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

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July 21, 1994

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Washington, DC 20460

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

Dear Sir/Madam:

As a follow-up to our previous 8(e) submission on a commercial cationic mixture on 05/27/94 (No 8(e) Number ~~Has~~ Been Assigned), I am enclosing copies of three studies entitled "Static Acute Toxicity of CT-529-94 to *Daphnia magna*", "Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)" and "Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*) in Dilution Water Amended with 20 mg/L Humic Acid". These reports were received by CYTEC on July 21, 1994. These documents do 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.



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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Peggy Geimer, M.D.
Corporate Medical Director
Cytex Industries, Inc.
Five Garret Mountain Plaza
West Paterson, New Jersey 07424

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

AUG 16 1994

EPA acknowledges the receipt of information submitted by your organization on a "For Your Information" (FYI) voluntary basis. For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA FYI Document Control Number (e.g., FYI-00-0000) assigned by EPA to your submission(s). Please cite this number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests" .

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Please address any further correspondence with the Agency related to this FYI submission to:

Document Processing Center (7407)
Attn: FYI Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan
Terry R. O'Bryan
Risk Analysis Branch

Enclosure

13047 A

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7200 E. ABC Lane, Columbia, MO 65202

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

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

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JUL 21 1994

P. A. VERNON

STUDY TITLE

Static Acute Toxicity of CT-529-94 to *Daphnia magna*

DATA REQUIREMENT

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

AUTHOR

Marc C. Sword
Biologist II

STUDY SUBMITTED ON

July 18, 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 #41673

Page 1 of 75

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC Laboratories' Final Report #41673, entitled " Static Acute Toxicity of CT-529-94 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
ABC Laboratories' Study Director

7-18-94

Date

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed Study #41673, "Static Acute Toxicity of CT-529-94 to *Daphnia magna*," for Cytec Industries, West Paterson, New Jersey.

The following inspections/audit were conducted on this study.

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Reported to Study Director</u>	<u>Date Reported to Management</u>
05-24-94	0-Hour Biological Observations/Daphnia Addition	05-24-94	05-24-94
06-03-94	Daphnia Addition	06-03-94	06-03-94
06-22-94	TOC Analysis at ES&S Facility	06-22-94	06-22-94
06-22-94	Draft Report	06-22-94	06-24-94
07-13-94	Final Report	07-13-94	07-14-94

The undersigned conducted the draft and final report audits. The audits indicate the report is an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.



Greg Veltri
Quality Assurance Officer II

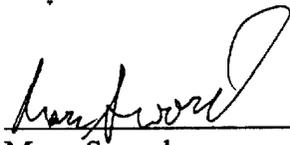
7/18/94

Date

SIGNATURE PAGE

Submitted by: ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
Columbia, Missouri 65202
(314) 474-8579

Prepared by:

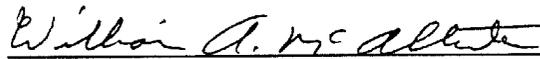


7-18-94

Marc Sword
Biologist II/Study Director

Date

Approved by:



7-18-94

William A. McAllister
Manager, Environmental Toxicology

Date



7-18-94

Jon E. Rhodes
Team Leader, Environmental Toxicology

Date



7/18/94

Greg Veltri
Quality Assurance Officer II

Date

:amk

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INVERTEBRATE ACUTE TOXICITY COMPENDIUM

Subject: "Static Acute Toxicity of CT-529-94 to *Daphnia magna*," ABC Laboratories' Final Report #41673

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-529-94

Nominal Test Concentrations: Control, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6,
and 10 mg/L

Dilution Water: 152 mg/L as CaCO₃ (hardness),
166 mg/L as CaCO₃ (total alkalinity),
8.3 (pH),
320 μMhos/cm (conductivity)

Experimental Test Dates: Start – June 3, 1994
Termination – June 5, 1994

Length of Study: 48 Hours

Results: 48-Hour EC₅₀ = 0.37 mg/L (95% confidence interval = 0.30 to 0.45 mg/L)

48-Hour NOEC = 0.10 mg/L

Slope of 48-Hour Dose-Response Line = 3.9

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 nine concentration and control static toxicity test to determine the toxicity of CT-529-94 to *Daphnia magna*. Range-finding tests were conducted from May 17, 1994, to May 20, 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-529-94 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. 9405 as approved by a representative of Cytec Industries on April 27, 1994, and by ABC Laboratories' study director on May 24, 1994. The definitive study was conducted from June 3, 1994, to June 5, 1994.

I. Test Material

A. Compound Receipt

The CT-529-94 test material (batch 31) was received from Cytec Industries on April 21, 1994 and was assigned ABC reference #TS-7238. The sample was a cloudy liquid and was stored at room temperature. The expiration date was given as February 1995. 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 0.1002 g of test substance to 100 mL of hard blended water. The concentration of the standard was 1.0 mg/mL.

- The preliminary concentrations were prepared by adding appropriate aliquots of 1.0-, 3.2-, or 32-mg/L working standards to the test chambers.

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 39 to 43 footcandles (423 to 467 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 CaCO₃ (Table I). The water was filtered with a sediment filter prior to use. The hard blended water was biologically aged before use. 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-AA₁ on June 3, 1994. The adults from this culture had been cultured for 13 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 averaged 53 footcandles (569.8 lux).

B. Test Design

1. Biological

The first range-finding test was conducted from May 17, 1994, to May 19, 1994, at the nominal concentrations of 0.010, 0.032, 0.10, 0.32, 1.0, and 3.2 mg/L with a concurrent control chamber. Five daphnids were added to each test chamber. All solutions were clear during the test.

The control, 0.010, 0.032, and 0.10 mg/L chambers had no immobility or abnormal/sublethal effects noted during the test. Cumulative immobility in the 0.32, 1.0, and 3.2 mg/L concentrations was 20, 0, and 60% at 48 hours. The abnormal effects of daphnids on bottom of the test chamber, trailing extraneous material, and/or quiescence were also noted in the 0.32, 1.0, and 3.2 mg/L test concentrations.

The second range-finding test was performed from May 18, 1994, to May 20, 1994, at the nominal concentrations of 10 and 32 mg/L with a concurrent control chamber. There were 5 daphnids added to each control and test concentration. Total immobility was observed in the 10 and 32 mg/L solutions by 48 hours, and the solutions were observed to be slightly cloudy during the test. The control daphnids were normal, and the control solution was clear during the test.

The nine definitive test concentrations were selected after consultation with the study sponsor. The definitive concentrations ranged in a logarithmic series from 0.10 to 10 mg/L, with two replicates per concentration. Duplicate hard blended water controls were also included. Ten *Daphnia magna* (first instar less than 24 hours old) per replicate beaker were used for the definitive bioassay, for a total of 20 *Daphnia magna* per concentration or control. Daphnids were randomly added to test beakers within 30 minutes of test solution preparation. Random assignment was accomplished by placing 10 neonates into each of 20 hard blended water filled (~5-10 mL) scintillation vials that had been labeled 1-20. A computer generated randomization table assigned a number to each specific test replicate. The daphnids were transferred from the scintillation vials to each appropriate beaker at 0 hour according to the randomization table.

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.

The first attempt of the definitive test, performed from May 24, 1994, to May 26, 1994, was not successful due to complete mortality in the control A replicate at 48 hours.

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 48 hours. Temperature was monitored continuously with a datalogger and thermistor probe. A 200 mL aliquot of the 1.0 L parent control solution was sampled at 0 hour for total organic carbon (TOC) analysis. The pH of the sample was adjusted to <2.0 with sulfuric acid. A backup sample was collected in the same manner. The primary sample was delivered to Engineering Surveys and Services in Columbia, Missouri, for TOC analysis. The backup sample was retained in a refrigerator at ABC Laboratories.

IV. Biological Data Analysis

Statistical analysis of the concentration vs. effect data (immobility) was performed using a computerized EC₅₀ program developed by Stephan et al. (4). This program is designed to calculate the EC₅₀ 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 EC₅₀ (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 EC₅₀ 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-529-94 was completed on June 5, 1994. An examination of the culture records for this test indicated that the *Daphnia magna* were in good condition for testing. Duplicate replicates of the control, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg/L nominal concentrations were tested.

Table II presents the water quality parameters measured during the definitive test. Temperature was 21 and 20°C when measured at 0 and 48-hours, respectively. The pH values ranged from 8.4 to 8.7. Dissolved oxygen concentrations ranged from 7.1 to 8.2 mg/L during the test. These values represented 84 to 94% saturation at 21 and 20°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 control and test solutions were clear with no visible surface film or precipitate throughout the study. TOC measured in triplicate on 0-hour control dilution water was <1.0, <1.0, and <1.0 mg/L (\bar{x} = <1.0 mg/L).

Table III presents the individual immobility and behavioral observations for daphnids exposed to CT-529-94. 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, surfacing, trailing extraneous material, and/or daphnids on bottom of the test chambers were observed in the 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg/L test concentrations during the test. The 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg/L test concentrations elicited 40, 20, 55, 100, 100, 100, 100, and 100% immobility, respectively at 48 hours.

All results were based on the nominal concentrations of CT-529-94. The 48-hour no-observed effect concentration was 0.10 mg/L, the lowest concentration tested, based on the lack of abnormal effects or immobility in this concentration. The 24- and 48-hour EC_{50} values were >3.2 and 0.37 mg/L, respectively (Table IV). The slope of the 48-hour dose-response line was 3.9 as calculated by least squares regression analysis of percent immobility transformed to probits versus log concentration (Figure 1).

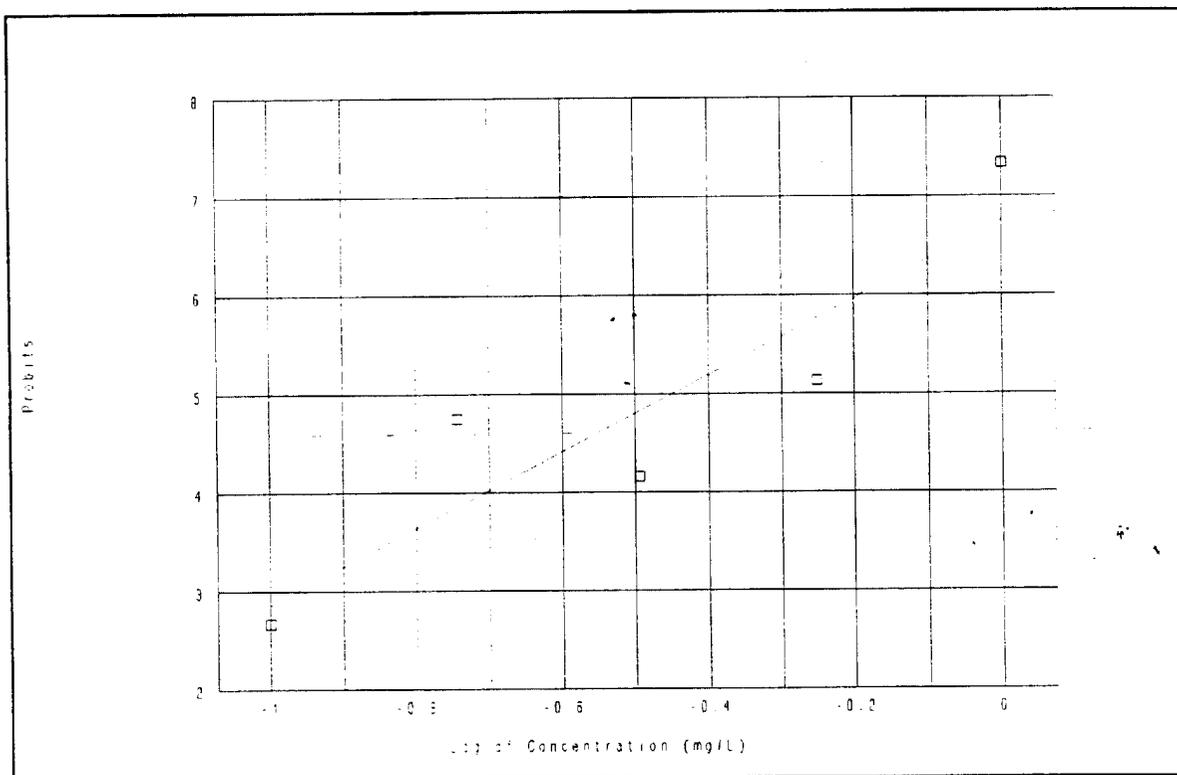


Figure 1: The 48-Hour Dose-Response Line for *Daphnia magna* Exposed to CT-529-94

The study was conducted following 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 Aged Hard Blended Water Used by
ABC Laboratories' Environmental Toxicology Division

Dilution Water Screen ^a		Monthly Screens ^b (mg/L)	
Hardness	152 mg/L (as CaCO ₃)	Total Organic Carbon	< 1.0 mg/L
Alkalinity	166 mg/L (as CaCO ₃)	Suspended Solids	0.0-0.2 mg/L
pH	8.3		
Conductivity	320 μ Mhos/cm		
Biyearly Screens ^c			
Elements (mg/L)		Chlorinated Hydrocarbons (μ g/L)	
Aluminum	< 0.20	DDE	< 0.01
Arsenic	< 0.01	DDD	< 0.01
Boron	0.250	DDT	< 0.01
Cadmium	< 0.0005	Dieldrin	< 0.01
Chromium	< 0.001	Endrin	< 0.01
Cobalt	< 0.05	α -BHC	< 0.01
Copper	< 0.02	β -BHC	< 0.01
Fluoride	0.9	γ -BHC-Lindane	< 0.01
Iron	< 0.1	Δ -BHC	< 0.01
Lead	< 0.003	Heptachlor	< 0.01
Mercury	0.00032	Heptachlor Epoxide	< 0.01
Nickel	< 0.05	Aldrin	< 0.01
Selenium	< 0.005	Chlordane	< 0.3
Silver	< 0.0005	Methoxychlor	< 0.05
Zinc	< 0.04	Toxaphene	< 4.0
		Endosulfan Sulfate	< 0.03
		Endosulfan I	< 0.01
		Endosulfan II	< 0.01
		HCB	< 0.01
		Mirex	< 0.01
		Telodrin	< 0.01
		PCBs	< 1.0
Organophosphate (μ g/L)		Miscellaneous (mg/L)	
Diazinon	< 0.1	Chemical Oxygen Demand	< 10
Methyl Parathion	< 0.02	Un-Ionized Ammonia	< 0.1
Ethyl Parathion	< 0.02	Nitrite N	< 0.02
Malathion	< 0.05	Nitrate N	0.17
Ronnel	< 0.01	Total Phosphorus as P	< 0.05
Ethion	< 0.02	Chlorine Residual	< 0.02
Trithion	< 0.05		
Vapona	< 2.0		

^a Represents the values measured on dilution water used for the test

^b Represents the values obtained from the screens of May 1994

^c Represents the values obtained from the screen of January 1994

Note: Results of the chemical analyses supporting these data are on file at ABC Laboratories.

TABLE II

Water Quality Measurements During the Static Acute
Toxicity Test of CT-529-94 to *Daphnia magna*

Nominal Test Concentration (mg/L)	Water Quality					
	0 Hour			48 Hours		
	Temp. ^a °C	DO ^b mg/L	pH ^c	Temp. °C	DO mg/L	pH
Control A	21	7.3	8.4	20	8.1	8.4
Control B	21	7.1	8.4	20	8.1	8.4
0.10 A	21	7.5	8.5	20	8.2	8.5
0.10 B	21	7.2	8.5	20	8.2	8.5
0.18 A	21	7.4	8.6	20	8.1	8.5
0.18 B	21	7.3	8.6	20	8.2	8.5
0.32 A	21	7.7	8.7	20	8.0	8.5
0.32 B	21	7.8	8.7	20	8.1	8.5
0.56 A	21	7.9	8.7	20	8.2	8.6
0.56 B	21	7.9	8.7	20	8.0	8.5
1.0 A	21	7.9	8.7	20	8.1	8.5
1.0 B	21	7.9	8.7	20	8.1	8.5
1.8 A	21	7.9	8.7	20	8.1	8.5
1.8 B	21	8.0	8.7	20	8.1	8.5
3.2 A	21	8.0	8.7	20	8.0	8.5
3.2 B	21	7.9	8.7	20	8.2	8.5
5.6 A	21	8.0	8.7	20	7.9	8.5
5.6 B	21	7.9	8.7	20	8.1	8.5
10 A	21	8.0	8.7	20	8.1	8.5
10 B	21	8.0	8.7	20	8.0	8.5

^a Temperature measured with a mercury thermometer

^b Dissolved oxygen concentrations – YSI Dissolved Oxygen System Model 54 ARC

^c pH – Beckman Model Φ 34 pH meter with a Beckman Model 39841 probe

NOTES: Dissolved oxygen saturation values corrected for altitude at the test temperatures of 20 and 21°C are 8.7 and 8.5 mg/L, respectively.

TOC measured in triplicate on 0-hour control dilution water was <1.0, <1.0, and <1.0 mg/L (\bar{x} = <1.0 mg/L).

