treatment levels. Mortality was mostly observed at the highest treatment level.

Table 3: Effect of Dimethyl Disulfide on frond number of Duckweed, *Lemna gibba*

TWA and (Nominal) Concentrations (mg a.i./L)	Initial frond number/test solution	frond number at			
		3 days	5 days	7 days	
				frond number	% inhibition
Negative control	12	38	71	116	N/A
3.2 (3.1)	12	35	65	113	2.6
5.6 (6.3)	12	32	59	105	8.9
12 (13)	12	33	60	103	11

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

TWA and (Nominal) Concentrations (mg a.i./L)	Initial frond number/test solution	frond number at			
		3 days	5 days	7 days	
				frond number	% inhibition
24 (25)	12	27	43	73	37
48 (50)	12	17	18	19	84
95 (100)	12	16	16	16	86
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Effect of Dimethyl Disulfide on growth of Duckweed, *Lemna gibba*

TWA and (Nominal) Concentrations mg ai/L	Initial frond number/test solution	Growth rate (days ⁻¹ , mean)	Growth rate % Inhibition	Biomass, dry weight (mg, mean)	Biomass % Inhibition
Negative control	12	0.324	N/A	9.5	N/A
3.2 (3.1)	12	0.320	1.2	9.1	4.6
5.6 (6.3)	12	0.310	4.3	8.8	7.7
12 (13)	12	0.307	5.3	8.7	8.4
24 (25)	12	0.258	20	7.8	18
48 (50)	12	0.065	80	4.4	53
95 (100)	12	0.044	87	1.2	87
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

N/A- not applicable

Table 5: Statistical endpoint values.*

Statistical Endpoint	Frond No.	Growth rate (Frond no.)	Biomass
NOAEC or EC ₀₅ (mg a.i./L)	5.5	5.5	5.5
LOAEC (mg a.i./L)	12	12	12
IC ₅₀ or EC ₅₀ (mg a.i./L) (95% C.I.)	31 (25-34)	36 (33-38)	46 (41-50)

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Statistical Endpoint	FronD No.	Growth rate (FronD no.)	Biomass
Other (IC ₂₅ /EC ₂₅)	N/A	N/A	N/A
Reference chemical NOAEC IC ₅₀ /EC ₅₀	N/A	N/A	N/A

*Study author-reported values

B. REPORTED STATISTICS:

Day 7 EC50 values were determined using linear interpolation with treatment response and exposure concentration data. The data were tested for normality using Shapiro-Wilks' Test, and for homogeneity of variance using Levene's Test. Treatment group means were compared to the control using ANOVA and Dunnett's t-test. The NOAEC and LOAEC were determined from the statistical analyses and evaluation of concentration-response patterns. All statistical analyses were conducted using Toxstat Version 3.5.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer tested the normality of the data using the Chi-square and Shapiro Wilks tests and homogeneity of variance using the Hartley and Bartlett's test. If the data met the assumptions of ANOVA, the NOAEC values were determined using the parametric Williams' and Dunnett's tests. If the data did not meet the assumptions of ANOVA, the NOAEC values were determined using the non-parametric Steele's or Kruskal-Wallis test and visual interpretation of the data. The ECx values and probit slopes were determined using the probit analysis. All analyses were conducted using the reviewer calculated time weighted average mean-measured concentrations and Nuthatch statistical software.

Values input for growth rate were multiplied by 1000 to eliminate means with a zero value.

FronD density

EC₀₅: 6.0 mg a.i./L 95% C.I.: 3.3 to 11 mg a.i./L
 EC₅₀: 28 mg a.i./L 95% C.I.: 22 to 36 mg a.i./L
 NOAEC: 3.2 mg a.i./L
 Probit Slope: 2.45 ± 0.302

Growth rate

EC₀₅: 9.7 mg a.i./L 95% C.I.: 5.8 to 16 mg a.i./L
 EC₅₀: 34 mg a.i./L 95% C.I.: 28 to 42 mg a.i./L
 NOAEC: 12 mg a.i./L
 Probit Slope: 2.98 ± 0.383

Biomass (Dry weight)

EC₀₅: 17 mg a.i./L 95% C.I.: 14 to 21 mg a.i./L
 EC₅₀: 48 mg a.i./L 95% C.I.: 44 to 52 mg a.i./L
 NOAEC: 12 mg a.i./L
 Probit Slope: 3.69 ± 0.258

D. STUDY DEFICIENCIES:

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

There were no study deficiencies that would impact the outcome of the study.

E. REVIEWER'S COMMENTS:

The reviewer's results were similar to the study authors', and probit slopes were additionally calculated for all endpoints. Therefore, the reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

At test initiation and termination, the pH of the solutions ranged from 7.8 to 8.0 and 8.3 to 9.0, respectively, well above the pH suggested by OPPTS guidelines of 7.5. However, the pH varied similarly across all treatment and control levels.

