Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Algae (*Navicula pelliculosa*)

PMRA Submission Number {.....} EPA MRID Number 47471206

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	355006
OECD Data Point	{.....}
EPA MRID	47471206
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:** Moncie V Wright
Staff Scientist, Cambridge Environmental **Date:** 09/10/08

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

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

Secondary Reviewer(s): Valerie Woodard **Date:** 3-23-09
{EPA/OECD/PMRA}

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Company Code	{.....}	[For PMRA]
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Date Evaluation Completed:

CITATION: Minderhout, T., Kendall, T.Z. and H.O. Krueger. 2008. Dimethyl Disulfide: A 96-Hour Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Unpublished study performed by Wildlife International, Easton, Maryland. Laboratory Project No.: 524A-124A. Study sponsored by Arkema, Inc., Philadelphia, Pennsylvania. Study completed July 1, 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 diatom *Navicula pelliculosa* were exposed to Dimethyl Disulfide at nominal concentrations of 0 (negative control), 2.3, 3.9, 6.5, 11, 18, and 30 mg a.i./L under static conditions. Initial-measured concentrations were <1.00 (<LOQ; control), 2.76, 5.10, 7.05, 11.9, 18.8, and 32.4 mg a.i./L.

The % growth inhibition in cell density, in the treated algal culture as compared to the negative control, ranged from -54 to -88% in the first five treatment levels, and was 94% at the highest treatment level. The % growth inhibition in biomass, in the treated algal culture as compared to the negative control, ranged from -28 to -32% in the first four treatment groups, and was 8.2 to 96% in the highest two treatment groups. The % growth inhibition in growth rate, in the treated algal culture as compared to the negative control, ranged from -10 to -14% in all but the highest treatment group, which was 63%.

The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of 18.8 and 22 mg a.i./L, respectively.

There was adherence of cells to the test chambers in the negative control and first five treatment groups. Aggregation/flocculation of algae was observed in the negative control and the 2.0 and 3.9 mg a.i./L mean-measured treatment groups. There were no noticeable changes in cell morphology as compared to the control.

This toxicity study is classified as scientifically sound and satisfies the guideline requirement for a Tier II nonvascular plant toxicity study with the freshwater diatom, *Navicula pelliculosa.*

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density

EC_{05}: 19 mg a.i./L	95% C.I.: 13 to 26 mg a.i./L
EC_{50}: 24 mg a.i./L	95% C.I.: 20 to 29 mg a.i./L
NOAEC: 18.8 mg a.i./L	
Probit Slope: 14.4 ± 4.29	

Biomass (Area under the growth curve)

EC_{05}: 15 mg a.i./L	95% C.I.: 14 to 18 mg a.i./L
EC_{50}: 22 mg a.i./L	95% C.I.: 20 to 23 mg a.i./L
NOAEC: 18.8 mg a.i./L	
Probit Slope: 10.9 ± 1.18	

Growth rate

EC_{05}: 22 mg a.i./L	95% C.I.: 12 to 42 mg a.i./L
EC_{50}: 30 mg a.i./L	95% C.I.: 26 to 34 mg a.i./L
NOAEC: 18.8 mg a.i./L	
Probit Slope: 12.8 ± 10.3	

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 Day 2 to study termination (range 7.9-10.7).
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 96-hour measured concentrations yielded recoveries of 54 to 70.5% of the nominal test concentrations, indicating that dimethyl disulfide was not 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 diatom, *Navicula pelliculosa*

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 Texas at Austin, Texas
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**):	7.4-7.5 8.2-10.7 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-38.7 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:	 4 N/A 4	 *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: Initial Measured:	0 (Negative control), 2.3, 3.9, 6.5, 11, 18, and 30 mg a.i./L <1.00 (<LOQ; control), 2.76, 5.10, 7.05, 11.9, 18.8, and 32.4 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.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	 23.6-24.7°C Continuous 3850 - 4590 lux Cool-white fluorescent lighting	 *EPA temperature: <u>Skeletonema</u>: 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.*
<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 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.* $44x10^4$ 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 44 x 10^4 cells/mL in the negative control, yielding inhibitions of -68, -71, -88, -86, -54, and 94% when compared to the negative control at the initial-measured 2.76, 5.10,

7.05, 11.9, 18.8, and 32.4 mg a.i./L treatment levels, respectively.

Biomass (area under the growth curve) averaged 184.4 x 10^5 cells · days/mL in the negative control, yielding inhibitions of -28, -30, -20, -32, 8.2, and 96% when compared to the negative control.

Growth rate averaged 0.0465 $hours^{-1}$ in the negative control, yielding inhibitions of -12, -12, -14, -14, -10, and 63% when compared to the negative control.

The study author used the mean-measured concentrations for calculations of endpoint values. Based on cell density, the 96-hour NOAEC and EC_{50} values were 15 and 20 mg a.i./L. Based on biomass, the 96-hour NOAEC and EC_{50} values were 15 and 18 mg a.i./L. Based on growth rate, the 96-hour NOAEC and EC_{50} values were 15 and 25 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 17, 16, and 20 mg a.i./L, respectively.

There was adherence of cells to the test chambers in the negative control and first five treatment groups. Aggregation/flocculation of algae was observed in the negative control and the 2.0 and 3.9 mg a.i./L mean-measured treatment groups. There were no noticeable changes in cell morphology as compared to the control.

Table 3: Effect of Dimethyl Disulfide on algal growth (*Navicula pelliculosa*)

Initial 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	3.2	16.2	37.3	44	N/A
2.76 (2.3)	0.5	2.2	19.4	41.4	73.7	-68
5.10 (3.9)	0.5	2.0	21.6	40.3	75.1	-71
7.05 (6.5)	0.5	2.1	17.9	32.9	82.5	-88
11.9 (11)	0.5	1.9	17.6	42.4	81.9	-86
18.8 (18)	0.5	2.7	10.5	25.2	67.8	-54
32.4 (30)	0.5	1.0	1.3	0.8	2.7	94

Table 4: Effect of Dimethyl Disulfide on algal growth (*Navicula pelliculosa*)

Initial 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	0.0465	N/A	184.4	N/A
2.76 (2.3)	0.5	0.0520	-12	235.8	-28
5.10 (3.9)	0.5	0.0522	-12	239.3	-30
7.05 (6.5)	0.5	0.0532	-14	221.6	-20
11.9 (11)	0.5	0.0531	-14	242.7	-32
18.8 (18)	0.5	0.0511	-10	169.4	8.2
32.4 (30)	0.5	0.0172	63	6.6	96

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)	15	15	15
EC_{50} (mg a.i./L)	20	25	18
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	20 (16-24)	25 (22-29)	18 (16-19)
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.