8EHQ-0594-12995

CYTEC

ORIGINAL **Contains No CBI**

CYTEC INDUSTRIES INC.
Five Garret Mountain Plaza
West Paterson, NJ 07424
Tel. (201) 357-3100

B

8EHQ-94-12995
PDCN: 88940000237

94 MAY 13 AM 11:22
RECEIVED
OPPT CBIC

May 9, 1994

OPPT Document Processing Center (TS-790)
Office of Pollution Prevention and Toxics (OPPT)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Attention: **SECTION 8(E) COORDINATOR**

Dear Sir/Madam:

As a follow-up to our previous 8(e) submission on a commercial anionic mixture on 04/13/94 (No 8(e) Number Has Been Assigned), I am enclosing a copy of the study entitled "Static Acute Toxicity of CT-546-94 to *Daphnia mahna*". This report was received by us on May 9,1994. This document does not contain confidential business information.

If you have any questions please contact me at (201) 357-3375.

Sincerely,

Patricia Ann Vernon
Associate Toxicologist
Toxicology & Product Stewardship Dept.

8EHQ-94-12995
SP001 05/20/94

89940000205

RECEIVED

RECEIVED
6-16-94

74pp.

7200 E. ABC Lane, Columbia, MO 65202

Tel: 314/474-8579 Fax: 314/443-9033

"Working for You"

SPONSOR

Cytec Industries
5 Garret Mountain Plaza
West Paterson, New Jersey 07424

STUDY TITLE

Static Acute Toxicity of CT-546-94 to *Daphnia magna*

DATA REQUIREMENT

U.S. EPA-TSCA, 40 CFR, Part 797
Guideline 797.1300 and
OECD Guideline No. 202 (Acute Immobilization)

AUTHORS

Marc C. Sword
Biologist II

Warren Railton
Laboratory Technician II

STUDY SUBMITTED ON

May 4, 1994

PERFORMING LABORATORY

ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

ABC LABORATORIES' PROJECT ID

Final Report #41494

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC Laboratories' Final Report #41494, entitled " Static Acute Toxicity of CT-546-94 to *Daphnia magna*," for Cytec Industries, West Paterson, New Jersey.

ABC's study director for the above test herein confirms that the study was conducted in compliance with the U.S. EPA Good Laboratory Practice Standards (1); Toxic Substances Control Act (40 CFR, Part 792), and the Principles of Good Laboratory Practice Standards (2); OECD Guidelines for Testing of Chemicals, Annex 2.

No analytical confirmation of test concentrations was done nor was the stability or purity of the test material under test conditions investigated. This was the responsibility of the study sponsor. The sponsor was also responsible for retaining samples of the test substance.

This statement of compliance does not extend to ancillary analyses performed by the following laboratory: fish food contaminant screen conducted by Lancaster Laboratories, Lancaster, Pennsylvania. Results of these analyses are kept on file at ABC Laboratories.

All data in support of this study, original and certified exact copies, were provided to Cytec Industries with the final report. A copy of the report was retained at ABC Laboratories, Inc.

Marc Sword 5/4/94

Marc Sword Date
ABC Laboratories' Study Director

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed Study #41494, "Static Acute Toxicity of CT-546-94 to *Daphnia magna*," for Cytec Industries, West Paterson, New Jersey.

The following inspections/audits were conducted on this study.

Date of Inspection	Phase Inspected	Date Reported to Study Director	Date Reported to Management
03-09-94	48 Hour Biological Observations	03-09-94	04-26-94
03-21-94	Draft Report & Raw Data	03-21-94	03-23-94
05-04-94	Final Report	05-04-94	05-04-94

The undersigned conducted the report audits. These audits indicated that the report submitted was an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.

L. Melinda Anielewski-Beeler 5/4/1994

L. Melinda Anielewski-Beeler Date
Quality Assurance Officer II

SIGNATURE PAGE

Submitted by:

ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
P.O. Box 1097
Columbia, Missouri 65205
(314) 474-8579

Prepared by:

Marc Sword 5/4/94
Marc Sword Date
Biologist II/Study Director

Warren Railton (WAM) 5-4-94
Warren Railton Date
Laboratory Technician II

Approved by:

William A. McAllister 5-4-94
William A. McAllister Date
Manager, Environmental Toxicology

Jon E. Rhodes 5-4-94
Jon E. Rhodes Date
Team Leader, Environmental Toxicology

L. Melinda Anielewski Beeler 050494
L. Melinda Anielewski Beeler Date
Quality Assurance Officer II

:dsh

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INVERTEBRATE ACUTE TOXICITY COMPENDIUM

Subject: "Static Acute Toxicity of CT-546-94 to *Daphnia magna*," ABC Laboratories' Final Report #41494

Sponsor: Cytec Industries

Test Facility: ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
Columbia, Missouri 65205
(314) 474-8579

Location of Original Raw Data and Final Report:

Cytec Industries
West Paterson, New Jersey

Test Material: CT-546-94

Nominal Test Concentrations: Control, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/L

Dilution Water: 158 mg/L as $CaCO_3$ (hardness),
160 mg/L as $CaCO_3$ (total alkalinity),
8.3 (pH),
320 μMhos/cm (conductivity)

Experimental Test Dates: Start – March 7, 1994
Termination – March 9, 1994

Length of Study: 48 Hours

Results: 48-Hour EC_{50} = 0.15 mg/L (95% confidence interval = 0.10 to 0.18 mg/L)

48-Hour NOEC = 0.10 mg/L

Slope of 48-Hour Dose-Response Line = 9.24

Test Species: *Daphnia magna*

Source of Organisms: ABC Laboratories' In-house Culture

Age of Organisms at Study Initiation: Neonates (<24 hours old)

METHODS AND MATERIALS

Cytec Industries contracted the Environmental Toxicology division of ABC Laboratories, Inc., to conduct a five concentration and control static toxicity test to determine the toxicity of CT-546-94 to *Daphnia magna*. Range-finding tests were conducted from February 23, 1994 to March 3, 1994, to determine the concentration range for the definitive toxicity test. The primary objective of this study was to estimate the acute toxicity of CT-546-94 to *Daphnia magna* by determining the EC_{50}, if possible, and examining abnormal/sublethal responses during the course of the test. This was accomplished by exposing 20 *Daphnia magna* to each concentration and dilution water control. *Daphnia magna* are used for this testing because they are representative of chemically sensitive freshwater invertebrates. Each chamber was monitored for immobility and sublethal/behavioral responses every 24 hours over a 48-hour exposure duration.

The static *Daphnia magna* toxicity test was conducted following the procedures outlined in ABC Protocol No. AMCY 7806 as approved by a representative of Cytec Industries on February 13, 1994, and by ABC Laboratories' study director on February 21, 1994. The definitive study was conducted from March 7, 1994, to March 9, 1994.

I. Test Material

A. Compound Receipt

The CT-546-94 test material (lot #10138134) was received from Cytec Industries on February 9, 1994 and was assigned ABC reference #TS-7090. The sample was a viscous, milky liquid and was stored at room temperature. Sample purity was not specified and all test concentrations were prepared based on total product. This sample was used to prepare all preliminary and definitive test concentrations.

B. Preparation of Test Solutions

The definitive test concentrations were obtained by transferring appropriate aliquots of a primary standard of the substance to 1000 mL volumetric flasks to achieve the final test concentrations. Then 200 mL aliquots were transferred into the 250 mL test chambers. The primary standard was prepared by adding 3.000 g of test substance to 297 mL of hard blended water and stirring with a stainless steel paddle for approximately 20 minutes. The concentration of the standard was 10 mg/mL.

The preliminary concentrations were prepared as above or by adding direct aliquots of the 10 mg/mL standard to the test chambers, or by making a serial dilution of the 10 mg/mL standard.

II. Test Organisms

Daphnia magna were obtained from an in-house daphnid culture that ABC Laboratories has maintained since 1977. The primary culture was obtained from the Columbia National Fisheries Research Laboratory (CNFRL), Columbia, Missouri, in 1977. A trace of the daphnid strain indicated that CNFRL acquired their culture from the U.S. Fish & Wildlife Service Fish Control Laboratory, LaCrosse, Wisconsin, in 1960 and they obtained their culture from Pennsylvania State University in 1954 (3).

All daphnids were cultured and tested in a temperature-controlled area at 20 (±2)°C. The lighting was maintained at 58 to 65 footcandles (629 to 695 lux) on a 16-hour daylight photoperiod. Light reading measurements are kept on file at ABC Laboratories. The daphnid cultures were maintained in 1.5-L glass containers and were cultured in hard blended water. Hard blended water is a combination of well water and reverse osmosis water blended to a hardness of 130 to 160 mg/L as $CaCO_3$ (Table I). The water was filtered with a sediment filter prior to use. The hard blended water was biologically aged before use. The total organic carbon content of this water has been less than 2.0 mg/L when measured. During the holding period, the daphnids were fed a suspension of at least one algae species cultured at ABC: *Selenastrum capricornutum* and/or *Ankistrodesmus falcatus* at least every 3 days. Along with the algae, the daphnids were fed a supplement consisting of fish food (Zeigler Bros., Inc., Gardners, PA) and Fleischmann's active dry yeast. Results of the analysis of the fish food are kept on file at ABC Laboratories.

First-instar daphnids (<24 hours old) were selected for the test. To provide test daphnids that were <24 hours old, the adult daphnids were isolated by transferring to a fresh culture water/food suspension the previous day.

All test daphnids were provided by ABC culture #94-L_2 on March 7, 1994. The adults from this culture had been cultured for 18 days and were considered acceptable with no signs of stress, disease, or physical damage. Since the culturing and testing parameters of temperature, dilution water, and lighting were the same, no acclimation period was necessary. Test daphnids were not fed during the test.

III. Test Method

A. Test Vessel and Test Apparatus

The static *Daphnia magna* toxicity test was conducted in 250-mL glass beakers that had a 200 mL test volume. The dimensions of the beakers were mean depth 8.8 cm and mean diameter 6.3 cm. The mean depth and mean surface diameter for the test solution in the test vessels were each 6.3 cm. All test vessels were covered with loose-fitting petri dish covers to minimize evaporation and prevent

contamination during the study. These vessels were kept at 20 (± 2)°C in a temperature-controlled waterbath. The lighting was maintained on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods. The light intensity over the test area was 53 footcandles (565.7 lux).

B. Test Design

1. Biological

The first range-finding experiment was conducted from February 23. 1994, to February 25, 1994, at the nominal concentrations of 0.10, 1.0. 10 and 100 mg/L with a concurrent control chamber. Five Daphnia magna were added to each test chamber. The 10 and 100 mg/L test solutions were observed to be cloudy, while the control, 0.10 and 1.0 mg/L test solutions were clear at 0 hour.

100% immobility was observed in the 0.10, 1.0 and 10 mg/L chambers at 48 hours. The control and 100 mg/L chambers had no immobility although all daphnids in the 100 mg/L chamber showed the abnormal effects of tending to the bottom of the test chambers and quiescence. The control daphnids were all normal.

Because of the inconsistent effects between the 100 mg/L chamber and the other chambers, a second range-finding test was conducted at the nominal concentrations of 0.0010, 0.010, 0.10, 1.0, 10, 100 and 1000 mg/L. Total immobility was observed in the 100 and 1000 mg/L solutions at 48 hours. There were 4 and 3 immobilities in the 1.0 and 10 mg/L test chambers, respectively. There were no immobilities or abnormal effects observed in the control or the 0.0010, 0.010, and 0.10 mg/L test concentration during the test. The control and 0.0010, 0.010, 0.10, 1.0 and 10 mg/L test solutions were observed to be clear throughout the preliminary test. However, the 100 mg/L was observed to be cloudy and the 1000 mg/L test chamber was very cloudy throughout the test period.

The five definitive test concentrations were selected after consultation with the study sponsor. The duplicate definitive concentrations ranged in a logarithmic from 0.10 to 1.0 mg/L. Duplicate hard blended water controls were also included. Ten *Daphnia magna* (first instar less than 24 hours old) per beaker were used for the definitive bioassay. Daphnids were impartially added to test beakers within 30 minutes of test solution preparation.

All concentrations were observed at 0, 24, and 48 hours for immobility and other abnormal effects such as quiescence, and tending to the bottom of the test chambers.

2. Chemical and Physical

Water chemistry parameters of temperature, dissolved oxygen, and pH were performed in both replicates of all test concentrations at 0 hour before the addition of organisms and at 24 and 48 hours. Temperature was monitored continuously with a datalogger and thermistor probe.

IV. Biological Data Analysis

Statistical analysis of the concentration vs. effect data (immobility) was performed using a computerized EC_{50} program developed by Stephan et al. (4). This program is designed to calculate the EC_{50} statistic and its 95% confidence limits using the binomial, moving average, and probit tests. Three different methods of analyzing the data are used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the EC_{50} (4, 5), although all three models are valid. However, if no immobility occurred or if a dose response could not be demonstrated over a reasonable range (<37 to >63%), the EC_{50} and/or its 95% confidence limits could not be calculated. The 48-hour dose-response slope was calculated by least-squares regression analysis of percent immobility transformed to probits versus log concentration.

RESULTS AND DISCUSSION

The 48-hour static *Daphnia magna* test with CT-546-94 was completed on March 9, 1994. An examination of the culture records for this test indicated that the *Daphnia magna* were in good condition for testing.

Table II presents the water quality parameters measured during the definitive test. Temperature was 19°C, 20°C and 19°C when measured at 0, 24 and 48-hours, respectively. The pH values ranged from 8.2 to 8.4. Dissolved oxygen concentrations ranged from 7.6 to 8.5 mg/L during the test. These values represented 87 to 96% saturation at 20 and 19°C, respectively. The dissolved oxygen was considered adequate for testing (6); therefore, the test chambers were not aerated during the test. Temperature measured continuously in the waterbath during the test was within the protocol specified temperature range. All controls and test solutions were clear with no visible surface film or precipitate throughout the study.

Table III presents the individual immobility and behavioral observations for daphnids exposed to CT-546-94. No immobility or abnormal effects were observed in the control or 0.10 mg/L test concentrations during the study. The abnormal effects of immobility, quiescence, and/or

daphnids on bottom of the test chambers were observed in the 0.18, 0.32, 0.56, and 1.0 mg/L test concentrations during the test. The 0.18, 0.32, 0.56, and 1.0 mg/L concentrations elicited 80, 100, 95, and 100% immobility, respectively at 48 hours.