The reviewer independently calculated the time-weighted average of the six test concentrations, and used those numbers in the statistical analyses. These values were nearly identical to the mean-measured values.

The in-life portion of the test was conducted from February 22 to 29, 2008.

Periodic water screening analysis for potential contaminants was not conducted under any accepted GLP standards.

F. CONCLUSIONS:

The study is acceptable. The 7-Day NOAEC and EC₅₀ values for frond density, the most sensitive endpoint, were 3.2 and 28 mg a.i./L, respectively.

Frond density

EC ₀₅ : 6.0 mg a.i./L	95% C.I.: 3.3 to 11 mg a.i./L
EC ₅₀ : 28 mg a.i./L	95% C.I.: 22 to 36 mg a.i./L
NOAEC: 3.2 mg a.i./L	
Probit Slope: 2.45 ± 0.302	

Growth rate

EC ₀₅ : 9.7 mg a.i./L	95% C.I.: 5.8 to 16 mg a.i./L
EC ₅₀ : 34 mg a.i./L	95% C.I.: 28 to 42 mg a.i./L
NOAEC: 12 mg a.i./L	
Probit Slope: 2.98 ± 0.383	

Biomass (Dry weight)

EC ₀₅ : 17 mg a.i./L	95% C.I.: 14 to 21 mg a.i./L
EC ₅₀ : 48 mg a.i./L	95% C.I.: 44 to 52 mg a.i./L
NOAEC: 12 mg a.i./L	
Probit Slope: 3.69 ± 0.258	

Endpoint(s) Affected: frond density, growth rate, and biomass

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

III. REFERENCES:

1. U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (Draft), OPPTS Number 850.4400: *Aquatic Plant Toxicity Test using Lemna spp., Tiers I and II*. Washington, D.C.
2. Organization for Economic Cooperation and Development. Working Draft of a Proposal for a New Guideline 221A *Lemna sp.* Growth Inhibition Test. Circulated 9 April 2004.
3. ASTM Standard Guide 1415-91E. 1991. *Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3*. American Society for Testing and Materials. Philadelphia, PA.
4. Microsoft Corporation, Microsoft Excel 2000. Copyright 1985-1989.
5. West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.
6. Norberg-King, T.J. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. Version 2.0. U.S. EPA. National Effluent Toxicity Center. Duluth, Minnesota. Technical Report 03-93.

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Dimethyl disulfide & L. gibba frond no. (mg/L)
File: 1204f Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	6	8	0

Calculated Chi-Square goodness of fit test statistic = 5.7230
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Dimethyl disulfide & L. gibba frond no. (mg/L)
File: 1204f Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 505.333

W = 0.978

Critical W (P = 0.05) (n = 21) = 0.908

Critical W (P = 0.01) (n = 21) = 0.873

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

Dimethyl disulfide & L. gibba frond no. (mg/L)
File: 1204f Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 46.43
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Dimethyl disulfide & L. gibba frond no. (mg/L)
 File: 1204f Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 7.67
 Table Chi-square value = 16.81 (alpha = 0.01)
 Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
 Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Dimethyl disulfide & L. gibba frond no. (mg/L)
 File: 1204f Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	33853.905	5642.317	156.319
Within (Error)	14	505.333	36.095	
Total	20	34359.238		

Critical F value = 2.85 (0.05,6,14)
 Since F > Critical F REJECT Ho:All groups equal

Dimethyl disulfide & L. gibba frond no. (mg/L)
 File: 1204f Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	115.667	115.667		
2	3.258	112.667	112.667	0.612	
3	5.601	105.333	105.333	2.107	
4	11.8	102.667	102.667	2.650	*
5	24.436	73.000	73.000	8.698	*
6	48.2	19.000	19.000	19.706	*
7	95.314	16.333	16.333	20.250	*

Dunnnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Dimethyl disulfide & L. gibba frond no. (mg/L)
 File: 1204f Transform: NO TRANSFORMATION

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

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	Neg control	3			
2	3.258	3	12.411	10.7	3.000
3	5.601	3	12.411	10.7	10.333
4	11.8	3	12.411	10.7	13.000
5	24.436	3	12.411	10.7	42.667
6	48.2	3	12.411	10.7	96.667
7	95.314	3	12.411	10.7	99.333

Dimethyl disulfide & L. gibba frond no. (mg/L)
File: 1204f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	115.667	115.667	115.667
2	3.258	3	112.667	112.667	112.667
3	5.601	3	105.333	105.333	105.333
4	11.8	3	102.667	102.667	102.667
5	24.436	3	73.000	73.000	73.000
6	48.2	3	19.000	19.000	19.000
7	95.314	3	16.333	16.333	16.333

