Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Algae (*Anabaena flos-aquae*)

PMRA Submission Number {.....} EPA MRID Number 47471205

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	355006
OECD Data Point	{.....}
EPA MRID	47471205
EPA Guideline	OPPTS 850.5400 (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 Moncie V Wright **Signature:**
Staff Scientist, Cambridge Environmental **Date:** 09/10/08

Secondary Reviewer: Teri S. Myers Teri S Myers **Signature:**
Senior Scientist, Cambridge Environmental **Date:** 11/04/08

Primary Reviewer: Edward Odenkirchen **Date:** {.....}
{EPA/OECD/PMRA}

Secondary Reviewer(s): Valerie Woodard **Date:** {.....} 3-30-09
{EPA/OECD/PMRA}

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

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

Date Evaluation Completed:

CITATION: Minderhout, T., Kendall, T.Z. and H.O. Krueger. 2008. Dimethyl Disulfide: A 96-Hour Toxicity Test with the Freshwater Alga (*Anabaena flos-aquae*). Unpublished study performed by Wildlife International, Easton, Maryland. Laboratory Project No.: 524A-123. Study sponsored 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 nonvascular 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 meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the freshwater blue-green algae *Anabaena flos-aquae* were exposed to Dimethyl Disulfide at nominal concentrations of 0 (negative control), 0.15, 0.38, 0.96, 2.4, 6.0, and 15 mg a.i./L under static conditions. Mean-measured concentrations were <0.100 (<LOQ; control), 0.17, 0.34, 0.80, 1.9, 5.5, and 14 mg a.i./L.

The % growth inhibition in cell density, in the treated algal culture as compared to the negative control, ranged from 36 to 98%. The % growth inhibition in biomass, in the treated algal culture as compared to the negative control, ranged from 13 to 94%. The % growth inhibition in growth rate, in the treated algal culture as compared to the negative control, ranged from 9.5 to 72%.

The most sensitive endpoint was cell density, with NOAEC and EC_{50} values of <0.17 and 0.32 mg a.i./L.

There was no adherence to cells to any of the test chambers and aggregations were not observed in any of the treatment groups. Enlarged cells were observed at the three highest test concentrations.

This toxicity study is classified as scientifically sound and satisfies the guideline requirement for a Tier II nonvascular plant toxicity study with the freshwater blue-green algae, *Anabaena flos-aquae*.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: 0.011 mg a.i./L 95% C.I.: 0.0011 to 0.10 mg a.i./L
EC_{50}: 0.32 mg a.i./L 95% C.I.: 0.11 to 0.90 mg a.i./L
NOAEC: <0.17 mg a.i./L
Probit Slope: 1.12 ± 0.211

Biomass (Area under the growth curve)
EC_{05}: 0.021 mg a.i./L 95% C.I.: 0.0032 to 0.14 mg a.i./L
EC_{50}: 0.56 mg a.i./L 95% C.I.: 0.23 to 1.4 mg a.i./L
NOAEC: 0.17 mg a.i./L
Probit Slope: 1.16 ± 0.196

Growth rate
EC_{05}: 0.48 mg a.i./L 95% C.I.: 0.15 to 1.6 mg a.i./L
EC_{50}: 6.7 mg a.i./L 95% C.I.: 4.7 to 9.4 mg a.i./L
NOAEC: 0.17 mg a.i./L
Probit Slope: 1.44 ± 0.256

Endpoint(s) Effected: Cell density, biomass, and growth rate

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following US EPA Series 850 - Ecological Effects Test Guidelines (draft), OPPTS No. 850.5400, OECD Guidelines for the Testing of Chemicals 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*, Official Journal of the European Communities No. L383, Method C.3 *Algal Inhibition Test*, and ASTM Standard 1218-90E *Standard Guide for Conducting Static 96-Hour Toxicity Test with Microalgae*. The following deviations from OPPTS 850.5400 were noted:

1. The physicochemical properties of the test material were not reported.
2. Pretest health of the test species was not reported.
3. OPPTS guidelines require that the pH for this species stay within a range of 7.5 ± 0.1. The pH in this study was above the recommended range from study initiation (range 7.6-7.8) to termination (range 8.9-10.0).
4. OPPTS guidelines recommend that the test begin with 10,000 cells/mL per vessel, but vessels in this study contained 5,000 cells/mL at test initiation.

