Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Algae (*Skeletonema costatum*)

PMRA Submission Number {........}   EPA MRID Number 47471207

Data Requirement:

PMRA DATA CODE	{............}
EPA DP Barcode	355006
OECD Data Point	{............}
EPA MRID	47471207
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   **Signature:**
Staff Scientist, Cambridge Environmental   **Date:** 09/11/08

Secondary Reviewer: 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:** {3-20-09}
{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 Marine Diatom (*Skeletonema costatum*). Unpublished study performed by Wildlife International, Easton, Maryland. Laboratory Project No.: 524A-125. 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 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 marine diatom *Skeletonema costatum* were exposed to Dimethyl Disulfide at nominal concentrations of 0 (negative control), 0.58, 1.3, 2.8, 6.2, 14, and 30 mg a.i./L under static conditions. Mean-measured concentrations were <0.300 (<LOQ; control), 0.48, 0.95, 2.6, 5.7, 12, and 27 mg a.i./L.

The % growth inhibition in cell density, in the treated algal culture as compared to the negative control, ranged from 22 to 98%. The % growth inhibition in biomass, in the treated algal culture as compared to the negative control, ranged from 29 to 96%. The % growth inhibition in growth rate, in the treated algal culture as compared to the negative control, ranged from 5.8 to 94%.

The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of <0.48 and 1.2 mg a.i./L, respectively.

There was no aggregation/flocculation of algae in the control or treatment groups. Adherence of cells to the test chambers was observed in the negative control and the first three test concentrations.

This toxicity study is classified as scientifically sound and the guideline requirement for a Tier II nonvascular plant toxicity study with the marine diatom, *Skeletonema costatum*.

Results Synopsis

Test Organism: *Skeletonema costatum*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: 0.18 mg a.i./L   95% C.I.: 0.051 to 0.64 mg a.i./L
EC_{50}: 1.3 mg a.i./L   95% C.I.: 0.69 to 2.5 mg a.i./L
NOAEC: <0.48 mg a.i./L
Probit Slope: 1.91 ± 0.328

Biomass (Area under the growth curve)
EC_{05}: 0.084 mg a.i./L   95% C.I.: 0.017 to 0.42 mg a.i./L
EC_{50}: 1.2 mg a.i./L   95% C.I.: 0.55 to 2.6 mg a.i./L
NOAEC: <0.48 mg a.i./L
Probit Slope: 1.43 ± 0.238

Growth rate
EC_{05}: 0.58 mg a.i./L   95% C.I.: 0.14 to 2.4mg a.i./L
EC_{50}: 3.9 mg a.i./L   95% C.I.: 2.0 to 7.3 mg a.i./L
NOAEC: 0.95 mg a.i./L
Probit Slope: 2.01 ± 0.449

Endpoint(s) Effected: Cell density, biomass, and growth rate
I. MATERIALS AND METHODS

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Algae (*Skeletonema costatum*)

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 8.0 ± 0.1. The pH in this study ranged above the recommended maximum (8.3-8.5) at study termination for all but the two highest treatment concentrations.
4. OPPTS guidelines recommend that the test begin with 77,000 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 time 0 measured concentrations yielded recoveries of 76.8 to 106% of nominal test concentrations, and the 96-hour measured concentrations yielded recoveries of 69.5 to 82.6% 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 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: Marine diatom, *Skeletonema costatum*

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: CCMP 1332
Source: In-house cultures originally obtained from CCMP-Provasoli-Guillard
                        National Center for the Culture of Marine Phytoplankton
Age of inoculum: At least 2 weeks.
Method of cultivation: Grown under test conditions (saltwater 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:	Continuous.	
Culturing media and conditions: (same as test or not)	Saltwater algal medium Appeared to be same as test; temperature, lighting, and incubation facility were not reported.	*EPA recommends two week acclimation period.*
Health: (any mortality observed)	Not reported.	*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	Static.	*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).*
Renewal rate for static renewal	N/A	
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**):	8.0 8.0-8.5 Yes. None 30 ppt	*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	
Dilution water 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 30 ppt 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 saltwater 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 77,000 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		