Dimethyl disulfide & L. gibba frond no. (mg/L)
File: 1204f 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
Neg control	115.667				
3.258	112.667	0.612		1.76	k= 1, v=14
5.601	105.333	2.106	*	1.85	k= 2, v=14
11.8	102.667	2.650	*	1.88	k= 3, v=14
24.436	73.000	8.698	*	1.89	k= 4, v=14
48.2	19.000	19.706	*	1.90	k= 5, v=14
95.314	16.333	20.250	*	1.91	k= 6, v=14

s = 6.008

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	6.0	3.3	11.	0.12	0.55
EC10	8.5	5.0	14.	0.11	0.60
EC25	15.	10.	22.	0.079	0.68

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

EC50 28. 22. 36. 0.051 0.78

Slope = 2.45 Std.Err. = 0.302

!!!Poor fit: p < 0.001 based on DF= 4.00 14.0

1204F : Dimethyl disulfide & L. gibba frond no. (mg/L)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. - Pred.	Pred. %Control	%Change
0.00	3.00	116.	116.	0.0737	100.	0.00
3.26	3.00	113.	114.	-1.67	98.9	1.09
5.60	3.00	105.	111.	-5.31	95.7	4.28
11.8	3.00	103.	95.1	7.54	82.3	17.7
24.4	3.00	73.0	64.8	8.20	56.1	43.9
48.2	3.00	19.0	32.9	-13.9	28.4	71.6
95.3	3.00	16.3	11.3	5.05	9.77	90.2

Dimethyl disulfide & L. gibba growth rate (mg/L)

File: 1204g Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	6	8	0

Calculated Chi-Square goodness of fit test statistic = 5.7230

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Dimethyl disulfide & L. gibba growth rate (mg/L)

File: 1204g Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1802.000

W = 0.953

Critical W (P = 0.05) (n = 21) = 0.908

Critical W (P = 0.01) (n = 21) = 0.873

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

Dimethyl disulfide & L. gibba growth rate (mg/L)

File: 1204g Transform: NO TRANSFORMATION

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 11.08
 Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)
 Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
 Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Dimethyl disulfide & L. gibba growth rate (mg/L)
 File: 1204g Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 2.71
 Table Chi-square value = 16.81 (alpha = 0.01)
 Table Chi-square value = 12.59 (alpha = 0.05)
 Average df used in calculation ==> df (avg n - 1) = 2.00
 Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Dimethyl disulfide & L. gibba growth rate (mg/L)
 File: 1204g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	274634.286	45772.381	355.613
Within (Error)	14	1802.000	128.714	
Total	20	276436.286		

Critical F value = 2.85 (0.05,6,14)
 Since F > Critical F REJECT Ho:All groups equal

Dimethyl disulfide & L. gibba growth rate (mg/L)
 File: 1204g Transform: NO TRANSFORMATION

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	323.667	323.667		
2	3.258	319.333	319.333	0.468	
3	5.601	310.000	310.000	1.475	
4	11.8	306.333	306.333	1.871	
5	24.436	257.667	257.667	7.125	*
6	48.2	65.333	65.333	27.888	*
7	95.314	43.667	43.667	30.227	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Dimethyl disulfide & L. gibba growth rate (mg/L)
File: 1204g 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	Neg control	3			
2	3.258	3	23.436	7.2	4.333
3	5.601	3	23.436	7.2	13.667
4	11.8	3	23.436	7.2	17.333
5	24.436	3	23.436	7.2	66.000
6	48.2	3	23.436	7.2	258.333
7	95.314	3	23.436	7.2	280.000

Dimethyl disulfide & L. gibba growth rate (mg/L)
File: 1204g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	323.667	323.667	323.667
2	3.258	3	319.333	319.333	319.333
3	5.601	3	310.000	310.000	310.000
4	11.8	3	306.333	306.333	306.333
5	24.436	3	257.667	257.667	257.667
6	48.2	3	65.333	65.333	65.333
7	95.314	3	43.667	43.667	43.667

Dimethyl disulfide & L. gibba growth rate (mg/L)
File: 1204g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISOTONIZED	CALC.	SIG	TABLE	DEGREES OF
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Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

IDENTIFICATION	MEAN	WILLIAMS	P=.05	WILLIAMS	FREEDOM
Neg control	323.667				
3.258	319.333	0.468		1.76	k= 1, v=14
5.601	310.000	1.475		1.85	k= 2, v=14
11.8	306.333	1.871		1.88	k= 3, v=14
24.436	257.667	7.125	*	1.89	k= 4, v=14
48.2	65.333	27.888	*	1.90	k= 5, v=14
95.314	43.667	30.227	*	1.91	k= 6, v=14

s = 11.345

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	9.7	5.8	16.	0.10	0.60
EC10	13.	8.3	20.	0.090	0.65
EC25	20.	15.	28.	0.066	0.73
EC50	34.	28.	42.	0.043	0.81

