

Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Sheepshead

Minnow (*Cyprinodon variegates*)

PMRA Submission Number N/A

EPA MRID Number 47307504

Data Requirement:	PMRA Data Code:	N/A
	EPA DP Barcode:	348310
	OECD Data Point:	N/A
	EPA MRID:	47307504
	EPA Guideline:	850.1075

Test material:	Dimethyl Disulfide (DMDS)	Purity: 99.6%
Common name:	Dimethyl Disulfide	
Chemical name:	IUPAC: Not reported	
	CAS name: Not reported	
	CAS No.: 624-92-0	
	Synonyms: None reported	

Primary Reviewer: Aviva L. Kafka
Staff Scientist, Cambridge Environmental Inc.

Signature:
Date: 03/21/08

Aviva L. Kafka

Secondary Reviewer: Teri S. Myers
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Date: 03/30/2010

Date: {.....}

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

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Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Minderhout, T., Kendall, T.Z. & Krueger, H.O. 2007. Dimethyl Disulfide: A 96-Hour Static-Renewal Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegates*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory report number 524A-117. Study sponsored by Arkema, Inc. Study completed on October 2, 2007.



Data Evaluation Report on the Acute Toxicity of {TAI or EUP} to Fish {Name of Species}

PMRA Submission Number {.....}

EPA MRID Number {.....}

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.

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EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, Sheepshead Minnow (*Cyprinodon variegates*) were exposed to dimethyl disulfide at nominal concentrations of 0 (negative control), 2.6, 4.3, 7.2, 12 and 20 mg a.i./L under static renewal conditions. The mean measured concentrations were <2.00 (<LOQ; negative control), 2.3, 4.0, 6.9, 10 and 18 mg a.i./L. The LC₅₀ was calculated using the binomial method because there are less than two concentrations with mortality between 0 and 100 percent. The 96-h LC₅₀ (95% C.I.) was 5.6 (4.0-6.9) mg a.i./L. The NOAEC value was <2.3 mg a.i./L, based on sublethal effects observed at all levels; the EC₅₀ was determined to be 3.7 (2.3-6.9) mg a.i./L, based on sublethal effects.

Cumulative mortality was 0% in the negative control, 2.3 and 4.0 mg a.i./L treatment groups. Cumulative mortality was 90% in the 6.9 mg a.i./L treatment group, and 100% in the 10 and 18 mg a.i./L treatment groups. Signs of toxicity (lethargy, loss of equilibrium, lying on the bottom and surfacing) were observed during the test and were considered treatment related in all treatment groups. Based on the results of this study, dimethyl disulfide would be classified as moderately toxic to Sheepshead Minnow (*Cyprinodon variegatus*) on an acute toxicity basis in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as acceptable and does satisfy the guideline requirement for an acute estuarine/marine fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean weight or length): 0.10 g (0.08-0.15 g); 2.0 cm (1.8-2.2 cm)

Test Type (Flow-through, Static, Static Renewal): Static Renewal (every 24 hours)

LC₅₀: 5.6 mg a.i./L 95% C.I.: 4.0-6.9 mg a.i./L

NOAEC: <2.3 mg a.i./L Probit Slope: N/A

EC₅₀: 3.7 (2.3-6.9) mg a.i./L

Most sensitive endpoint(s) affected: Signs of toxicity (lethargy, loss of equilibrium, lying on the bottom and surfacing)

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the American Society for Testing and Materials (ASTM), ASTM Standard E729-80 and the U.S. Environmental Protection Agency Series 850- Ecological Effects Test Guidelines, OPPTS No. 850.1075 (draft, 1996), *Fish Acute Toxicity Test, Freshwater and Marine*. The following deviations from OPPTS 850.1075 are noted:

Total organic carbon and particulate matter of the water used in the test chambers were not reported. Furthermore, the results from the periodic screening analysis of the dilution water indicated that the following elements exceeded the maximum allowable concentration (0.001 mg/L): aluminum (<0.200 mg/L), iron (<0.200 mg/L), zinc (<0.0200 mg/L), arsenic (<0.0200 mg/L), chromium (<0.0150 mg/L), cobalt (<0.0050 mg/L), copper (<0.0100 mg/L), lead (<0.0150 mg/L) and nickel (<0.0100 mg/L).