All results were based on the nominal concentrations of 0.10, 0.18, 0.32, 0.56, and 1.0 mg/L. The 48-hour no-observed effect concentration was 0.10 mg/L, the lowest concentration tested. The 24- and 48-hour EC_{50} values were > 1.0 and 0.15 mg/L, respectively (Table IV). The slope of the 48-hour dose-response line was 9.24 as calculated by least squares regression analysis (Figure 1).

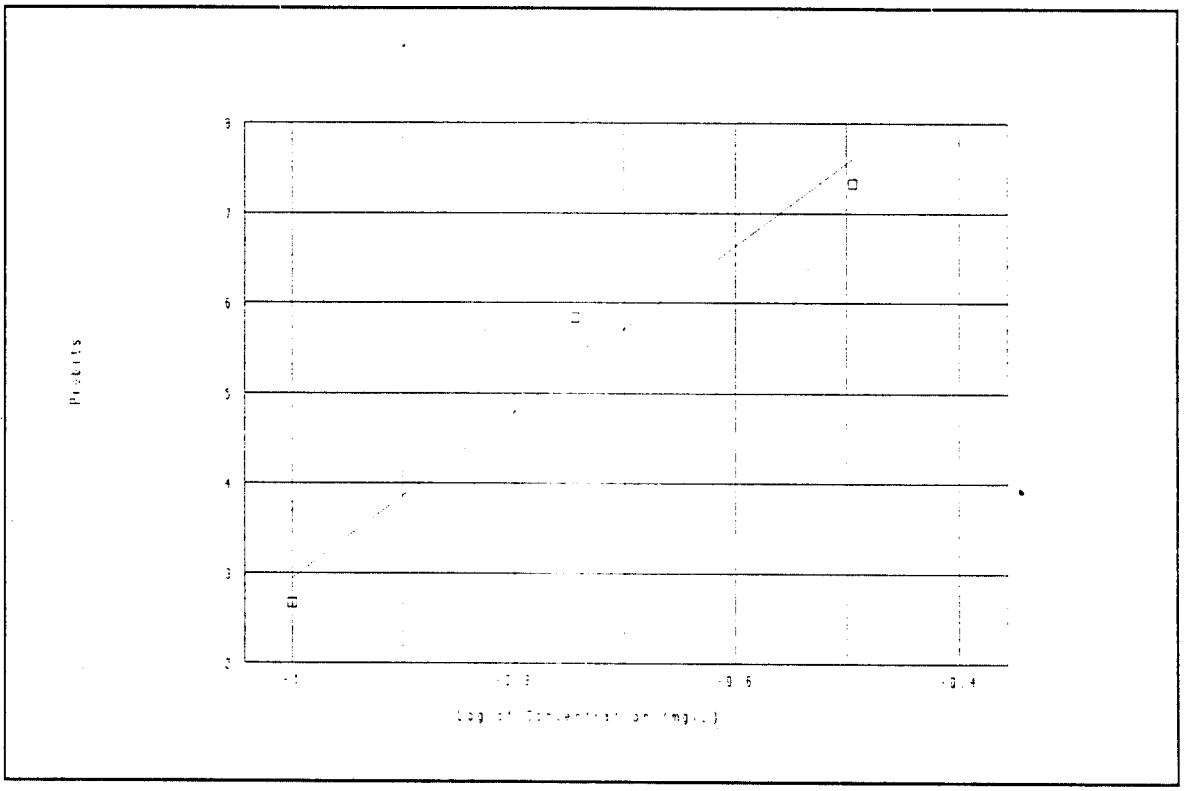


Figure 1: The 48-Hour Dose-Response Line for *Daphnia Magna* Exposed to CT-564-94

The study was conducted following the Good Laboratory Practice regulations (1, 2) and the final report was reviewed by ABC Laboratories' Quality Assurance Unit. All data in support of this study, original and certified exact copies, were provided to Cytec Industries with the final report. A copy of the report was retained at ABC Laboratories, Inc.

TABLE I

Chemical Characteristics of Hard Blended Water Used by
ABC Laboratories' Environmental Toxicology Division

Dilution Water Screen[b]		Monthly Screens	
Hardness	158 mg/L (as $CaCO_3$)	Total Organic Carbon	< 1.0 mg/L
Alkalinity	160 mg/L (as $CaCO_3$)	Suspended Solids	0.8-1.4 mg/L
pH	8.3		
Conductivity	320 μMhos/cm		

Quarterly Screens[c]

Elements			Chlorinated Hydrocarbons	
Aluminum	<0.20	mg/L	Aldrin	<0.010 μg/L
Arsenic	<0.010	μg/L	Chlordane	<0.30 μg/L
Boron	0.250	mg/L	DDE	<0.010 μg/L
Cadmium	<0.00050	mg/L	DDD	<0.010 μg/L
Chromium	<0.0010	mg/L	DDT	<0.010 μg/L
Cobalt	<0.050	mg/L	Dieldrin	<0.010 μg/L
Copper	<0.020	mg/L	α-BHC	<0.010 μg/L
Fluoride	0.90	mg/L	β-BHC	<0.010 μg/L
Iron	0.10	mg/L	γ-BHC	<0.010 μg/L
Lead	<0.0030	μg/L	Δ-BHC	<0.010 μg/L
Mercury	<0.00032	μg/L	Endrin	<0.010 μg/L
Nickel	<0.050	mg/L	Heptachlor	<0.010 μg/L
Selenium	<0.0050	μg/L	H.E.	<0.010 μg/L
Silver	<0.00050	μg/L	Methoxychlor	<0.050 μg/L
Zinc	<0.040	mg/L	Toxaphene	<4.0 μg/L

Organophosphate Insecticides		PCB	
Diazinon	<0.10 μg/L	PCB	<1.0 μg/L
Methyl Parathion	<0.020 μg/L		
Ethyl Parathion	<0.020 μg/L		
Ronnel	<0.010 μg/L	Miscellaneous	
Malathion	<0.050 μg/L	Chlorine, Residual	<0.020 mg/L
Endosulfan I	<0.010 μg/L	Un-ionized Ammonia	<0.10 mg/L
Endosulfan II	<0.010 μg/L	COD	10 mg/L
Endosulfan	<0.030 μg/L		
Vapona	<2.0 μg/L		

[a] Hard blended dilution water is a mixture of reverse osmosis and well water to achieve a final hardness (as $CaCO_3$) of ~130-160 mg/L.
[b] Represents the values measured on dilution water the day of the test
[c] Represents the values obtained from the screen of January 1994

Note: Chemical analyses supporting these data are on file at ABC Laboratories.

TABLE II

Water Quality Measurements During the Static Acute Toxicity Test of CT-546-94 to *Daphnia magna*

	Water Quality								
	0 Hour			24 Hours			48 Hours		
Nominal Test Conc. (mg/L)	Temp.[a] °C	DO[b] mg/L	pH[c]	Temp. °C	DO mg/L	pH	Temp. °C	DO mg/L	pH
Control A	19	8.4	8.3	20	7.9	8.2	19	8.1	8.2
Control B	19	8.4	8.3	20	7.9	8.3	19	8.2	8.2
0.10 A	19	8.4	8.3	20	7.9	8.3	19	8.2	8.2
0.10 B	19	8.5	8.3	20	7.9	8.3	19	8.0	8.3
0.18 A	19	8.4	8.3	20	7.9	8.3	19	8.1	8.3
0.18 B	19	8.4	8.3	20	7.9	8.3	19	8.2	8.3
0.32 A	19	8.4	8.3	20	7.9	8.3	19	8.2	8.3
0.32 B	19	8.4	8.3	20	7.9	8.3	19	8.2	8.3
0.56 A	19	8.5	8.3	20	7.8	8.3	19	8.0	8.3
0.56 B	19	8.5	8.3	20	7.8	8.3	19	8.0	8.3
1.0 A	19	8.5	8.3	20	7.6	8.4	19	8.0	8.3
1.0 B	19	8.5	8.3	20	7.6	8.4	19	7.9	8.3

[a] Temperature measured with a mercury thermometer

[b] Dissolved oxygen concentrations – YSI Dissolved Oxygen System Model 54 ARC

[c] pH – Beckman Model Φ34 pH meter with a Beckman Model 39841 probe

NOTE: Dissolved oxygen saturation values corrected for altitude at the test temperatures of 19 and 20°C are 8.9 and 8.7 mg/L, respectively.

TABLE III

Immobility and Behavioral Observations During the Static Acute Toxicity Test of CT-546-94 to *Daphnia magna*

Nominal Test Conc. (mg/L)	Number Placed in Test Vessel	24 Hours		48 Hours	
		Imm.	Observations	Imm.	Observations
Control A	10	0	10 N; CLR	0	10 N; CLR
Control B	10	0	10 N; CLR	0	10 N; CLR
0.10 A	10	0	10 N; CLR	0	10 N; CLR
0.10 B	10	0	10 N; CLR	0	10 N; CLR
0.18 A	10	0	10 N; CLR	6	2 OB,Q; 2 N; CLR
0.18 B	10	0	10 N; CLR	10	---
0.32 A	10	0	10 N; CLR	10	---
0.32 B	10	0	10 N; CLR	10	---
0.56 A	10	1	9 N; CLR	9	1 OB, Q; CLR
0.56 B	10	0	10 N; CLR	10	---
1.0 A	10	0	10 OB, Q; CLR	10	---
1.0 B	10	0	10 OB, Q; CLR	10	---

Key to Abbreviations: Imm. = Immobility; N = Normal; OB = On Bottom of the Test Chamber; Q = Quiescent; CLR = Clear Solution

TABLE IV

Static Acute Toxicity of CT-546-94 to *Daphnia magna*

	EC_{50} in mg/L	
Compound	24-Hour	48-Hour
CT-546-94[a]	>1.0[b]	0.15[c] (0.10 and 0.18)[d]

N = 20 daphnids per concentration

[a] Bioassay conducted at 20 (±2)°C

[b] Insufficient immobility for the calculation of an EC_{50}

[c] The EC_{50} was calculated using the binomial method.

[d] 95% confidence limits

The 48-hour no-observed effect concentration was 0.10 mg/L, the lowest concentration tested.

REFERENCES

(1) U.S. Environmental Protection Agency. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792).

(2) Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2. C(31) 30(Final):7-28.

(3) Personal Communication, Herman O. Sanders, 1979, U.S. Department of the Interior.

(4) Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W. Andrews. 1978. A Computer Program for Calculating an LC_{50}. U.S. Environmental Protection Agency. Duluth, Minnesota, pre-publication manuscript, August 1978.

(5) Stephan, C.E. 1977. Methods for Calculating an LC_{50}, p. 65-84. In F.L. Mayer and J.L. Hamelink (Eds.). *Aquatic Toxicology and Hazard Evaluation.* ASTM Special Technical Publication 634. ASTM. Philadelphia.

(6) Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians.* Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009.

RAW DATA APPENDICES

ABC Laboratories Study #41494

Static Acute Toxicity of CT-546-94 to *Daphnia magna*

NOTE: Some of the records that appear in these raw data appendices have been provided as photocopies of original records on file at ABC Laboratories. This has been done by necessity for certain data that are used commonly in several studies at ABC Laboratories. Such records include organism culture records and hard blended water chemical screen results.

The following abbreviations may have been used in the raw data:

C – Calculation error

R – Recording error

E – Entry error

F – Form change

S – Spelling error

T – Transcription error

W – Writeover

D – Date error

PROJECT PERSONNEL

This is a listing of the personnel who participated in various phases of this study. For official signatures of these individuals, please refer to the following pages.

Marc Sword
Biologist II/Study Director

Warren Railton
Laboratory Technician II

John Rhodes
Team Leader, Environmental Toxicology

"This is an exact copy of
The original document"
By Warren Railton date 3-11-94

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

NAME	SIGNATURE	INITIAL	DATE
William J. Adams	William J. Adams	WJA	2/9/94
William A. McAllister	William A. McAllister	WAM	2-4-94
Alan D. Forbis	Alan D. Forbis	AF	2-4-94
Jon E. Rhodes	Jon E. Rhodes	JR	2-4-94
James B. Bussard	James B. Bussard	JBB	2-9-94
Tom Leak	Tom Leak	TL	2-4-94
Charles E. Jameson	Charles E. Jameson	CEJ	2-4-94
Timothy J. Madsen	Timothy J. Madsen	TJM	02-04-94.
Stephen L. Hicks	Stephen L. Hicks	SLH	2-7-94
Scott J. Voney	Scott J. Voney	SJV	2-7-94
Paul Cohle	Paul Cohle	PC	2-4-94
Doug Gledhill	Doug Gledhill	DG	2-7-94
Hugh Murrell	Hugh Murrell	HM	2-4-94
Ryan Warbritton	Ryan Warbritton	RW	2-4-94
Amy Adams	Amy Adams	AA	2-8-94
Robert Pezold	Robert G. Pezold	RGP	2-4-94
Marc C. Sword	Marc Sword	MCS	2/4/94
Tammy Strawn	Tammy Strawn	TS	2-7-94
Dorothy C. England	Dorothy C. England	DCE	2/7/94
Warren Railton	Warren Railton	WR	2-4-94
Jane H. Bowman	Jane Bowman	JHB	2-4-94
Janelle L. Downing	Janelle L. Downing	JLD	2-4-94

Note: This list includes all personnel of the Environmental Toxicology division and is not study specific. Primary project personnel will be identified separately for each project.

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

NAME	SIGNATURE	INITIAL	DATE
Laurie L. Roesel	Laurie L Roesel	LLR	2-7-94
Michelle A. Muckerman	Michelle A. Muckerman	mm	2/7/94
Jamie L. Veltri	Jamie L. Veltri	JLV	2/7/94
John Bucksath	John Bucksath	JDB	2/7/94
Kathryn Konering	Kathryn Konering	KK	2-8-94
Bret Hurshman	Bret A Hurshman	BAH	2-8-94
Luke Stuerman	Luke Stuerman	LMS	2/8/94
Gerald A. Nothdurft	Gerald A Nothdurft	GAN	2-7-1994
Yuan Yang	Yuan Yang	YY	2-7-94
Jianping Liu	Jianping Liu	JL	2/8/94
Karen March	Karen L March	KLM	2-8-94
David Burgess	David Burgess	DB	2-8-94
Debbie Jameson	Debbie Jameson	dlj	2-7-94
Anita M. Klick	Anita M Klick	AMK	2-9-94
Donna S. Hoek	Donna S Hoek	DSH	2/7/94
John Ingersoll	[illegible]	JI	2.8.94
Edward Harper	Edward Harper	EDH	2-8-94

Note: This list includes all personnel of the Environmental Toxicology division and is not study specific. Primary project personnel will be identified separately for each project.