TABLE III

Immobility and Behavioral Observations During the Static
Acute Toxicity Test of CT-529-94 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	0	10 N
Control B	10	0	10 N	0	10 N
0.10 A	10	0	10 N	0	10 N
0.10 B	10	0	10 N	0	10 N
0.18 A	10	0	3 TR; 1 SUR; 6 N	4	2 OB/TR; 4 OB
0.18 B	10	1	4 TR; 5 N	4	1 TR; 5 OB
0.32 A	10	0	1 SUR/TR; 6 OB/TR; 3 OB	2	6 OB; 1 TR; 1 N
0.32 B	10	0	4 SUR/TR; 3 OB/TR; 3 OB	2	1 OB/TR; 7 OB
0.56 A	10	0	2 SUR/TR; 3 SUR; 5 OB	5	5 OB
0.56 B	10	0	4 SUR/TR; 2 OB/TR; 3 OB; 1 N	6	1 SUR/TR; 3 OB
1.0 A	10	0	5 SUR/TR; 5 OB/TR	10	---
1.0 B	10	0	6 SUR/TR; 4 OB/TR	10	---
1.8 A	10	0	2 SUR/TR; 6 OB/TR; 2 OB	10	---
1.8 B	10	0	6 OB/TR; 4 OB	10	---
3.2 A	10	0	6 OB/TR; 4 OB	10	---
3.2 B	10	1	5 OB/TR; 4 OB	10	---
5.6 A	10	4	6 OB/TR	10	---
5.6 B	10	5	5 OB/TR	10	---
10 A	10	2	7 OB/TR; 1 OB	10	---
10 B	10	9	1 OB/TR	10	---

Key to Abbreviations: Imm. = Immobility; N = Normal; OB = On Bottom of the Test Chamber; SUR = Surfacing; TR = Trailing Extraneous Material

Note: All control and test concentrations were clear during the test, with no precipitate or surface film.

TABLE IV

Static Acute Toxicity of CT-529-94 to *Daphnia magna*

<u>Compound</u>	<u>EC₅₀ in mg/L</u>	
	<u>24-Hour</u>	<u>48-Hour</u>
CT-529-94 ^a	> 3.2 ^b	0.37 ^c (0.30 and 0.45) ^d

N = 20 daphnids per concentration

^a Bioassay conducted at 20 (±2)°C

^b Insufficient immobility for the calculation of an EC₅₀

^c The EC₅₀ was calculated using the moving average method.

^d 95% confidence limits

The 48-hour no-observed effect concentration was 0.10 mg/L, the lowest concentration tested, based on the lack of immobility or abnormal/sublethal effects noted at this concentration.

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 #41673

Static Acute Toxicity of CT-529-94 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

John Rhodes
Team Leader, Environmental Toxicology

Marvin D. Hoffman
Biologist I

Jane Bowman
Biologist III

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

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The original document"

By Kevin Sword date 6-22-94

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

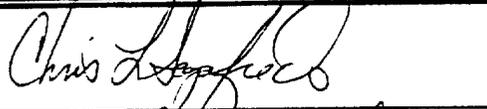
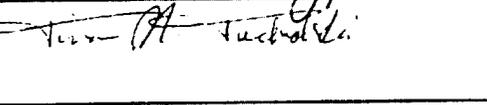
ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

NAME	SIGNATURE	INITIAL	DATE
Laurie L. Roesel	<i>Laurie L. Roesel</i>	LAR	2-7-94
Michelle A. Muckerman	<i>Michelle A. Muckerman</i>	MM	2/7/94
Jamie L. Veltri	<i>Jamie L. Veltri</i>	JLV	2/7/94
John Bucksath	<i>John Bucksath</i>	JDB	2/7/94
Kathryn Konering	<i>Kathryn Konering</i>	KK	2-8-94
Bret Hurshman	<i>Bret A. Hurshman</i>	BAH	2-8-94
Luke Stuerman	<i>Luke Stuerman</i>	LMS	2/8/94
Gerald A. Nothdurft	<i>Gerald A. Nothdurft</i>	GAN	2-7-1994
Yuan Yang	<i>Yuan Yang</i>	YY	2-7-94
Jianping Liu	<i>Jianping Liu</i>	JL	2/8/94
Karen March	<i>Karen March</i>	KM	2-8-94
David Burgess	<i>David Burgess</i>	DB	2-8-94
Debbie Jameson	<i>Debbie Jameson</i>	dj	2-7-94
Anita M. Klick	<i>Anita M. Klick</i>	AMK	2-9-94
Donna S. Hoek	<i>Donna S. Hoek</i>	DSH	2/7/94
John Ingersoll	<i>John Ingersoll</i>	JII	2-8-94
Edward Harper	<i>Edward Harper</i>	ESH	2-8-94
D. Abram	<i>D. Abram</i>	DA	5-3-94
Christopher J. Pope	<i>Christopher J. Pope</i>	CJP	5-24-94
Marvin D. Hoffman	<i>Marvin D. Hoffman</i>	MH MDH	6-15-94
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By *Diane Havel* date 6-22-94

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.

SIGNATURE & INITIAL IDENTIFICATION ENGINEERING SURVEYS AND SERVICES

NAME & TITLE	SIGNATURE	INITIAL	DATE
Chris Segafredo, Director of Environ- mental Services			30 Dec 93
Linda Adams, Laboratory Manager		LA	30 Dec 93
Rebecca Ann Bergfield, Chemist		RB	30 Dec 93
Tina M. Tucholski, Chemist		TMT	30 Dec 93
"This is an exact copy of The original document"			
By <u></u> date <u>6-22-94</u>			

Note: This list includes personnel of Engineering Surveys and Services Testing Laboratories located at 1113 Fay Street in Columbia, Missouri, who provided analyses in support of various studies performed by the Environmental Toxicology division of ABC Laboratories. This list of personnel is not study specific. Primary project personnel will be identified separately for each project.

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION

Definitive Preliminary Screen Flow-Through Static

Test Material: LT 529-94 Protocol No.: 9405

Study Director: Marci Szwed Study #: 41673

Test Species: *Daphnia magna* [] (Lot # 94-AA₁) Waterbath #: #6
 Dilution Water: Hard Blended [] # Instar/Vessel: 10
 Date Initiated: 6-3-94 Time: 3:48 P.M. By: MCS Date Terminated: 6-5-94

Test Conc. (mg/L)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
Control A	0	10N, 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	3TR, 1SUR, 6A; CLR	4	2TR, 0B, 7OB; CLR
0.18 B	0	10N, CLR	1	4TR, 5M; CLR	4	1TR, 5OB; CLR
0.32 A	0	10N, CLR	0	1SUR/TR, 6TR, 0B, 3OB; CLR	2	6OB, 1TR, 1N; CLR
0.56 ^{0.32} B	0	10N, CLR	0	4SUR/TR, 3OB/TR, 3OB; CLR	2	1TR, 0B, 7OB; CLR
0.56 A	0	10N, CLR	0	2SUR/TR, 3SUR, 5OB; CLR	5	5OB; CLR
0.56 B	0	10N, CLR	0	4SUR/TR, 3TR, 0B, 3OB, 1N; CLR	6	1SUR/TR, 3OB; CLR
1.0 A	0	10N, CLR	0	5SUR/TR, 5OB/TR; CLR	10	-; CLR
1.0 B	0	10N, CLR	0	6SUR/TR, 4OB/TR; CLR	10	-; CLR
1.8 A	0	10N, CLR	0	2SUR/TR, 6OB/TR, 2OB; CLR	10	-; CLR
1.8 B	0	10N, CLR	0	6OB/TR, 4OB; CLR	10	-; CLR
ID/Date/Time	MCS 6-3-94 3:56 P.M.		MCS 6-4-94 3:50 P.M.		MCS 6-5-94 4:20 P.M.	
Anal. ID Date/Time	—		—		—	

Comments: N = Normal, SUR^{CS} = 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
 DE MDH 6-2-94
 Note: Trailing Ext. Material, some daphnids sticking to bottom, each other, or side of Baskel, MCS
 Surfacing daphnids dropped below water surface with drop of test solution MCS 6-4-94 6-4-94

Reviewed by: Jon Rhodes Date: 6-20-94
 Study Director: Marci Szwed Date: 6/7/94 + 6-20-94

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION						
<input checked="" type="checkbox"/> Definitive <input type="checkbox"/> Preliminary <input type="checkbox"/> Screen <input type="checkbox"/> Flow-Through <input checked="" type="checkbox"/> Static						
Test Material: <u>CT 529-94</u>			Protocol No.: <u>9405</u>			
Study Director: <u>MARC Sward</u>			Study #: <u>416723</u>			
Test Species: <input checked="" type="checkbox"/> <i>Daphnia magna</i> [] _____ (Lot # <u>94-AA, 1</u>) Waterbath #: <u>6</u> Dilution Water: <input checked="" type="checkbox"/> Hard Blended [] _____ # Instar/Vessel: <u>10</u> Date Initiated: <u>6-3-94</u> Time: <u>3:45 P.M.</u> By: <u>MCS</u> Date Terminated: <u>6-5-94</u>						
Test Conc. (mg/L)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
3.2 A	0	ION, CLR	0	6 OB/TR, 4 OB, j CLR	10	- j CLR
3.2 B	0	ION, CLR	1	5 TR/OB, 4 OB, j CLR	10	- j CLR
5.6 A	0	ION, CLR	4	6 TR/OB j CLR	10	- j CLR
5.6 B	0	ION, CLR	5	5 TR/OB j CLR	10	- j CLR
10.0 A	0	ION, CLR	2	7 TR/OB 1 OB j CLR	10	- j CLR
10.0 B	0	ION, CLR	9	1 TR/OB j CLR	10	- j CLR
ID/Date/Time	<u>MCS 6-3-94 3:56 P.M.</u>		<u>MCS 6-4-94 3:50 P.M.</u>		<u>MCS 6-5-94 4:20 P.M.</u>	
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 DE MOH 6-2-94 DE MCS 6-7-94 Note: Trailing extraneous material (TR), some daphnids sticking to bottom of chamber or each other or side of beaker. MCS, 6-4-94						
Reviewed by: <u>Tom T. Jones</u>			Date: <u>6-20-94</u>			
Study Director: <u>Marc Sward</u>			Date: <u>6/7/94 + 6-20-94</u>			

WATER QUALITY

Test Material: CT 529-94 Protocol No.: 9405

Study Director: MARC SWEED Study #: 41673

Concentration (mg/L)	6-3-94 ^{WLS-MDH} Day 0			6-3-94 ^{WCS-MDH} Day 0			6-5-94 Day 2			6-5-94 Day 2		
	Temp °C	DO mg/L	pH	Temp °C	DO mg/L	pH	Temp °C	DO mg/L	pH	Temp °C	DO mg/L	pH
Control	21	7.3	8.4	21	7.1	8.4	20	8.1	8.7	20	8.1	8.4
0.10	21	7.5	8.5	21	7.2	8.5	20	8.2	8.5	20	8.2	8.5
0.18	21	7.4	8.6	21	7.3	8.6	20	8.1	8.5	20	8.2	8.5
0.32	21	7.7	8.7	21	7.5	8.7	20	8.0	8.5	20	8.1	8.5
0.56	21	7.9	8.7	21	7.9	8.7	20	8.2	8.6	20	8.0	8.5
1.0	21	7.9	8.7	21	7.9	8.7	20	8.1	8.5	20	8.1	8.5
1.8	21	7.9	8.7	21	8.0	8.7	20	8.1	8.5	20	8.1	8.5
3.2	21	8.0	8.7	21	7.9	8.7	20	8.0	8.5	20	8.2	8.5
5.6	21	8.0	8.7	21	7.9	8.7	20	7.9	8.5	20	8.1	8.5
10.0	21	8.0	8.7	21	8.0	8.7	20	8.1	8.5	20	8.0	8.5

- * Temperature measured with a mercury thermometer.
- ° Dissolved Oxygen probe (YSI Dissolved Oxygen System Model 54 A), ABC material control # 1905-730
- ° pH: Beckman 034 pH meter, ABC material control 163-640 used with a Beckman Model 39841 probe

Remarks: Note: 0.10mg/L water (Clean @ ~ 5.0 before depth and addition WCS 6-3-94)
 Light - Day 2: West end of W86 557.1 lux, East end 582.4 lux. YSI Model 9200A Dissolved Oxygen/Phosphore
 Model 01-189, ABC MAT CONT #163-341, Photometric model probe, serial # PH 4721, WCS 6-5-94
 Information Added 6-20-94 MDH

Prepared by: Marc Sweed Date: 6-3-94
 Reviewed by: Jon R. [Signature] Date: 6-20-94

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: AF Hd Blend A

TEMPERATURE: 24.1 °C

DO: 8.0 mg/L

pH: 8.3

ALKALINITY:* 166 mg/L

HARDNESS:* 152 mg/L

CONDUCTIVITY: 320 µmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE Mercury Digital

<u>METER TYPE</u>	<u>BRAND</u>	<u>MODEL</u>	<u>MATERIAL CODE</u>
-------------------	--------------	--------------	----------------------

DISSOLVED OXYGEN	<input checked="" type="checkbox"/> YSI <input type="checkbox"/>	54A	1905-730
------------------	---	-----	----------

pH Meter:	<input checked="" type="checkbox"/> BECKMAN <input type="checkbox"/>	φ34	163-640
Probe:	BECKMAN	39841	-----

CONDUCTIVITY:	<input checked="" type="checkbox"/> YSI <input type="checkbox"/>	33	1905-520
---------------	---	----	----------

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as CaCO₃)

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 The original document
 by handwritten date 6-20-94
 ③ EMS 6-20-94

Analysis by: [Signature] Date: 6-5-94

Reviewed by: Jane Bowman Date: 6-19-94

Continuous Temperature Graph

Test Material: CT-529-94

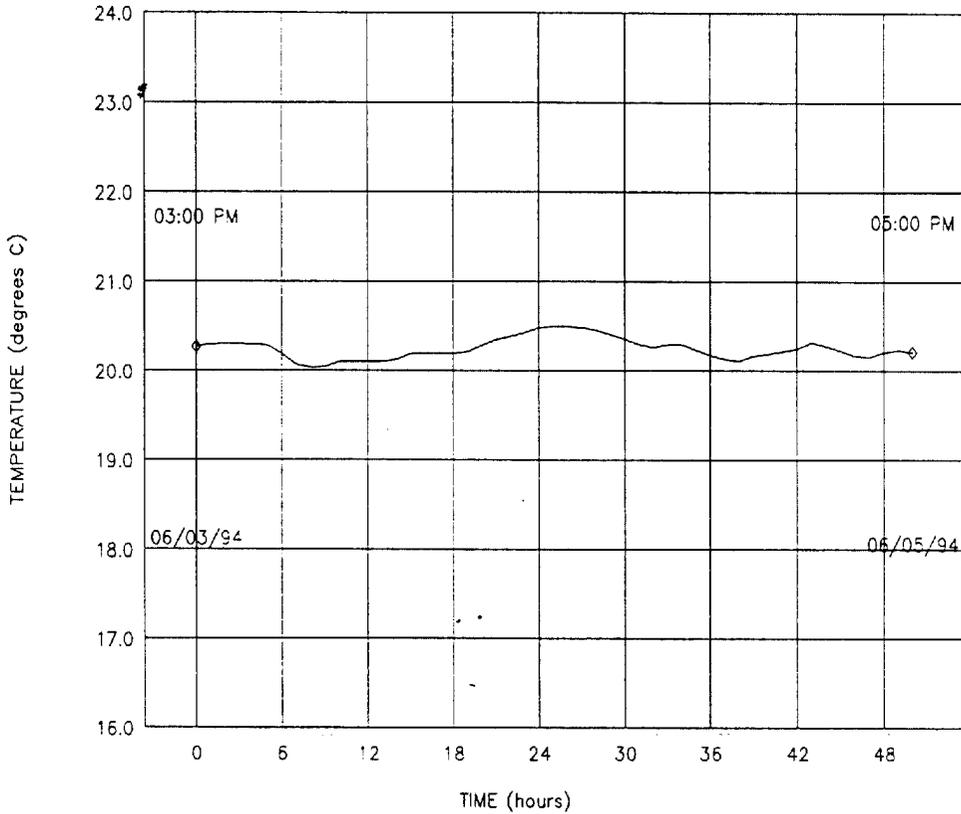
ABC Study Number: 41673

Protocol Number: 4405

Lab Form No: AQ9 (4/15/91)
ABC Laboratories
P.O.Box 1097, 7200 East ABC Lane
Columbia, Missouri 65205

Data Logger
Material Cont. No.: 1905-1025

Waterbath No. 6
DEFINITIVE TEMPERATURE RECORDING



Comments: _____

Prepared By: *Dana Howard* Date: 6/20/94
Reviewed By: *Jane Bowring* Date: 6-22-94
Approved By: *Dana Howard* Date: 6-22-94

SAMPLE COLLECTION FOR TOC ANALYSIS

Test Material: CT 529-94 Protocol No.: 9405
 Study Director: MARC Sward Study #: 41673

Date/ID: Mani Hoffman 6-3-94

For each control or test level sampled, approximately ^{200 ml} 100 mL of dilution water were drawn from ^{parent control solution} mid-chamber level of each replicate with a volumetric pipette and ^{place in} composited into an amber bottle for a total volume of approximately 200 mL. A second composite sample was collected in the same manner to retain as a back-up sample for each control or test level sampled. The samples were collected at 0 hour before ^{analysis} fish addition. Samples were then prepared for total organic carbon (TOC) analysis by adding sulfuric acid until the pH was < 2.0. The back-up sample(s) were then placed into a refrigerator at approximately 3°C for storage and the primary sample(s) were delivered to Engineering Surveys and Services Testing Laboratory in Columbia, MO, for TOC analysis.