Parameter	Details	Remarks
		Criteria
Nominal: Measured:	0 (Negative control), 0.58, 1.3, 2.8, 6.2, 14, and 30 mg a.i./L <0.300 (<LOQ; control), 0.48, 0.95, 2.6, 5.7, 12, and 27 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.*
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:	19.0-20.4°C 16L:8D 3760 to 4870 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 using a particle counter (Coulter Electronics, Inc.). 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.* 43x10⁴ 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 43×10^4 cells/mL in the negative control, yielding inhibitions of 30, 22, 81, 87, 98, and 98% when compared to the negative control at the mean-measured 0.48, 0.95, 2.6, 5.7,

12, and 27 mg a.i./L treatment levels, respectively.

Biomass (area under the growth curve) averaged 97.6×10^5 cells · days/mL in the negative control, yielding inhibitions of 37, 29, 67, 90, 96, and 93% when compared to the negative control.

Growth rate averaged 0.0464 hours^{-1} in the negative control, yielding inhibitions of 8.1, 5.8, 37, 60, 89, and 94% when compared to the negative control.

The study authors used the mean-measured concentrations for calculations of endpoint values. For cell density and biomass, they calculated EC5s and EC10s and substituted them for the NOAEC since the original NOAECs they obtained were below the lowest treatment level. However, the original NOAECs are reported in this section.

Based on cell density, the 96-hour NOAEC and EC_{50} values were <0.48 and 1.3 mg a.i./L. Based on biomass, the 96-hour NOAEC and EC_{50} values were <0.48 and 1.2 mg a.i./L. Based on growth rate, the 96-hour NOAEC and EC_{50} values were 0.95 and 3.9 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 1.4, 1.2, and 3.6 mg a.i./L, respectively.

There was no aggregation/flocculation of algae in the control or treatment groups. Adherence of cells to the test chambers was observed in the negative control and the first three test concentrations.

Table 3: Effect of Dimethyl Disulfide on algal growth (*Skeletonema costatum*)

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	1.4	3.2	16.3	43	N/A
0.48 (0.58)	0.5	1.1	2.8	8.3	30.1	30
0.95 (1.3)	0.5	1.6	2.8	10	33.5	22
2.6 (2.8)	0.5	0.9	1.5	8.7	8.2	81
5.7 (6.2)	0.5	0.9	0.8	1.2	5.7	87
12 (14)	0.5	1.1	1.0	0.8	0.9	98
27 (30)	0.5	1.9	1.5	0.8	0.7	98

Table 4: Effect of Dimethyl Disulfide on algal growth (*Skeletonema costatum*)

Mean-Measured and (Nominal) Concentrations (mg a.i./L)	Initial Cell Density (x10⁴ cells/mL)	Mean Growth Rate (hours⁻¹)		Mean Biomass (Area Under the Growth Curve) (x 10⁵ cells * days/mL)	
		0-96 Hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	0.5	0.0464	N/A	97.6	N/A
0.48 (0.58)	0.5	0.0426	8.1	61.3	37
0.95 (1.3)	0.5	0.0437	5.8	70.5	29
2.6 (2.8)	0.5	0.0291	37	32.4	67
5.7 (6.2)	0.5	0.0184	60	9.4	90
12 (14)	0.5	0.0051	89	3.9	96
27 (30)	0.5	0.0030	94	6.9	93

Table 5: Statistical endpoint values.*

Statistical Endpoint	Cell Density	Growth rate	Biomass (Area under the growth curve)
NOAEC or EC₀₅ (mg a.i./L)	<0.48	0.95	<0.48
EC₅₀ (mg a.i./L)	1.3	3.9	1.2
IC₅₀ or EC₅₀ (mg a.i./L) (95% C.I.)	1.3 (0.69-2.5)	3.9 (2.0-7.3)	1.2 (0.55-2.6)
Other (IC₂₅/EC₂₅)	N/A	N/A	N/A
Reference chemical, if used NOAEC IC₅₀/EC₅₀	N/A	N/A	N/A

*Study author-reported values

B. REPORTED STATISTICS:

The EC₅₀ values and their 95% 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. The study authors calculated EC₅s and EC₁₀s for the endpoints where the NOAEC was below the lowest test concentration and

substituted those values for the NOAEC.

