Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide TC to Fish (*Danio rerio*)

PMRA Submission Number {.....} EPA MRID Number 470528-15

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	D338050
OECD Data Point	{.....}
EPA MRID	470528-15
EPA Guideline	OPPTS 850.1075 (72-1a)

Test material: Dimethyl Disulfide TC **Purity:** 998.89 g/kg (99.9%)
Common name: DMDS
Chemical name: IUPAC: Dimethyl Disulfide
CAS name: Dimethyl Disulfide
CAS No.: 624-92-0
Synonyms: None Reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 05/22/07

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 05/31/07

Secondary Reviewer: James Felkel 10/30/08 **Date:** {.....}
{EPA/OECD/PMRA}

Secondary Reviewer(s): {.....} **Date:** {.....}
{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: {dd-mm-yyyy}

CITATION: Scheerbaum, Dirk. 2007. Dimethyl Disulfide TC: Fish (Zebrafish), Acute Toxicity Test, Semi-Static, 96 H. Unpublished study performed by Dr. U Noack-Laboratorien, Kathe-Paulus-Str 1, D-31157 Sarstedt. Laboratory report number FAZ106301. Study sponsored by Arkema/Thiochemistry Business Unit, Departement Securite Environnement Produit, France. Study completed January 8, 2007.

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 fish. 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.

This Data Evaluation Record may have been revised by the Environmental Fate and Effects Division subsequent to signing by Cambridge Environmental Inc. personnel.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, Zebrafish (*Danio rerio*) were exposed to Dimethyl Disulfide TC at initial measured concentrations of <0.010 (<LOQ; control), 5.30, 10.6, 22.3, 40.2 and 89.1 mg ai/L under static-renewal conditions; nominal concentrations were 0 (negative control), 6.25, 12.5, 25, 50 and 100 mg/L. The 96-h LC_{50} was 7.50 mg ai/L. The NOAEC value, based on mortality and sub-lethal effects, was 5.30 mg ai/L. Sub-lethal effects (i.e., lethargy, fish lying on the side, missing escape reflex, fish lying on abdomen and hyperventilation) were observed in the groups exposed to initial measured concentrations of 10.6, 22.3 and 40.2 mg ai/L of Dimethyl Disulfide. Based on the results of this study, Dimethyl Disulfide TC would be classified as moderately toxic to Zebrafish (*Danio rerio*) in accordance with the classification system of the U.S. EPA.

The study is considered scientifically sound and is classified ACCEPTABLE for an acute freshwater fish study. The NOAEC and LC_{50} values were 5.30 and 7.50 mg ai/L, respectively.

Results Synopsis

Test Organism Size/Age (mean weight or length): Mean Weight- 0.23 g; Mean Length- 2.59 cm
Test Type (Flow-through, Static, Static Renewal): Static-Renewal

LC_{50}: 7.50 mg ai/L 95% C.I.: 5.30-10.6 mg ai/L
NOAEC: 5.30 mg ai/L Probit Slope: N/A
EC_{50}: Not Determined
Endpoint(s) Affected: Mortality and Sub-Lethal Effects

I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: This study was reported to be conducted following guidelines outlined in EPA OPPTS Draft Guideline 850.1075 and that this guideline is "in accordance" with EC, OECD and Japanese (METI) guidelines.

The following deviations from OPPTS 850.1075 guidelines are noted in the present review:

The pH values of the dilution water (7.05-7.61) fell below the recommended range (7.2-7.6) and average fish weight at study initiation (0.23 g) was lower than the minimum recommended (0.5 g).

These deviations are not considered to impact the acceptability of the study. Fish were reportedly fed daily during the acclimation period (except for 24 hours prior to test initiation), and thus fish were apparently old enough to be actively feeding. Zebrafish are allowed by the draft 850 guidelines (4/96).

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided; the study was declared to be conducted in compliance with the present OECD, EC and German principles of Good Laboratory Practice.