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; 1989), OECD Principles of GLP (ENV/MC/CHEM (98) 17) 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 averaged 0 and 96-hour measured concentrations yielded recoveries of 79 to 113% of the nominal test concentrations, indicating that dimethyl disulfide was relatively stable to very 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 chemicals were stored at ambient temperature.

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.**	

2. Test organism:

Name: Freshwater blue-green algae, *Anabaena flos-aquae*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: Not reported.
Source: In-house cultures originally obtained from the University of Toronto, Canada
Age of inoculum: At least 2 weeks.
Method of cultivation: Grown under test conditions (freshwater algal medium).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was not conducted.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous. Freshwater algal medium Appeared to be same as test; temperature, lighting, and incubation facility were not reported. Not reported.	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system Static/static renewal Renewal rate for static renewal	Static. N/A	*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 on a mechanical shaker in a temperature-controlled environmental chamber.	
Duration of the test	96 hours.	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 300 mL 300 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name		

Parameter	Details	Remarks *Criteria*
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	7.6-7.8 8.9-10.0 Yes. $NaHCO_3$ N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) 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, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
<u>Dilution water</u> source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Purified well water. Adjusted to 7.5 ± 0.1 N/A Filter-sterilized. Not reported. Not reported. ND-12,100 mg/L ND Not reported.	*EPA pH: <u>Skeletonema costatum</u>= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Test material was added directly to the freshwater algal medium.	
Aeration or agitation	Agitation at 100 rpm	

Parameter	Details	Remarks *Criteria*
Initial cells density	*Ca.* 5.0 x 10^3 cells/mL	OPPTS guidelines (850.5400) recommend that the test begin with 10,000 cells/mL for this species. *EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates Control: Solvent control: Treatments:	 3 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal: Measured:	 0 (Negative control), 0.15, 0.38, 0.96, 2.4, 6.0, and 15 mg a.i./L <0.100 (<LOQ; control), 0.17, 0.34, 0.80, 1.9, 5.5, and 14 mg a.i./L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples collected at 0 and 96 days, along with calibration standards and quality control samples, were analyzed using HPLC with UV (200 nm) detection.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	23-24.1°C Continuous 1940 - 2340 lux Cool-white fluorescent lighting	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density, biomass (area under the growth curve), and growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell counts were conducted daily on all replicate vessels of each test concentration and the control using a hemacytometer and a microscope. Growth rate and biomass were determined using calculations of cell density.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	None.	
Indicate whether there was an exponential growth in the control	Yes. After 96 hours, the mean cell density was *ca.* 148.7×10^4 cells/mL in the negative control.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours of exposure, cell density averaged 148.7×10^4 cells/mL in the negative control, yielding inhibitions of 36, 64, 62, 78, 92, and 98% when compared to the negative control at the mean-measured 0.17, 0.34, 0.80, 1.9, 5.5, and 14 mg a.i./L treatment levels, respectively.

Biomass (area under the growth curve) averaged 324.1 x 10^5 cells · days/mL in the negative control, yielding inhibitions of 13, 55, 55, 70, 88, and 94% when compared to the negative control.

Growth rate averaged 0.0592 $hours^{-1}$ in the negative control, yielding inhibitions of 9.5, 19, 18, 28, 45, and 72% when compared to the negative control.

The study author used the mean-measured concentrations for calculations of endpoint values. All endpoints had a reported NOAEC of 0.17 mg a.i./L. Based on cell density, the 96-hour EC_{50} value was reported to be 0.31 mg a.i./L. Based on biomass, the 96-hour EC_{50} values were reported to be 0.55 mg a.i./L. Based on growth rate, the 96-hour EC_{50} value was reported to be 6.7 mg a.i./L.

The study author also calculated 72-hour EC_{50} values for all endpoints. Cell density, biomass, and growth rate had EC_{50} values of 0.32, 1.2, and 5.1 mg a.i./L, respectively.

There was no adherence to cells to any of the test chambers. Aggregations were not observed in any of the treatment groups. Enlarged cells were observed at the three highest test concentrations.