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}: 0.18 mg a.i./L	95% C.I.: 0.051 to 0.64 mg a.i./L
EC_{50}: 1.3 mg a.i./L	95% C.I.: 0.69 to 2.5 mg a.i./L
NOAEC: <0.48 mg a.i./L	
Probit Slope: 1.91 ± 0.328	

Biomass (Area under the growth curve)
EC_{05}: 0.084 mg a.i./L	95% C.I.: 0.017 to 0.42 mg a.i./L
EC_{50}: 1.2 mg a.i./L	95% C.I.: 0.55 to 2.6 mg a.i./L
NOAEC: <0.48 mg a.i./L	
Probit Slope: 1.43 ± 0.238	

Growth rate
EC_{05}: 0.58 mg a.i./L	95% C.I.: 0.14 to 2.4mg a.i./L
EC_{50}: 3.9 mg a.i./L	95% C.I.: 2.0 to 7.3 mg a.i./L
NOAEC: 0.95 mg a.i./L	
Probit Slope: 2.01 ± 0.449	

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 authors' results were in agreement. However, the reviewer provided probit slopes, therefore 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 March 28 to April 1, 2008.

F. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of <0.48 and 1.2 mg a.i./L, respectively.

Test Organism: *Skeletonema costatum*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density

EC_{05}: 0.18 mg a.i./L   95% C.I.: 0.051 to 0.64 mg a.i./L
EC_{50}: 1.3 mg a.i./L   95% C.I.: 0.69 to 2.5 mg a.i./L
NOAEC: <0.48 mg a.i./L
Probit Slope: 1.91 ± 0.328

Biomass (Area under the growth curve)

EC_{05}: 0.084 mg a.i./L   95% C.I.: 0.017 to 0.42 mg a.i./L
EC_{50}: 1.2 mg a.i./L   95% C.I.: 0.55 to 2.6 mg a.i./L
NOAEC: <0.48 mg a.i./L
Probit Slope: 1.43 ± 0.238

Growth rate

EC_{05}: 0.58 mg a.i./L   95% C.I.: 0.14 to 2.4mg a.i./L
EC_{50}: 3.9 mg a.i./L   95% C.I.: 2.0 to 7.3 mg a.i./L
NOAEC: 0.95 mg a.i./L
Probit Slope: 2.01 ± 0.449

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. 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.
8. Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry*. 11: 1485-1494.
9. Cohen, Jacob. 1977. Statistical Power Analysis for the Behavioral Sciences. Academic Press, New York.
10. U.S. Environmental Protection Agency. 1994. Pesticide Reregistration Rejection Rate Analysis. Ecological Effects. EPA 738-R-94-035.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Dimethyl disulfide & S. costatum 96-hr cell density
File: 1207c        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 & S. costatum 96-hr cell density
File: 1207c        Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
------------------------------------------------------------------------

D =  254.453

W =    0.940

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 & S. costatum 96-hr cell density
File: 1207c        Transform: NO TRANSFORMATION

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

Calculated H statistic (max Var/min Var) =   613.00
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 & S. costatum 96-hr cell density
File: 1207c   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic =   18.47
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 FAIL homogeneity test at 0.01 level. Try another transformation.