Test Levels Sampled:

- Control
- Humic Acid Control
- Vehicle Blank
- Level 1
- Level 2
- Level 3
- Level 4
- Level 5

Sulfuric Acid

Lot #: 85921

Supplier: Fisher

pH meter: Beckman Model ϕ 34 with Beckman Model 39841 pH probe
 ABC Material Control #163-640

Refrigerator ABC Material Control #166-12

Notes: ① Fmcs 6-3-94

Prepared by: Mani Hoffman Date: 6-3-94

Reviewed by: Marc Sward Date: 6/20/94

**ENGINEERING SURVEYS AND SERVICES
TESTING LABORATORY**

1113 Fay Street • Columbia, Missouri 65201 • (314) 449-2646
802 El Dorado Drive • Jefferson City, Missouri 65101 • (314) 636-3303

Date 24 June 1994
Lab No. 4625

Project: ABC Laboratories - 41673 RECEIVED
Columbia, Missouri JUN 28 AM 7 13 Date Received: 7 June 1994
Location: _____
Sample No./ Description: 1312 / Study 41673, control 0 hour, 6-3-94

TEST RESULTS

PARAMETER	SAMPLE NUMBER		DETECTION LIMIT
	METHOD	1312	
Total Organic Carbon, mg/l	5310 B	ND	1.00
Total Organic Carbon, mg/l	5310 B	ND	1.00
Total Organic Carbon, mg/l	5310 B	ND	1.00

Note: Sample secured and delivered to lab by others.

ND = None Detected

Method number from Standard Methods for the Examination of Water & Wastewater, current edition, unless otherwise noted.

CC: 1 Sword

ENGINEERING SURVEYS AND SERVICES
BY:

Chris L. Sagafredo
Chris L. Sagafredo
ABC LABS 041570

pg 0029

COMPOUND PREPARATIONS: <input type="checkbox"/> Preliminary <input checked="" type="checkbox"/> Definitive				
Test Material: <u>CT 529-94</u>		Protocol No.: <u>9405</u>		
Study Director: <u>MARC Sward</u>		Study #: <u>41673</u>		
Purity % <u>N/A</u>		ABC Ref #: <u>TS 7238</u>		(Batch/Lot #: <u>31</u>)
Prep. of Primary Standard/Weighed by: <u>M Hoffman</u> Date: <u>6-3-94</u>				
Nominal Target Weight <u>0.1000</u> g Dil. Vol. <u>100</u> mL of <u>Hard Blend</u>				
Actual Gross Weight <u>0.11002</u> g Concentration <u>1.0</u> mg/mL				
Tare Weight <u>0.0000</u> g				
Net Weight <u>0.10002</u> g Balance checked with Class S weights:				
Adjusted Net Weight <u>0.1000</u> g + <u>0.0000</u> g = <u>1.0001</u> g				
Corrected for Purity <u>N/A</u> 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: <u>MDH</u> Time: <u>2:20 pm</u> Date: <u>6-3-94</u>				
Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Vol. (L)	Final Conc. (mg/L)	
1.0	0	1.0	Control	
1.0	0.10	1.0	0.10	
1.0	0.18	1.0	0.18	
1.0	0.32	1.0	0.32	
1.0	0.56	1.0	0.56	
1.0	1.00	1.0	1.0	
1.0	1.80	1.0	1.8	
Remarks:	Balance: <input checked="" type="checkbox"/> Sartorius R 300 S: ABC Material # <u>1905-475</u>			
	<input type="checkbox"/> Other: _____			
Each Conc.: <u>2</u> Replicate <u>250</u> mL test vessels w/ <u>200</u> mL test vol.				
Control(s), Description: <u>Control receives 200ml of HARD Blend H₂O</u>				
*Lot # of vehicle used: <u>N/A</u>				
Reviewed by: <u>Marc Sward</u>			Date: <u>6/7/94</u>	
Study Director: <u>Marc Sward</u>			Date: <u>6/7/94</u>	

OBSERVATIONS AND/OR REMARKS FORM

Test Material: Various Protocol No. Various

Study Director: Various Study #: Various

2-11-93/KRT
 DE KRT 2-11-93
 Ten Pyrex brand beakers were chosen in an impartial manner from the supply of 250ml standard static beakers. Each of the 10^① 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:

<u>Beaker #</u>	<u>Depth</u>	<u>Width</u>
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

③ This supply of beakers is used for daphnia static testing. KRT 2-11-93

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By Kurt Sworel date 6-20-93

$\bar{X} (\pm S.D.)$ 6.3cm (±0.10) 6.3 (±0.05)

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

OBSERVATIONS AND/OR REMARKS FORM

Test Material: Various Protocol No.: Various

Study Director: Various Study #: Various

8-10-93 *gus* 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.

<u>Beaker #</u>	<u>Depth cm</u>	<u>Diameter cm</u>
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

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By *Luwa Sword* date *6-20-94*

NOTE: Individual entries must be dated and initialed.

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

Study Director: *Luwa Sword (signed copy)* Date: *6-20-94*

RANDOM GROUP ASSIGNMENT		PAGE 1
TEST MATERIAL :	C7-529-94	Analytical BioChemistry Labs 7200 East ABC Lane P.O. Box 1097 Columbia, MO 65205
STUDY NUMBER :	41673	
TEST GROUP :	Daphnia placement	
DATE :	06-03-1994	

Group # 1

n	ID	Concn (mg/L) mcs 6-3-94
1	4B	0.56
2	7B	3.2
3	9B	10
4	6B	1.8
5	2B	0.18
6	3A	0.32 0.32 (3)
7	4A	0.56
8	8B	5.6
9	5A	1.0
10	1B	0.1
11	6A	1.8
12	9A	10
13	CA	Control
14	CB	Control
15	5B	1.0
16	8A	5.6
17	3B	0.32
18	7A	3.2
19	2A	0.18
20	1A	0.1

Daphnids were removed from culture and placed in 20 scintillation vials labelled 1-20. Each vial received 10 daphnids. Each vial had approximately 10 ml of dilution water. The daphnids were then ⁽¹⁾transferred ⁽²⁾transferred to their respective test chambers following the above random table as a guide, mcs 6-3-94

(2) Transferred/removed with a large bore pipette, mcs 6-3-94
 (1) W mcs 6-3-94 (3) E mcs 6-3-94

DATA ENTERED BY: Marc Szwed DATE: 6-3-94
 REVIEWED BY: Jon Rhodes DATE: 6-20-94
 STUDY DIRECTOR: Marc Szwed DATE: 6-7-94

INVERTEBRATE CULTURE RECORD

Species: Daphnia magna Lot #: 94-AA
 Date Initiated: 5/21/94 # Used to Initiate: 10 or 15
 Lot # Initiated from: 94-AA Culture Volume: ~1500 mL
 Food Lot # A20 S20 A21 S21 A22 S22

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	-Neonates Present	Comments
5-21-94 <u>kw</u>	20	-	✓	-	15	
5-22 <u>kw</u>	20	-	✓	-	-	DEW-22-94
5-23 <u>kw</u>	20	✓	✓	15	0	
5-24 <u>kw</u>	20	-	✓	-	1	
5-25 <u>kw</u>	20	✓	✓	15	0	
5-26 <u>kw</u>	20	-	✓	-	-	
5-27 <u>AA</u>	20	✓	✓	15	0	
5-28 <u>SLH</u>	20	-	✓	-	1	
5-29 <u>SLH</u>	20	✓	✓	15	200	
5-30 <u>SLH</u>	20	-	✓	-	-	
5-31 <u>AA</u>	20	✓	✓	15	400	
6-1 <u>kw</u>	20	-	✓	-	-	
6-2 <u>kw</u>	20	✓	✓	15	0	
6-3 <u>kw</u>	20	-	✓	-	-	Removed a 200 for 41673 mod

*This is an exact copy of
 The original document*

Date received 6-20-94
 By

* Temperature measured with a mercury thermometer
 Note: Cultures may be fed one or more of the following:
 -0.5-1.0 mL of a 5.0 mg/mL suspension of trout chow yeast
 -1.0 mL suspension of dried algae (spirulina and/or chlorella)
 -1.5-4.0 mL of a 1.0 * 10⁸ cells/mL concentration of live algae (The algae consists of *Selenastrum* sp., *Ankistrodesmus* sp., *Chlamydomonas* sp., and/or *Chlorella* sp., and *Nitzschia* sp.)

Prepared by: [Signature] Date: 5-21-94
 Reviewed by: [Signature] Date: 6/20/94

INVERTEBRATE CULTURE RECORD

Species: Daphnia magna Lot #: 94-AA1
 Date Initiated: 5-21-94 # Used to Initiate: 10 or 15
 Lot # Initiated from: 94-AA Culture Volume: ~1500 mL
 Food Lot # 94 A22, S22 A23 S23 A24, S24

Date/ID	Temp.* °C	Changed Over	Fed	No. Adult Live	~Neonates Present	Comments
6-4 AA	20	✓	✓	15	200	
6-5 A	20	-	✓	-	-	
6-6 AA	20	✓	✓	15	900	
6-7 AA	20	-	✓	-	-	
6-8 AA	20	✓	✓	15	0	
6-9 AA	20	-	✓	-	-	Removed a 140 for 41677 MDH
6-10 AA	20	✓	✓	15	400	
6-11 AA	20	-	✓	-	-	
6-12 AA	20	✓	✓	15	450	
6-13 AA	20	-	✓	-	-	
6-14 AA	20	✓	✓	15	20	
6-15 AA	20	-	✓	-	-	
6-16 AA	-	-	-	-	-	terminated

This is an exact copy of
 The original document
 BY [Signature] date 6-20-94

* Temperature measured with a mercury thermometer
 Note: Cultures may be fed one or more of the following:
 -0.5-1.0 mL of a 5.0 mg/mL suspension of trout chow yeast
 -1.0 mL suspension of dried algae (spirulina and/or chlorella)
 -1.5-4.0 mL of a 1.0 * 10⁸ cells/mL concentration of live algae (The algae consist of *Selenastrum* sp., *Ankistrodesmus* sp., *Chlamydomonas* sp., and/or *Chlorella* sp., and *Nitzschia* sp.)

Prepared by: Amy Adams Date: 6-4-94
 Reviewed by: [Signature] Date: 6/20/94

EC ₅₀ Calculations	
ABC Study No. : 41673	Analytical Bio-Chemistry Labs
Compound Name : CT-529-94	7200 East ABC Lane
Species : Daphnia magna	P.O. Box 1097
Exposure Period: 24 Hours	Columbia, Missouri 65205
Study Director : Marc Sword	06-13-1994
	Page # 1

—RESULTS CALCULATED USING THE BINOMIAL METHOD—

CONC. µg/L	NUMBER EXPOSED	NUMBER IMMOBILE	PERCENT IMMOBILE	BINOMIAL PROB. (PERCENT)
10	20	11	55	41.19011975000094
5.6	20	9	45	41.19011975000094
3.2	20	1	5	2.002714205001754D-03
1.8	20	0	0	LESS THAN 0.001
1	20	0	0	LESS THAN 0.001
.56	20	0	0	LESS THAN 0.001
.32	20	0	0	LESS THAN 0.001
.18	20	1	5	2.002714205001754D-03
.1	20	0	0	LESS THAN 0.001

AT A CONFIDENCE LEVEL OF 95 PERCENT THE BINOMIAL TEST SHOWS THAT THE EC₅₀ IS ABOVE 3.2 µg/L

THE USEFULNESS OF ANY EC₅₀ 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.

AN APPROXIMATE EC₅₀ OF 7.48331356048584 µg/L IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN 5.6 AND 10

—RESULTS CALCULATED USING THE MOVING AVERAGE METHOD—

SPAN	G	EC ₅₀ , µg/L	95 PERCENT CONFIDENCE LIMITS	
			LOWER	UPPER
2	.2921844063278295	7.48331356048584	5.73049259185791	12.36324214935303
1	10.2756398023306	7.48331356048584	0	+INFINITY

PREPARED BY: Marc Sword
 REVIEWED BY: Jan Rhoads

DATE: 6/13/94
 DATE: 6-20-94

EC ₅₀ Calculations	
ABC Study No. : 41673	Analytical Bio-Chemistry Labs
Compound Name : CT-529-94	7200 East ABC Lane
Species : <i>Daphnia magna</i>	P.O. Box 1097
Exposure Period: 24 Hours	Columbia, Missouri 65205
Study Director : Marc Sword	06-13-1994
	Page # 2

—RESULTS CALCULATED USING THE PROBIT METHOD—

—RESULTS CALCULATED USING THE PROBIT METHOD—

ITER	G	H	CHI-SQUARE	PROBABILITY
7	3.024796581190769	11.49328084618432	80.45296592329024	(0.001

BECAUSE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.821309900524677

95 PERCENT CONFIDENCE LIMITS = -1.346301980569668 AND 4.988921781619043

EC₅₀ = 10.4204797744751 mg/L

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Method Reported: Binomial [] Moving Average [] Probit

Note: Method selected is that which gives the narrowest confidence limits for EC₅₀.

PREPARED BY: Marc Sword DATE: 6-13-94
 REVIEWED BY: Jon Rhodes DATE: 6-20-94

ABC LABS/041673

PS 0038

EC ₅₀ Calculations	
ABC Study No. : 41673	Analytical Bio-Chemistry Labs
Compound Name : CT-529-94	7200 East ABC Lane
Species : <i>Daphnia magna</i>	P.O. Box 1097
Exposure Period: 48 Hours	Columbia, Missouri 65205
Study Director : Marc Sword	06-13-1994

Page # 1

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

CONC. µg/L	NUMBER EXPOSED	NUMBER IMMOBILE	PERCENT IMMOBILE	BINOMIAL PROB. (PERCENT)
10	20	20	100	LESS THAN 0.001
5.6	20	20	100	LESS THAN 0.001
3.2	20	20	100	LESS THAN 0.001
1.8	20	20	100	LESS THAN 0.001
1	20	20	100	LESS THAN 0.001
.56	20	11	55	41.19011975000094
.32	20	4	20	.5900960178526391
.18	20	0	40	25.1722135618361
.1	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT .1 AND 1 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 EC₅₀ OF .51911652008816528 µg/L IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN .32 AND .56

---RESULTS CALCULATED USING THE MOVING AVERAGE METHOD---

SPAN	G	EC ₅₀ , µg/L	95 PERCENT CONFIDENCE LIMITS	
			LOWER	UPPER
5	3.1622052117469880-02	.3693223595619202	.2993587051524353	.4496173256642578
4	5.1752505411583760-02	.3692564362247986	.3113115429878235	.4451240599155426
3	.1605152378504331	.4155237125283466	.3132273256778717	.5472689270973206
2	.0975383807734645	.4915169775485992	.4091192483901978	.5734658241271973
1	.7542519358847143	.5191168189048767	.4060352444648743	2.321701282223267

PREPARED BY:

Marc Sword

DATE:

6/13/94

REVIEWED BY:

Jon Rhodes

DATE:

6-20-94

EC ₅₀ Calculations	
ABC Study No. : 41673	Analytical Bio-Chemistry Labs
Compound Name : CT-529-94	7200 East ABC Lane
Species : <i>Daphnia magna</i>	P.O. Box 1097
Exposure Period: 48 Hours	Columbia, Missouri 65205
Study Director : Marc Sword	06-13-1994
	Page # 2

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

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

ITER	G	H	CHI-SQUARE	PROBABILITY
5	.2927760491277674	2.569279110083679	17.98495377058576	1.203740780643381D-02

BECAUSE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.120622243009044

95 PERCENT CONFIDENCE LIMITS = 1.432091585341803 AND 4.809152900676285

EC₅₀ = .3778246641159058 mg/L

95 PERCENT CONFIDENCE LIMITS = .2265533953905106 AND .6088950037956238

Method Reported: [] Binomial Moving Average [] Probit

Note: Method selected is that which gives the narrowest confidence limits for EC₅₀.

PREPARED BY: Marc Sword

DATE: 6/13/94

REVIEWED BY: Jon Rhoads

DATE: 6-20-94

DOSE-RESPONSE CALCULATIONS

Test Material: CT-529-94ABC Study Number: 41673Protocol Number: 9405Study Director: Marc SwardData 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.	Calc. Probits	Calc. Probits
1	0.000	100.0	7.33	6.75
0.56	-0.252	55.0	5.13	5.77
0.32	-0.495	20.0	4.16	4.82
0.18	-0.745	40.0	4.75	3.85
0.1	-1.000	0.0	2.67	2.85

Regression Output:

Constant	6.74881
Std Err of Y Est	0.82248
R Squared	0.82288
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	3.89504
Std Err of Coef.	1.04329

$$Y = 3.895 X + (6.749)$$

$$\text{Dose Response Slope} = 3.90$$

$$\text{Correlation Coefficient (r)} = 0.91$$

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.