Table 3: Effect of Dimethyl Disulfide on algal growth (*Anabaena flos-aquae*)

Mean-measured and (Nominal) Concentrations (mg a.i./L)	Initial cell Density (x 10^4 cells/mL)	Cell density (x 10^4 cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					cell count	% inhibition
Negative control	0.5	4.9	11.1	46.5	148.7	N/A
0.17 (0.15)	0.5	6.2	8.7	56.3	95.3	36
0.34 (0.38)	0.5	4.7	10.0	21.3	53.0	64
0.80 (0.96)	0.5	9.7	3.9	20.7	57.0	62
1.9 (2.4)	0.5	2.6	5.4	17.8	33.0	78
5.5 (6.0)	0.5	3.3	4.6	3.7	12.4	92
14 (15)	0.5	4.0	3.0	0.8	2.7	98

Table 4: Effect of Dimethyl Disulfide on algal growth (*Anabaena flos-aquae*)

Mean-Measured and (Nominal) Concentrations (mg a.i./L)	Initial Cell Density (x10^4 cells/mL)	Mean Growth Rate ($hours^{-1}$)		Mean Biomass (Area Under the Growth Curve) (x 10^5 cells * days/mL)	
		0-96 Hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	0.5	0.0592	N/A	324.1	N/A
0.17 (0.15)	0.5	0.0534	9.5	281.2	13
0.34 (0.38)	0.5	0.0480	19	146	55
0.80 (0.96)	0.5	0.0483	18	146.5	55
1.9 (2.4)	0.5	0.0429	28	97.4	70
5.5 (6.0)	0.5	0.0327	45	38.6	88
14 (15)	0.5	0.0165	72	17.9	94

Table 5: Statistical endpoint values.*

Statistical Endpoint	Cell Density	Growth rate	Biomass (Area under the growth curve)
NOAEC or EC_{05} (mg a.i./L)	0.17	0.17	0.17
EC_{50} (mg a.i./L)	0.31	6.7	0.55
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	0.31 (0.11-0.90)	6.7 (4.8-9.6)	0.55 (0.23-1.4)
Other (IC_{25}/EC_{25})	N/A	N/A	N/A
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

* Study author-reported values

B. REPORTED STATISTICS:

The EC_{50} values and their confidence limits were determined by non-linear regression or linear interpolation using the SAS System for Windows, Version 8.02 and TOXSTAT® Version 3.5. The data were tested for normality using Shapiro-Wilks' Test, and for homogeneity of variance using Levene's Test. The treatment groups were then compared to the negative control using ANOVA and Dunnett's test. The NOAEC was determined based on the results of the statistical analyses and an evaluation of the concentration-response pattern. All statistical determinations were made at the 95% confidence level.

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 mean-measured concentrations and Nuthatch statistical software.

Cell density values were divided by 10,000 and biomass values were divided by 100,000 for ease of data input, and growth rate values were multiplied by 10,000 to eliminate means with a zero value.

Cell density

EC_{05}: 0.011 mg a.i./L	95% C.I.: 0.0011 to 0.10 mg a.i./L
EC_{50}: 0.32 mg a.i./L	95% C.I.: 0.11 to 0.90 mg a.i./L
NOAEC: <0.17 mg a.i./L	
Probit Slope: 1.12 ± 0.211	

Biomass (Area under the growth curve)

EC_{05}: 0.021 mg a.i./L	95% C.I.: 0.0032 to 0.14 mg a.i./L
EC_{50}: 0.56 mg a.i./L	95% C.I.: 0.23 to 1.4 mg a.i./L
NOAEC: 0.17 mg a.i./L	
Probit Slope: 1.16 ± 0.196	

Growth rate

EC_{05}: 0.48 mg a.i./L	95% C.I.: 0.15 to 1.6 mg a.i./L
EC_{50}: 6.7 mg a.i./L	95% C.I.: 4.7 to 9.4 mg a.i./L
NOAEC: 0.17 mg a.i./L	
Probit Slope: 1.44 ± 0.256	

D. STUDY DEFICIENCIES:

There were no study deficiencies that would change the results of the study.

E. REVIEWER=S COMMENTS:

The reviewer's and the study author's results were generally in agreement, but the reviewer's analysis detected significant reductions in cell density at all treatment levels. The reviewer's analysis also provided probit slope values, so the reviewer's results are presented in the Conclusions and Executive Summary sections of this DER.

The periodic water screening analysis for contaminants was not conducted according to any standards of GLP.

The in-life portion of the test was conducted from April 4 to 8, 2008.

F. CONCLUSIONS:

This study is acceptable. The most sensitive endpoint was cell density, with NOAEC and EC_{50} values of <0.17 and 0.32 mg a.i./L.