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

Dimethyl disulfide & S. costatum 96-hr cell density
File: 1207c   Transform: NO TRANSFORMATION

                          ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	5558.339	926.390	50.971
Within (Error)	14	254.453	18.175	
Total	20	5812.792		

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

Dimethyl disulfide & S. costatum 96-hr cell density
File: 1207c   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	43.000	43.000		
2	0.48	30.133	30.133	3.696	*
3	0.95	33.567	33.567	2.710	*
4	2.6	8.233	8.233	9.988	*
5	5.7	5.667	5.667	10.725	*
6	12	0.867	0.867	12.104	*
7	27	0.700	0.700	12.152	*

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

Dimethyl disulfide & S. costatum 96-hr cell density
File: 1207c   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	0.48	3	8.807	20.5	12.867
3	0.95	3	8.807	20.5	9.433
4	2.6	3	8.807	20.5	34.767
5	5.7	3	8.807	20.5	37.333
6	12	3	8.807	20.5	42.133
7	27	3	8.807	20.5	42.300

Dimethyl disulfide & S. costatum 96-hr cell density
File: 1207c   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	43.000	43.000	43.000
2	0.48	3	30.133	30.133	31.850
3	0.95	3	33.567	33.567	31.850
4	2.6	3	8.233	8.233	8.233
5	5.7	3	5.667	5.667	5.667
6	12	3	0.867	0.867	0.867
7	27	3	0.700	0.700	0.700

Dimethyl disulfide & S. costatum 96-hr cell density
File: 1207c   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	43.000				
0.48	31.850	3.203	*	1.76	k= 1, v=14
0.95	31.850	3.203	*	1.85	k= 2, v=14
2.6	8.233	9.988	*	1.88	k= 3, v=14
5.7	5.667	10.725	*	1.89	k= 4, v=14
12	0.867	12.104	*	1.90	k= 5, v=14
27	0.700	12.152	*	1.91	k= 6, v=14

s =   4.263
Note: df used for table values are approximate when v > 20.

Estimates of EC%

```
----------------------------------------------------------------------
Parameter    Estimate       95% Bounds          Std.Err.    Lower Bound
                         Lower      Upper                   /Estimate
EC5            0.18      0.051      0.64         0.26         0.28
EC10           0.28      0.091      0.86         0.23         0.33
EC25           0.58      0.24       1.4          0.18         0.41
EC50           1.3       0.69       2.5          0.13         0.53


        Slope =    1.91  Std.Err. =    0.328


Goodness of fit: p =       0.19   based on DF=        4.0         14.
----------------------------------------------------------------------
1207C : Dimethyl disulfide & S. costatum 96-hr cell density
----------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
----------------------------------------------------------------------
    Dose      #Reps.     Obs.      Pred.       Obs.      Pred.      %Change
                         Mean      Mean       -Pred.    %Control

    0.00       3.00      43.0      43.0     -0.00652    100.        0.00
    0.480      3.00      30.1      34.3      -4.20       79.8      20.2
    0.950      3.00      33.6      26.1       7.50       60.6      39.4
    2.60       3.00      8.23      12.3      -4.04       28.5      71.5
    5.70       3.00      5.67      4.80       0.870      11.2      88.8
    12.0       3.00      0.867     1.43      -0.559      3.31      96.7
    27.0       3.00      0.700     0.260      0.440      0.604     99.4

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

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

Dimethyl disulfide & S. costatum 96-hr biomass mg/L
File: 1207b        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             9                 5              7          0

----------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   7.6969
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.



Dimethyl disulfide & S. costatum 96-hr biomass mg/L
File: 1207b        Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
----------------------------------------------------------------------

D = 1245.940
```

```
W =    0.969

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 & S. costatum 96-hr biomass mg/L
File: 1207b        Transform: NO TRANSFORMATION
```

Hartley test for homogeneity of variance

```
Calculated H statistic (max Var/min Var) =   28.19
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 & S. costatum 96-hr biomass mg/L
File: 1207b        Transform: NO TRANSFORMATION
```

Bartletts test for homogeneity of variance

```
Calculated B statistic =    7.51
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 & S. costatum 96-hr biomass mg/L
File: 1207b        Transform: NO TRANSFORMATION
```

                              ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	24294.718	4049.120	45.498

```
Within (Error)     14          1245.940          88.996
-----------------------------------------------------------------------
Total              20          25540.658
-----------------------------------------------------------------------
```