Data Entered By: Marc SwardDate: 6-20-94Reviewed By: Jane BourneDate: 6-22-94

COMPOUND RECEIPT

WVH
MC



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 ^①52~~4~~⁹-94

ABC SUPPLIED INFORMATION

Date Received: 04/21/94 Logged In By: RMF ABC Ref # TS-7238
Storage: Room Temp Total Weight: 436.9 g
Physical Description: Cloudy liquid
Remarks: _____

SUPPLIER PROVIDED INFORMATION

Firm: Cyttec
Address: 2715 Miller Road
City/State/Zip: Kalamazoo MI 49001 Phone: 616/349-6677
Batch/Lot No. 31 CAS # N/A Purity: N/A
Amount Declared: 250 ml Expiration Date: 2/95
Storage Instructions N/A
Other _____

① Compound mislabeled by Cyttec. Should be CT 529-94 as per
Patty Vernon on 4/26/94
RMF

**"This is an exact copy of
The original document"**

By Wendy Wood date 6-20-94

DAPHNID ACUTE TOXICITY BIOASSAY – IMMOBILIZATION						
<input type="checkbox"/> Definitive <input checked="" type="checkbox"/> Preliminary <input type="checkbox"/> Screen <input type="checkbox"/> Flow-Through <input checked="" type="checkbox"/> Static						
Test Material: <u>CT-529-94</u>		Protocol No.: <u>9405</u>				
Study Director: <u>Maic Sword</u>		Study #: <u>41673</u>				
Test Species: <input checked="" type="checkbox"/> <i>Daphnia magna</i> [] _____ (Lot # <u>94-4</u>) Waterbath #: <u>6</u> Dilution Water: <input checked="" type="checkbox"/> Hard Blended [] _____ # Instar/Vessel: <u>5</u> Date Initiated: <u>5-17-94</u> Time: <u>4:35 P.M.</u> By: <u>MCS</u> Date Terminated: <u>5-19-94</u>						
Test Conc. ($\mu\text{g/L}$)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
Control	0	SN, CLR	0	SN, CLR	0	SN, CLR
① 3.2 0.010	0	SN, CLR	0	SN, CLR	0	SN, CLR
① 1.0 0.032	0	SN, CLR	0	SN, CLR	0	SN, CLR
0.10	0	SN, CLR	0	SN, CLR	0	SN, CLR
0.32	0	SN, CLR	0	SN, CLR	1	3OB, 1OB, TR ②
1.0	0	SN, CLR	0	2OB, TR CLR 2OB, Q 1N	0	3OB 2OB, TR ②
3.2	0	3OB/Q, 2N, CLR	0	3OB, Q CLR 1TR, Q, 1Q	3	1OB, TR ② 1OB
ID/Date/Time	MCS 5-17-94 4:40 _{PM}		MCS 5-18-94 2:45 _{PM}		MDH 5/19/94 11:44 AM	
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 DEMCS 5-17-94 ② No solution obs. MCS 5-26-94						
Reviewed by: <u>John Brown</u>				Date: <u>5-26-94</u>		
Study Director: <u>Maic Sword</u>				Date: <u>5-26-94</u>		

COMPOUND PREPARATIONS: Preliminary Definitive

Test Material: CT-529-94 Protocol No.: 9405

Study Director: Marc Sword Study #: 41673

Purity % NA ABC Ref #: TS-7238 Batch/Lot #: 31

Prep. of Primary Standard/Weighed by: Marc Sword Date: 5/17/94
 Nominal Target Weight 0.0320 g Dil. Vol. 100 mL of D.I. H₂O
 Actual Gross Weight 0.0320 g Concentration 0.32 mg/mL
 Tare Weight 0.0000 g
 Net Weight 0.0320 g Balance checked with Class S weights:
 Adjusted Net Weight 0.0200 g + 0.0000 g = 0.0200 g
 Corrected for Purity _____ g (Class S) (tare) (final wt)

Prep. of Working Standard/Transferred by: Marc Sword Date: 5-17-94

Conc. of Parent Sol.	Aliq. Vol. (mL)	Dilution Vol. (mL)	Dilution Vehicle*	Final Concentration
0.32 mg/mL	3.125	1000	Hard Blended H ₂ O	1.0 mg/L
0.32 mg/mL	10	1000	Hard Blended H ₂ O	3.2 mg/L

Prep. of Test Conc./Transferred by: MCS Time: 4:30 PM Date: 5-17-94

Concentration of Standard (mg/mL)	Aliquot Volume (mL) (L)	Dilution Vol. (L)	Final Conc. (mg/L)
1.0	0.200	0.200	1.0
1.0	0.064	0.200	0.32
1.0	0.020	0.200	0.10
1.0	0.0064 0.0064	0.200	0.032
1.0	0.0020 0.0020	0.200	0.010
NA	NA	0.200	Control
3.2	0.200	0.200	3.2

Remarks: Balance: Sartorius R 300 S: ABC Material # 1905-475 *
 Other: _____

Each Conc.: 1 Replicate 250 mL test vessels w/ 200 mL test vol.
 Control(s), Description: Hard Blended Dilution Water Control, Tank (2)
 *Lot # of vehicle used: NA
 (1) F MCS 5-17-94 (2) E MCS 5-24-94
 (2) E MCS 5-17-94

Note: Stock sealed @ 100m temperature, Acute Hood, ABC Mat. Cont. #1905-685, MCS 5-17-94

Reviewed by: Jane Brown Date: 5-26-94
 Study Director: Marc Sword Date: 5-26-94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 / Protocol No. 9405

Study Director: Marc Sword Study #: 41673

Date: 6-20-94 Initials: MCS

The following 6 pages are not used as definitive test data. These data may be used as preliminary test data where appropriate. They are from an attempt of the definitive test that was not used for the following reason:

Control mortality

NOTE: Individual entries must be dated and initialed.

Reviewed by: Jane Brown Date: 6-22-94

Study Director: Marc Sword Date: 6-22-94

DAPHNID ACUTE TOXICITY BIOASSAY - IMMOBILIZATION

Definitive Preliminary Screen Flow-Through Static

Test Material: CT 529-94 Protocol No.: 9405

Study Director: MARC SWORD Study #: 41673

Test Species: *Daphnia magna* [] _____ (Lot # 94AA) Waterbath #: #6
 Dilution Water: Hard Blended [] _____ # Instar/Vessel: 10
 Date Initiated: 5-24-94 Time: 2:35PM By: MDH Date Terminated: 5/26/94

Test Conc. (mg/L)	Immobility and Behavioral Observations					
	0-Hour		24-Hour		48-Hour	
	Imm.	Obs.	Imm.	Obs.	Imm.	Obs.
Control A	0	10N, CLR	0	10N, CLR	10	-; CLR
Control B	0	10N, CLR	0	9N, 1TR, CLR	0	10N; CLR
0.10 A	0	11N, CLR	0	11N, CLR	0	11N; CLR
0.10 B	0	10N, CLR	0	10N, CLR	0	10N; CLR
0.18 A	0	10N, CLR	0	9N, 1TR, CLR	0	7OB, 3N; CLR
0.18 B	0	10N, CLR	0	10N, CLR	0	6OB, 4N; CLR
0.32 A	0	10N, CLR	0	7N, 3TR, OB, CLR	0	1TR, OB, 4OB; CLR
0.32 B	0	10N, CLR	0	5N, 5TR, OB, CLR	0	2TR, OB, 8OB; CLR
0.56 A	0	10N, CLR	0	2OB, 2N, 6TR, OB, CLR	0	3TR, OB, 7N, 7OB; CLR
0.56 B	0	10N, CLR	0	2N, 4OB, 4TR, OB, CLR	0	2TR, OB, 8OB; CLR
1.0 A	0	10N, CLR	0	2OB, TR-3N, 1TR-4OB, CLR	2	4TR, OB, 4OB; CLR
1.0 B	0	10N, CLR	0	3TR, OB, 2N-5OB, CLR	2	3TR, OB, 5OB; CLR
1.8 A	0	10N, CLR	0	3TR, OB, 1N, 6OB, CLR	10	-; CLR
1.8 B	0	10N, CLR	0	5TR, OB, 5OB, CLR	8	2OB; CLR
ID/Date/Time	MDH 5-24-94 2:50 PM		MDH 5-25 2:25 AM		MCS 3:00 P.M. 5/26/94	
Anal. ID Date/Time	N/A		N/A		N/A	

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
 Light Intensity Measurement = East 618.6 Lux 57.4 footcandles
 West 559.0 Lux 51.9 footcandles
 (1) E MCS 5/26/94 (2) 4 daphnids are in a clump, MCS 5/26/94 (3) W MCS 6-20-94

Reviewed by: Jon Plales Date: 6-20-94
 Study Director: Marc Sword Date: 6-20-94

COMPOUND PREPARATIONS: Preliminary Definitive

Test Material: CT 529-94 Protocol No.: 9405

Study Director: MARC SWORD Study #: 41673

Purity % N/A ABC Ref #: TS 7238 (Batch) Lot #: 31

Prep. of Primary Standard/Weighed by: MARVIN HOFFMAN Date: 5-24-94
 Nominal Target Weight 0.1000 g Dil. Vol. 100 mL of Hard blend
 Actual Gross Weight 0.1000 g Concentration 1.0 mg/mL
 Tare Weight 0.0000 g
 Net Weight 0.1000 g Balance checked with Class S weights:
 Adjusted Net Weight 0.1000 g + 0.0000 g = 0.1001 g
 Corrected for Purity N/A g (Class S) (tare) (final wt)

Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Vol. (L)	Final Conc. (mg/L)
<u>1.0</u>	<u>0</u>	<u>1.0</u>	<u>Control</u>
<u>1.0</u>	<u>0.10</u>	<u>1.0</u>	<u>0.10</u>
<u>1.0</u>	<u>0.18</u>	<u>1.0</u>	<u>0.18</u>
<u>1.0</u>	<u>0.32</u>	<u>1.0</u>	<u>0.32</u>
<u>1.0</u>	<u>0.56</u>	<u>1.0</u>	<u>0.56</u>
<u>1.0</u>	<u>1.0</u>	<u>1.0</u>	<u>1.0</u>
<u>1.0</u>	<u>1.8</u>	<u>1.0</u>	<u>1.8</u>
<u>1.0</u>	<u>3.2</u>	<u>1.0</u>	<u>3.2</u>
<u>1.0</u>	<u>5.6</u>	<u>1.0</u>	<u>5.6</u>
<u>1.0</u>	<u>10.0</u>	<u>1.0</u>	<u>10.0</u>

Prep. of Test Conc./Transferred by: MARVIN HOFFMAN Time: 2:05 PM Date: 5-24-94

Remarks: Balance: Sartorius R 300 S: ABC Material # 1905-475
 Other:

Each Conc.: 2 Replicate 250 mL test vessels w/ 200 mL test vol.
 Control(s), Description: Hard Blended Water
 *Lot # of vehicle used: N/A

Note: This data was not used as definitive test data. Int: MCS Date: 6-20-94

Reviewed by: Marc Sword Date: 5/24/94

Study Director: Marc Sword Date: 6/7/94

WATER QUALITY

Test Material: CT 529-94

Protocol No.: 9405

Study Director: MARC Sward

Study #: 41673

Concentration (mg/L)	MDH 5:25 AM		MDH 5:24 PM		MDH 5:25 PM		MDH 5:25 PM	
	Temp °C	DO mg/L	pH	Temp °C	DO mg/L	pH	Temp °C	DO mg/L
Control	21	7.7	8.3	21	7.8	8.3	21	7.9
0.10	21	7.8	8.3	21	7.8	8.3	21	7.8
0.18	21	7.8	8.3	21	7.7	8.3	21	8.0
0.32	21	7.8	8.3	21	7.9	8.3	21	8.1
0.56	21	7.7	8.3	21	7.7	8.3	21	8.1
1.0	21	7.8	8.3	21	7.9	8.3	21	8.1
1.8	21	7.9	8.3	21	7.8	8.3	21	8.2
3.2	21	7.9	8.3	21	7.9	8.3	21	8.1
5.6	21	7.8	8.3	21	7.9	8.3	21	8.2
10.0	21	7.7	8.3	21	7.8	8.3	21	8.1

- * Temperature measured with a mercury thermometer.
- ° Dissolved Oxygen probe (YSI Dissolved Oxygen System Model 54 A), ABC material control # 1905-730
- ° pH: Beckman 034 pH meter, ABC material control 163-640 used with a Beckman Model 39841 probe

Remarks: See water chemistry report 5-24-94
MDH 6-20-94 2:15 p.m. ACS 5-24-94
 Note: This data was not used as definitive test data. Int: ACS Date: 6-22-94

Prepared by: Marc Sward Date: 5-24-94
 Reviewed by: Jon Stiles Date: 6-20-94

ABC LABS 7041673

00051

RANDOM GROUP ASSIGNMENT		PAGE 1
TEST MATERIAL: CT-529-94	Analytical BioChemistry Labs	
STUDY NUMBER: 41673	7200 East ABC Lane	
TEST GROUP: DAPHNID PLACEMENT	P.O. Box 1097	
DATE: 05-24-1994	Columbia, MO 65205	

Group # 1

<u>n</u>	<u>ID</u> ①
1	6B
2	5B
3	2A 3A ②
4	4A
5	6A
6	3B
7	1A
8	9B
9	1B
10	2B
11	CA
12	CB
13	7A
14	7B
15	9A
16	4B
17	2A 2A ②
18	8A
19	5A
20	8B

Note: This data was not used as definitive test data. Int: MCS Date: 6-20-94

② These levels were inadvertently switched, MCS 5/24/94

① E MCS 5/24/94

(10 per replicate)
Daphnids are ~~plus~~ placed in scintillation vials numbered

1 - 20. Daphnids are then transferred to the test chambers

Following the above random placement + c/bk. MCS 5-24-94

① ID# Key: C = Control, 1 = 0.10, 2 = 0.18, 3 = 0.32, 4 = 0.56, 5 = 1.0, 6 = 1.8, 7 = 3.2, 8 = 5.6, and 9 = 10 (all in mg/L), MCS 5-24-94

DATA ENTERED BY: Mrs. Sworel DATE: 5/24/94

REVIEWED BY: Jon Stokes DATE: 6-20-94

STUDY DIRECTOR: Mrs. Sworel DATE: 6/7/94

SAMPLE COLLECTION FOR TOC ANALYSIS

Test Material: CT 529-94 Protocol No.: 9405

Study Director: MARC SWARD Study #: 41673

Date/ID: MDH 5-24-94

For each control or test level sampled, approximately ²⁰⁰100 mL of dilution water were drawn from ^{parent control solution} mid-chamber level of each replicate with a volumetric pipette and ^{placed} composited into an amber bottle for a total volume of approximately 200 mL. A second ^{composite} sample was collected in the same manner to retain as a back-up sample for each control or test level sampled. The samples were collected at 0 hour before ^{fish} addition. Samples were then prepared for total organic carbon (TOC) analysis by adding sulfuric acid until the pH was <2.0. The back-up sample(s) were then placed into a refrigerator at approximately 3°C for storage and the primary sample(s) were delivered to Engineering Surveys and Services Testing Laboratory in Columbia, MO, for TOC analysis.

Test Levels Sampled:

- Control
- Humic Acid Control
- Vehicle Blank
- Level 1
- Level 2
- Level 3
- Level 4
- Level 5

Sulfuric Acid

Lot #: 85-92-1

Supplier: Fisher

pH meter: Beckman Model ϕ 34 with Beckman Model 39841 pH probe
ABC Material Control #163-640

Refrigerator ABC Material Control #166-12

Notes: DF mcs 5/24/94
⊕ Information added 6-20-94 MDH

Note: This data was not used as definitive test data. Int: MCS Date: 6-20-94

Prepared by: Marie Hoffman Date: 5-24-94

Reviewed by: Jon Rhodes Date: 6-20-94

CYTEC

Order # 1738

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

ARC LABORATORIES

APR 26 1994

April 18, 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-529-94 Aquatic Toxicity Studies

Dear Dave:

You will receive, under separate cover, a material identified as CT-529-94. Please submit this material to the following tests at the following costs:

<u>Test</u>	<u>Protocol</u>	<u>Cost</u>	<u>Study #</u>
Acute Toxicity to Rainbow Trout	7601	██████████	→ 41672
Acute Toxicity to <u>Daphnia Magna</u>	7806	██████████	→ 41673
Acute Toxicity to Green Algae	OECD-201	██████████	→ 41674

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

Analytical Bio-Chemistry Laboratories, Inc.
Page 2
April 18, 1994

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-529-94 is an opaque liquid with an oily odor. This material is soluble in water but limited by viscosity. The following special handling conditions apply to this material: CAUSES EYE AND SKIN IRRITATION - Store at room temperature away from light and heat source. Avoid contact with iron, copper or aluminum equipment.

THE TOXICOLOGICAL PROPERTIES OF CT-529-94 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.

ABC LABS PO 41673

PS 0055

Analytical Bio-Chemistry Laboratories, Inc.
Page 3
April 18, 1994

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: APRIL 28, 1994

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.

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

pavABC

CALL MEMO

NAME: Patricia Ann Vernon
FIRM: Cytec Industries Inc.
STREET: 5 Garret Mountain Plaza
CITY: West Paterson **STATE:** NJ **ZIP:** 07424
PHONE: 201-357-3375 **FAX:** 201-357-3057

ABC CONTACT: Stephen L. Hicks
DATE: May 31, 1994

CIRCULATE TO: Study Personnel

TYPE OF WORK: DAP DAPFT DAPCHR DAPREN FISH
 ALG OTHER _____

COMPOUND: CT-529-94

NOTES:

I called Patty to confirm that test material received as CT-524-94, on April 21, 1994, should have been labeled CT-529-94. Patty did confirm that the correct name was CT-529-94.