Test Organism: *Anabaena flos-aquae*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density

EC_{05}: 0.011 mg a.i./L	95% C.I.: 0.0011 to 0.10 mg a.i./L
EC_{50}: 0.32 mg a.i./L	95% C.I.: 0.11 to 0.90 mg a.i./L
NOAEC: <0.17 mg a.i./L	
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Biomass (Area under the growth curve)

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NOAEC: 0.17 mg a.i./L	
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EC_{05}: 0.48 mg a.i./L	95% C.I.: 0.15 to 1.6 mg a.i./L
EC_{50}: 6.7 mg a.i./L	95% C.I.: 4.7 to 9.4 mg a.i./L
NOAEC: 0.17 mg a.i./L	
Probit Slope: 1.44 ± 0.256	

Endpoint(s) Effected: Cell density, biomass, and growth rate

III. REFERENCES:

1. OECD. 2006. OECD Guidelines for Testing of Chemicals, Guideline 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test.* Adopted 23 March 2006.
2. Official Journal of the European Communities. 1992. No. L383. Method C.3. *Algal Inhibition Test.*
3. U.S. EPA. 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400.
4. ASTM Standard Guide 1218-90E. 1990. *Standard Guide for conducting static 96-hour toxicity tests with microalgae.* American Society for Testing and Materials. Philadelphia, Pennsylvania.
5. The SAS System for Windows. 1999. Version 8.02. SAS Institute Inc. Cary, North Carolina.
6. West, Inc. and D.D. Gulley. TOXSTAT Version 3.5. Copyright 1996. Western EcoSystems Technology, Inc., Cheyenne, Wyoming.
7. Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry.* 11: 1485-1494.
8. Norberg-King, T.J. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. Version 2.0 U.S. Environmental Protection Agency. National Effluent Toxicity Assessment Center. Duluth, Minnesota. Technical report 03-93.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Dimethyl Disulfide & A. flos-aquae 96-hour Cell Density (mg/L)
File: 1205c 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	8	6	7	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 & A. flos-aquae 96-hour Cell Density (mg/L)
File: 1205c Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 10460.240

W = 0.967

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 & 96-hour Cell Density (mg/L)
File: 1205c Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 1584.06
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 & A. flos-aquae 96-hour Cell Density (mg/L)
File: 1205c Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 15.26
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 & A. flos-aquae 96-hour Cell Density (mg/L)
File: 1205c Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	46212.450	7702.075	10.308
Within (Error)	14	10460.240	747.160	
Total	20	56672.690		

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

Dimethyl Disulfide & A. flos-aquae 96-hour Cell Density (mg/L)
File: 1205c 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	148.667	148.667		
2	0.172	95.333	95.333	2.390	
3	0.343	53.000	53.000	4.286	*
4	0.798	57.000	57.000	4.107	*
5	1.92	33.000	33.000	5.183	*
6	5.47	12.400	12.400	6.106	*
7	13.55	2.667	2.667	6.542	*

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

Dimethyl Disulfide & A flos-aquae 96-hour Cell Density (mg/L)

File: 1205c 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	0.172	3	56.465	38.0	53.333
3	0.343	3	56.465	38.0	95.667
4	0.798	3	56.465	38.0	91.667
5	1.92	3	56.465	38.0	115.667
6	5.47	3	56.465	38.0	136.267
7	13.55	3	56.465	38.0	146.000

Dimethyl Disulfide & A flos-aquae 96-hour Cell Density (mg/L)
File: 1205c 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	148.667	148.667	148.667
2	0.172	3	95.333	95.333	95.333
3	0.343	3	53.000	53.000	55.000
4	0.798	3	57.000	57.000	55.000
5	1.92	3	33.000	33.000	33.000
6	5.47	3	12.400	12.400	12.400
7	13.55	3	2.667	2.667	2.667

Dimethyl Disulfide & A. flos-aquae 96-hour Cell Density (mg/L)
File: 1205c 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	148.667				
0.172	95.333	2.390	*	1.76	k= 1, v=14
0.343	55.000	4.197	*	1.85	k= 2, v=14
0.798	55.000	4.197	*	1.88	k= 3, v=14
1.92	33.000	5.183	*	1.89	k= 4, v=14
5.47	12.400	6.106	*	1.90	k= 5, v=14
13.55	2.667	6.542	*	1.91	k= 6, v=14

s = 27.334
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.011	0.0011	0.10	0.46	0.11

EC10	0.023	0.0032	0.16	0.40	0.14
EC25	0.079	0.018	0.35	0.31	0.22
EC50	0.32	0.11	0.90	0.22	0.35

Slope = 1.12 Std.Err. = 0.211

Goodness of fit: p = 0.67 based on DF= 4.0 14.