ABC LABORATORIES, INC.

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION

☒Definitive ☐Preliminary ☐Screen ☐Flow-Through ☒Static

Test Material: CT-5466-94 Protocol No.: AMCY 7506

Study Director: Marc Sword Study #: 411494

Test Species: [X] *Daphnia magna* [ ] _____ (Lot # 94-L2) Waterbath #: 6
Dilution Water: [X] Hard Blended [ ] _____ # Instar/Vessel: 10
Date Initiated: 3-7-94 Time: 3:15P.M. By: W. Rossiter Date Terminated: 3-9-94

Test Conc. (mg/L)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
Control A	0	10N ~~5NO~~ CLR	0	10N CLR	0	10N CLR
Control B	0	10N CLR	0	10N CLR	0	10N CLR
0.10 A	0	10N CLR	0	10N CLR	0	10N CLR
0.10 B	0	10N CLR	0	10N CLR	0	10N CLR
0.18 A	0	10N CLR	0	10N CLR	6	2OB.C 2N CLR
0.18 B	0	10N CLR	0	10N CLR	10	— CLR
0.32 A	0	10N CLR	0	10N CLR	10	— CLR
0.32 B	0	10N CLR	0	10N CLR	10	— CLR
0.56 A	0	10N CLR	1	9N CLR	9	10B.C CLR
0.56 B	0	10N CLR	0	10N CLR	10	— CLR
1.0 A	0	10N CLR	0	10OB,C CLR	10	— CLR
1.0 B	0	10N CLR	0	10OB,C CLR	10	— CLR
ID/Date/Time	WR/3-7-94/3:15P.M.		WR/3-8-94/3:55P.M.		WR/3-9-94/3:00P.M.	
Anal. ID Date/Time	—		—		—	

Comments: N = Normal, SUR = Surfacing, C = Clumping, CT = Coated with Extraneous Material, ERR = Erratic Movement, TR = Trailing Extraneous Material, Q = Quiescent, OB = On Bottom, P = Precipitate, CLR = Clear, CLD = Cloudy/Colored Solution, SF = Surface Film
Note: Data loggers started recording at 3:15 P.M. on 3-7-94 WR 3-7-94
Light measurement over the test chambers at 0 hour was 5-3 ~~LUX~~ FC WR 3-7-94
OE WR 3-7-94 Li-Cor, model LI-189 meter #16 3-341 with 93-92 sensor used to measure light intensities

Reviewed by: Marc Sword Date: 3/10/94

Study Director: Marc Sword Date: 3/10/94

FORM AQ106 (3/25/93)

ABC LABORATORIES, INC.

WATER QUALITY

Test Material: CT-546-94 Protocol No.: AMY 7806

Study Director: Marc Sward Study #: 414/94

Concentration (mg/L)	3-7-94 Day 0 WR						3-8-94 Day 1 WR						3-9-94 Day 2 WR					
	Temp[a] °C	DO[b] mg/L	pH[c]	Hard.[d]	Total Alk.[d]	[e]	Temp[a] °C	DO[b] mg/L	pH[c]	Hard.[d]	Total Alk.[d]	[e]	Temp[a] °C	DO[b] mg/L	pH[c]	Hard.[d]	Total Alk.[d]	[e]
Control A	19	8.4	8.3	—	—	—	20	7.9	8.2	—	—	—	19	8.1	8.2	—	—	—
Control B	19	8.4	8.3	—	—	—	20	7.9	8.3	—	—	—	19	8.2	8.2	—	—	—
0.10 A	19	8.4	8.3	—	—	—	20	7.9	8.3	—	—	—	19	8.2	8.2	—	—	—
0.10 B	19	8.5	8.3	—	—	—	20	7.9	8.3	—	—	—	19	8.0	8.3	—	—	—
0.18A	19	8.4	8.3	—	—	—	20	7.9	8.3	—	—	—	19	8.1	8.3	—	—	—
0.18B	19	8.4	8.3	—	—	—	20	7.9	8.3	—	—	—	19	8.2	8.3	—	—	—
0.32 A	19	8.4	8.3	—	—	—	20	7.9	8.3	—	—	—	19	8.2	8.3	—	—	—
0.32 B	19	8.4	8.3	—	—	—	20	7.9	8.3	—	—	—	19	8.2	8.3	—	—	—
0.56 A	19	8.5	8.3	—	—	—	20	7.8	8.3	—	—	—	19	8.0	8.3	—	—	—
0.56 B	19	8.5	8.3	—	—	—	20	7.8	8.3	—	—	—	19	8.0	8.3	—	—	—
1.0 A	19	8.5	8.3	—	—	—	20	7.6	8.4	—	—	—	19	8.0	8.3	—	—	—
1.0 B	19	8.5	8.3	—	—	—	20	7.6	8.4	—	—	—	19	7.9	8.3	—	—	—

a Temperature measured with a mercury thermometer
b Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
c pH : Beckman Φ34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
d Hardness and total alkalinity (mg/L as $CaCo_3$) analyzed using titrimetric method adopted from Standard Methods
e Other: —

Remarks: —

Prepared by: Duncan Patton Date: 3-7-94

Reviewed by: Marc Sward Date: 3/10/94

FORM # AQ141 (01/11/94)

ABC LABORATORIES, INC.

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hard Blended A

TEMPERATURE: 21.1 °C

DO: 8.4 mg/L

pH: 8.3

ALKALINITY:* 160 mg/L

HARDNESS:* 158 mg/L

CONDUCTIVITY: 320 µmhos/cm

OTHER: _____

INSTRUMENTS:

TEMPERATURE ☒ Mercury ☐ Digital

METER TYPE		BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	☒	YSI	54ARC	1905-730
	☐			
pH Meter:	☒	BECKMAN	Φ34	163-640
Probe:		BECKMAN	39841	------
	☐			
CONDUCTIVITY:	☒	YSI	33	1905-520
	☐			

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as $CaCO_3$)

"This is an exact copy of The original document"

By Lisa Sword date 3/8/94

Analysis by: _____ Date: 3.7.94

Reviewed by: Lisa Sword Date: 3/8/94

FORM # AQ-17 (9/15/93)

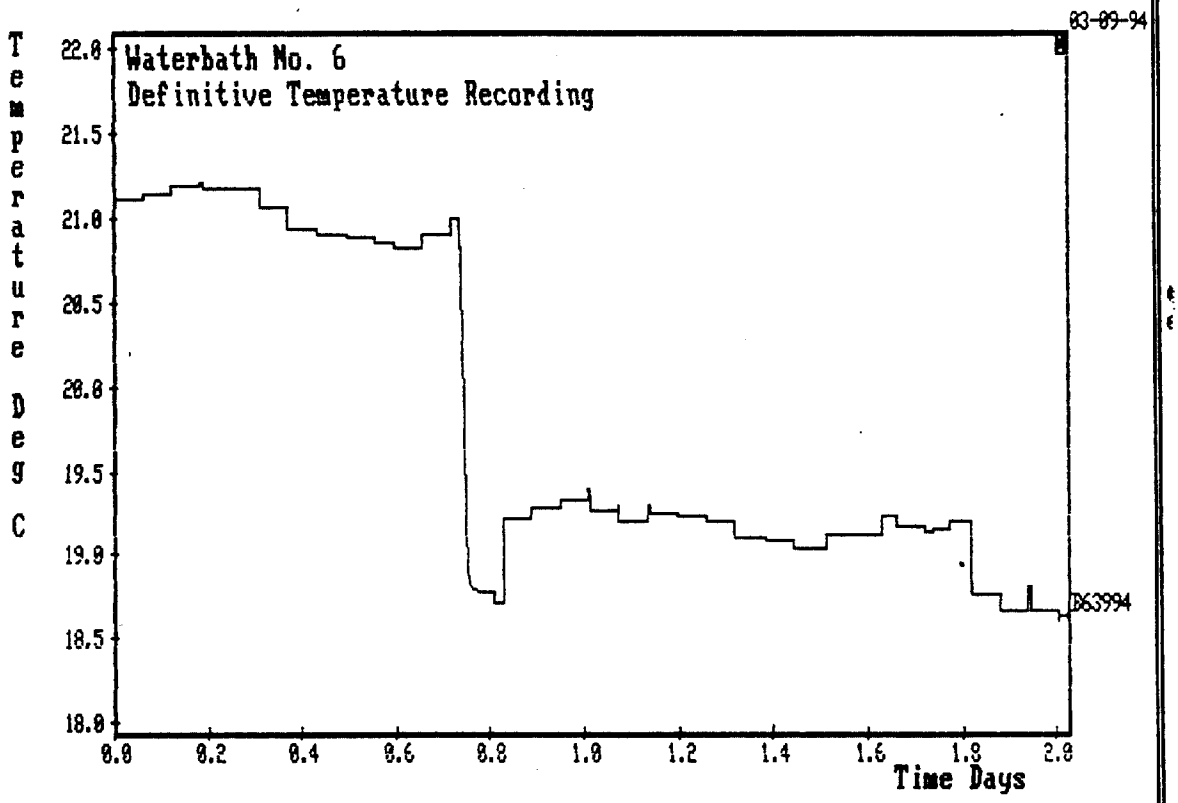
ABC LABORATORIES, INC.

CONTINUOUS TEMPERATURE GRAPH

Test Material: CT-546-94 Protocol No.: AMCY 7506

Study Director: Marc Sword Study #: 41494

Data Logger Material Cont. No.: 163 -830



Comments:

Prepared by: William Rankin Date: 3-9-94

Study Director: Marc Sword Date: 3/11/94

Form AQ9 (1/7/92) (FDIII)

ABC LABORATORIES, INC.

COMPOUND PREPARATIONS: ☐ Preliminary ☒ Definitive

Test Material: CT-546-94 Protocol No.: AMCY 7800

Study Director: Mark Sigward Study #.: 41494

Purity % N/A ABC Ref #: TS-7090 Batch/Lot #: ① ~~N/A~~ 10135134

Prep. of Primary Standard/Weighed by: Warren Rattler Date: 3-7-94

Nominal Target Weight 3.000 g Dil. Vol. 300 mL of Hard Blended H_2O *

Actual Gross Weight 3.000 g Concentration 10 mg/mL

Tare Weight 0.000 g

Net Weight 3.000 g Balance checked with Class S weights:

Adjusted Net Weight 2.000 g + 0.000 g = 2.001 g

Corrected for Purity N/A g (Class S) (tare) (final wt)

Prep. of Working Standard/Transferred by: Warren Rattler Date: 3-7-94

Conc. of Parent Sol.	Aliq. Vol. (mL)	Dilution Vol. (mL)	Dilution Vehicle*	Final Concentration
10 mg/mL	1.0	1000	Hard Blended H_2O	0.010 mg/mL

Prep. of Test Conc./Transferred by: W. Rattler Time: 3:00 PM Date: 3-7-94

Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Vol. (L)	Final Conc. (mg/L)
0.010	.10	1.0	0.10 A+B
0.010	.13	1.0	0.13 A+B
0.010	.32	1.0	0.32 A+B
0.010	.56	1.0	0.56 A+B
0.010	100	1.0	1.0 A+B

Remarks: Balance: ☐ Sartorius R 300 S: ABC Material #

☒ Other: Mettler PM460 ABC Mat. Cert. # 1905-1220

Each Conc.: Double Replicate 250 mL test vessels w/ 200 mL test vol.

Control(s), Description: Hard Blended H_2O

*Lot # of vehicle used: N/A

① E WR 3-23-94

Reviewed by: Mark Sigward Date: 3/10/94

Study Director: Mark Sigward Date: 3/10/94

FORM # 410 (09/23/92)

ABC LABORATORIES, INC.

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-564-94 Protocol No. AMCY 7806

Study Director: Marc Sword Study #: 41494

3-7-94 WR Stock solution was prepd using the method listed on this page. Solution was made from 2:00p.m. to 2:30 p.m.

OVERHEAD STIRRER METHOD

SEE FIGURE 2

EQUIPMENT

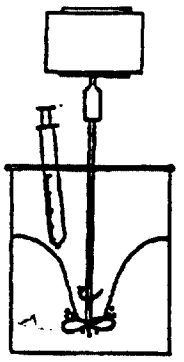
Variable Speed Overhead Mixer, equipped with a 3 blade propeller (1 1/2 - 2 inch diameter)
600 ml Beaker (Do not substitute other size beakers.)
Unused 3 ml Plastic Syringe

PROCEDURE

1. The propeller should be at the 100 ml mark on the beaker.
2. Add 297 gm/ml of water to the beaker.
3. Fill the syringe with 3.0 gm of emulsion. If you cannot weigh the product, then fill the syringe to the 3.0 ml mark.
4. Adjust the RPM of the mixer so that that the fluid vortex reaches down to the propeller on the stir shaft.
5. Add the emulsion to the shoulder of the vortex in one continuous squirt, being careful not to immerse the tip of the syringe.
6. Turn down the speed after 30 seconds to minimize the vortex.
7. Continue mixing for 15-20 minutes.
8. The resulting emulsion solution strength is 1.0%. No further mixing is needed. The solution may be diluted to the desired working concentration.

FIGURE 2

OVERHEAD STIRRER METHOD



NOTE: SYRINGE TIP SHOULD NOT CONTACT WATER.