**"This is an exact copy of
The original document"**

By A Hicks date 5-31-94

DEFINITIVE CONCENTRATION NOTIFICATION

Test Material: CT-529-94 Protocol No.: 9405

Study Director: Marc Sword Study #: 41673

Firm: Cytec Industries

Phone: (201) 357-3375

Sponsor's Study Representative: Patricia A. Vernon

Person Notified: Same (X) Other:

Date of Notification: 5-23-94

Notified by: Marc Sword

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

Vehicle: NA

<u>Test Material</u>	<u>ABC #</u>	<u>Test Species</u>	<u>Test Conc. (mg/L)</u>
CT-529-94	41673	Daphnia magna	Control, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg/L

Reviewed by: Jon Rhodes Date: 5-24-94

Study Director: Marc Sword Date: 5-24-94



"Working for You"

PROTOCOL ALTERATION NOTIFICATION

STUDY TITLE:	"Static Acute-Toxicity of CT-529-94 to <i>Daphnia magna</i> "		
PROTOCOL NO.:	9405	ALTERATION NO.:	1
LABORATORY:	ABC Laboratories, Inc.	LAB STUDY NO.:	41673
SPONSOR:	Cytec Industries	EFFECTIVE DATE:	June 23, 1994

DEVIATION:

- Protocol Section: Test Procedure - Chemical and Physical

Section 5.5.1 of the protocol states that water quality parameters (pH, dissolved oxygen, and temperature) will be measured at 0, 24, and 48 hours in all test chambers. Water quality parameters were not measured at 24 hours.

Reason:

OECD guideline 202 does not require 24 hour measurements, therefore, the sponsor requested that the measurements not be made.

Effect on Study: Deletes 24 hour water quality measurements from the study design.

STUDY DIRECTOR'S SIGNATURE:	<i>Marjorie</i>	DATE:	6/23/94
SPONSOR REPRESENTATIVE'S SIGNATURE	<i>Patricia Dowling</i>	DATE:	7/1/94



"Working for You"

PROTOCOL COVER SHEET

The protocol for ABC Laboratories study #41673 consists of copies of the following information:

ABC Laboratories Protocol: 9405

Study Authorization Letter Dated: April 18, 1994

Test Material: CT-529-94

Species: Daphnia magna

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

The proposed experimental start date is May 24, 1994

The proposed experimental completion date is May 26, 1994

Protocol Approval

ABC Laboratories' Study Director

Name (Signed): Marc C. Sword Date: 5/24/94

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

The above information has been collected and compiled into the protocol for study #41673. The signature date above constitutes the "Study Initiation Date".

7200 E. ABC Lane, Columbia, MO 65202

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



"Working for You"

ABC LABORATORIES

MAY 4 1994

ABC BLANKET PROTOCOL NO. 9405

(Revised April 22, 1994 for Cytex Industries)

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

ABC Study # _____

Test Substance _____

PROTOCOL APPROVAL

ABC Laboratories' Study Director

Name (signed) and Date: To be completed on the protocol cover sheet for each individual study

Name/Title (typed): To be completed on the protocol cover sheet for each individual study

Sponsor Representative

Name (signed): Patricia Ann Vernon Date: 4/27/94

Name/Title (typed): Patricia Ann Vernon, Associate Toxicologist

(Other sponsor-required signatures may be added below.)

TEST-SPECIFIC INFORMATION

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

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 substance. The sponsor also agrees to accept any and all of the test substance that remains unused at the end of testing and to assume responsibility for its proper disposal.

Testing Facility

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, MO 65202

Phone: (314) 474-8579
Fax: (314) 443-9089

Study Sponsor

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

Phone: (201) 357-3375
Fax #: (201) 357-3057

Test Substance(s) To be completed on the protocol cover sheet for each individual study
(name(s) used in report and correspondence)

Note: Written confirmation of percent purity along with specific activity and molecular weight, where applicable, must be provided.

Analytical Confirmation (Please check the appropriate statement.)

Analytical confirmation will be performed by ABC Laboratories, Inc.

Sponsor requests no analytical confirmation.

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

Test Concentrations Definitive test concentrations will be specified in a notification form or protocol alteration.

Test Organism

Species: *Daphnia magna*

Supplier: In-house culture

Experimental Dates

Proposed starting date: To be completed on the protocol cover sheet for each individual study

Proposed termination date: To be completed on the protocol cover sheet for each individual study

GLP Compliance (Check the most appropriate):

EPA-FIFRA _____; EPA-TSCA X ; FDA _____; OECD X ; Other _____

Special Instructions:

Special Instructions for may be included on the protocol cover sheet for each individual study.

This protocol may be used as the base protocol for *Daphnia magna* acute toxicity tests in dilution water amended with humic acid. If humic acid is added to the study design, the concentration of humic acid will be included on the protocol cover sheet for the individual study or in an amendment in a protocol alteration form. Studies adding humic acid to the study design will include appropriate humic acid control replicates during the definitive test.

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 OBJECTIVE

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 substance 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. *Daphnia magna* were chosen for this testing because they are representative of freshwater invertebrate species.

3.0 TEST SUBSTANCE

A letter of authorization for the study should be sent with the test substance or previous to its shipment and should contain the following chemical/physical properties of the compound if available: name of test substance, batch/lot number, physical description, purity, stability, suggested storage conditions, water and organic solvent solubility, available toxicity information, and handling precautions. The following parameters are of particular importance as prestudy information in the consideration process for analytical confirmation of test solutions: water solubility, vapor pressure, hydrolysis rate and photolysis rate. If analytical confirmation is desired, the study sponsor must submit

an appropriate analytical method to ABC. A MSDS 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 substance will be returned to the study sponsor at study completion. Archival of retention samples will be the sponsor's responsibility.

4.0 RANGE-FINDING STUDY

- 4.1 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 the preliminary test will be used to set concentration levels for the definitive bioassay.
- 4.2 Test Daphnids. The test lot of *Daphnia magna* will be obtained from an in-house culture. The taxonomic key presented by Pennak (11) will be used to periodically document the species of the culture daphnids. All test daphnids will be held in a controlled-temperature area at 20 (\pm 2) °C. The lighting will be 50-80 footcandles (approximately 533 to 866 lux) 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 use. The source of the fish food will be provided in the report text. First-instar daphnids (\leq 24 hours old) will be selected for testing and will not be fed during the test.
- 4.3 Exposure System. The range-finding test will be conducted in 250-mL glass beakers containing 200 mL of solution. These test vessels will be placed in a temperature-controlled area with temperature maintained at 20 (\pm 2) °C. The dilution water used in toxicity testing at ABC Laboratories will be ABC well water prepared to a total hardness of between 130 to 160 mg/L as CaCO₃. The specific water hardness will be achieved by blending naturally hard ABC well water with ABC well water that has been demineralized by reverse osmosis (R.O.). Before this water is used as test dilution water, it will be passed through a series of filters to reduce particulate matter and organic carbon content.
- 4.4 Test Procedure. The range-finding procedure is as follows:
 - 4.4.1 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.

- 4.4.2 The range-finding test will be initiated by exposing groups of five daphnids to at least three widely spaced toxicant concentrations, usually spaced by a factor of 10. If acute toxicity information is available from previous studies or if the study sponsor indicates that the material is basically nontoxic, only one preliminary test concentration may be used before the definitive test (e.g., 100 mg/L) to confirm this information. The test daphnids will be placed in the test chambers within 30 minutes after solution preparations. The initial toxicant concentrations most often used are 1, 10, and 100 mg/L. ABC's numerous static tests have shown that a significant percentage of the compounds tested have aquatic toxicities that fall within this range. No preliminary test will be performed if the sponsor requests a specific concentration regime.
- 4.4.3 After 24 hours of exposure, the test chambers will be observed for daphnid immobilization and/or adverse behavioral effects. A record will be maintained of immobilization and abnormal behavior at each observation. Based upon 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. For example, if the exposure results in total immobilization, new solutions will be prepared below the lowest initial concentration until no immobilization or partial immobilization is reached. In the converse situation, if no immobilization is observed, new solutions will be added at concentrations normally spaced by a factor of 10 above the highest initial level until immobilization is noted (to a maximum of 100 mg/L). In this manner, a bracket is formed for the toxic range of the compound. However, if no immobility is observed at preliminary test levels in the range of 100-1000 mg/L, additional concentrations may not be added.
- 4.4.4 The range-finding 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.
- 4.4.5 Results of the range-finding study will be used to set the concentration range of the definitive study.

5.0 DEFINITIVE STUDY

- 5.1 General. Following the range-finding study, the definitive test will be conducted by the procedures described below.
- 5.2 Test Daphnids. Aspects concerning the culture and acclimation of test daphnids will be the same as discussed in the range-finding study.
- 5.3 Exposure System. The exposure system for the definitive study will be the same as outlined in the range-finding study, except that duplicate test chambers per concentration will be used in the definitive biological test. A continuous record of the water bath temperature will be maintained and presented in the raw data of the toxicity report.
- 5.4 Test Procedure – Biological. The basic test procedure for the definitive bioassay will be as follows:
- 5.4.1 Test daphnids will be selected from a subculture of first-instar (≤ 24 hours old) individuals that have been cultured in the same water source as the test dilution water.
- 5.4.2 The definitive test will consist of duplicate replicates of at least five toxicant concentrations and duplicate dilution water controls with 10 daphnids each for a total of 20 daphnids per concentration or control. As an alternate test design, the definitive test will be conducted with triplicate test chambers at 100 or 1000 mg/L or at the water solubility limits of the test substance and triplicate dilution water control chambers. The definitive test concentrations will be selected after discussing preliminary results with the study sponsor and will follow a geometric or logarithmic series in which the ratio is between 1.5 and 2.0. Ten test daphnids will be impartially placed in each test chamber within 30 minutes after solution preparations, unless special, lengthy solution preparation (such as stirring overnight) is required. The method of preparation will be documented, if special preparation is needed.
- If a vehicle (organic solvent) is used to prepare test solutions, additional vehicle blank chambers will be used that will receive an aliquot of the solvent equivalent to the highest amount used in the test chambers. The vehicle concentration in any test chamber will not exceed 0.5 mL/L and, if possible, will be less than 0.1 mL/L. The vehicle used will be one of the following: dimethylformamide, triethylene glycol, methanol, acetone, or ethanol.

- 5.4.3 As an alternate test design, 4 replicate test chambers containing 5 daphnids each may be used.
- 5.4.4 The test chambers will be observed for immobilization and adverse behavioral effects at 24 and 48 hours (± 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, 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 $\leq 35\%$ and at least one concentration with immobilization of $\geq 65\%$ (8). The only exception to this is when maximum exposure testing occurs at the water solubility limit of the test substance or at a concentration of 100 mg/L or greater, where the test is designed to confirm no toxicity (or minimal toxic response, $< 50\%$) at these levels.

5.5 Test Procedure – Chemical and Physical.

- 5.5.1 Water quality parameters of temperature, dissolved oxygen, and pH will be monitored during the test. Measurements of these parameters will be made at 0, 24, and 48 hours of testing in each test concentration. At 0-hour, water chemistry will be performed on solutions before daphnids are distributed to testing chambers. Water chemistry will be performed at 24 and 48 hours on all test chambers. The continuous record of the water bath temperature maintained for the definitive study will be included in the raw data report. If appropriate, the 0 hour control/dilution water and/or humic acid control water may be analyzed for total organic carbon (TOC) concentration. If TOC is analyzed, the following location is added to the designation of testing facility in the "Test Specific Information" section of the protocol as the laboratory performing TOC analysis:

Engineering Surveys and Services
1113 Fay Street
Columbia, Missouri 65201

Phone: (314) 449-2646

- 5.5.2 In addition to the above water quality measurements for each test, the following water parameters will be measured at least weekly in the control/dilution water used for static daphnid testing: hardness, alkalinity,

pH, and conductivity. The nearest measurement prior to or on the initiation date of the study will be provided in the report.

5.5.3 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 wants to adjust test solutions, this must be authorized in writing before test initiation. If the sponsor wants aeration or pH adjustment, ABC recommends that the concentrations of test substance be measured by an appropriate analytical method to determine if these procedures have affected nominal exposure levels.

5.6 Test Procedure – Analytical Confirmation. The EPA suggests that test solutions be chemically analyzed to determine exact concentrations of the test substance versus nominal exposure levels. If the sponsor requests, all test concentrations will be analyzed at least at 0 and 48 hours in each test chamber. The measured test concentrations should not vary more than $\pm 20\%$ among replicate test chambers. The Test-Specific Information page will indicate who will be performing the analytical confirmation or if the sponsor wishes that no confirmation be performed. The specific method will be provided by the sponsor and 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. An analytical sampling scheme is attached, see Table I.

5.7 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, if possible. These values will be determined by a computer program developed by Stephan et al. (12). This program evaluates the data with a binomial, moving average, and probit analysis. If possible, the slope of the 48-hour dose-response line will be calculated for the 48-hour observation period. The slope will be calculated by a computer program that transforms percent immobility to probit values versus log of the concentration. A 48-hour no-observed effect level will be determined based on the absence of any abnormal effects or immobility.

6.0 DATA MAINTENANCE/REPORTING

6.1 Records to be Maintained. Records to be maintained will include, but not be limited to, compound receipts; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC);

material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until finalization of the study. Upon completion of the project, all raw data specifically for this study will be submitted to the sponsor as part of the final report.

6.2 Report. A final report containing all original raw data and/or certified copies of certain raw data records will be submitted to the sponsor. A copy of the report and associated raw data will be kept on file in ABC Laboratories' archives. The final report will include, but not be limited to, the following:

- 6.2.1 Study dates, name, and address of test facility.
- 6.2.2 Objectives and test procedures as stated in approved protocol.
- 6.2.3 A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
- 6.2.4 Description of test substance (date of receipt, storage conditions, purity, physical characteristics, and method of preparing stock and/or test solutions).
- 6.2.5 Description of test conditions during the study (vehicles used, dilution water, test temperature, lighting, pH, DO).
- 6.2.6 Description of methods used during the study.
- 6.2.7 Description of test organisms (source, culture techniques, etc.).
- 6.2.8 Summary of the data and a statement of the conclusions drawn from any data analyses, if appropriate.
- 6.2.9 Location of raw data.
- 6.2.10 List and signatures of study personnel.
- 6.2.11 GLP compliance statement by study director and a statement by ABC Laboratories' Quality Assurance Unit.
- 6.2.12 An appendix or separate raw data report will contain the original raw data or certified copies of raw data, letter of test authorization (if available), protocol alterations, the approved protocol, and the analytical

method appendix (if analytical confirmation is performed by ABC Laboratories).

7.0 DEFINITIVE TEST CONCENTRATION NOTIFICATION

After discussion with a representative of the study sponsor, definitive test concentrations will be specified in a notification form. This form will be signed and dated by the study director and will be attached to the protocol before the start of the definitive study. If after discussion with a sponsor representative it is determined that the definitive test concentrations are to be changed, the new concentrations will be specified in another notification form. The study director will sign and date the new form and will document on the form the reason for the change in definitive test concentrations.

8.0 PROTOCOL ALTERATIONS

The study director, upon approval of the sponsor representative, may make other alterations to this protocol. Proposed alterations, except for those which involve the test concentrations (see Definitive Test Concentration Notification above), will take the form of a written Protocol Alteration describing the alteration, the reason for the alteration, and the effect on the study, if any. All alterations will be signed and dated by both the study director and the sponsor representative. The signed Protocol Alteration Notification form will be maintained with the protocol.

Should a significant problem develop while the study is in progress, the study director will notify the sponsor representative as soon as practical to discuss the problem and any corrective actions taken. Upon verbal authorization from the sponsor representative, the study director will proceed with any further actions deemed appropriate. If the sponsor representative cannot be reached, the study director will proceed with the appropriate modifications and will notify the sponsor representative as soon as possible.

In the event of protocol deviations, an attempt will be made to notify the sponsor representative within a reasonable period of time. A written description of the deviation(s) will be submitted on a Protocol Alteration Notification form to the sponsor representative. All deviations will be signed and dated by both the study director and the sponsor representative.

9.0 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 guidelines listed in this protocol. The results of these inspections will be reported to the study director and ABC management. The draft and final reports will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures used

were followed. A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the study director and management.

10.0 GLP COMPLIANCE

This study will be conducted in accordance with U.S. EPA Good Laboratory Practice Standards; Toxic Substances Control (40 CFR, Part 792). The report will contain a statement attesting to that fact.

TABLE I
ANALYTICAL SAMPLING SCHEME

Sample	Sampling Time ^b (hrs)	
	0	48
Stock ^a	1x	---
Control	1x	1x
Vehicle Blank	1x	1x
Test Level 1	1x	1x
Test Level 2	1x	1x
Test Level 3	1x	1x
Test Level 4	1x	1x
Test Level 5	1x	1x
Total Samples and Fortifications ^c	18	17

^a The stock solution will be analyzed at study initiation.

^b At 0 hour extra solution for each control and test level will be prepared for collection of the 0 hour analytical samples. At 48 hours a composite of the two replicates from each control and test level will be analyzed.

^c Three QC fortifications bracketing the test levels will be prepared on each sample day.

11.0 REFERENCES

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- (12) Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for calculating an LC₅₀. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August 1978.

SPONSOR

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

RECEIVED

JUL 21 1994

P. A. VERNON

STUDY TITLE

Static Acute Toxicity of CT-529-94
to Rainbow Trout (*Oncorhynchus mykiss*)

DATA REQUIREMENT

OECD Guideline 203

AUTHORS

Marc C. Sword
Biologist II/Study Director

REPORT SUBMITTED ON

July 18, 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 #41672

Page 1 of 77

STUDY COMPLIANCE STATEMENT

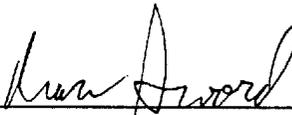
Study Compliance Statement for ABC Laboratories' Final Report #41672 entitled, "Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)," for Cytec Industries, West Paterson, New Jersey.

ABC Laboratories' study director for the above test herein confirms that the study was conducted in compliance with the OECD's Principles of Good Laboratory Practices (1); OECD Guidelines for Testing of Chemicals, Annex 2.