These deviations do not affect the acceptability of this study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with GLP standards as published by the U.S. Environmental Protection Agency (40 CFR, Part 160 and 792, 17 August 1989); OECD Principles of GLP (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999), with the following exception: periodic analyses of saltwater for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

A. MATERIALS:

1. Test material Dimethyl Disulfide

Description: Liquid

Lot No./Batch No. : 05.03.06

Purity: 99.6%

Stability of compound under test conditions: With the exception of one sample, the measured concentrations at all levels and time periods exceeded 70% of nominal values, showing the stability of the test material under test conditions.

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

Storage conditions of test chemicals: The test chemical, dimethyl disulfide, was stored in ambient conditions.

Physicochemical properties of dimethyl disulfide.

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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:

Species: Sheepshead Minnow (*Cyprinodon variegates*)
*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: 60 ± 5 days

Weight at study initiation: 0.10 g (0.08-0.15 g) *EPA recommends: mean 0.5 - 5 g.*

Length at study initiation: 2.0 cm (1.8-2.2 cm) *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: Aquatic Bio Systems, Fort Collins, Colorado *EPA recommends that all organisms be from the same source*

B. STUDY DESIGN:

1. Experimental Conditions

- a. Range-finding study: Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of exploratory range finding toxicity data.
- b. Definitive Study

Table 1: Experimental Parameters

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Parameter	Details	Remarks
		Criteria
<u>Acclimation</u>		Pre-test mortality is not reported.
Period:	14 days minimum	<i>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.</i>
Conditions: (same as test or not)	Same	
Feeding:	Fed a commercially prepared diet supplied by Ziegler Brothers Inc., Gardners, Pennsylvania and Artemia from Brine Shrimp Direct, Ogden, Utah.	
Health: (any mortality observed)	The fish were not fed during the final 48 hours before the test, nor during the 96-hour toxicity test.	
Duration of the test	96 hours	<i>The recommended test duration is 96 hours.</i>
<u>Test condition</u>		
Static/flow-through	Static renewal	<i>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.</i>
Type of dilution system - for flow-through method.	N/A	
Renewal rate for static renewal	24 hours	
Aeration, if any	None reported.	<i>Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.</i>

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Parameter	Details	Remarks
		Criteria
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass 1 gallon 3.8 L	Test chambers were approximately 1 gallon glass jars with Teflon-lined lids. Depth of the test water in a representative test chamber was approximately 24 cm. 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:	Natural seawater collected from Indian River Inlet, Delaware	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.

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Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u> Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	Not reported 8.0-8.2 5.3-7.7 mg/L (>60% saturation) Not reported Not reported See Reviewer's Comments None detected 12100 mg/L (Total Cl) 22.1-22.4°C 20-21% Before and after every 24-hour renewal period.	----- <u>Hardness:</u> EPA recommends 40 - 48 mg/L as CaCO ₃ (OECD recommends 10 - 250 mg/L) <u>pH:</u> 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) <u>Dissolved Oxygen:</u> 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). <u>Temperature:</u> 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). <u>Salinity:</u> 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.
<u>Number of replicates/groups:</u> control: solvent control: treated ones:	2 N/A 2/level	----- Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.
<u>Number of organisms per replicate /groups:</u> control: solvent control: treated ones:	10 N/A 10*	*Replicate B of 4.3 mg a.i./L treatment level contained nine fish. ----- Number of organisms per replicate should be ≥ 10/concentration; OECD guideline recommends at least 7 fish/concentration.