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword Date: 3/11/94

Study Director: Marc Sword Date: 3/11/94

FORM # 55 (11/25/91) (FDI)

COMPOUND RECEIPT

Lab Form No. 352

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.
P.O. Box 1097 • Columbia, MO 65205
Shipping Address: 7200 East ABC Lane, Columbia, MO 65202
(314) 474-8579 • Answer Back (ABCLAB UD)
FAX (314) 443-9033

Compound CT 546-94

ABC SUPPLIED INFORMATION

Date Received: 02/09/94 (1) Logged In By: RMF ABC Ref # TS-7090

Storage: Room Temp Total Weight: 994.6 g

Physical Description: Viscous milky liquid

Remarks:

SUPPLIER PROVIDED INFORMATION

Firm: Cytec

Address: 1937 West Main Street

City/State/Zip Stamford CT 06902 Phone: 203/321-2200

Batch/Lot No. 10138134 CAS # N/G Purity: N/G

Amount Declared: 500 mL Expiration Date: 7/94

Storage Instructions N/G

Other

(1) (E) 2/9/94 RMF

"This is an exact copy of
The original document"

By Anna Sword date 3/10/94

ABC LABORATORIES, INC.

OBSERVATIONS AND/OR REMARKS FORM

Test Material: Various Protocol No.: Various

Study Director: Various Study #: Various

8-10-93 JMB

Ten Pyrex brand beakers were chosen in an impartial manner from the supply of 250 mL beakers used for daphnid static testing. The depth and diameter of each beaker was measured from the inside of each of the 10 beakers. Depth was measured using a standard cm/in ruler and the diameter was measured using calipers.

Beaker #	Depth cm	Diameter cm
1	8.8	6.3
2	8.8	6.3
3	8.8	6.3
4	8.8	6.2
5	8.7	6.4
6	8.8	6.3
7	8.8	6.3
8	8.8	6.3
9	8.8	6.3
10	8.8	6.2
Mean ± SD	8.8 ± 0.03	6.3 ± 0.06

"This is an exact copy of The original document"

By [signature] date 3/23/94

NOTE: Individual entries must be dated and initialed.

Reviewed by: Jane Bowman Date: 8-10-93

Study Director: [signature] (signed copy) Date: 3/23/94

FORM # 55 (11/25/91) (FDI)

ABC LABORATORIES, INC.

OBSERVATIONS AND/OR REMARKS FORM

Test Material: Various Protocol No. Various

Study Director: Various Study #: Various

2-11-93/KRT

①E KRT 2-11-93

Ten Pyrex brand beakers were chosen in an impartial manner from the supply of 250mL standard static beakers.② Each of the 10 ~~mL~~ ① beakers was filled to the 200mL graduation, which is the standard volume used in daphnia static tests. The depth and width of the volume of water were measured and were as shown below:

Beaker #	Depth	Width
1	6.2cm	6.4cm
2	6.3cm	6.3cm
3	6.1cm	6.4cm
4	6.1cm	6.3cm
5	6.3cm	6.3cm
6	6.3cm	6.4cm
7	6.2cm	6.3cm
8	6.3cm	6.4cm
9	6.3cm	6.3cm
10	6.4cm	6.3cm
$\bar{X}$ (±S.D.)	6.3cm (0.10)	6.3 (±0.05)

② This supply of beakers is ① used for daphnia static testing. KRT 2-11-93

"This is an exact copy of The original document" By [illegible] date 2-11-93

NOTE: Individual entries must be dated and initialed.

Reviewed by: [signature] Date: 2/11/93

Study Director: [signature] Date: 2/11/93 / 2-11-93

FORM # 55 (11/25/91) (FDI)

123LA03P041494

PG 0030

EC_{50} Calculations	
ABC Study No. : 41494 Compound Name : CT-546-94 Species : Daphnia Magna Exposure Period: 24 hours Study Director : Marc Sword	Analytical Bio-Chemistry Labs 7200 East ABC Lane P.O. Box 1097 Columbia, Missouri 65205 03-11-1994 Page # 1

——RESULTS CALCULATED USING THE BINOMIAL METHOD——

CONC. µg/L	NUMBER EXPOSED	NUMBER IMMOBILE	PERCENT IMMOBILE	BINOMIAL PROB. (PERCENT)
1	20	0	0	LESS THAN 0.001
.56	20	1	5	2.002714205001754D-03
.32	20	0	0	LESS THAN 0.001
.18	20	0	0	LESS THAN 0.001
.1	20	0	0	LESS THAN 0.001

AT A CONFIDENCE LEVEL OF 95 PERCENT THE BINOMIAL TEST SHOWS THAT THE EC_{50} IS ABOVE 1 µg/L

THE USEFULLNESS OF ANY EC_{50} CALCULATED FROM THIS DATA IS QUESTIONABLE BECAUSE A CONCENTRATION EFFECT RELATIONSHIP HAS NOT BEEN DEMONSTRATED OVER A REASONABLE RANGE (E.G. (37 TO)63) OF PERCENT IMMOBILE.

NEITHER THE APPROXIMATE EC_{50} CALCULATION NOR THE MOVING AVERAGE METHOD CAN BE USED WITH THIS SET OF DATA. EITHER THE HIGHEST CONCENTRATION IMMOBILIZED LESS THAN 50 PERCENT OR THE LOWEST IMMOBILIZED MORE THAN 50%. IF THE PROBIT SLOPE IS NEGATIVE ENTER THE DATA AGAIN USING NUMBER UNAFFECTED INSTEAD OF NUMBER IMMOBILIZED.

WHEN THERE ARE LESS TWO DIFFERENT CONCENTRATIONS AT WHICH THE PERCENT IMMOBILE IS BETWEEN 0 AND 100, THE PROBIT METHOD CANNOT GIVE ANY STATISTICALLY SOUND RESULTS.

① D WR 3-11-94

Method Reported: [ ] Binomial [ ] Moving Average [ ] Probit

Note: Method selected is that which gives the narrowest confidence limits for EC_{50}.

PREPARED BY: Warren Patton DATE: 3-11-94 ~~3-10-94~~ ②

REVIEWED BY: Marc Sword DATE: 3/11/94

123LASS#041494

PB 0031

EC_{50} Calculations

ABC Study No. : 41494	Analytical Bio-Chemistry Labs
Compound Name : CT-546-94	7200 East ABC Lane
Species : Daphnia Magna	P.O. Box 1097
Exposure Period: 48 hours	Columbia, Missouri 65205
Study Director : Marc Sword	03-11-1994 Page # 1

----RESULTS CALCULATED USING THE BINOMIAL METHOD----

CONC. ug/L	NUMBER EXPOSED	NUMBER IMMOBILE	PERCENT IMMOBILE	BINOMIAL PROB. (PERCENT)
1	20	20	100	LESS THAN 0.001
.56	20	19	95	2.0027142050017540-03
.32	20	20	100	LESS THAN 0.001
.18	20	16	80	.5908960178526391
.1	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT .1 AND .18 CAN BE USED AS
STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS BECAUSE
THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THIS LIMIT IS >95 PERCENT

AN APPROXIMATE EC_{50} OF .1499374359846115 ug/L IS OBTAINED BY
NONLINEAR INTERPOLATION BETWEEN .1 AND .18

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS SET OF
DATA BECAUSE NO SPAN PRODUCES MOVING AVERAGES WHICH BRACKET
50 PERCENT IMMOBILE AND ALSO USES TWO CONCENTRATIONS WHICH HAVE
PERCENT IMMOBILE BETWEEN 0 AND 100 PERCENT.

----RESULTS CALCULATED USING THE PROBIT METHOD----

ITER	G	H	CHI-SQUARE	PROBABILITY
7	7.82198686411175	19.49961028120466	58.49883084361398	<0.001

(1) D WR 3-11-94

PREPARED BY: Warren Ruetter DATE: 3-11-94 ~~3-10-94~~

REVIEWED BY: Marc Sword DATE: 3/11/94

ABCLAB#041494

EC_{50} Calculations	
ABC Study No. : 41494 Compound Name : CT-546-94 Species : Daphnia Magna Exposure Period: 48 hours Study Director : Marc Sword	Analytical Bio-Chemistry Labs 7200 East ABC Lane P.O. Box 1097 Columbia, Missouri 65205 03-11-1994 Page # 2

----RESULTS CALCULATED USING THE PROBIT METHOD---- (continued)

BECAUSE THE PROBABILITY IS LESS THAN 0.05, RESULTS
CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD
NOT BE USED.
SLOPE = 5.7255709117775815
95 PERCENT CONFIDENCE LIMITS =-10.28759922624878 AND 21.73874104980041

EC_{50} = .1601783186197281 ug/L
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

(1) D WR 3-11-94
Method Reported: [X] Binomial [ ] Moving Average [ ] Probit

Note: Method selected is that which gives the narrowest confidence limits for EC_{50}.

PREPARED BY: Warren Rankin DATE: 3-11-94 ~~3-10-94~~ (C)

REVIEWED BY: Marc Sword DATE: 3/11/94

ABCLAB041494

DOSE-RESPONSE CALCULATIONS

Test Material: ~~CT-564-94~~ CT-546-94

ABC Study Number: 41494

Protocol Number: AMCV 7506

Study Director: Marc Sword

Data at 48 Hours of Exposure.

The following calculations provide a least squares estimate of the slope of the dose response line.

$$Y = m X + b$$

where: Y = Percent Mortality in Probits
b = Y-intercept
X = Log Concentration
m = Slope of Dose Response Line

Actual Conc.	Log Conc.	Percent Mort.	Probits	Calc. Probits
0.1	-1.000	0.0	2.67	2.94
0.18	-0.745	80.0	5.84	5.30
0.32	-0.495	100.0	7.33	7.60

Regression Output:

Constant	12.1752
Std Err of Y Est	0.66552
R Squared	0.96090
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	9.23647
Std Err of Coef.	1.86316

Y = 9.236 X + (12.175)

Dose Response Slope = 9.24

Correlation Coefficient (r) = 0.98

NOTE: These results are only intended to illustrate the dose-response line and determine the slope for this acute toxicity data. Probits for 0 and 100% mortality were set at 2.67 and 7.33, respectively. All other probits were obtained from: Finney, D. J. 1964. Statistical Methods in Biological Assay, 2nd Edition. Griffin, London.

GENR 3-23-94 GD WR 3-11-94

Data Entered By: Warren Ryder Date: 3-11-94

Reviewed By: Marc Sword Date: 3/11/94

ABC LAB# 041494

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Daphnia magna* Lot #: 94-L2

Date Initiated: 2-16-94 # Used to Initiate: 10 or 15

Lot # Initiated from: 94-L1 Culture Volume: ~1500 mL

Food Lot # 94 A8 S8 A9 S9 C7

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
3-2-94 KW	20	✓	✓	10	500	
3-3 KW	20	—	✓	—	—	
3-4 KW	20	✓	✓	10	450	
3-5 SLH	20	—	✓	—	—	
3-6 SLH	20	✓	✓	10	10	Used ~120 neonates for study # 41494 on 3-7-94
3-7 KW	20	—	✓	—	—	
3-8 KW	20	✓	✓	10	350	Reviewed Protocol 3/8/94

"This is an exact copy of The original document"

By [signature] date 3/8/94

* Temperature measured with a mercury thermometer

Note: Cultures may be fed one or more of the following:
- 1.0 mL of a 5.0 mg/mL suspension of trout chow yeast
- 1.0 mL suspension of dried algae (spirulina and/or chlorella)
- 3.0-4.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of *Selenastrum* sp., *Ankistrodesmus* sp., *Chlamydomonas* sp., and/or *Chlorella* sp., and *Nitzchia* sp.)

Prepared by: [signature] Date: 3-2-94

Reviewed by: _____ Date: _____

FORM # 396a (10/20/93)

ABCLAB041494

PB 0335

ABC LABORATORIES, INC.

INVERTEBRATE CULTURE RECORD

Species: *Daphnia magna* Lot #: 94-L2

Date Initiated: ①L2-16-94 # Used to Initiate: 10 or 15

Lot # Initiated from: 94-L1 Culture Volume: ~1500 mL

Food Lot # A757 A858

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
~~L2~~ ① 2-16-94 KW	20	—	✓	—	15	①EPW 2-16-94
2-17 KW	20	—	✓	—	—	
2-18 KW	20	✓	✓	15	0	
2-19 KW	20	—	✓	—	—	
2-20 AA	20	✓	✓	15	0	
2-21 AA	20	—	✓	—	—	
2-22 KW	20	✓	✓	15→10	0	Trimmed to 10
2-23 KW	20	—	✓	—	—	
2-24 KW	20	✓	✓	10	<50	
2-25 KW	20	—	✓	—	—	
2-26 KW	20	✓	✓	10	300	
2-27 DM	20	—	✓	—	—	
2-28 KW	20	✓	✓	10	0	Ready to Dap
3-1 KW	20	—	✓	—	—	

"This is an exact copy of The original document" 3/8/94 date By

* Temperature measured with a mercury thermometer

Note: Cultures may be fed one or more of the following:
- 1.0 mL of a 5.0 mg/mL suspension of trout chow yeast
- 1.0 mL suspension of dried algae (spirulina and/or chlorella)
- 3.0-4.0 mL of a 1.0 * 10^8 cells/mL concentration of live algae (The algae consists of *Selenastrum* sp., *Ankistrodesmus* sp., *Chlamydomonas* sp., and/or *Chlorella* sp., and *Nitzchia* sp.)

Prepared by: [signature] Date: 2-16-94

Reviewed by: [signature] Date: 3/8/94

FORM # 396a (10/20/93)

ABC LABS#041494

ABC LABORATORIES, INC.

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION

☐Definitive ☒Preliminary ☐Screen ☐Flow-Through ☒Static

Test Material: CT-546-94 Protocol No.: AMCY 7806

Study Director: Marc Sword Study #: 4124/94

Test Species: [X] *Daphnia magna* [ ] _____ (Lot # 94-A2) Waterbath #: 6
Dilution Water: [X] Hard Blended [ ] _____ # Instar/Vessel: 5
Date Initiated: 2-23-94 Time: 3:00 P.M. By: W. Rueter Date Terminated: 2-25-94

Test Conc. (MG/L)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
0.10	0	5N CLR	0	5N CLR	5	– CLR
1.0	0	" CLR	0	2 SUR① LOB IN CLR	5	– CLR
10	0	" CLD	0	5 SUR① CLD	5	– CLD
100	0	" CLD	0	5OB CLD	0	5OB;Q CLD
Control	0	" CLR	0	5N CLR	0	5N CLR
ID/Date/Time	WR/2-23-94/3:00P.M.		WR/2-24-94/4:05P.M.		WR/2-25-94/3:40P.M.	
Anal. ID Date/Time						

Comments: N = Normal, SUR = Surfacing, C = Clumping, CT = Coated with Extraneous Material, ERR = Erratic Movement, TR = Trailing Extraneous Material, Q = Quiescent, OB = On Bottom, P = Precipitate, CLR = Clear, CLD = Cloudy/Colored Solution, SF = Surface Film
① A drop of solution was dropped on each surfacing daphnia and they immediately sank to the bottom of the chamber WR 2-24-94

Reviewed by: Marc Sword Date: 2/28/94

Study Director: Marc Sword Date: 2/28/94

FORM AQ106 (3/25/93)

ABC LAB#C4194

ABC LABORATORIES, INC.