Neither the stability or purity of the test substance under test conditions was investigated. This was the responsibility of the study sponsor. The sponsor was responsible for retaining a sample 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 report, original and certified exact copies, were provided to Cytec Industries with the final report. A copy of the raw data and final report, along with facility records, was retained at ABC Laboratories, Inc.



Marc C. Sword
ABC Laboratories Study Director

7-18-94
Date

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed Study #41672, "Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)," for Cytex Industries, West Paterson, New Jersey.

The following inspections/audits were conducted on this study.

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Reported to Study Director</u>	<u>Date Reported to Management</u>
05-11-94	Addition of Fish	05-11-94	05-11-94
05-23-94	0-Hour Total Organic Carbon Analysis	05-23-94	05-23-94
06-14-94	Draft Report and Raw Data	06-14-94	06-15-94
07-13-94	Final Report	07-13-94	07-14-94

The undersigned conducted the report audits. The audits indicate the report is an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.

Draft Report: Lori A. Hoffman (by MDN) 7-18-94
Lori A. Hoffman, M.S. Date
Quality Assurance Specialist

Final Report: Greg Veltri 7/18/94
Greg Veltri Date
Quality Assurance Officer II

SIGNATURE PAGE

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Prepared by:

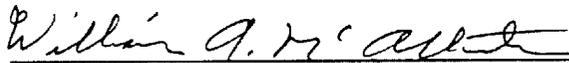


Marc C. Sword
Biologist II, Study Director

7-18-94

Date

Approved by:



William A. McAllister
Manager, Environmental Toxicology

7-18-94

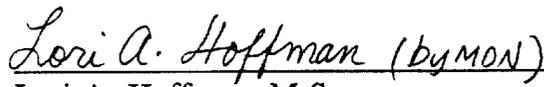
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Jon E. Rhodes
Team Leader, Environmental Toxicology

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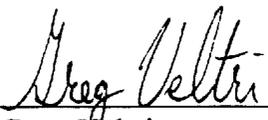
Date



Lori A. Hoffman, M.S.
Quality Assurance Specialist

7-18-94

Date



Greg Veltri
Quality Assurance Officer II

7/18/94

Date

:amk

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FISH ACUTE TOXICITY COMPENDIUM

Subject: "Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)," ABC Laboratories' Study Number 41672

Sponsor: Cytec Industries

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

Location of Original Raw Data and Final Report:

Cytec Industries
West Paterson, New Jersey

Test Substance: CT-529-94

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

Dilution Water: 144 mg/L as CaCO₃, (total hardness)
152 mg/L as CaCO₃, (total alkalinity)
8.1 (pH)
340 μMhos/cm (conductivity)

Experimental Test Dates: Start – May 11, 1994
Termination – May 15, 1994

Length of Study: 96 Hours

Results: 96-Hour LC₅₀ = 0.42 mg/L (95% C.I. = 0.32 to 0.56 mg/L)

96-Hour NOEC = 0.10 mg/L

96-Hour Slope of the Dose-Response Line = 19

Test Species: Rainbow Trout (*Oncorhynchus mykiss*)

Source of Organisms: Mt. Lassen Trout Farm
Red Bluff, California

Mean ± SD Wet Weight: 0.48 ± 0.12 g

Mean ± SD Standard Length: 35 ± 3 mm

Standard Length Range: 31 to 38 mm

SUMMARY

The acute toxicity of CT-529-94 to rainbow trout (*Oncorhynchus mykiss*) was assessed using the methods outlined by the protocol for this study. Water quality parameters of temperature, dissolved oxygen, and pH were measured throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following nominal concentrations of CT-529-94: 0.10, 0.18, 0.32, 0.56, and 1.0 mg/L. During the test, all control and test solutions were clear with no precipitate or surface film. Ten fish, with a mean \pm SD wet weight of 0.48 ± 0.12 g, a mean \pm SD standard length of 35 ± 3 mm, and a standard length range of 31 to 38 mm were exposed to each test concentration and control.

The results of the four day static fish toxicity study using CT-529-94 are summarized below. The 24-, 48-, and 72-hour LC₅₀ values were also determined.

<u>Test Substance</u>	<u>96-Hour LC₅₀</u> <u>(95% C.I.)</u>
CT-529-94	0.42 mg/L (0.32 to 0.56 mg/L)

The slope of the 96-hour dose-response line was 19. The 96-hour no-observed effect concentration was 0.10 mg/L, which was based on the lack of greater than 10% mortality or abnormal effects at this concentration. One fish was observed to have the effects of labored respiration and sitting on the bottom of the test chamber. However, the effects were transient and 10% effects or mortality are acceptable in the control fish. Therefore the effects seen in the 0.10 mg/L chamber were not considered in the estimation of the no observed effect concentration. Abnormal effects of mortality, loss of equilibrium, labored respiration, surfacing, fish on bottom of the test chamber, and/or quiescence were observed during the 96-hour exposure period in the 0.18, 0.32, 0.56, and 1.0 mg/L test concentrations.

INTRODUCTION

This static acute toxicity test was performed by the Environmental Toxicology division of ABC Laboratories, Inc., Columbia, Missouri, for Cytec Industries. The test was conducted from May 11, 1994, to May 15, 1994, as authorized in a letter of April 18, 1994. The purpose of this test was to determine the 24-, 48-, 72-, and 96-hour LC₅₀ values for CT-529-94 to rainbow trout (*Oncorhynchus mykiss*). A range-finding study was conducted from April 27, 1994, to May 1, 1994, to determine the concentration range for the definitive test. The study was conducted following the procedures outlined in ABC Protocol No. 7601 as approved by a representative of Cytec Industries on February 28, 1994 and by the study director on May 11, 1994.

METHODS AND MATERIALS

The procedures for static bioassays, as described in *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians* (2) and *Standard Methods for the Examination of Water and Wastewater* (3), were used in this experiment.

I. Test Fish

The 60 rainbow trout (lot #494) used in the test were obtained from Mt. Lassen Trout Farm, in Red Bluff, California. The rainbow trout were received as green eggs and milt and were fertilized on January 27, 1994, at ABC Laboratories. After hatching, the fish were reared and maintained in hard blended water and were fed newly hatched brine shrimp (*Artemia* sp.) and/or a commercially available fish food (salmon starter) daily. The fish food was received from Zeigler Bros. Inc. in Gardners, PA, and the brine shrimp was received from Aquarium Products in Glen Burnie, Maryland. All fish food was analyzed for contaminants and the results of those analyses are on file at ABC Laboratories.

The fish were held in ABC hard blended water at the approximate test temperature for approximately 10 weeks post hatch before the test. All test fish were held on a 16-hour daylight photoperiod with a 30-minute transition period and were observed for at least fourteen days prior to testing. Fish culture techniques were basically those described by Brauhn and Schoettger (4). A daily record of fish observations during the holding period, along with any prophylactic or therapeutic disease treatments, was maintained.

A subplot of fish was acclimated to the test temperature approximately 48 hours before the test. The fish were not fed during the acclimation and test periods. Weight and length measurements were made on the control group of fish at the termination of the test. The rainbow trout (*Oncorhynchus mykiss*) used as the control group for this experiment had a mean \pm SD wet weight of 0.48 ± 0.12 g, a mean \pm SD standard length of 35 ± 3 mm, and a standard length range of 31 to 38 mm. This gave a test chamber loading biomass of 0.32 g/L for the definitive study. The control group was representative of all the fish used in the definitive study.

II. Test Substance

The CT-529-94 sample (batch #31) was received from Cytec Industries on April 21, 1994, in good condition and was assigned ABC reference #TS-7238. The sample upon receipt was observed to be a cloudy liquid and was stored at room temperature. Sample purity was not specified. The expiration date given was February 1995. All test concentrations were based on the total substance, i.e. not corrected for sample purity. All standard weights and dilution values were recorded.

The definitive test concentrations were obtained by transferring appropriate aliquots of a working standard of the substance to the test chambers. The working standard was prepared by adding 0.150 g of test substance to 1000 mL of hard blended water. The test substance was weighed into a glass weigh boat, then quantitatively transferred to a 1-L volumetric flask by rinsing the weigh boat into the flask with ~500 mL of hard blended water. The solution was sonicated for ~15 minutes, then the flask was brought to volume with hard blended water. The resulting nominal concentration of the standard was 0.150 mg/mL. Appropriate aliquots of the standard were transferred to the test chambers at 0-hour and the solutions were stirred with a glass rod.

III. Test Water

The static toxicity test was conducted in five gallon glass vessels containing 15 liters of hard blended water. This dilution water was prepared by blending naturally hard well water with well water that had been demineralized by reverse osmosis (R.O.). This blended water was prepared to yield a total hardness of 130-160 mg/L as CaCO₃. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.0 mg/L and pH 8.2. Table I provides results of the most recent screening for selected water chemical/physical parameters and contaminants in blended dilution water. The test vessels were kept in a waterbath at 12 (\pm 2)^oC. Waterbath temperature was continuously recorded using a datalogger and thermistor probe.

IV. Biological Test Procedure

A range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 0.10, 1.0, 10, and 100 mg/L. Based on the results of preliminary testing, 5 concentrations of the test substance, ranging in a logarithmic series from 0.10 to 1.0 mg/L, were selected for the definitive bioassay. A dilution water control was also included. Ten fish were added to each control and test chamber in an impartial manner within 30 minutes after addition of test substance. All test organisms were observed once every 24 hours for mortality and abnormal (sublethal) effects. Dead individuals, if any, were removed from the test chambers after each 24-hour observation. The test was on a 16-hour daylight photoperiod with a 30-minute transition period. Light intensity measured over the waterbath during the test averaged 63 footcandles (678 lux). Light measurement data are kept on file at ABC Laboratories.

Temperature (°C), dissolved oxygen (mg/L), and pH were measured at 0, 24, 48, 72, and 96 hours of testing in all test chambers. Approximately 200 mL of control water were collected at 0 hour before fish addition with a volumetric pipette from mid-chamber level for analysis of total organic carbon (mg/L TOC). The sample was placed in an amber bottle and pH was reduced to <2.0 with sulfuric acid. The sample was delivered to Engineering Surveys and Services in Columbia, Missouri for TOC analysis. The sample was analyzed in triplicate. A backup sample collected in the same manner was retained at ABC Laboratories under refrigeration.

V. Biological Data Analysis

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC₅₀ program developed by Stephan et al. (5). If possible, this program calculated the LC₅₀ statistic and its 95 percent confidence limits using the binomial, moving average, and probit tests. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<37 to >63%), an LC₅₀ and/or its 95 percent confidence limits could not be calculated. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC₅₀ (5, 6) although all three models are valid. The dose-response line was calculated using least squares regression analysis of percent mortality transformed to probits versus log concentration. All calculations were based on the nominal concentrations of CT-529-94.

RESULTS AND DISCUSSION

The definitive test was successfully completed on May 15, 1994. An examination of the fish culture and acclimation records for this test indicated that the fish were in good condition for testing.

Water quality data are presented in Table II. The dissolved oxygen concentrations ranged from 6.8 to 9.0 mg/L during the test. These values represented 67 and 89% saturation at 13°C, respectively, and were considered adequate for testing (2), therefore, the test chambers were not aerated during testing. The pH values ranged from 7.7 to 8.3. Temperature measured with a mercury thermometer ranged from 12.1 to 13.1 °C. Temperature measured continuously in the waterbath during the test was within the protocol specified temperature range. During the test, all control and test solutions were clear.

Total Organic Carbon (TOC) measured in triplicate on control water collected at 0 hour before fish were added to the test chambers was <1.0, <1.0, and <1.0 mg/L (\bar{x} = <1.0 mg/L).

The 0.56 and 1.0 mg/L test concentrations both elicited 100% cumulative mortality at 96 hours. No mortality was observed in the control, 0.10, 0.18, or 0.32 mg/L test concentrations. Mortality and abnormal/sublethal effects data are presented in Table III. The abnormal effects of mortality, loss of equilibrium, labored respiration, surfacing, fish on bottom of the test chamber, and/or quiescence were observed in the 0.10, 0.18, 0.32, 0.56, and 1.0 mg/L test

concentrations during the 96-hour exposure period. All control fish and 90% of the 0.10 mg/L test concentration fish were normal throughout the exposure period.

The results of the 96-hour static toxicity test with rainbow trout (*Oncorhynchus mykiss*) exposed to CT-529-94 are presented in Table IV. The 24-, 48-, 72-, and 96-hour LC_{50} values for CT-529-94 were 0.70, 0.42, 0.42, and 0.42 mg/L, respectively. The no-observed effect concentration (NOEC) was 0.10 mg/L based on the lack of greater than 10% mortality or abnormal effects at this concentration. One fish was observed to have the effects of labored respiration and sitting on the bottom of the test chamber. However, the effects were transient and 10% effects or mortality are acceptable in the control fish. Therefore the effects seen in the 0.10 mg/L chamber were not considered in the estimation of the no observed effect concentration. The slope of the 96-hour dose-response line for rainbow trout exposed to CT-529-94 was 19 as calculated by least squares regression analysis of percent mortality transformed to probits versus log concentration (Figure 1).

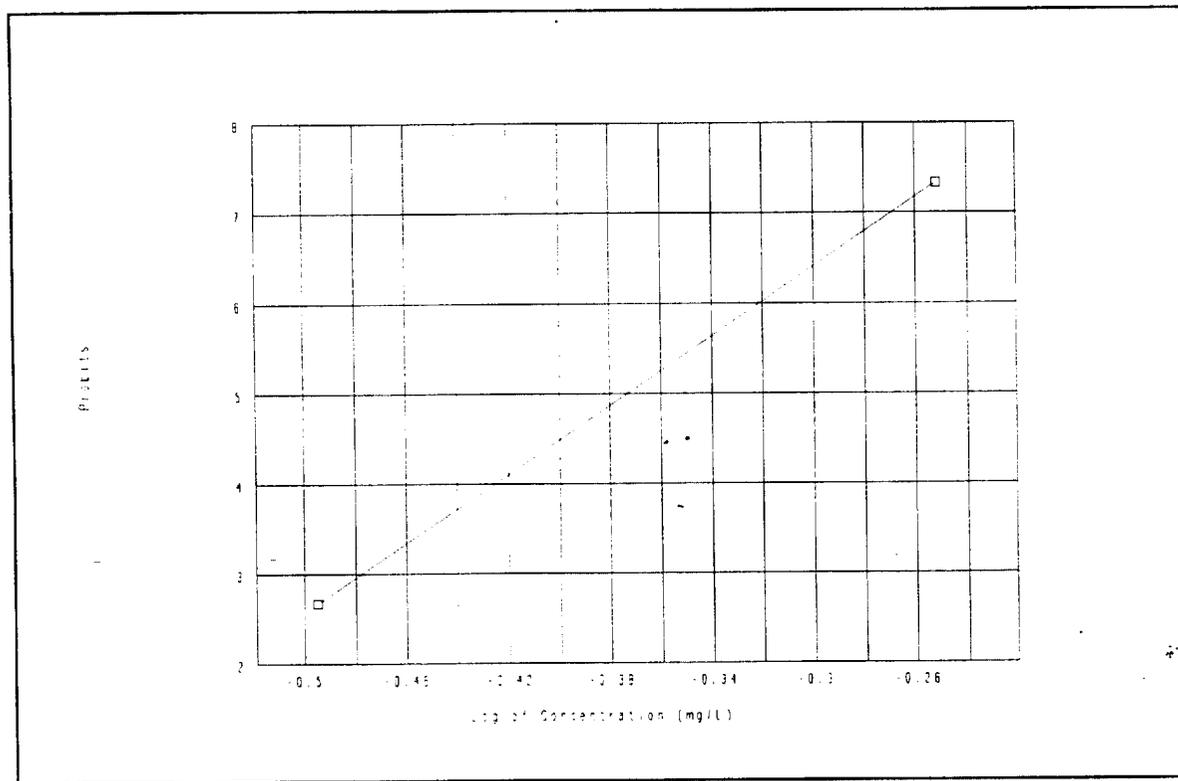


Figure 1: The 96-Hour Dose-Response Line for Rainbow Trout (*Oncorhynchus mykiss*) Exposed to CT-529-94

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

TABLE I

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

Dilution Water Screen ^a		Monthly Screens (mg/L) ^b	
Hardness	144 mg/L (as CaCO ₃)	Total Organic Carbon	< 1.00
Alkalinity	152 mg/L (as CaCO ₃)	Suspended Solids	0.2
pH	8.1		
Conductivity	340 μ Mhos/cm		
Biyearly Screens ^c			
Elements (mg/L)		Chlorinated Hydrocarbons (μ g/L)	
Aluminum	< 0.20	DDE	< 0.01
Arsenic	< 0.01	DDD	< 0.01
Boron	0.246	DDT	< 0.01
Cadmium	< 0.0005	Dieldrin	< 0.01
Chromium	< 0.001	Endrin	< 0.01
Cobalt	< 0.050	α -BHC	< 0.01
Copper	< 0.020	β -BHC	< 0.01
Fluoride	0.8	γ -BHC-Lindane	< 0.01
Iron	< 0.10	Δ -BHC	< 0.01
Lead	< 0.003	Heptachlor	< 0.01
Mercury	0.00024	Heptachlor Epoxide	< 0.01
Nickel	< 0.05	Aldrin	< 0.01
Selenium	< 0.005	Chlordane	< 0.3
Silver	< 0.0005	Methoxychlor	< 0.05
Zinc	< 0.04	Toxaphene	< 4.0
		Endosulfan Sulfate	< 0.03
		Endosulfan I	< 0.01
		Endosulfan II	< 0.01
		HCB	< 0.01
		Mirex	< 0.01
		Telodrin	< 0.01
		PCBs	< 1.0
Organophosphate (μ g/L)		Miscellaneous (mg/L)	
Diazinon	< 0.1	Chemical Oxygen Demand	< 10
Methyl Parathion	< 0.02	Un-Ionized Ammonia	< 0.1
Ethyl Parathion	< 0.02	Nitrite N	< 0.02
Malathion	< 0.05	Nitrate N	0.16
Ronnel	< 0.01	Total Phosphorus	< 0.05
Ethion	< 0.02	Chlorine Residual	< 0.02
Trithion	< 0.05		
Vapona	< 2.0		

^a Represents the values measured on dilution water used for the test

^b Represents the values obtained from the screens of May 1994

^c Represents the values obtained from the screen of January 1994

Note: Results of the chemical analyses supporting these data are on file at ABC Laboratories.