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

Dimethyl disulfide & S. costatum 96-hr biomass mg/L
File: 1207b   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	97.633	97.633		
2	0.48	61.267	61.267	4.721	*
3	0.95	70.467	70.467	3.527	*
4	2.6	32.433	32.433	8.465	*
5	5.7	9.367	9.367	11.459	*
6	12	3.900	3.900	12.169	*
7	27	6.867	6.867	11.784	*

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

Dimethyl disulfide & S. costatum 96-hr biomass mg/L
File: 1207b   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	0.48	3	19.488	20.0	36.367
3	0.95	3	19.488	20.0	27.167
4	2.6	3	19.488	20.0	65.200
5	5.7	3	19.488	20.0	88.267
6	12	3	19.488	20.0	93.733
7	27	3	19.488	20.0	90.767

Dimethyl disulfide & S. costatum 96-hr biomass mg/L
File: 1207b   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	97.633	97.633	97.633
2	0.48	3	61.267	61.267	65.867

```
          3               0.95    3      70.467      70.467      65.867
          4                2.6    3      32.433      32.433      32.433
          5                5.7    3       9.367       9.367       9.367
          6                 12    3       3.900       3.900       5.383
          7                 27    3       6.867       6.867       5.383
```

Dimethyl disulfide & S. costatum 96-hr biomass mg/L
File: 1207b   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	97.633				
0.48	65.867	4.124	*	1.76	k= 1, v=14
0.95	65.867	4.124	*	1.85	k= 2, v=14
2.6	32.433	8.465	*	1.88	k= 3, v=14
5.7	9.367	11.459	*	1.89	k= 4, v=14
12	5.383	11.976	*	1.90	k= 5, v=14
27	5.383	11.976	*	1.91	k= 6, v=14

s =   9.434
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.084	0.017	0.42	0.33	0.20
EC10	0.15	0.037	0.62	0.29	0.24
EC25	0.40	0.13	1.2	0.23	0.33
EC50	1.2	0.55	2.6	0.16	0.46

        Slope =   1.43 Std.Err. =   0.238

!!!Poor fit: p =   0.024 based on DF=   4.0   14.

1207B : Dimethyl disulfide & S. costatum 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	97.6	98.5	-0.867	100.	0.00
0.480	3.00	61.3	70.5	-9.19	71.5	28.5
0.950	3.00	70.5	55.0	15.5	55.8	44.2
2.60	3.00	32.4	31.2	1.24	31.7	68.3
5.70	3.00	9.37	16.5	-7.17	16.8	83.2
12.0	3.00	3.90	7.61	-3.71	7.73	92.3
27.0	3.00	6.87	2.67	4.20	2.71	97.3

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

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

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

Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g        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            9                 5              7           0

--------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   7.6969
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.



Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g        Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------

D = 51464.000

W =    0.848

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
--------------------------------------------------------------------

Data FAIL normality test. Try another transformation.

Warning -  The two homogeneity tests are sensitive to non-normal data and
           should not be performed.



Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g        Transform: NO TRANSFORMATION

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

Calculated H statistic (max Var/min Var) =   251.61
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 & S. costatum 96-hr Growth rate mg/L
File: 1207g   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic =   18.97
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 FAIL homogeneity test at 0.01 level. Try another transformation.

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

Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	609724.952	101620.825	27.644
Within (Error)	14	51464.000	3676.000	
Total	20	661188.952		

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

Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g   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	463.667	463.667		
2	0.48	426.333	426.333	0.754	
3	0.95	437.000	437.000	0.539	
4	2.6	291.333	291.333	3.481	*

```
    5                         5.7     184.000              184.000          5.649   *
    6                          12      51.000               51.000          8.336   *
    7                          27      30.000               30.000          8.760   *
-----------------------------------------------------------------------------------
Dunnett table value =  2.53      (1 Tailed Value, P=0.05,  df=14,6)
```

Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g   Transform: NO TRANSFORMATION

```
        DUNNETTS TEST    -    TABLE 2 OF 2              Ho:Control<Treatment
---------------------------------------------------------------------------------
                              NUM OF   Minimum Sig Diff   % of      DIFFERENCE
GROUP      IDENTIFICATION     REPS     (IN ORIG. UNITS)  CONTROL   FROM CONTROL
-----  --------------------  ------   -----------------  -------   ------------
    1           Neg control     3
    2                  0.48     3           125.246        27.0        37.333
    3                  0.95     3           125.246        27.0        26.667
    4                   2.6     3           125.246        27.0       172.333
    5                   5.7     3           125.246        27.0       279.667
    6                    12     3           125.246        27.0       412.667
    7                    27     3           125.246        27.0       433.667
---------------------------------------------------------------------------------
```

Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g   Transform: NO TRANSFORMATION

```
        WILLIAMS TEST   (Isotonic regression model)    TABLE 1 OF 2
---------------------------------------------------------------------------------
GROUP                                  ORIGINAL     TRANSFORMED     ISOTONIZED
          IDENTIFICATION      N          MEAN          MEAN           MEAN
------  --------------------  ---      ----------   -----------    -----------
    1           Neg control    3        463.667       463.667        463.667
    2                  0.48    3        426.333       426.333        431.667
    3                  0.95    3        437.000       437.000        431.667
    4                   2.6    3        291.333       291.333        291.333
    5                   5.7    3        184.000       184.000        184.000
    6                    12    3         51.000        51.000         51.000
    7                    27    3         30.000        30.000         30.000
---------------------------------------------------------------------------------
```

Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
File: 1207g   Transform: NO TRANSFORMATION

```
        WILLIAMS TEST   (Isotonic regression model)    TABLE 2 OF 2
---------------------------------------------------------------------------------
                   ISOTONIZED   CALC.      SIG      TABLE      DEGREES OF
    IDENTIFICATION    MEAN      WILLIAMS   P=.05    WILLIAMS    FREEDOM
------------------- ----------- ---------- ----- ----------- -------------
        Neg control    463.667
               0.48    431.667    0.646               1.76     k= 1, v=14
               0.95    431.667    0.646               1.85     k= 2, v=14
                2.6    291.333    3.481       *       1.88     k= 3, v=14
                5.7    184.000    5.649       *       1.89     k= 4, v=14
                 12     51.000    8.336       *       1.90     k= 5, v=14
```

```
                    27    30.000    8.760      *      1.91      k= 6, v=14
-----------------------------------------------------------------------------
s =   60.630
Note: df used for table values are approximate when v > 20.
```

```
 Estimates of EC%
---------------------------------------------------------------------------
Parameter   Estimate    95% Bounds        Std.Err.   Lower Bound
                        Lower     Upper               /Estimate
EC5           0.58      0.14       2.4     0.30        0.24
EC10          0.89      0.26       3.1     0.26        0.29
EC25          1.8       0.69       4.6     0.20        0.39
EC50          3.9       2.0        7.3     0.13        0.53

       Slope =    2.01  Std.Err. =    0.449
```

```
Goodness of fit: p =     0.88   based on DF=       4.0      14.
---------------------------------------------------------------------------
1207G : Dimethyl disulfide & S. costatum 96-hr Growth rate mg/L
---------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
---------------------------------------------------------------------------
    Dose     #Reps.    Obs.     Pred.     Obs.      Pred.    %Change
                       Mean     Mean     -Pred.    %Control
    0.00     3.00      464.     464.     -0.150     100.      0.00
    0.480    3.00      426.     448.     -21.4      96.5      3.46
    0.950    3.00      437.     412.      24.7      88.9      11.1
    2.60     3.00      291.     294.     -2.83      63.4      36.6
    5.70     3.00      184.     170.      14.1      36.6      63.4
    12.0     3.00      51.0     74.6     -23.6      16.1      83.9
    27.0     3.00      30.0     20.7      9.26      4.47      95.5
```