A. REPORTED MATERIALS:

1. Test material Dimethyl Disulfide TC

Description: Light Yellow Liquid

Lot No./Batch No. : 17-08-04

Purity: 998.89 g/kg (99.9%)

Stability of compound under test conditions: New solutions (containing live fish) were analyzed at 0 and 72 hours, while aged solutions (containing live fish) were analyzed at 24 and 72 hours. The aged solutions at 24 hours yielded recoveries of 63-77% of the initial measured concentrations. Only the two lowest treatment levels (nominal 6.25 and 12.5 mg/L) were analyzed at 72 and 96 hours; complete mortality was observed at the three remaining levels (nominal 25, 50 and 100 mg/L). The aged solutions at 96 hours yielded recoveries of 70 and 94% of the 72-hour measured values at the nominal 6.25 and 12.5 mg/L treatment levels, respectively.

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

Storage conditions of test chemicals: Stored at room temperature, protected from light and moisture.

Physicochemical properties of Dimethyl Disulfide TC.

Parameter	Values	Comments

Parameter	Values	Comments
Water solubility at 20EC	Insoluble @ 20°C	
Vapor pressure	20°C: 28 hPa (mbar) 25°C: 38 hPa (mbar)	
UV absorption	Not Reported	
PKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: Zebrafish (*Danio rerio*) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: Juvenile

Weight at study initiation: Mean- 0.23 g; range not reported *EPA recommends: mean 0.5 - 5 g.*

Length at study initiation: Mean- 2.59 cm; range not reported; however, the study author stated that the longest fish was not more than twice the length of the shortest fish *EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ± 1.0 cm for bluegill and 5.0 ± 1.0 cm for rainbow trout*

Source: *EPA recommends that all organisms be from the same source*

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 96-hour range-finding test was conducted using nominal concentrations of 0 (negative control), 10 and 100 mg/L. The test was stopped after 48 hours due to no mortality in the control or 10 mg/L treatment group; complete mortality was observed in the 100 mg/L treatment group at the first observation period at 24 hours.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		
Period:	At least 12 days	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Conditions: (same as test or not)	Same as test	
Feeding:	Fish were fed daily (4% of the fish body weight per feeding day) with Stör perlets; SERA GmbH, D-52518 Heinsberg. Fish were not fed within 24 hours of test initiation.	
Health: (any mortality observed)	Only healthy and normal fish were used. Mortality was <5% prior to test initiation.	
Duration of the test	96 Hours	*The recommended test duration is 96 hours.*
Test condition		
Static/flow-through	Static Renewal	
Type of dilution system - for flow-through method.	N/A	
Renewal rate for static renewal	Test solutions were renewed daily.	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	None	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass 14.5 L 13.5 L	Due to the high volatility of the test item, it was directly applied to the test vessels. The study author also reported that, with regard to the high volatility of the test item, a reduced gas phase above the water was set up. *Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Local tap water was filtered on activated charcoal and aerated for at least 24 hours to remove chlorine.	 *Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf)* *Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	54-64 mg/L as $CaCO_3$ 7.05-7.61 76-100% saturation See Reviewer's Comments Not Reported See Reviewer's Comments See Reviewer's Comments <0.01 mg/L (0 Hrs, Control) 21.5-22.7°C N/A Temperature, DO and pH were measured in all test vessels containing live fish before and after each renewal period. Temperature was also measured continuously in the control vessel.	*Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ∃ 60% during first 48 hrs and ∃ 40% during second 48 hrs; flow-through: ∃ 60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 1 N/A 1	*Recommended number of replicates includes a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 N/A 10	*Number of organisms per replicate should be ∃ 10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	<0.8 g/L	*Recommended static conditions are # 0.8 g/L at # 17EC and # 0.5 g/L at > 17EC. Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: nominal: measured:	0 (negative control), 6.25, 12.5, 25, 50 and 100 mg/L <0.010 (<LOQ; control), 5.30, 10.6, 22.3, 40.2 and 89.1 mg ai/L	Samples from the three highest treatment levels were not analyzed at 72 and 96 hours due to complete mortality. The measured concentrations from the old solutions at 24 hours yielded recoveries of 69-77% of nominal, while the measured concentrations from the aged solutions at 96 hours (from the two lowest treatment levels), yielded recoveries of 70-94% of the initial measured concentrations at 72 hours. As measured values were not determined before and after each renewal period for each treatment level, the reviewer was unable to calculate the time-weighted average or mean-measured concentrations. Further, the measured values in the aged solutions at 24 hours ranged <70% of the initial measured concentrations at 0 hours. Therefore, the reviewer reported the 0 hour measured concentrations as the "measured" values in the DER; additionally, the reviewer used these concentrations for all reporting and statistical analyses.
Solvent (type, percentage, if used)	N/A; a solvent was not used	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	12-16 hours of illumination with a 15-30 minute transition period	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the definitive test.	*Fish should not feed during the study.*