1205C : Dimethyl Disulfide & A. flos-aquae 96-hour Cell Density (mg/L)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	149.	147.	1.69	100.	0.00
0.172	3.00	95.3	90.6	4.77	61.6	38.4
0.343	3.00	53.0	71.2	-18.2	48.4	51.6
0.798	3.00	57.0	48.0	8.98	32.7	67.3
1.92	3.00	33.0	28.0	4.95	19.1	80.9
5.47	3.00	12.4	12.3	0.143	8.34	91.7
13.6	3.00	2.67	5.02	-2.36	3.42	96.6

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

Dimethyl disulfide & A. flos-aquae 96-hr Biomass mg/L
File: 1205b 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	8	6	7	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 & A. flos-aquae 96-hr Biomass mg/L
File: 1205b Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 49809.552

W = 0.918

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 & A. flos-aquae 96-hr Biomass mg/L
File: 1205b        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
-------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =  127.89
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 & A. flos-aquae 96-hr Biomass mg/L
File: 1205b       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
-------------------------------------------------------------------------------

Calculated B statistic =   13.83
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 & A. flos-aquae 96-hr Biomass mg/L
File: 1205b        Transform: NO TRANSFORMATION

                              ANOVA TABLE
-------------------------------------------------------------------------------

SOURCE             DF             SS                MS            F
-------------------------------------------------------------------------------
Between             6         240504.081        40084.013       11.266

Within (Error)     14          49809.552         3557.825
-------------------------------------------------------------------------------
Total              20         290313.633
```

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

Dimethyl disulfide & A. flos-aquae 96-hr Biomass mg/L
File: 1205b 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	324.120	324.120		
2	0.172	281.160	281.160	0.882	
3	0.343	145.960	145.960	3.658	*
4	0.798	146.520	146.520	3.647	*
5	1.92	97.440	97.440	4.654	*
6	5.47	38.640	38.640	5.862	*
7	13.55	17.880	17.880	6.288	*

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

Dimethyl disulfide & A. flos-aquae 96-hr Biomass mg/L
File: 1205b 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	0.172	3	123.216	38.0	42.960
3	0.343	3	123.216	38.0	178.160
4	0.798	3	123.216	38.0	177.600
5	1.92	3	123.216	38.0	226.680
6	5.47	3	123.216	38.0	285.480
7	13.55	3	123.216	38.0	306.240

Dimethyl disulfide & A. flos-aquae 96-hr Biomass mg/L
File: 1205b 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	324.120	324.120	324.120
2	0.172	3	281.160	281.160	281.160
3	0.343	3	145.960	145.960	146.240
4	0.798	3	146.520	146.520	146.240
5	1.92	3	97.440	97.440	97.440
6	5.47	3	38.640	38.640	38.640
7	13.55	3	17.880	17.880	17.880

Dimethyl disulfide & A. flos-aquae 96-hr Biomass mg/L
File: 1205b 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	324.120				
0.172	281.160	0.882		1.76	k= 1, v=14
0.343	146.240	3.652	*	1.85	k= 2, v=14
0.798	146.240	3.652	*	1.88	k= 3, v=14
1.92	97.440	4.654	*	1.89	k= 4, v=14
5.47	38.640	5.862	*	1.90	k= 5, v=14
13.55	17.880	6.288	*	1.91	k= 6, v=14

s = 59.648
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.021	0.0032	0.14	0.39	0.15
EC10	0.044	0.0084	0.23	0.34	0.19
EC25	0.15	0.041	0.53	0.26	0.28
EC50	0.56	0.23	1.4	0.18	0.41

Slope = 1.16 Std.Err. = 0.196

Goodness of fit: p = 0.43 based on DF= 4.0 14.

1205B : Dimethyl disulfide & A. flos-aquae 96-hr Biomass mg/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	324.	329.	-5.08	100.	0.00
0.172	3.00	281.	238.	42.8	72.4	27.6
0.343	3.00	146.	197.	-50.7	59.7	40.3
0.798	3.00	147.	141.	5.34	42.9	57.1
1.92	3.00	97.4	87.9	9.55	26.7	73.3
5.47	3.00	38.6	41.2	-2.54	12.5	87.5
13.6	3.00	17.9	17.8	0.104	5.40	94.6

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

Dimethyl Disulfide & A. flos-aquae 96-hr Growth Rate
File: 1205g Transform: NO TRANSFORMATION