Slope = 2.98 Std.Err. = 0.383

!!!Poor fit: p < 0.001 based on DF= 4.00 14.0

1204G : Dimethyl disulfide & L. gibba growth rate (mg/L)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	324.	325.	-1.29	100.	0.00
3.26	3.00	319.	325.	-5.26	99.9	0.114
5.60	3.00	310.	322.	-11.9	99.1	0.940
11.8	3.00	306.	298.	8.33	91.7	8.30
24.4	3.00	258.	218.	39.5	67.1	32.9
48.2	3.00	65.3	108.	-42.5	33.2	66.8
95.3	3.00	43.7	30.5	13.1	9.39	90.6

Dimethyl Disulfide & L. gibba 7-day Biomass (mg/L)

File: 1204b Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	6	8	0

Calculated Chi-Square goodness of fit test statistic = 5.7230

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

Dimethyl Disulfide & *L. gibba* 7-day Biomass (mg/L)
File: 1204b Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 4.187

W = 0.936

Critical W (P = 0.05) (n = 21) = 0.908

Critical W (P = 0.01) (n = 21) = 0.873

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

Dimethyl Disulfide & *L. gibba* 7-day Biomass (mg/L)
File: 1204b Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 19.19
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Dimethyl Disulfide & *L. gibba* 7-day Biomass (mg/L)
File: 1204b Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 6.99
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Dimethyl Disulfide & *L. gibba* 7-day Biomass (mg/L)
File: 1204b Transform: NO TRANSFORMATION

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	172.256	28.709	96.017
Within (Error)	14	4.187	0.299	
Total	20	176.443		

Critical F value = 2.85 (0.05,6,14)
 Since F > Critical F REJECT Ho:All groups equal

Dimethyl Disulfide & L. gibba 7-day Biomass (mg/L)
 File: 1204b 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	Neg control	9.500	9.500		
2	3.258	9.067	9.067	0.971	
3	5.601	8.767	8.767	1.643	
4	11.8	8.700	8.700	1.792	
5	24.436	7.833	7.833	3.733	*
6	48.2	4.433	4.433	11.348	*
7	95.314	1.200	1.200	18.590	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Dimethyl Disulfide & L. gibba 7-day Biomass (mg/L)
 File: 1204b 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	Neg control	3			
2	3.258	3	1.130	11.9	0.433
3	5.601	3	1.130	11.9	0.733
4	11.8	3	1.130	11.9	0.800
5	24.436	3	1.130	11.9	1.667
6	48.2	3	1.130	11.9	5.067
7	95.314	3	1.130	11.9	8.300

Dimethyl Disulfide & L. gibba 7-day Biomass (mg/L)
 File: 1204b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	9.500	9.500	9.500
2	3.258	3	9.067	9.067	9.067
3	5.601	3	8.767	8.767	8.767
4	11.8	3	8.700	8.700	8.700
5	24.436	3	7.833	7.833	7.833
6	48.2	3	4.433	4.433	4.433
7	95.314	3	1.200	1.200	1.200

Dimethyl Disulfide & L. gibba 7-day Biomass (mg/L)
File: 1204b 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
Neg control	9.500				
3.258	9.067	0.971		1.76	k= 1, v=14
5.601	8.767	1.642		1.85	k= 2, v=14
11.8	8.700	1.792		1.88	k= 3, v=14
24.436	7.833	3.733	*	1.89	k= 4, v=14
48.2	4.433	11.347	*	1.90	k= 5, v=14
95.314	1.200	18.589	*	1.91	k= 6, v=14

s = 0.547

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	17.	14.	21.	0.044	0.81
EC10	21.	18.	26.	0.038	0.83
EC25	31.	27.	36.	0.027	0.88
EC50	48.	44.	52.	0.017	0.92

Slope = 3.69 Std.Err. = 0.258

Goodness of fit: p = 0.64 based on DF= 4.0 14.

1204B : Dimethyl Disulfide & L. gibba 7-day Biomass (mg/L)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	9.50	9.05	0.448	100.	0.00
3.26	3.00	9.07	9.05	0.0145	100.	0.000853
5.60	3.00	8.77	9.05	-0.283	100.	0.0301
11.8	3.00	8.70	8.94	-0.237	98.7	1.27
24.4	3.00	7.83	7.76	0.0729	85.7	14.3
48.2	3.00	4.43	4.45	-0.0158	49.1	50.9

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....}

EPA MRID Number 47471204

95.3 3.00 1.20 1.20 0.00114 13.2 86.8