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Parameter	Details	Remarks
		Criteria
Biomass loading rate	0.27 g/L of test/control solution	<i>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.</i>
<u>Test concentrations:</u> nominal: measured:	0 (negative control), 2.6, 4.3, 7.2, 12 and 20 mg a.i./L <LOQ (2.00 mg a.i./L), 2.3, 4.0, 6.9, 10 and 18 mg a.i./L	
Solvent (type, percentage, if used)	N/A	<i>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.</i>
Lighting	Photoperiod of 16 hours light and 8 hours darkness	Photoperiod was controlled by an automatic timer. 30-minute transition period of low light intensity provided at the beginning and end of the 16-hr light period to avoid sudden changes in lighting. Light intensity at test initiation, measured by a SPER Scientific Model 840006C light meter, was 152 lux at the surface of the water of one representative test chamber. <i>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.</i>
Feeding	Fish were not fed 2 days prior to the test or during the test	<i>Fish should not feed during the study.</i>
<u>Recovery of chemical</u> Frequency of determination Level of quantization Level of detection	New solution= 0 and 72 hours Old solution= 0, 24 and 96 hours 2.00 mg a.i./L Not reported	

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Parameter	Details	Remarks
		<i>Criteria</i>
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

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2. Observations:

Table 2: Observations

Parameter	Details	Remarks
		<i>Criteria</i>
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and biological observations of the Sheepshead minnow (e.g., loss of equilibrium, surfacing, lethargy, lying on the bottom)	Death defined as the lack of movement by the exposed organism (i.e., absence of gill movement and reaction to gentle prodding).
Observation intervals	0, 24, 48, 72 and 96 hours	<i>Observation intervals should be a minimum of every 24 hours.</i>
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Cumulative mortality after 96 hours was 0% in the negative control, and mean-measured 2.3 and 4.0 mg a.i./L treatment groups. Cumulative mortality was 90% in the 6.9 mg a.i./L, and 100% in the 10 and 18 mg a.i./L treatment groups. The 96-hour LC₅₀ and the NOAEC for mortality were 5.6 and 4.0 mg a.i./L.

EPA requires that control or solvent mortality not exceed 10%. OECD requires that maximum-allowable control or solvent control mortality is 10% (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing.

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Table 3: Effect of Dimethyl Disulfide on Mortality of Sheepshead Minnow (*Cyprinodon variegates*).

Treatment (mg a.i./L) Mean measured and (nominal)	No. of fish at start of study	Observation period					
		24 Hours		72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control (dilution water only), if used	20	0	0	0	0	0	0
2.3 (2.6)	20	0	0	0	0	0	0
4.0 (4.3)	20	0	0	0	0	0	0
6.9 (7.2)	20	0	0	6	30	18	90
10 (12)	20	0	0	20	100	20	100
18 (20)	20	20	100	20	100	20	100
NOAEC	<2.3 mg a.i./L						
LC ₅₀	5.6 mg a.i./L						
Positive control, if used mortality: LC ₅₀ :	N/A						

N/A- Not Applicable

B. NON-LETHAL TOXICITY ENDPOINTS:

There were no sub-lethal effects observed in the control treatment group. Sub-lethal effects were noted in all subsequent treatment levels. Loss of equilibrium was observed in the 2.3 mg a.i./L treatment group at 72 hours. Loss of equilibrium was observed in the 4.0 mg a.i./L treatment group at 1.5, 24, 48, 72 and 96 hours. Surfacing and lying on the bottom was also observed in the 4.0 mg a.i./L treatment group at 72 hours. Surfacing and lethargy was additionally noted in the 4.0 mg a.i./L treatment group at 96 hours. Loss of equilibrium was observed in the 6.9 mg a.i./L treatment group at 48 and 72 hours. Lying on the bottom was also observed in the 6.9 mg a.i./L treatment group at 72 hours. Lethargy was additionally observed in the 6.9 mg a.i./L treatment group at 96 hours. Surfacing was observed in the 10 mg a.i./L treatment group at 24 hours. Lying on the bottom was noted in the 10 mg a.i./L treatment group at 48 hours.