TABLE II

Water Quality Measurements During the Static Acute Toxicity Test
of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)

Nominal Test Conc. (mg/L)	Water Quality								
	0 Hour			24 Hours			48 Hours		
	Temp. ^a °C	DO ^b mg/L	pH ^c	Temp. °C	DO mg/L	pH	Temp. °C	DO mg/L	pH
Control	12.5	9.0	8.2	12.9	8.0	8.0	12.1	8.0	7.9
0.10	12.9	8.9	8.3	12.9	8.1	8.1	12.3	8.1	7.9
0.18	13.1	8.8	8.3	12.9	8.2	8.2	12.4	8.1	8.1
0.32	13.0	8.8	8.3	12.8	7.9	8.1	12.3	7.6	8.0
0.56	12.9	8.8	8.3	12.8	7.8	8.1	12.3	7.9	8.1
1.0	12.8	8.8	8.3	12.9	7.3	8.2	12.3	8.6	8.2

^a Temperature measured with a digital thermometer built into Beckman Model Φ 34 pH meter[†]

^b Dissolved oxygen concentrations – YSI Dissolved Oxygen System Model 54 ARC

^c pH – Beckman Model Φ 34 pH meter with a Beckman Model 39841 probe

NOTE: Dissolved oxygen saturation values corrected for altitude at the test temperatures of 12 and 13°C are 10.3 and 10.1 mg/L, respectively.

Total Organic Carbon (TOC) measured in triplicate on control water collected at 0 hour before fish were added to the test chambers was <1.0, <1.0, and <1.0 mg/L (\bar{x} = <1.0 mg/L).

TABLE II (continued)

Water Quality Measurements During the Static Acute Toxicity Test
of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)

Nominal Test Conc. (mg/L)	Water Quality					
	72 Hours			96 Hours		
	Temp. ^a °C	DO ^b mg/L	pH ^c	Temp. °C	DO mg/L	pH
Control	* 12.8	7.7	7.7	13.0	7.6	7.7
0.10	13.0	7.8	7.8	13.0	7.7	7.8
0.18	13.0	7.9	8.0	13.0	7.9	8.0
0.32	13.0	6.9	7.8	13.0	6.8	7.8
0.56	13.0	7.9	8.0	13.1	7.8	8.1
1.0	13.0	8.7	8.2	13.1	8.7	8.3

^a Temperature measured with a digital thermometer built into Beckman Model Φ 34 pH meter ^t

^b Dissolved oxygen concentrations – YSI Dissolved Oxygen System Model 54 ARC

^c pH – Beckman Model Φ 34 pH meter with a Beckman Model 39841 probe

NOTE: Dissolved oxygen saturation value corrected for altitude at the test temperature of 13°C is 10.1 mg/L.

TABLE III

Mortality and Behavioral Observations During the Static Acute Toxicity Test of
CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)

Nominal Test Conc. (mg/L)	Number Placed in Test	24 Hours		48 Hours		72 Hours		96 Hours	
		Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations
Control	10	0	10 N	0	10 N	0	10 N	0	10 N
0.10	10	0	10 N	0	1 LR/OB; 9 N	0	10 N	0	1 OB; 9 N
0.18	10	0	4 LR; 6 N	0	4 LR; 6 N	0	1 LR; 9 N	0	1 OB; 9 N
0.32	10	0	4 LR/OB/Q; 6 LR	0	4 LR/OB/Q; 5 LR; 1 LR/OB	0	3 LR/OB/Q; 3 SUR/LR; 4 LR	0	1 OB/LR; 9 LR
0.56	10	1	2 SUR/LR; 3 LR; 2 LR/OB; 2 LOE/LR	10	---	10	---	10	---
1.0	10	10	---	10	---	10	---	10	---

Key to Observations: N = Normal; LOE = Loss of Equilibrium; Q = Quiescent; SUR = Surfacing; LR = Labored Respiration; OB = Fish on Bottom of Test Chamber

TABLE IV

Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)

Test Substance	LC ₅₀ in mg/L			
	24-Hour	48-Hour	72-Hour	96-Hour
CT-529-94 ^a	0.70 ^b	0.42 ^b	0.42 ^b	0.42 ^b
	(0.56 and 1.0) ^c	(0.32 and 0.56) ^c	(0.32 and 0.56) ^c	(0.32 and 0.56) ^c

N = 10 fish per concentration

^a Toxicity test as conducted at $12 \pm 2^\circ\text{C}$, mean \pm SD fish wet weight and standard length, 0.48 ± 0.12 g and 35 ± 3 mm, standard length range 31 to 38 mm.

^b The LC₅₀ was calculated using the binomial method.

^c 95% confidence limits.

The 96-hour no-observed effect concentration was 0.10 mg/L, based on the lack of greater than 10% mortality or abnormal effects at this concentration.

REFERENCES

- (1) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30(Final):7-28.
- (2) Committee on Methods for Toxicity Tests with Aquatic Organisms (C. E. Stephan, Chairman). 1975. *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians*. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p.
- (3) American Public Health Association. 1980. *Standard Methods for the Examination of Water and Wastewater*. 15th ed. Washington, DC. 1134 p.
- (4) Brauhn, J. L. and Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May, 1975. 45 p.
- (5) 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.
- (6) Stephan, C. 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.

RAW DATA APPENDICES

ABC Laboratories Study # 41672

Static Acute Toxicity of CT-529-94
to Rainbow Trout (*Oncorhynchus mykiss*)

NOTE: Some of the records that appear in this raw data appendix 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 acclimation records, organism culture logs, and blended water chemical screen results.

Some of the following abbreviations may have been used in the raw data:

E - Entry Error

S - Spelling Error

F - Form Change

W - Writeover

R - Recording Error

T - Transcription Error

C - Calculation Error

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.

March Sword
Biologist II, Study Director

Jane Bowman
Biologist III

Dorothy England
Biologist II

Marvin D. Hoffman
Biologist I

Warren Railton
Laboratory Technician II

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

**"This is an exact copy of
The original document"**

NAME	SIGNATURE ^{over pencil}	INITIAL ⁶⁻¹⁵⁻⁹⁴	DATE
William J. Adams	<i>William J. Adams</i>	WJA	2/9/94
William A. McAllister	<i>William A. McAllister</i>	WAM	2-4-94
Alan D. Forbis	<i>Alan D. Forbis</i>	AF	2-4-94
Jon E. Rhodes	<i>Jon E. Rhodes</i>	JR	2-4-94
James B. Bussard	<i>James Bussard</i>	JBB	2-9-94
Tom Leak	<i>Tom Leak</i>	TL	2-4-94
Charles E. Jameson	<i>Charles E. Jameson</i>	CEJ	2-4-94
Timothy J. Madsen	<i>Timothy J. Madsen</i>	TJM	02-04-94
Stephen L. Hicks	<i>Stephen L. Hicks</i>	SLH	2-7-94
Scott J. Voney	<i>Scott J. Voney</i>	SV	2-7-94
Paul Cohle	<i>Paul Cohle</i>	PC	2-4-94
Doug Gledhill	<i>Doug Gledhill</i>	DG	2-7-94
Hugh Murrell	<i>Hugh Murrell</i>	HM	2-4-94
Ryan Warbritton	<i>Ryan Warbritton</i>	RW	2-4-94
Amy Adams	<i>Amy Adams</i>	AA	2-8-94
Robert Pezold	<i>Robert Pezold</i>	RGP	2-4-94
Marc C. Sword	<i>Marc C. Sword</i>	MCS	2/4/94
Tammy Strawn	<i>Tammy Strawn</i>	TS	2-7-94
Dorothy C. England	<i>Dorothy C. England</i>	DCE	2/7/94
Warren Railton	<i>Warren Railton</i>	WR	2-4-94
Jane H. Bowman	<i>Jane Bowman</i>	JHB	2-4-94
Janelle L. Downing	<i>Janelle L. Downing</i>	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	<i>Laurie L. Roesel</i>	LAR	2-7-94
Michelle A. Muckerman	<i>Michelle A. Muckerman</i>	MM	2/7/94
Jamie L. Veltri	<i>Jamie L. Veltri</i>	JLV	2/7/94
John Bucksath	<i>John Bucksath</i>	JDB	2/7/94
Kathryn Konering	<i>Kathryn Konering</i>	KK	2-8-94
Bret Hurshman	<i>Bret A. Hurshman</i>	BAH	2-8-94
Luke Stuerman	<i>Luke Stuerman</i>	LMS	2/8/94
Gerald A. Nothdurft	<i>Gerald A. Nothdurft</i>	GAN	2-7-1994
Yuan Yang	<i>Yuan Yang</i>	YY	2-7-94
Jianping Liu	<i>Jianping Liu</i>	JL	2/8/94
Karen March	<i>Karen March</i>	KM	2-8-94
David Burgess	<i>David Burgess</i>	DB	2-8-94
Debbie Jameson	<i>Debbie Jameson</i>	dj	2-7-94
Anita M. Klick	<i>Anita M. Klick</i>	ANK	2-9-94
Donna S. Hoek	<i>Donna S. Hoek</i>	DSTH	2/7/94
John Ingersoll	<i>John Ingersoll</i>	J	2-8-94
Edward Harper	<i>Edward Harper</i>	EJH	2-8-94
D. Abram	<i>D. Abram</i>	DA	5-3-94
Christopher J. Pope	<i>Christopher J. Pope</i>	CJP	5-24-94
Marvin D. Hoffman	<i>Marvin D. Hoffman</i>	MH MDH	6-15-94

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The original document"**

By Wen Anzou date 6/15/94

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.

SIGNATURE & INITIAL IDENTIFICATION ENGINEERING SURVEYS AND SERVICES

NAME & TITLE	SIGNATURE	INITIAL	DATE
Chris Segafredo, Director of Environ- mental Services	<i>Chris Segafredo</i>	<i>CS</i>	30 Dec 93
Linda Adams, Laboratory Manager	<i>Linda L. Adams</i>	LA	30 Dec 93
Rebecca Ann Bergfield, Chemist	<i>Rebecca Bergfield</i>	RB	30 Dec 93
Tina M. Tucholski, Chemist	<i>Tina M. Tucholski</i>	TMT	30 Dec 93
This is an exact copy of The original document"			
By <u><i>Ann Award</i></u> date <u><i>6-15-94</i></u>			

Note: This list includes personnel of Engineering Surveys and Services Testing Laboratories located at 1113 Fay Street in Columbia, Missouri, who provided analyses in support of various studies performed by the Environmental Toxicology division of ABC Laboratories. This list of personnel is not study specific. Primary project personnel will be identified separately for each project.

MORTALITY AND BEHAVIORAL OBSERVATIONS - FISH

Test Material: CT-529-94 Protocol No.: 7601 Definitive
 Study Director: Marc Sword Study #: 41672 Preliminary
 Test Species: *L. macrochirus* *O. mykiss* *P. promelas*
 No./Vessel: 10 Dil. Water: Blended Fish Lot #: 494 Fish Fed: NO
 Acclimation Mortality: 0 Waterbath: 1 Date Initiated: 5-11-94
 Compound Added: 2:00 pm Fish Added: 2:25 PM Date Terminated: 5-15-94

Test Conc. mg/L (ppm)	24 Hours		48 Hours			72 Hours			96 Hours		
	Dead	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.
Control	0	10 N	0	0	10 N	0	0	10 N	0	0	10 N
0.10	0	10 N	0	0	1 LR/OB 9 N	0	0	10 N	0	0	1 OB 9 N
0.18	0	4 LR 6 N	0	0	4 LR 6 N	0	0	1 LR 9 N	0	0	1 OB 9 N
0.32	0	4 LR/OB/Q 6 LR	0	0	4 LR/OB/Q 1 LR/OB 5 LR	0	0	3 LR/OB/Q 3 SUR/LR 4 LR	0	0	1 OB/LR 9 LR
0.56	1	2 SUR/LR 2 LR/OB 2 LOE/LR 3 LR	9	10	—	0	10	—	0	10	—
1.00	10	—	0	10	—	0	10	—	0	10	—

Observer Date/Time: JAD 5-12-94 2:35 pm MDH 5-13-94 2:20 pm JAD 5-13-94 5-14-94 1:50 pm JMD 5-15-94 2:05 pm

REMARKS: N = Normal; LOE = Loss of Equilibrium; Q = Quiescent; SUR = Surfacing; ES = Erratic Swimming; DK = Dark Discoloration; OB = On Bottom; LR = Labored Respiration

QA Procedure Audit ① JAD 5-14-94
② MDH 5-16-94

Prepared by: Marvin Hoffman Date: 5-11-94
 Reviewed by: Marc Sword Date: 5-26-94
 Approved by: Marc Sword Date: 5-26-94

WATER QUALITY

Test Material: CT-529-94 Protocol No.: 7601
 Study Director: Marc Sward Study #: 416767 41672-
 Date: 5-13-94 2 Day MDH 2:11PM

Concentration (mg/L)	0 Day MH 5-11-94						5-13-94 2 Day MDH					
	Temp °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e	Day	Temp °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e	
Control	12.5	9.0	8.2	-	-	JHD	12.1	8.0	7.9	-	-	
0.10	12.9	8.9	8.3	-	-	JHD	12.3	8.1	7.9	-	-	
0.18	13.1	8.8	8.3	-	-	JHD	12.4	8.1	8.1	-	-	
0.32	13.0	8.8	8.3	-	-	JHD	12.3	7.6	8.0	-	-	
0.56	12.9	8.8	8.3	-	-	JHD	12.3	7.9	8.1	-	-	
1.00	12.8	8.8	8.3	-	-	JHD	12.3	8.1	8.2	-	-	

a Temperature measured with a mercury thermometer. C
 b Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54 ARC) ABC material control #1905-730
 c pH: Beckman 34 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
 d Hardness and total alkalinity (mg/L as CaCO₃) analyzed using titrimetric method adopted from Standard Methods
 e Other: 5-11-94
 ① Beckman meter has built in digital thermometer, MCS-15-94
 ② classification MCS 5-26-94
 ③ date recorded on pages 8, assuming water is Dissolved Oxygen, assigning value of 80, MCS 6/13/94

Remarks: DF MDH 5-12-94 Entry made on 5-12-94 MDH 5-13-94 Entry made on 5/13/94
 Prepared by: Marc Sward Date: 5-11-94
 Reviewed by: Marc Sward Date: 5-26-94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 Protocol No. 7601Study Director: MARC SWORD Study #: 41672

5-11-94 MDH Checked out Material (CT-529-94) for Compound preparation. 0.150 g of CT-529-94 was weighed out into a glass ~~tare~~ weigh boat on a Calibrated Mettler balance. The glass weigh boat was rinsed into a 1000 ml flask with \approx 500 ml of DI H₂O. The flask was then placed into the sonicator for approximately 15 minutes. The flask was labeled with; Study #, Compound, Study Director & concentration level. When flask was removed from Sonicator \approx 500 ml more of DI H₂O was added to bring Volumetric flask up to a volume of 1000 ml. Test chambers were labeled with; Study #, Concentrations, Study Director, and Compound #. TOC (2 bottles) were collected from the Control level at 1:55 pm. AT 2:00 pm the Standard was aliquoted into the test chambers. All of the solutions in the test chambers appear clear with no precipitate.

5-12-94 JHB all solution clear with no visible precipitate or surface film.