COMPOUND PREPARATIONS (Preliminary)

Test Material: CT-546-94 Protocol No: 7601, AMCY780c
Study Director: Marc Sword Study #: 41493; 41494
Lot/Batch # 10138134 Purity N/A % ABC Ref. # TS-7090

PREPARATION OF PRIMARY STANDARD

Weighed by: Warren Rattler Date: 2-23-94
Target Weight 3.000 g Dilution Volume 300 mL of Hard Blended H_2O
Gross Weight 3.000 g Concentration 10 mg/mL
Tare Weight 0.000 g Balance calibrated with Class C weights:
Net Weight 3.000 g 2.000 g + 0.000 g = 2.001 g
Adj. Net Wet N/A g* (Class C) (tare) (final wt.)
Balance Used: Mettler PM 460 ABC material control no.: 1905-1020

PREPARATION OF ~~WORKING STANDARD~~ Preliminary Test Concentrations

Transferred by: Warren Rattler Date: 2-23-94

	Conc. of ~~Primary~~ Working Std. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (~~mL~~ L)	Final Concentration (~~mg/mL~~ mg/L)
1	10	0.020	0.20	0.10 (2)
2	10	0.020	0.20	1.0 (2)
3	10	0.20	0.20	10 (2)
4	10	2.0	0.20	100 (2)

~~PREPARATION OF CONCENTRATED WORKING STANDARD~~

~~Weighed by:~~ ~~Date:~~
~~Target Weight~~ ~~g~~ ~~Dilution Volume~~ ~~mL of~~
~~Gross Weight~~ ~~g~~ ~~Concentration~~ ~~mg/mL~~
~~Tare Weight~~ ~~g~~ ~~Balance calibrated with Class C weights:~~
~~Net Weight~~ ~~g~~ ~~g + g = g~~
~~Adj. Net Weight~~ ~~g*~~ ~~(Class C) (tare) (final wt.)~~
~~Balance Used:~~ ~~ABC material control no.:~~

PREPARATION OF PRELIMINARY TEST CONCENTRATIONS

Transferred by: Warren Rattler Date: 2-23-94

	Conc. of Working Std. (mg/mL)	Aliquot Volume (mL)	Dilution Volume (L)	Final Conc. (mg/L)
1	10	0.15	15	0.10 (4) (3)
2	10	1.5	15	1.0 (3)
3	10	15	15	10 (3)
4	10	150	15	100 (3)

Remarks: (1) F WR 2-23-94 (4) E WR 2-23-94
(2) These were used for study # 41494 WR 2-23-94
(3) These were used for study # 41493 WR 2-23-94
*Corrected for purity

"This is an exact copy of The original document"
By Warren Rattler date 2-24-94

Prepared by: Warren Rattler Date: 2-23-94
Reviewed by: Marc Sword Date: 2/24/94

Form AQ111 (07/23/92)

ABC LABS#041494

ABC LABORATORIES, INC.

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-546-94 Protocol No. 7611 and AMC475C6

Study Director: Marc Sword Study #: 411463 and 411464

2-23-94 WR The dosing solution used for both ~~growth~~ numbers studies was prepared using the method listed on this page. Dosing Solution was prepared from 2:15 p.m. to 2:35 p.m.

OVERHEAD STIRRER METHOD

SEE FIGURE 2

EQUIPMENT

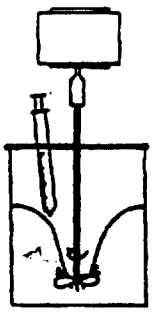
Variable Speed Overhead Mixer, equipped with a 3 blade propeller (1 1/2 - 2 inch diameter)
600 ml Beaker (Do not substitute other size beakers.)
Unused 3 ml Plastic Syringe

PROCEDURE

1. The propeller should be at the 100 ml mark on the beaker.
2. Add 297 gm/ml of water to the beaker.
3. Fill the syringe with 3.0 gm of emulsion. If you cannot weigh the product, then fill the syringe to the 3.0 ml mark.
4. Adjust the RPM of the mixer so that that the fluid vortex reaches down to the propeller on the stir shaft.
5. Add the emulsion to the shoulder of the vortex in one continuous squirt, being careful not to immerse the tip of the syringe.
6. Turn down the speed after 30 seconds to minimize the vortex.
7. Continue mixing for 15-20 minutes.
8. The resulting emulsion solution strength is 1.0%. No further mixing is needed. The solution may be diluted to the desired working concentration.

FIGURE 2

OVERHEAD STIRRER METHOD



NOTE: SYRINGE TIP SHOULD NOT CONTACT WATER.

"This is an exact copy of The original document"

By Donna Parker date 2-24-94

@EWR 2-24-94

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword Date: 2/24/94

Study Director: Marc Sword Date: 2/24/94

FORM # 55 (11/25/91) (FDI)

ABC LABS#041494

PG 0039

ABC LABORATORIES, INC.

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION

☐Definitive ☒Preliminary ☐Screen ☐Flow-Through ☒Static

Test Material: CT-546-94 Protocol No.: AMCV 7506

Study Director: Mark Sweet Study #: 41494

Test Species: [X] *Daphnia magna* [ ] _____ (Lot # 94-62) Waterbath #: 6
Dilution Water: [X] Hard Blended [ ] _____ # Instar/Vessel: 5
Date Initiated: 3-1-94 Time: 4:30 P.M. By: W. Rucker Date Terminated: 3/3/94

Test Conc. (mg/L)	Immobility and Behavioral Observations							
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
Control	0	5N CLR	0	5N CLR	0	5N CLR
0.0010	0	5N CLR	0	5N CLR	0	5N CLR
0.010	0	5N CLR	0	5N CLR	0	5N CLR
0.10	0	5N CLR	0	5N CLR	0	5N CLR
1.0	0	5N CLR	0	5N CLR	4	1SUR CLR
10	0	5N CLR	0	5N CLR	3	1SUR 1OB CLR
10C	0	5N CLD	5	— CLD	5	— CLD
1000	0	5N VCLD	5	— VCLD①	5	— VCLD②
ID/Date/Time	WR/3-1-94/4:30 P.M.		WR/3-2-94/4:40 P.M.		MCS/1:30 P.M./3-3-94	
Anal. ID Date/Time	—		—		—	

Comments: N = Normal, SUR = Surfacing, C = Clumping, CT = Coated with Extraneous Material, ERR = Erratic Movement, TR = Trailing Extraneous Material, Q = Quiescent, OB = On Bottom, P = Precipitate, CLR = Clear, CLD = Cloudy/Colored Solution, SF = Surface Film
VCLD = Very Cloudy, Daphnia impossible to observe. ② same obs as ① MCS 3/3/94
① Daphnia observed on the bottom of the chamber by looking underneath the beaker. Using a Pasteur pipet, they were observed immobile WR 3-2-94

Reviewed by: Jon Rhodes Date: 3-8-94

Study Director: Mark Sweet Date: 3/8/94

FORM AQ106 (3/25/93)

ABC LABS#041494

ABC LABORATORIES, INC.

COMPOUND PREPARATIONS: ☒ Preliminary ☐ Definitive

Test Material: CT-546-94 Protocol No.: AMCV 7406

Study Director: Marc Sinnard Study #.: 41494

Purity % N/A ABC Ref #: TS-7090 Batch/Lot #: 10133134

Prep. of Primary Standard/Weighed by: W. Rankin Date: 3-1-94

Nominal Target Weight 3.000 g Dil. Vol. 300 mL of Hard Blended H_2O

Actual Gross Weight 3.000 g Concentration 10 mg/mL

Tare Weight 0.000 g

Net Weight 3.000 g Balance checked with Class S weights:

Adjusted Net Weight 2.000 g + 0.000 g = 2.001 g

Corrected for Purity N/A g (Class S) (tare) (final wt)

Prep. of Working Standard/Transferred by: _____ Date: _____

Conc. of Parent Sol.	Aliq. Vol. (mL)	Dilution Vol. (mL)	Dilution Vehicle*	Final Concentration

Prep. of Test Conc./Transferred by: W. Rankin Time: 4:20 P.M. Date: 3-1-94

Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Vol. (L)	Final Conc. (mg/L)
0.010 ②	0.10 ~~0.10~~ ~~5.0~~ ①	1.0	0.010
0.010 ②	⑥ ~~1.0~~ 0.5	① ~~1.0~~ 0.50	0.10
0.010 ②	⑥ ~~1.0~~ 5	⑥ ~~1.0~~ 0.50	0.10
0.010 ②	① ~~1.0~~ 50	⑥ ~~1.0~~ 0.50	1.0
10	1.0	① ~~1.0~~ 1.0	10* ④
10	⑥ ~~1.0~~ 5	① ~~1.0~~ 0.50	100
10	① ~~1.0~~ 50	① ~~1.0~~ 0.50	1000

Remarks: Balance: ☐ Sartorius R 300 S: ABC Material #_____

☒ Other: Mettler PM 460 Delta Range 1905-1000

Each Conc.: ~~Single~~ Replicate 250 mL test vessels w/ 200 mL test vol.

Control(s), Description: Hard Blended H_2O

*Lot # of vehicle used:

④ This was used as the 0.010 mg/mL standard labeled ②, MCS 3/23/94

EWR 3-1-94

② This standard is the 10* mg/L Final Concentration that was previously made for this study ③ WR 3-5-94 ③ concurrently on 3/1/94, MCS 3/23/94

Reviewed by: Jan Rhodes Date: 3-8-94

Study Director: Marc Sinnard Date: 3/8/94

FORM # 410 (09/23/92)

ABC LABS# 041494

PB 0041

ABC LABORATORIES, INC.

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-546-94 Protocol No. AIMCY 7SCC

Study Director: Marc Sweed Study #: 41494

3-1-94 WR Stock solution was made using the method listed on this page. Solution was made from ~3:30 P.M. to ~4:00 p.m.

OVERHEAD STIRRER METHOD

SEE FIGURE 2

EQUIPMENT

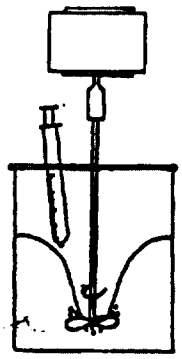
Variable Speed Overhead Mixer, equipped with a 3 blade propeller (1 1/2 - 2 inch diameter)
600 ml Beaker (Do not substitute other size beakers.)
Unused 3 ml Plastic Syringe

PROCEDURE

1. The propeller should be at the 100 ml mark on the beaker.
2. Add 297 gm/ml of water to the beaker.
3. Fill the syringe with 3.0 gm of emulsion. If you cannot weigh the product, then fill the syringe to the 3.0 ml mark.
4. Adjust the RPM of the mixer so that that the fluid vortex reaches down to the propeller on the stir shaft.
5. Add the emulsion to the shoulder of the vortex in one continuous squirt, being careful not to immerse the tip of the syringe.
6. Turn down the speed after 30 seconds to minimize the vortex.
7. Continue mixing for 15-20 minutes.
8. The resulting emulsion solution strength is 1.0%. No further mixing is needed. The solution may be diluted to the desired working concentration.

FIGURE 2

OVERHEAD STIRRER METHOD



NOTE: SYRINGE TIP SHOULD NOT CONTACT WATER.

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sweed Date: 3/11/94

Study Director: Marc Sweed Date: 3/11/94

FORM # 55 (11/25/91) (FDI)

ABC LABS# 041494 pg 0042

CYTEC A BUSINESS UNIT OF AMERICAN CYANAMID COMPANY

MSDS No: 662

MATERIAL SAFETY DATA

DATE: 08/23/9

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME: CT-546-94

SYNONYMS: Modified polyacrylamide in water-in-oil emulsion

CHEMICAL FAMILY: Modified polyacrylamide

MOLECULAR FORMULA: Polymer

MOLECULAR WGT: Polymer

AMERICAN CYANAMID COMPANY, FIVE GARRET MOUNTAIN PLAZA, WEST PATERSON, NEW JERSE 07424, USA - 201/357-3100
EMERGENCY PHONE: For emergency involving spill, leak, fire, exposure or accident call CHEMTREC: 1-800/424-9300.

2. COMPOSITION/INFORMATION ON INGREDIENTS

OSHA REGULATED COMPONENTS

COMPONENT	CAS. NO.	%	TWA/CEILING	REFERE
Petroleum distillate hydrotreated light	064742-47-8	25.5-28	400 ppm	OSHA
Ammonia	007664-41-7	0.5-1.9	35 ppm STEL 25 ppm	OSHA/AC ACGIH

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW

APPEARANCE AND ODOR: White opaque liquid; ammonia odor

STATEMENTS OF HAZARD:
WARNING! CAUSES EYE AND SKIN IRRITATION
VAPOR IRRITATING

POTENTIAL HEALTH EFFECTS

EFFECTS OF OVEREXPOSURE:
The estimated acute oral (rat) LD50, acute dermal (rabbit) LD50 and 4-hour inhalation (rat) LC50 value this material are >5,000 mg/kg, >2,000 mg/kg and >20 mg/L, respectively.
Direct contact with this material may cause moderate eye and skin irritation.
Inhalation overexposure may cause irritation of the respiratory tract and eyes.
Refer to Section 11 for toxicology information on the OSHA regulated components of this product.

4. FIRST AID MEASURES

In case of skin contact, remove contaminated clothing without delay. Flush skin thoroughly with water. Do r reuse clothing without laundering.
In case of eye contact, immediately irrigate with plenty of water for 15 minutes. Obtain medical attention if irritation persists.
exposure. Administer oxygen if there is difficulty in breathing.