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Table 4: Sub-lethal Effect of Dimethyl Disulfide on Sheepshead Minnow (*Cyprinodon variegates*)

Treatment (mg a.i./L) measured and (nominal) concentrations	Observation period		
	24 Hours	72 Hours	96 Hours
	% affected	% affected	% affected
Control (dilution water only), if used	0	0	0
2.3 (2.6)	0	20% loss of equilibrium	0
4.0 (4.3)	0	5% surfacing 5% loss of equilibrium 32% lying on the bottom	26% surfacing 37% lethargy
6.9 (7.2)	0	14% loss of equilibrium 86% lying on the bottom	100% lethargy
10 (12)	0	--	0
18 (20)	10% surfacing	--	0
NOAEC	<2.3 mg a.i./L		
LOAEC	2.3 mg a.i./L		
EC ₅₀	Not reported		
Positive control, if used % sublethal effect: EC ₅₀ :	N/A		

C. REPORTED STATISTICS:

The mortality data were analyzed using the computer program of C.E. Stephan. The program was designed to calculate the LC₅₀ value and the 95% confidence interval by probit analysis, the moving average method and binomial probability with nonlinear interpolation. The binomial probability method was used in this study to calculate the 24, 48, 72 and 96-hour LC₅₀ values. The no-mortality concentration and NOEC were determined by visual interpretation of the mortality and observation data.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer used the binomial probability method to calculate the LC₅₀ using Toxanal statistical software. The probit and moving average methods were not used because there were less than two concentrations with mortality between 0 and 100 percent. The NOAEC was determined visually, based on sublethal effects. The reviewer also determined the EC₅₀ using sublethal effects data.

LC₅₀: 5.6 mg a.i./L 95% C.I.: 4.0-6.9 mg a.i./L

NOAEC: <2.3 mg a.i./L Probit Slope: N/A

EC₅₀: 3.7 (2.3-6.9) mg a.i./L

Most sensitive endpoint(s) affected: Signs of toxicity (lethargy, loss of equilibrium, lying on the bottom and surfacing)

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E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's.

The results from the periodic screening analysis of the dilution water indicated that the following elements exceeded the maximum allowable concentration (0.001 mg/L): aluminum (<0.200 mg/L), iron (<0.200 mg/L), zinc (<0.0200 mg/L), arsenic (<0.0200 mg/L), chromium (<0.0150 mg/L), cobalt (<0.0050 mg/L), copper (<0.0100 mg/L), lead (<0.0150 mg/L) and nickel (<0.0100 mg/L).

G. CONCLUSIONS:

This study is classified as acceptable and does fulfill the requirements for an acute estuarine/marine fish toxicity study. The 96-hour LC₅₀ was 5.6 mg a.i./L. The NOAEC was <2.3 mg a.i./L. Based on the results of this study, Dimethyl Disulfide would be classified as moderately toxic to Sheepshead minnow (*Cyprinodon variegates*) on an acute toxicity basis.

LC₅₀: 5.6 mg a.i./L 95% C.I.: 4.0-6.9 mg a.i./L

NOAEC: <2.3 mg a.i./L Probit Slope: N/A

EC₅₀: 3.7 (2.3-6.9) mg a.i./L

Most sensitive endpoint(s) affected: Signs of toxicity (lethargy, loss of equilibrium, lying on the bottom and surfacing)

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine*.

U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure: *Acute Toxicity Test for Estuarine and Marine Organisms (Estuarine Fish 96-Hour Acute Toxicity Test)*. Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-006. Washington, D.C.

ASTM Standard E729-96. 1996. *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians*. American Society for Testing and Materials.

Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

Finney, D.J. 1971. *Statistical Methods in Biological Assay*. Second edition. Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews*. Vol. II, No. 2. Pp. 115-145.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
18	20	20	100	9.536742E-05
10	20	20	100	9.536742E-05
6.9	20	18	90	2.012253E-02
4	20	0	0	9.536742E-05
2.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 4 AND 6.9 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 5.573415

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.

Calculation of EC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
6.9	20	20	100	9.536742E-05
4	19	12	63.15789	17.96417
2.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.3 AND 6.9 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 3.665595

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.