Parameter	Details	Remarks *Criteria*
Recovery of chemical Frequency of determination Level of quantization Level of detection	 Analytical verification was conducted on new solutions at 0 and 72 hours and on aged solutions at 24 and 96 hours. 0.010 mg ai/L Not Reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used.	
Other parameters, if any	None Reported	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	6, 24, 48, 72 and 96 hours	*Observation intervals should be a minimum of every 24 hours.*
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. REPORTED MORTALITY:

By test termination (96 hours), mortality was 0% in the negative control and initial measured 5.30 mg ai/L treatment group and 100% in the initial measured 10.6, 22.3, 40.2 and 89.1 mg ai/L treatment groups. Complete mortality was observed in the initial measured 10.6, 22.3, 40.2 and 89.1 mg ai/L treatment groups after 48, 24, 24 and 6 hours of exposure, respectively. The study author's analyses yielded NOAEC and LC_{50} values of 3.30 and 5.01 (3.30-7.59) mg ai/L, respectively, based on the geometric mean measured test concentrations. The study author's NOAEC value corresponded to the initial measured concentration of 5.30 mg ai/L.

Table 3: Effect of Dimethyl Disulfide on Mortality of *Danio rerio*.

Initial Measured Concentrations (mg ai/L)	No. of Fish at Start of Study	Observation Period					
		Day 1		Day 2		Day 4	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
<0.010 (Negative Control)	10	0	0	0	0	0	0
5.30	10	0	0	0	0	0	0
10.6	10	6	60	10	100	10	100
22.3	10	10	100	10	100	10	100
40.2	10	10	100	10	100	10	100
89.1	10	10	100	10	100	10	100
NOAEC*	3.30 mg ai/L						
LC_{50}*	5.01 (3.30-7.59) mg ai/L						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

* The study author's toxicity values were derived using the geometric mean-measured concentrations. The reviewer's results are based on the initial measured concentrations.
N/A-Not Applicable as a positive control was not used

B. REPORTED NON-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in the negative control or initial measured 5.30 mg ai/L treatment group at any point during the definitive test. Within 6 hours of exposure, all surviving fish in the initial measured 10.3, 22.3 and 40.2 mg ai/L treatment groups were exhibiting symptoms of toxicity; all fish in the highest treatment group were dead within 6 hours. Effects at the 10.6 mg ai/L level included fish lying on their side and missing the escape reflex. At the 22.3 and 40.2 mg ai/L treatment groups, fish were observed lying on their abdomen, missing the escape reflex and hyperventilating.

Table 4: Sub-lethal Effect of Dimethyl Disulfide on *Danio rerio*.

Initial Measured Concentrations (mg ai/L)	Observation Period		
	Endpoints at 6 Hrs	Endpoints at 24 Hours	Endpoints at 96 Hours
	% Affected	% Affected	% Affected
<0.010 (Negative Control)	A.N.	A.N.	A.N.
5.30	A.N.	A.N.	A.N.
10.6	10%-Lying on side 90%- Missing escape reflex	50%-Lethargic 50%- Missing escape reflex	--
22.3	60%-Missing escape reflex 40%-Lying on abdomen 40%-Hyperventilating	--	--
40.2	50%-Missing escape reflex 50%-Lying on abdomen 50%-Hyperventilating	--	--
89.1	--	--	--
NOAEC*	3.30 mg ai/L		
LOAEC*	7.59 mg ai/L		
EC_{50}	Not Determined		
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A