5. FIRE FIGHTING MEASURES

FLAMMABLE PROPERTIES

FLASH POINT: >212 F; 100 C
METHOD: Closed Cup

FLAMMABLE LIMITS
(% BY VOL): Not available

AUTOIGNITION TEMP: Not available

DECOMPOSITION TEMP: Not available

EXTINGUISHING MEDIA AND FIRE FIGHTING INSTRUCTIONS

Use water spray, carbon dioxide or dry chemical to extinguish fires. Use water to keep containers cool. Wear self-contained, positive pressure breathing apparatus and full fire-fighting protective clothing. See Section 8 (Exposure Controls/Personal Protection) for special protective clothing.

6. ACCIDENTAL RELEASE MEASURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED

Where exposure level is not known, wear NIOSH approved positive pressure self-contained respirator. Where exposure level is known, wear NIOSH approved respirator suitable for level of exposure. In addition to the protective clothing/equipment in Section 8 (Exposure Controls/Personal Protection), wear rain suit. Spills of thi product are very slippery. Spilled material should be absorbed onto an inert material and scooped up. The are should be thoroughly flushed with water and scrubbed to remove residue. If slipperiness remains, apply more dry-sweeping compound.

7. HANDLING AND STORAGE

Avoid contact with eyes, skin, and clothing. Avoid breathing vapor. Keep container closed. Use with adequate ventilation.

To avoid product degradation and equipment corrosion, do not use iron, copper or aluminum containers or equipment.

OSHA regulations (29 CFR 106.a.14), require that the flashpoint of materials of this type be determined by the Pensky-Martens Closed Tester method. The test for this product indicates it has a flashpoint greater than 200F (93.3C). Another method indicates a potential for flash at approximately 154F (67.8C); therefore, caution shou be exercised in storage and handling.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

ENGINEERING CONTROLS AND PERSONAL PROTECTIVE EQUIPMENT (PPE)

Where this material is not used in a closed system, good enclosure and local exhaust ventilation should be provided to control exposure. Food, beverages, and tobacco products should not be carried, stored, or consumed where this material is in use. Before eating, drinking, or smoking, wash face and hands with soap and water. Avoid skin contact. Protective clothing such as impervious gloves, apron, workpants, long sleeve work shirt, or disposable coveralls are recommended to prevent skin contact. For operations where eye or fac contact can occur, wear eye protection such as chemical splash proof goggles or face shield. Eyewash equipment and safety shower should be provided in areas of potential exposure. Where exposures are below the Permissible Exposure Limit (PEL), no respiratory protection is required. Where exposures exceed the PEL use respirator approved by NIOSH for the material and level of exposure. See "GUIDE TO INDUSTRIAL RESPIRATORY PROTECTION" (NIOSH).

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AND ODOR: White opaque liquid; ammonia odor

BOILING POINT: Similar to water

MELTING POINT: Similar to water

VAPOR PRESSURE: Similar to water

SPECIFIC GRAVITY: 1.015-1.035

VAPOR DENSITY: Similar to water

% VOLATILE (BY WT): 65-75

pH: 9.5-11.0; (9.5-10.5 upon dilution)

SATURATION IN AIR (% BY VOL): Not available

EVAPORATION RATE: Not available

SOLUBILITY IN WATER: Limited by viscosity

10. STABILITY AND REACTIVITY

STABILITY: Stable
CONDITIONS TO AVOID: None known

POLYMERIZATION: Will Not Occur
CONDITIONS TO AVOID: None known

INCOMPATIBLE MATERIALS: None known

HAZARDOUS DECOMPOSITION PRODUCTS: Thermal decomposition or combustion may produce carbon monoxide, carbon dioxide, oxides of nitrogen, sulfur and ammonia.

11. TOXICOLOGICAL INFORMATION

Toxicological information on the OSHA regulated components of this product is as follows:

Acute overexposure to petroleum distillate vapors may cause eye and throat irritation. On direct skin contact, petroleum distillate may produce a severe skin irritation. Prolonged repeated exposure to petroleum distillate vapor may cause central nervous system damage as well as heart and blood disorders. The oral LD50 in the rat for various distillates ranges from 4.5 to greater than 25 ml/kg, and the inhalation LC50 in rats is about 15000 ppm. Aspiration of petroleum distillate may cause chemical pneumonitis. Overexposure to vapor may cause dizziness, drowsiness, headache, and nausea.

Ammonia vapor can cause respiratory tract and eye irritation. Direct contact with ammonia solutions causes irreversible eye damage, mucous membrane swelling and skin burns. The LC50 in rats by inhalation after one hour exposure is 7338 ppm. Single dose oral administration of ammonia solution to rats at 350 mg/kg produced no toxic effects.

12. ECOLOGICAL INFORMATION

No aquatic LC50, BOD, or COD data available.

OCTANOL/H_2O PARTITION COEF.: Not available

13. DISPOSAL CONSIDERATIONS

Disposal must be made in accordance with applicable governmental regulations.

14. TRANSPORT INFORMATION

	D.O.T. SHIPPING INFORMATION	IMO SHIPPING INFORMATION
SHIPPING NAME:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID. N.O.S.	NOT APPLICABLE/NOT REGULATED

HAZARD CLASS/ PACKING GROUP:	9 III	Not Applicable
UN NUMBER:	UN3082	Not Applicable
IMDG PAGE:	Not Applicable	Not Applicable
D.O.T. HAZARDOUS SUBSTANCES:	(PRODUCT REPORTABLE QUANTITY) AMMONIA (5,263 lbs)	Not Applicable
TRANSPORT LABEL REQUIRED:	Miscellaneous	None Required

	ICAO/IATA	TRANSPORT CANADA
SHIPPING NAME:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID. N.O.S.	NOT APPLICABLE/NOT REGULATED
HAZARD CLASS:	9	Not Applicable
SUBSIDIARY CLASS:	—	Not Applicable
UN / ID NUMBER:	3082	Not Applicable
PACKING GROUP:	—	Not Applicable
TRANSPORT LABEL REQUIRED:	Miscellaneous	None Required
PACKING INSTR:	PASSENGER 906 CARGO 906	Not Applicable
MAX NET QTY:	PASSENGER NO LIMIT CARGO NO LIMIT	Not Applicable

ADDITIONAL TRANSPORT INFORMATION

TECHNICAL NAME (N.O.S.):	(Contains ammonia)
COMMENTS:	DOT - This material is not regulated in packages containing less than the reportable quantity. The amount of product which contains the reportable quantity is shown above (see DOT Hazardous Substances). ICAO/IATA - This material is not regulated in packages containing less than the reportable quantity. The amount of product which contains the reportable quantity is shown above (see DOT Hazardous Substances).

15. REGULATORY INFORMATION

INVENTORY INFORMATION

US TSCA:	This product is manufactured in compliance with all provisions of the Toxic Substances Control Act, 15 U.S.C. This product contains a chemical substance that is subject to export notification under Section 12 (b) of the Toxic Substances Control Act, 15 U. S. C.
CANADA DSL:	Components of this product have been reported to Environment Canada in accordance with subsection 25 of the Canadian Environmental Protection Act and are included on the Domestic Substances List.

EEC EINECS: All components of this product are included on the European Inventory of Existing Chemical Substances [EINECS] in compliance with Council Directive 67/548/EEC, Amended 79/831/EEC.

OTHER ENVIRONMENTAL INFORMATION The following components are defined as toxic chemicals subject to reporting requirements of Section 313 of Title III and of 40 CFR 372 or subject to other EPA regulations.

COMPONENT	CAS. NO.	%	TPQ(lbs)	RQ(lbs)	S313	RCRA	TSCA 12
Isopropanol	000067-63-0	0.020-0.023	NONE	NONE	NO	NONE	YES
Ammonia	007664-41-7	0.5-1.9	100	100	YES	NONE	NO

PRODUCT CLASSIFICATION UNDER SECTION 311 OF SARA				
ACUTE (Y)	CHRONIC (N)	FIRE (N)	REACTIVE (N)	PRESSURE (N)

16. OTHER INFORMATION

NFPA HAZARD RATING (National Fire Protection Association)

Fire
1
Health 2 0 Reactivity
—
Special

FIRE: Materials that must be preheated before ignition can occur.

HEALTH: Materials which on intense or continued exposure could cause temporar incapacitation or possible residual injury unless prompt medical treatment is given

REACTIVITY: Materials which in themselves are normally stable, even under fire exposure conditions, and which are not reactive with water.

REASON FOR ISSUE:

Revised Section 15

Marvin A. Friedman, Ph.D., Director of Toxicology and Product Stewardship

ORIGINAL

FEBRUARY 3, 1994

Post-it™ brand fax transmittal memo 7671 | # of pages ▸ 7

To Mark Sword	From Patti Vernon
Co. ABC Labs	Co. Cytec Industries
Dept.	Phone # (201) 357-3375
Fax # (314) 443-9089	Fax # (201) 357-3057

TO: MARK SWORD
ABC LABS
FAX# (314) 443-9089

FROM: PATTI VERNON
CYTEC INDUSTRIES

SUBJECT: CT-546-94 SOLUTION MAKE-UP

A 0.5 - 1.0% SOLUTION OF THIS PRODUCT SHOULD BE INITIALLY PREPARED IN WATER. THIS IS TO INSURE PROPER BREAKING OF THE EMULSION. FURTHER DILUTIONS CAN BE MADE FROM THIS STOCK.

DUE TO THE HIGH VISCOSITY OF THIS PRODUCT THERE ARE SEVERAL METHODS FOR MAKING UP THE SOLUTION. A MAGNETIC STIR PLATE CAN BE USED IF THE SOLUTION STRENGTH IS 0.5%. ANY HIGHER SOLUTION STRENGTH WILL REQUIRE MORE MIXING ENERGY THAN A MAG STIRRER CAN PROVIDE. WE RECOMMEND USING EITHER A BRAUN MR 30/40 (OR EQUIVALENT MODEL) HAND BLENDER OR VARIABLE SPEED OVERHEAD STIR MOTOR WITH PROPELLER BLADE TO SET UP SOLUTIONS GREATER THAN 1.0%.

ATTACHED YOU WILL FIND SEVERAL PAGES DETAILING THE MAKE UP BY EACH OF THE ABOVE MENTIONED METHODS. DIRECTIONS SHOULD BE FOLLOWED ESPECIALLY IN REGARD TO MIX TIMES.

IF YOU ENCOUNTER ANY PROBLEMS PLEASE CALL LEEANN SCHECHTER AT (203) 321-2386.

TALK TO YOU SOON.

Patti

PATTI VERNON

MAGNETIC STIRRER METHOD

SEE FIGURE 4

EQUIPMENT

Magnestir 1250 Labline Stir Plate (or equivalent)
A 2 1/2 inch X 1/2 inch Teflon Coated Spin Bar
(The size of the spin bar is important; smaller spin bars generally yield inferior results.)
600 ml Beaker (Do not substitute other size beakers.)
Unused 3 ml Plastic Syringe

PROCEDURE

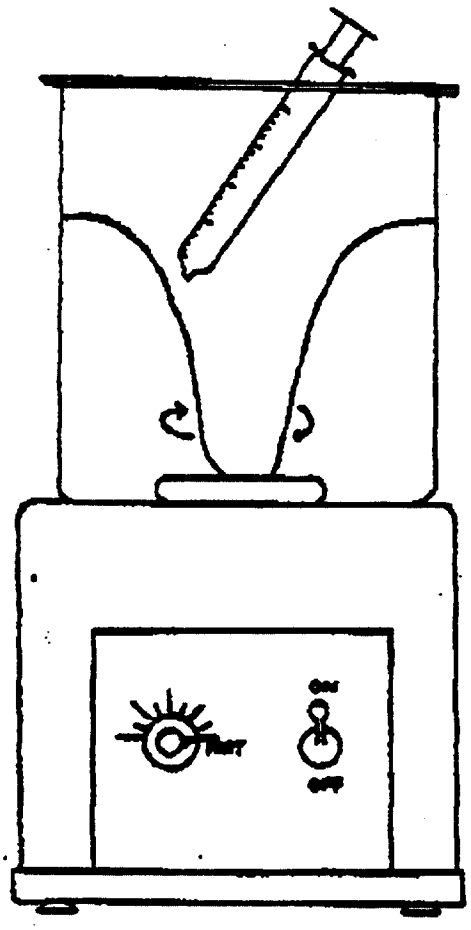
1. Add 297 gm/ml of water to the beaker.
2. Fill the syringe with ~~3.0~~ 1.5 gm of emulsion. If you cannot weigh the product, then fill the syringe to the ~~3.0~~ 1.5 ml mark.
3. Turn on the stir plate and adjust the speed of the spin bar so that the fluid vortex reaches down to fully contact it.
4. Add the emulsion to the shoulder of the vortex in one continuous squirt, being careful not to immerse the tip of the syringe.
5. Turn down the speed after 30 seconds to minimize the vortex.
6. Continue mixing for 20-30 minutes.
7. The resulting emulsion solution strength is ~~1.0~~ 0.5%. No further mixing is needed. The solution may be diluted to the desired working concentration.

Note: Although in common use, this is the least desireable way to prepare a solution from an emulsion. It is important to use a spin bar large enough and a stir plate with enough torque to insure the proper initial mixing energy.

CYANAMID

FIGURE 4

MAGNETIC STIR PLATE



NOTE: SYRINGE TIP SHOULD NOT CONTACT WATER.

TOTAL P.08

BRAUN MR 30/40 HAND BLENDER METHOD

SEE FIGURE 3

EQUIPMENT

Braun MR 30 or MR 40 Hand Blender (or equivalent)
(Available at kitchen section of department stores)
Small "C" Clamp (optional)
600 ml Beaker (Do not substitute other size beakers.)
Unused 3 ml Plastic Syringe

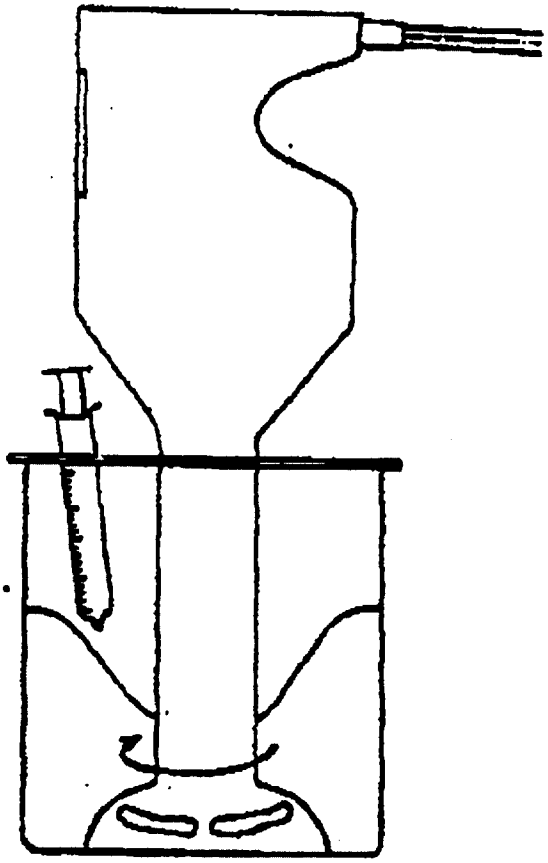
PROCEDURE

1. Add 297 gm/ml of water to the beaker.
2. Fill the syringe with 3.0 gm of emulsion. If you cannot weigh the product, then fill the syringe to the 3.0 ml mark.
3. Turn on the blender. A small "C" clamp can be used to hold down the spring-loaded push button.
4. Add the emulsion to the shoulder of the vortex in one continuous squirt, being careful not to immerse the tip of the syringe. Note: At times either tilting or holding the blender slightly off the bottom of beaker will enhance mixing.
5. Continue mixing for 15-20 seconds. Do not exceed 20 seconds!
6. The resulting emulsion solution strength is 1.0%. No further mixing is needed. The solution may be diluted to the desired working concentration.
7. Although usable immediately, additional quiescent aging (i.e. 30 minutes) may enhance performance of some products, especially those containing little or no charge.

CYANAMID

FIGURE 3

BRAUN MR 30/40 HAND BLENDER

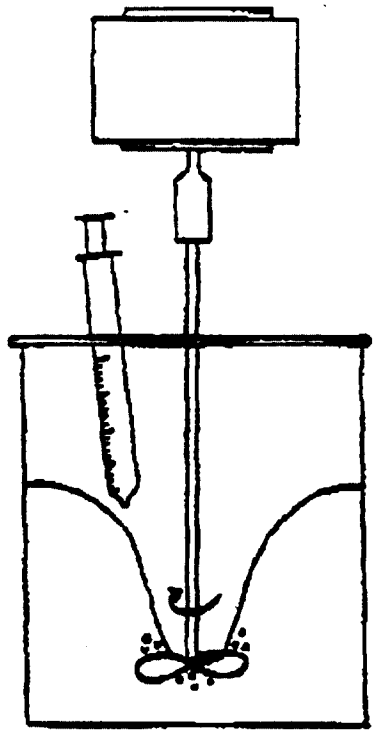


NOTE: SYRINGE TIP SHOULD NOT CONTACT WATER.

CYANAMID

FIGURE 2

OVERHEAD STIRRER METHOD



NOTE: SYRINGE TIP SHOULD NOT CONTACT WATER.

OVERHEAD STIRRER METHOD

SEE FIGURE 2

EQUIPMENT

Variable Speed Overhead Mixer, equipped with a 3 blade propeller (1 1/2 - 2 inch diameter)
600 ml Beaker (Do not substitute other size beakers.)
Unused 3 ml Plastic Syringe

PROCEDURE

1. The propeller should be at the 100 ml mark on the beaker.
2. Add 297 gm/ml of water to the beaker.
3. Fill the syringe with 3.0 gm of emulsion. If you cannot weigh the product, then fill the syringe to the 3.0 ml mark.
4. Adjust the RPM of the mixer so that that the fluid vortex reaches down to the propeller on the stir shaft.
5. Add the emulsion to the shoulder of the vortex in one continuous squirt, being careful not to immerse the tip of the syringe.
6. Turn down the speed after 30 seconds to minimize the vortex.
7. Continue mixing for 15-20 minutes.
8. The resulting emulsion solution strength is 1.0%. No further mixing is needed. The solution may be diluted to the desired working concentration.

CYTEC

CYTEC INDUSTRIES INC.
Five Garret Mountain Plaza
West Paterson, NJ 07424
Tel. (201) 357-3100

ABC LABORATORIES

FEB 4 1994

February 3, 1994

Analytical Bio-Chemistry Laboratories, Inc.
Interstate 70 East
7200 East ABC Lane
P.O. Box 1097
Columbia, MO 65205

Attention: Dave Burgess, Client Services Representative

Reference: CT-546-94 Aquatic Toxicity Studies

Dear Dave:

You will receive, under separate cover, a material identified as CT-546-94. Please submit this material to the following tests at the following costs:

Test	Protocol	Cost
Acute Toxicty to Fathead Minnow	7601	$1,800.00
Acute Toxicity to Daphnia Magna	7806	$1,600.00

Additional Cost May Be Incurred If TOC Measurements Can Be Performed Successfully.

RANGE FINDING SHOULD BEGIN WITH 0.1, 1, 10 and 100 MG/L.

These studies shall be governed by our master study contract dated January 21, 1985, which is incorporated herein by reference, and the Protocols on file, which are identified above.

The test material, CT-546-94 is a white opague emulsion with ammonia odor. This material is **soluble** in water but **limited by viscosity (</= 1% in water)**. The following special handling conditions apply to this material: **CAUSES EYE AND SKIN IRRITATION; VAPOR IRRITATING; MATERIAL IS VERY SLIPPERY** - Store at room temperature away from light and heat source. Avoid contact with iron, copper or aluminum equipment.

THE TOXICOLOGICAL PROPERTIES OF CT-546-94 HAVE NOT BEEN FULLY INVESTIGATED, SAFE HANDLING PROCEDURES SHOULD BE EMPLOYED. SEE ATTACHED MATERIAL SAFETY DATA SHEET FOR ADDITIONAL INFORMATION.

Please have all draft and final reports, invoices, and test material information sent to my attention. The Cytec Representative to whom all technical questions regarding this project should be addressed is P. A. Vernon. My office number is (201) 357-3375. Please inform me of your receipt of this material.

If you agree with the above, kindly sign and return to me a copy of this letter being submitted to you in duplicate. Our agreement in this matter will commence as of your date of acceptance.

Sincerely,

CYTEC INDUSTRIES

By: Patricia Ann Vernon
Name: Patricia Ann Vernon
Title: Associate Toxicologist

ACCEPTED: FEB 4, 1994

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.

By: David Burgess
Name: DAVID BURGESS
Title: CLIENT SERVICE REPRESENTATIVE

pavABC

7200 E. ABC Lane, Columbia, MO 65202

Tel: 314/474-8579 Fax: 314/443-9033

ABC LABORATORIES
APR 4 1994

"Working for You"

PROTOCOL ALTERATION NOTIFICATION

STUDY TITLE: "Static Acute Toxicity of CT-546-94 to *Daphnia magna*"	
PROTOCOL NO.: AMCY 7806	**ALTERATION NO.:** 1
LABORATORY: ABC Laboratories, Inc.	**LAB STUDY NO.:** 41494
SPONSOR: Cytec Industries	**EFFECTIVE DATE:** March 11, 1994

DEVIATION:

1. Protocol Sections: DEFINITIVE STUDY - Test Procedure - Chemical and Physical

   The protocol specifies that water quality parameters of temperature, dissolved oxygen, and pH will be measured at 0 and 48 hours in all test chambers. These parameters were also measured at 24 hours in all test chambers.

   Reason:

   The sponsor requested the measurements.

   Effect on Study:

   Determined 24-hour water quality.

STUDY DIRECTOR'S SIGNATURE: [signature] **DATE:** 3/11/94

SPONSOR REPRESENTATIVE'S SIGNATURE Patricia Amlen **DATE:** 3/31/94

ABC LABS#041494

7200 E. ABC Lane, Columbia, MO 65202

Tel: 314/474-8579 Fax: 314/443-9033

"Working for You"

PROTOCOL ALTERATION NOTIFICATION

STUDY TITLE: "Static Acute Toxicity of CT-546-94 to *Daphnia magna*"

PROTOCOL NO.: AMCY 7806 **ALTERATION NO.:** 2

LABORATORY: ABC Laboratories, Inc. **LAB STUDY NO.:** 41494

SPONSOR: Cytec Industries **EFFECTIVE DATE:** March 23, 1994

AMENDMENTS:

1. Protocol Sections: Header on pages 2-12

   The header of the protocol is incorrect, it lists the protocol no. as TSCA 797.1300. This is amended to the proper no., AMCY 7806.

   Reason:

   Editing error.

   Effect on Study:

   None.

2. Protocol Sections: Page 4.

   The protocol incorrectly lists Alan D. Forbis as the Aquatic Toxicology Manager. The Manager is now William A. McAllister.

   Reason:

   Change in ABC Laboratories' internal management structure.

   Effect on Study:

   None.

STUDY DIRECTOR'S SIGNATURE: Mark Arnold **DATE:** 3/23/94

SPONSOR REPRESENTATIVE'S SIGNATURE Patricia Donleavy **DATE:** 3/31/94

7200 E. ABC Lane, Columbia, MO 65202

Tel: 314/474-8579 Fax: 314/443-9033

"Working for You"

PROTOCOL COVER SHEET

The protocol for ABC Laboratories study number 41494 consists of copies of the following information:

ABC Laboratories Protocol: 7806

Study Authorization Letter Dated: February 3, 1994

Test Material: CT-546-94

Species: *Daphnia magna*

Supplier: ABC Laboratories (in-house culture)
Columbia, Missouri

The proposed experimental start date is February, 1994

The proposed experimental completion date is March, 1994

Protocol Approval

ABC Laboratories' Study Director

Name (Signed): Marc Sword Date: 2/21/94

Name (Typed): Marc C. Sword Title: Biologist II

The above information has been collected and compiled into the protocol for study number 41494. The signature date above constitutes the "Study Initiation Date."

ABC LABORATORIES, INC.

DEFINITIVE CONCENTRATION NOTIFICATION

Test Material: CT-546-94 Protocol No.: 7601

Study Director: Marc Sword Study #: 41494

Firm: Cytec Industries

Phone: (201) 357-3375

Sponsor's Study Representative: Patricia Ann Vernon

Person Notified: Same (X) Other:

Date of Notification: 3/4/94

Notified by: Marc Sword

Notification of preliminary testing results with the following definitive test levels agreed upon:

Vehicle: NA

Test Material	ABC #	Test Species	Test Conc. (mg/L)
CT-546-94	41494	Daphnia Magna	0.10, 0.18, 0.32, 0.56, and 1.0 mg/L

Reviewed by: Jon Rhodes Date: 3-4-94

Study Director: Marc Sword Date: 3/4/94

FORM # 186a (5/28/92) (FDII)

ABC LABS#041494

PE 0061

RECEIVED
'92 FEB 18 AM 8 42
ABC LABS

ABC PROTOCOL NO. AMCY 7806

(Revised February 7, 1992, American Cyanamid Blanket Protocol)

Static Bioassay Procedure for Determining the Acute Toxicity of Chemical Substances to *Daphnia magna*

ABC Study Number __________

Test Material ______________________

pg 0062

This protocol complies with EPA-TSCA 40 CFR,
Part 797, Guideline No. 797.1300.
and
OECD Guideline No. 202 (Acute Immobilization)

ABC LABS# 041494

7200 E. ABC Lane ■ P.O. Box 1097 ■ Columbia, MO 65205 U.S.A. ■ 314-474-8579 ■ Fax 314-443-9033
Prince William Quay ■ Berry Head Road ■ Suite 7 ■ Brixham, Devon ■ TQ5-9BW, U.K. ■ (0) 803-852244 ■ Fax (0) 803-852165

The following information and signatures are necessary in order to be in compliance with Good Laboratory Practice regulations and/or ABC Laboratories' policy.

TO BE COMPLETED BY THE STUDY SPONSOR

Study Sponsor

Company: American Cyanamid
One Cyanamid Plaza
Wayne, New Jersey 07470

Phone: 201-831-2534

Fax: 201-831-2821

Sponsor Identification Number (if applicable)

Number: _____

Test Material (all test and reference substances)

Name(s): _____
(name(s) used in report and correspondence)

Note: Written confirmation of percent purity or active ingredient, specific activity, and molecular weight, where applicable, must be provided.

Purity Correction (Please check appropriate statement.)

_____ Test concentrations will be based on purity or active ingredient.

__X__ Test concentrations will be based on total product.

Analytical Confirmation (Please check appropriate statement.)

_____ Analytical confirmation will be performed by ABC Laboratories.

__X__ Sponsor requests no analytical confirmation.

_____ Analytical samples will be collected and shipped to sponsor for analysis.

Special Instructions and/or Comments (if applicable)

Protocol Approval (The following must be signed by the appropriate study personnel.)

Sponsor's Study Representative

Name (signed): Patricia Ann Vernon Date: February 13, 1992

Name/Title (typed): Patricia Ann Vernon, Toxicology Program Coordinator

The sponsor is responsible for providing a Material Safety Data Sheet (MSDS), if available, and any other information necessary for proper handling, shipping, and storage of the test material. The sponsor also agrees to accept any and all of the test material that remains unused at the end of testing and to assume responsibility for its proper disposal.

TO BE COMPLETED BY ABC LABORATORIES

Study Conducted by: ABC Laboratories
7200 E. ABC Lane
P.O. Box 1097
Columbia, Missouri 65205
314-474-8579

Test Concentrations (to be determined after preliminary testing and consultation with the sponsor's representative)

Definitive test concentrations will be specified in a notification form or amendment that will be included in the raw data with the protocol.

Test Organism

Test Species: *Daphnia magna*

Supplier: In-house culture

Experimental Dates

Proposed starting date of definitive study: _____

Proposed termination date of definitive study: _____

Protocol Approval (The following must be signed by the appropriate study personnel.)