A.N.- All fish appear normal and healthy
*- The study author's toxicity values were derived using the geometric mean-measured concentrations. The reviewer's results are based on the initial measured concentrations.
N/A- Not Applicable as a positive control was not used

C. REPORTED STATISTICS:

The LC_{50} value was calculated by sigmoidal dose-response regression. Calculation of the confidence intervals for LC_{50} was reportedly done using standard procedures according to Clopper and Pearson (1934). The concentrations leading to 0 and 100% mortality, as well as the NOAEC after 96 hours, were reportedly determined directly from the test results where applicable. All of the study author's toxicity values were determined using the geometric mean-measured concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The 96-hour LC_{50} (and 95% C.I.) was determined by analyzing the cumulative mortality using the binomial probability method via Toxanal Statistical software. The NOAEC value was visually determined based on the observed % mortality. As no quantitative sub-lethal measurements were taken, the reviewer was unable to determine an EC_{50} value. All toxicity values were determined based on the initial measured concentrations.

LC_{50}: 7.50 mg ai/L 95% C.I.: 5.30-10.6 mg ai/L
NOAEC: 5.30 mg ai/L
Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

The average fish weight in this study (0.23 g) was less than the minimum recommended (0.5 g) for acute freshwater fish toxicity studies. Because sensitivity can vary as a function of body weight, use of lower weight fish can limit the comparability of these results with those from other studies that tested fish within the weight recommendation.

F. REVIEWER'S COMMENTS:

The study author's 95% C.I. associated with the 96-hour LC_{50} (3.30-7.59 mg ai/L) was narrower than the reviewer's (5.30-10.6 mg ai/L); however, the study author conducted all analyses using the geometric mean-measured concentrations, while the reviewer used the initial measured concentrations due to low recoveries at 24 hours (<70% of 0-hour measurements in the nominal 50 and 100 mg/L treatment groups). Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

Samples from the three highest treatment levels were not analyzed at 72 and 96 hours due to complete mortality. The measured concentrations from the old solutions at 24 hours yielded recoveries of 69-77% of nominal, while the measured concentrations from the aged solutions at 96 hours (from the two lowest treatment levels), yielded recoveries of 70-94% of the initial measured concentrations at 72 hours. As measured values were not determined before and after each renewal period for each treatment level, the reviewer was unable to calculate the time-weighted average or mean-measured concentrations. Furthermore, the measured concentrations of the aged solutions at 24 hours ranged <70% of the initial measured concentrations at 0 hours. Therefore, the reviewer reported the 0 hour measured concentrations as the "measured" values in the DER; additionally, the reviewer used these concentrations for all reporting and statistical analyses.

QC samples (fortified levels of 10, 100 and 100,000 µg/L) were analyzed to determine the accuracy, precision and specificity of the analytical methodology. The mean recovery rates were 99, 102 and 89% of nominal for the 10, 100 and 100,000 µg/L samples, respectively.

An analysis of the tap water was provided in the study report; however, it is reported in German. Therefore, the reviewer is unsure about which analytes were detected and at which concentrations.

The in-life portion of the definitive toxicity test was conducted from September 11 to September 15, 2006.

G. CONCLUSIONS:

The study is considered scientifically sound and is classified ACCEPTABLE for an acute freshwater fish study. The NOAEC and LC_{50} values were 5.30 and 7.50 mg ai/L, respectively.

LC_{50}: 7.50 mg ai/L 95% C.I.: 5.30-10.6 mg ai/L
NOAEC: 5.30 mg ai/L Probit Slope: N/A
EC_{50}: Not Determined
Endpoint(s) Affected: Mortality and Sub-Lethal Effects

III. REFERENCES:

EPA OPPTS Draft Guideline 850.1075 (1996).

Clopper and Pearson (1934): Biometrika 26: 404-413 cited in GraphPad Prism Statistics Guide 4.0

SANCO/3029/99 rev. 4, Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (Part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 (11/07/00).

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
89.1	10	10	100	9.765625E-02
40.2	10	10	100	9.765625E-02
22.3	10	10	100	9.765625E-02
10.6	10	10	100	9.765625E-02
5.3	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 5.3 AND 10.6 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7.495333

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.