ABC Laboratories' Study Director

Name (signed): _____ Date: _____

Name (typed): _____ Title: _____

ABC Laboratories' Aquatic Toxicology Manager: Alan D. Forbis

1.0 INTRODUCTION

Aquatic toxicity tests have been used extensively to assess the environmental effects of chemical substances. Aquatic bioassays are required by federal laws such as the Toxic Substances Control Act (TSCA) (1), Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (2), and the Clean Water Act of 1977 (3). Testing guidelines have been presented for determining the aquatic toxicity of pesticides regulated by FIFRA (4) and other chemical substances that fall under the jurisdiction of TSCA (5). In TSCA's Premanufacture Notification (PMN) process, chemical manufacturers are required to submit acute aquatic toxicity test data.

With TSCA's testing guidelines in mind, as well as the Good Laboratory Practice regulations (6) that complement them, ABC Laboratories, Inc. (ABC) has prepared the following protocol to assist PMN submitters in generating data on the acute toxicity of their products to freshwater organisms.

The static bioassay method presented here was patterned after procedures that were formulated by the American Society for Testing and Materials (7) and the U.S. Environmental Protection Agency (8, 9, 10).

2.0 OBJECTIVES

The primary objective of the toxicity test described herein is to evaluate the acute toxicity of a chemical substance to *Daphnia magna* under static conditions. Acute toxicity is evaluated by determining EC_{50} levels of the toxicant during a 48-hour exposure period. An EC_{50} is the approximate concentration of the test material that produces a 50% immobilization effect on test daphnids after prescribed intervals. As used in this protocol, immobilization is defined as the lack of movement except for minor activity of the appendages. The method is designed to yield EC_{50} values following 24, and 48 hours of exposure.

3.0 TESTING FACILITY

The study will be conducted by the Aquatic Toxicology Programs division of ABC Laboratories, Inc., 7200 E. ABC Lane, P.O. Box 1097, Columbia, Missouri 65205, 314-474-8579.

4.0 TEST MATERIAL

A letter of authorization for the study should be sent with the test material or previous to its shipment and should contain the following chemical/physical properties of the compound if available: name of test material, batch/lot number, physical description, purity, stability; suggested storage conditions, water and organic solvent solubility,

available toxicity information, and handling precautions. Of particular importance as prestudy information in the consideration process for analytical confirmation of test solutions are the following parameters: water solubility, vapor pressure, hydrolysis rate and photolysis rate. **A Material Safety Data Sheet must be sent before or with the sample shipment.**

Characterization, stability and solubility studies will be the sponsor's responsibility unless contracted to ABC Laboratories, Inc. The test material will be returned to the study sponsor at study completion. Archiving a retention sample will therefore be the sponsor's responsibility.

5.0 RANGE-FINDING STUDY

5.1 General. For most chemical substances, the approximate toxic level to aquatic organisms is not known. Because this information is essential before a definitive toxicity test can be conducted, ABC routinely performs range-finding tests for static bioassays with daphnids. The information derived from these preliminary tests will be used to set concentration levels for the definitive bioassay described in section 6.0.

5.2 Test Daphnids. The test lot of *Daphnia magna* will be obtained from an in-house culture. All test daphnids will be held in a controlled-temperature area at 20 ±2°C. The lighting will be 50-80 foot-candles on a 16-hour daylight photoperiod, with 15-30 minute dawn/dusk transition periods. During the holding period, they will be fed algae supplemented with a suspension of commercial fish food and yeast at least every 3 days. Fish food will be analyzed for contaminants before its use. First-instar daphnids (≤24 hours old) will be selected for testing and will not be fed during the test.

5.3 Test System. The range-finding test will be conducted in 250-mL glass beakers containing 200 mL of solution. Test chambers will be labeled with a black marking pen as to the concentration and study number. These test vessels will be placed in a temperature-controlled area with temperature maintained at 20 ±2°C. The test system will be identified by concentration, study number, and replicate.

Water used in the test will be ABC hard blended water with a hardness of 130-160 mg/L as $CaCO_3$. This water will be analyzed for contaminants quarterly.

5.4 Test Procedure. The range-finding procedure is as follows:

5.4.1 Test daphnids will be selected from a subculture of first-instar ($\leq$24 hours old) individuals that have been cultured in the same water source and under the same condition as those to be followed in the experiment. No acclimation period will be necessary.

5.4.2 The range-finding test will be initiated by exposing at least five daphnids to at least one or more toxicant concentrations usually spaced apart by a factor of 10 and up to 100 mg/L. The test daphnids will be placed in the test chambers in an impartial manner within 30 minutes after solution preparations.

5.4.3 After 24 and 48 hours of exposure, the test chambers will be observed for daphnid immobilization and adverse behavioral effects. A record will be maintained of immobilization and abnormal behavior at each observation. Based on this observation, additional test concentrations may be added at levels above or below the initial concentrations. This procedure will be followed until a toxic range is determined.

5.4.4 The preliminary test will be conducted for 24 to 48 hours, the exact duration dependent upon the results of the initial concentrations tested. In most cases, a preliminary test for 48 hours at three toxicant concentrations is sufficient to determine the toxic range.

5.4.5 Results of the range-finding study will be used to set the concentration range of the definitive study described in section 6.0.

6.0 DEFINITIVE STUDY

6.1 General. Following the preliminary range-finding study discussed in section 5.0, the definitive test will be conducted by the procedures described below.

6.2 Test Daphnids. Aspects concerning the culture and acclimation of test daphnids will be the same as discussed in section 5.2.

6.3 Test System. The test system for the definitive study will be the same as outlined in section 5.3, except that duplicate test chambers per concentration will be used in the definitive biological testing. A continuous record of the test area temperature will be maintained and presented in the raw data at the toxicity report.

6.4 Test Procedure – Biological. The basic test procedure for the definitive bioassay will be as follows:

6.4.1 Test daphnids will be selected from a subculture of first-instar ($\leq$24 hours old) individuals that have been cultured in the same water source as the test dilution water.

6.4.2 The definitive test will consist of at least five toxicant concentrations and a dilution water control. If a solvent is used to prepare test solutions, an additional vehicle blank chamber will be used that will receive an aliquot of the solvent equivalent to the highest amount used in the test chambers. The solvent concentration in any test chamber will not exceed 0.5 mL/L and, if possible, will be less than 0.1 mL/L. The solvent used will be one of the following: dimethylformamide, triethylene glycol, methanol, acetone, or ethanol. Ten test daphnids will be impartially placed in each test chamber (20 per concentration in duplicate replicates) within 30 minutes after solution preparations.

6.4.3 As alternate test designs, more replicate test chambers containing five daphnids each may be used, if the sponsor authorizes.

6.4.4 The test chambers will be observed for immobilization and adverse behavioral effects at 24 and 48 hours ($\pm$1-hour from time of test initiation). A record will be maintained for immobilization and abnormal behavior for each concentration tested. Immobilization is defined as the lack of spacial movement (swimming). Daphnids with only minor appendage movements will be considered immobile. If more than 10% of the daphnids are immobilized in the control population or if more than 5% of the daphnids in the control are observed to be floating, the test will be deemed unacceptable and the study will be terminated. The concentration range used must yield at least one concentration with immobilization of $<$35% and at least one concentration with immobilization of $>$65% (8). The only exception to this is when maximum exposure testing occurs at the water solubility limit of the test material or 1,000 mg/L, where the test is designed to confirm no toxicity (or minimal toxic response, $<$50%) at these levels.

6.5 Test Procedure – Chemical and Physical.

6.5.1 Water quality parameters of temperature, dissolved oxygen and pH will be monitored during the test. Measurements of these parameters will be made at 0 and 48 hours of testing in each test chamber. At 0-hour, water chemistry will be performed on solutions before daphnids are distributed

to testing chambers. Water chemistry will be performed at 48 hours on all test chambers. Dissolved oxygen values in exposure solutions will be considered impacted by the compound if they have been reduced by 2.0 mg/L as compared to the control solution. The degree of significance for pH levels will be ± 1.0 pH unit deviation from the control solution. All water quality parameters will be listed as ranges for each concentration in the final report. The continuous record of the test area temperature maintained for the definitive study will be included in the raw data report.

6.5.2 In addition to the above water quality measurements for each test, the following water parameters will be measured weekly in the dilution water used for static daphnid testing: hardness, alkalinity, pH, and conductivity. These data will be provided in the report.

6.5.3 If the control dissolved oxygen level falls below 60% saturation at any point during routine water analyses, ABC's study director will deem the test unacceptable and terminate it. ABC recommends that the test solutions not be artificially aerated or pH adjusted since these practices are deemed questionable by regulatory agencies. If the study sponsor anticipates that the compound may create impacts on water quality and he wants to adjust test solutions, he must authorize this in writing before test initiation. If the sponsor wants aeration or pH adjustment, ABC recommends that the concentrations of test material be measured by an appropriate analytical method to determine if these procedures have affected nominal exposure levels (see section 6.6).

6.6 Test Procedure – Analytical Confirmation. The EPA suggests that test solutions be chemically analyzed to determine exact concentrations of the test material versus nominal exposure levels. All test concentrations will be analyzed at least at 0 and 48 hours. Page 2 indicates who will be performing the analytical confirmation or confirms if the sponsor wishes that no confirmation be performed. The sponsor will provide the specific method that will be validated before the definitive study is initiated if ABC is to perform analytical confirmation. The analytical method will be appended to this protocol after validation.

6.7 Analysis of Results. The results of the definitive study will be statistically analyzed for 24- and 48-hour EC_{50} values and their corresponding 95% confidence limits. These values will be determined by a computer program developed by Stephan et al. (11). This program evaluates the data with a binomial, moving average and probit analysis.

6.8 Report. One copy of the report will be submitted. The report will contain all original raw data and exact copies of some records. A copy of the report and associated raw data will be held on file in ABC's archives. At the sponsor's request additional copies will be provided at an additional cost. The report will include, but not be limited to, the following:

6.8.1 Study dates, name and address of test facility

6.8.2 Objectives and test methods

6.8.3 Description of the statistical methods used for data analysis

6.8.4 Description of test material (date of receipt, storage conditions, purity, physical characteristics, and method of preparing test concentrations)

6.8.5 Description of test design and test organisms

6.8.6 Summary of the data analysis, immobility observations and test water quality; signs of solubility problems such as precipitation or surface film

6.8.7 Location of raw data

6.8.8 List and signatures of study personnel

6.8.9 GLP compliance statement by study director and a statement by ABC's Quality Assurance Unit

6.8.10 Analytical data of test concentration measurements (if required)

6.8.11 Original raw data for immobility observations and water quality, analytical confirmation raw data (if performed), letters of authorized protocol changes, certified copies of supporting records, and the approved protocol

6.9 Data Retention. All original raw data generated in the preliminary and definitive studies will be provided to the study sponsor in the appendix to the final report. A copy will be retained in ABC's archives.

7.0 PROTOCOL CHANGES

If modifications of this protocol are deemed necessary, a written statement of any change(s) and reason(s) proposed by the study sponsor or ABC will be submitted to the other party. All agreed changes will be expressed in writing, signed, and dated by the

sponsor's representative and the ABC study director. The signed change(s) will be appended to the protocol and included with the final report. This protocol is subject to revision when deemed necessary in order to comply with changes in federal regulations and state-of-the-art methodology. The protocol for a specific study will not be changed while the study is in progress without discussion with the study sponsor.

8.0 SPONSOR AUTHORIZATIONS DURING THE STUDY

If a significant problem develops while the study is in progress, ABC will notify the sponsor's representative. The problem and suggested test modifications will be discussed by telephone. ABC will proceed with the changes deemed necessary upon the verbal authorization of the sponsor's representative. If the sponsor's representative cannot be reached, ABC's study director will proceed with the actions he/she deems necessary and will contact the sponsor at the end of the test to discuss the problem encountered and corrective actions taken. ABC will then submit a letter for written acknowledgment to the sponsor's representative that will be handled in the same manner discussed in section 7.0. Minor study problems will be corrected at the discretion of ABC's study director.

9.0 GLP COMPLIANCE

To be in compliance with EPA's Good Laboratory Practice (GLP) regulations, the report of the investigation conducted using this protocol will contain a statement that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards; Toxic Substances Control (40 CFR 792).

10.0 QUALITY ASSURANCE

ABC's Quality Assurance Unit will inspect one or more critical phases to assure that equipment, personnel, procedures and records conform to the Good Laboratory Practice regulations for individual laboratory studies. The results of these inspections will be reported to the study director and ABC management. The draft and final report will be reviewed for protocol and GLP compliance, as well as to ensure that the methods and standard operating procedures used were followed. A signed statement will be included in the report specifying the dates inspections were made and the dates inspections were reported to the study director and management.

11.0 REFERENCES

(1) U.S. Congress. 1976. Toxic Substances Control Act. Public Law 94-469. *Federal Register*, October 11, 1976, pp. 2003-2051.

(2) U.S. Congress. 1972. Federal Insecticide, Fungicide, and Rodenticide Act. Public Law 92-516. *Federal Register*, October 21, 1972.

(3) U.S. Congress. 1977. Clean Water Act of 1977. Public Law 95-217. *Federal Register*, December 27, 1977, pp. 1566-1611.

(4) U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. National Technical Information Service, PB83-153908, EPA 540/9-82-024, October 1982.

(5) U.S. Environmental Protection Agency. 1985. Toxic Substances Control Act Test Guidelines; Final Rules. *Federal Register*, September 27, 1985, 40 CFR Parts 796, 797, and 798, Vol. 50 (No. 188).

(6) U.S. Environmental Protection Agency. 1989. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). *Federal Register*, Vol. 54; No. 158, pp. 34043-34050.

(7) American Society for Testing and Materials. 1980. Standard practice for conducting basic acute toxicity tests with fish, macroinvertebrates and amphibians. May, 1980, ASTM Committee E-35.23. p. 25

(8) Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April 1975. p. 61

(9) U.S. Environmental Protection Agency. 1985. Toxic Substances Control Act Test Guidelines; Final Rules, Daphnid Acute Toxicity Test. *Federal Register* Vol. 50 (No. 188), September 27, 1985, 797.1300.

(10) U.S. Environmental Protection Agency. 1982. Environmental Effects Test Guidelines, Daphnid Acute Toxicity Test. EPA 560/6-82-002, EG-1, August 1982.

(11) Stephan, C. E., K. A. Busch, R. Smith, J. Burke and R. W. Andrew. 1978. A computer program for calculating an LC_{50}. U. S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August 1978.