5-13-94 MDH All Test JARS resemble the previous days observation

5-14-94 JHB all solutions appear same as on 5-12-94

5-15-94 JHD all solution appear same as on 5-12-94

DEMOMH 5-11-94 \oplus also labeled with date. mcs 6/11/94

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword Date: 5-26-94

Study Director: Marc Sword Date: 5-26-94

Continuous Temperature Graph

Test Material: CT-529-94

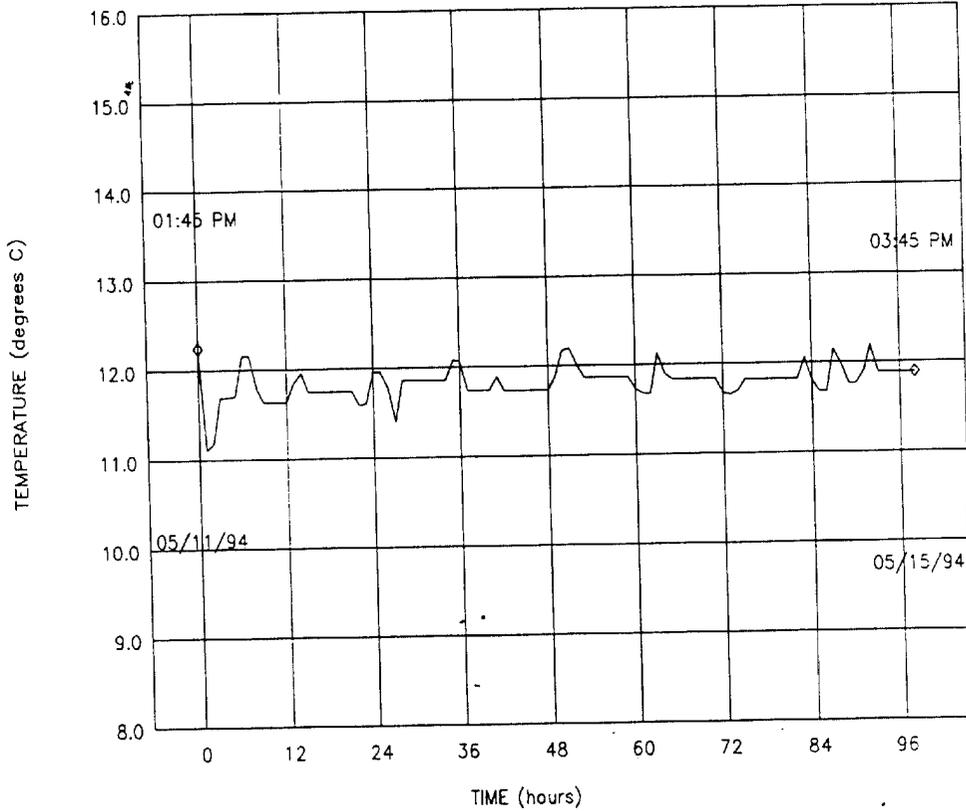
ABC Study Number: 41672

Protocol Number: 7601

Lab Form No: AQ9 (4/15/91)
ABC Laboratories
P.O.Box 1097, 7200 East ABC Lane
Columbia, Missouri 65205

Data Logger
Material Cont. No.: 1905-425

Waterbath No. 1
DEFINITIVE TEMPERATURE RECORDING



Comments: _____

Prepared By: *Kevin Sword* Date: 6/14/94
Reviewed By: *Jane Bowman* Date: 6-14-94
Approved By: *Kevin Sword* Date: 6-15-94

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: WAm Warm Hard Bleached

TEMPERATURE: 24.3 °C

DO: 8.2 mg/L

pH: 8.1

ALKALINITY:* 152 mg/L

HARDNESS:* 144 mg/L

CONDUCTIVITY: 340 μmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE Mercury Digital

METER TYPE	BRAND	MODEL	MATERIAL CODE
DISSOLVED OXYGEN	<input checked="" type="checkbox"/> YSI	54ARC	1905-730
pH Meter:	<input checked="" type="checkbox"/> BECKMAN	Φ34	163-640
Probe:	<input type="checkbox"/> BECKMAN	39841	-----
CONDUCTIVITY:	<input checked="" type="checkbox"/> YSI	33	1905-520
	<input type="checkbox"/>		

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as CaCO₃)

Analysis by: [Signature]

Date: 5-11-94

Reviewed by: Jane Bowman

Date: 5-17-94

SAMPLE COLLECTION FOR TOC ANALYSIS

Test Material: CT 529-94 Protocol No.: 7601

Study Director: MARC 'Sword Study #: 41672

Date/ID: 5-11-94 MDH ③

For each control or test level sampled, approximately ^{200 ①} ~~100~~ mL of dilution water were drawn from mid-chamber level of each replicate with a volumetric pipette and ^{placed ①} ~~composited~~ into an amber bottle ^① ~~for a total volume of approximately 200 mL.~~ A second ~~composite~~ sample was collected in the same manner to retain as a back-up sample for each control or test level sampled. The samples were collected at 0 hour before fish addition. Samples were then prepared for total organic carbon (TOC) analysis by adding sulfuric acid until the pH was <2.0. The back-up sample(s) were then placed into a refrigerator at approximately 3°C for storage and the primary sample(s) were delivered to Engineering Surveys and Services Testing Laboratory in Columbia, MO, for TOC analysis. *Note: Primary Sample also stored in refrigerator before delivery to ESS, mcs 5/11/94*

Test Levels Sampled:

- Control
- Humic Acid Control
- Vehicle Blank
- Level 1
- Level 2
- Level 3
- Level 4
- Level 5

Sulfuric Acid

Lot #: 8592-1

Supplier: Fisher Scientific

pH meter: Beckman Model ϕ 34 with Beckman Model 39841 pH probe
ABC Material Control #163-640

Refrigerator ABC Material Control #166-12

① FIMCS 5/11/94

Notes: ② Entries made on 5-16-94 MDH 5-16-94
③ Information added on 6-3-94 MDH 6-3-94

Prepared by: MARVIN HOFFMAN ② Date: 5-11-94 ②

Reviewed by: Marc 'Sword Date: 5-26-94

**ENGINEERING SURVEYS AND SERVICES
TESTING LABORATORY**

1113 Fay Street • Columbia, Missouri 65201 • (314) 449-2646
802 El Dorado Drive • Jefferson City, Missouri 65101 • (314) 636-3303

ARC LABORATORIES
JUN 01 1994

Date 26 May 1994
Lab No. 4614

Project: ABC Laboratories - 103678G
Location: Columbia, Missouri **Date Received:** 16 May 1994
Sample No./
Description: 903 / Study 41672, Control, 0 hour, 5/11/94
904 / Study 41679, Control, 0 hour, 5/12/94
905 / Study 41679, Humic Acid Control, 0 hour, 5/12/94
906 / Study 41484, Control, Day 0, 5/13/94

TEST RESULTS

PARAMETER	METHOD	SAMPLE NUMBER				DETECTION LIMIT
		903	904	905	906	
Total Organic Carbon, mg/l	5310 B	ND	2.62	14.5	ND	1.00
Total Organic Carbon, mg/l	5310 B	ND	2.62	14.0	ND	1.00
Total Organic Carbon, mg/l	5310 B	ND	2.86	13.9	ND	1.00

Note: Sample secured and delivered to lab by others.
Note: Test performed in accordance with accepted GLP procedure.

ND = None Detected

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The original document."
D. Munoz 6/13/94

Method number from Standard Methods for the Examination of Water & Wastewater, current edition, unless otherwise noted.

CC: 1 Sword

ENGINEERING SURVEYS AND SERVICES
BY: Chris L. Segafredo
ABC LABS#041612 PG 0031

WVA
MC

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 524-94 ^{① 9}

ABC SUPPLIED INFORMATION

Date Received: 04/21/94 Logged In By: RMF ABC Ref # TS-7238

Storage: Room Temp Total Weight: 436.9 g

Physical Description: Cloudy liquid

Remarks: _____

SUPPLIER PROVIDED INFORMATION

Firm: Cytec

Address: 2715 Miller Road

City/State/Zip: Kalamazoo MI 49001 Phone: 616/349-6677

Batch/Lot No. 31 CAS # N/G Purity: N/G

Amount Declared: 250 ml Expiration Date: 2/95

Storage Instructions N/G

Other _____

① Compound mislabeled by Cytec. Should be CT 529-94 as per
Patty Vernon on 4/26/94
RMF

**"This is an exact copy of
The original document"**

By David Wood date 6/14/94

LC ₅₀ Calculations	
ABC Study No. : 41672	Analytical Bio-Chemistry Labs
Compound Name : CT-529-94	7200 East ABC Lane
Species : Rainbow Trout	P.O. Box 1097
Exposure Period: 48 & 72 & 96 Hours	Columbia, Missouri 65205
Study Director : Marc Sword	05-19-1994

Page # 1

—RESULTS CALCULATED USING THE BINOMIAL METHOD—

CONC. µg/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1	10	10	100	9.765620529651642D-02
.56	10	10	100	9.765620529651642D-02
.32	10	0	0	9.765620529651642D-02
.18	10	0	0	9.765620529651642D-02
.1	10	0	0	9.765620529651642D-02

THE BINOMIAL TEST SHOWS THAT .32 AND .56 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 LC₅₀ OF .4233202040195465 µg/L IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN .32 AND .56

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE OR PROBIT METHOD CAN GIVE STATISTICALLY SOUND RESULTS.

Method Reported: Binomial Moving Average Probit

Note: Method selected is that which gives the narrowest confidence limits for LC₅₀.

PREPARED BY: Marc Sword DATE: 5/19/94
 REVIEWED BY: Jane Bowman DATE: 5-20-94

DOSE-RESPONSE CALCULATIONS

Test Material: CT-529-94ABC Study Number: 41672Protocol Number: 7601Study Director: Mark SwordData at 96 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
0.56	-0.252	100.0	7.33
0.32	-0.495	0.0	2.67

Regression

Constant 12.1582
R Squared 1
No. Observations 2

X Coefficient(s) 19.1739

Y = 19.174 X + (12.158)

Dose Response Slope = 19.17

Correlation Coefficient (r) = 1.0000

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.

Data Entered By: Mark SwordDate: 6/2/94Reviewed By: Jan BounaDate: 6-14-94

TEST ORGANISM MEASUREMENTS (10 Organisms)

Test Material: CT-529-94

Protocol No.: 7601

Study #: 41672

Study Director: Marc Sword

Test Species: Rainbow Trout

Lot #: 494

Source: Mt. Lassen Trout Farm

Group Measured: Control

Balance Used: Mettler PM460

ABC material control #: 1905-1020

SAMPLE	WET	STANDARD
	WEIGHT (g)	LENGTH (mm)
1	0.293	31
2	0.403	33
3	0.348	32
4	0.481	35
5	0.496	35
6	0.383	34
7	0.524	37
8	0.628	38
9	0.574	38
10	0.626	38

CALIBRATION

0.200 + 0.000 =	0.201
1.000 + 0.000 =	0.999
2.000 + 0.000 =	2.002
5.000 + 0.000 =	5.001
10.000 + 0.000 =	10.008

Class C weights used for calibration

MEAN WEIGHT (g)	STANDARD DEVIATION
0.476	0.116

MEAN LENGTH (mm)	STANDARD DEVIATION	LENGTH RANGE
35	3	31 = Min. 38 = Max.

Biomass Loading Rate = Total Weight of Fish: 4.756 g/15 L of water = 0.32 g/L

Entered by: Jane Bowman *JHB*

Date: 5-14-94

Checked by: *Marc Sword*

Date: 5-26-94

Study Director: *Marc Sword*

Date: 5-26-94

MORTALITY AND BEHAVIORAL OBSERVATIONS - FISH

Test Material: CP-524-94 Protocol No.: 7601 Definitive
 Study Director: Alvin Sward Study #: 41672 Preliminary
 Test Species: *L. macrochirus* *O. mykiss* *P. promelas*
 No./Vessel: 5 Dil. Water: Blended Fish Lot #: 494 Fish Fed: NO
 Acclimation Mortality: 0 Waterbath: 1 Date Initiated: 4-27-94
 Compound Added: 5:10 P.M. Fish Added: 5:15 P.M. Date Terminated: 5-1-94

Test Conc. mg/L (ppm)	24 Hours		48 Hours			72 Hours			96 Hours		
	Dead	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.
0.10	0	5N				0	0	5N	0	0	5N
1.0	5	-				0	5	-	0	5	-
10	5	-				0	5	-	0	5	-
100	5	-				0	5	-	0	5	-
Observer	WR		0			MCS			MCS		
Date/Time	4-28-94/3:45 P.M.					4/28/94 4:00 P.M.			5-1-94 4:15 P.M.		

REMARKS: N = Normal; LOE = Loss of Equilibrium; Q = Quiescent; SUR = Surfacing;
 ES = Erratic Swimming; DK = Dark Discoloration; OB = On Bottom; LR = Labored Respiration
 QA Procedure Audit No observations recorded on 4/28/94. MCS 4/30/94

Prepared by: Wayne Richter Date: 4-27-94
 Reviewed by: Alvin Sward Date: 5/4/94
 Approved by: Alvin Sward Date: 5/6/94

PREPARATION OF TEST CONCENTRATIONS (weight/volume)

Test Material: CT-529-94 Protocol No.: 7601
 Study Director: Marc Sward Study #: 41672

Purity: N/A % Batch/Lot No.: 31 ID No.: T5-7233

Sample Number	Target Weight (g)	Tare Weight (g)	Net Weight (g)	Adj. Net Weight* (g)	Dilution Volume (L)	Final Conc. (mg/L)
1	0.00150	0.00000	0.0015	-	15	0.10 (1)
2	0.015	0.000	0.015	-	15	1.0
3	0.150	0.000	0.150	-	15	10
4	1.500	0.000	1.500	-	15	100
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						

1.5 mL of acetone added to each test weight before addition to test chamber

1.5 mL of N/A added to vehicle blank test chamber

- * Corrected for purity or active ingredient of test compound
- Based on total product, i.e., not corrected for purity or active ingredient of test compound.

Balance Calibration: 1.000 g + 0.000 g = 0.999 g
 (Class C wt) (Tare) (Final wt)

Balance used: Mettler PM462 ABC material control #: 1905-1020

Sample weight was stirred into the dilution volume with a glass stir rod or stainless steel paddle.

Comments: (1) Balance used Sartorius R3005 ABC mat. cont. # 1021-43 W2 4-27-94
 (2) E W2 4-27-94

Weighed by: WARREN RAILTON ON 4/27/94 / Marc Sward Date: 5/6/94
 Reviewed by: Marc Sward Date: 5/6/94
 Study Director: Marc Sward Date: 5/6/94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 Protocol No. 7601

Study Director: Marc Sward Study #: 41672

4-27-94 WR The 1.0 and 0.10 mgk solutions are clear and the 10 and 100 mgk solutions are ^{cloudy} ~~clear~~ at 0 hour.

4-28-94 WR Same obs as 4-27-94

4-30-94 MCS 0.10 mg/L solution clear. Other solutions have been discarded due to ^{100%} ~~100%~~ mortality. No observations recorded on 4/29/94.

5-1-94 MCS Same obs as on 4/30/94

RE WR 4-27-94 © EMCS 5/6/94

NOTE: Individual entries must be dated and initialed.

Reviewed by: Barbara England Date: 5/4/94

Study Director: Marc Sward Date: 5/6/94

FISH ACCLIMATION RECORD

Species: Rainbow Trout Species Lot #: 494
 Acclimation Tank #: *Bath DP # Removed from Culture ^260
 Culture Tank/Aquarium: Living Stream Section #: LSB 1,2+3
 Date Removed: 5-9-94 Date Returned: 5-13-94
 Dilution Water Type: Hard Blended Well Salt

Acclimation Time (Hr)	Date	Obs.	Acclimation Temp.° (°C)	Acclimation Mortality	Number Used	Study Number
0	5/9/94	MDH	13°C	----	----	----
24	5/10/94	MDH	13°C	0		
48	5/11/94	MDH	13°C	0	60	41672
72	5/12/94	MDH	13°C	0	160	41679
96	5-13-94	MDH	13°C	0		

**This is an exact copy of
The original document"**

By James Howard date 6-15-94

Comments:

* Temperature measured using a mercury/digital thermometer

Remarks: Note all fish held without food during acclimation

Prepared by: Marie Hoffman Date: 5-9-94

Reviewed by: James Howard Date: 6-2-94

FISH ACCLIMATION RECORD

Species: Rainbow trout Species Lot #: 494
 Acclimation Tank #: FF # Removed from Culture ~175
 Culture (Tank/Aquarium/Living Stream) Section #: Tank 1
 Date Removed: 4-23-94 Date Returned: 4-29-94
 Dilution Water Type: Hard Blended Well Salt

Acclimation Time (Hr)	Date	Obs.	Acclimation Temp. ^a (°C)	Acclimation Mortality	Number Used	Study Number
0	4-23-94	AW	~12	----	----	----
24	4-24	AW	12	0		
48	4-25	JHB	① 15 13	0		
72	4-26	WR	13	0	15	41587
96	4-27	JHB	13	0	① 15 20	41672
					② 15 20	41679

**"This is an exact copy of
The original document"**

By Ann Sword date 6/14/94

Comments: ① E JHB 4-25 94
 ② E WR 4-27-94

^a Temperature measured using a mercury/digital thermometer

Remarks: Note - all fish held without food during acclimation

Prepared by: [Signature] Date: 4-23-94

Reviewed by: [Signature] Date: 5/2/94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 (Tank) Aquarium No: LSB Source: Mt. Lassen Trout Farm
 Water Type: Hard Food Lot No.: 41447 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: < 3800
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
5-5-94 <u>h</u>	0	7 3	11	Returned fish pulled on 4-30-94 that were not used for studies. MDH 5-5-94
5-6-94 <u>AA</u>	0	7 4	11	Returned those removed on 5-2 MCS
5-7-94 <u>DCE</u>	1	10	11	
5-8-94 <u>DCE</u>	0	10	11	
5-9 <u>h</u>	1	9 4	11	Removed 260 Fish for Acclimation MDH
5-10-94 <u>h</u>	0	7 4	11	
5-11-94 <u>h</u>	0	7 4	11	
5-12-94 <u>h</u>	0	7 4	11	
5-13-94 <u>h</u>	0	7 4	11	Returned acclimation fish pulled on 5-9-94 not used for study
5-14-94 <u>JHB</u>	1	11:45 AM + 8 PM	11	
5-15-94 <u>JHB</u>	0	11:30 AM	11	
5-16-94 <u>h</u>	0	7 5	12	"This is an exact copy of the original document"
5-17-94 <u>AA</u>	0	7 4	10	
5-18-94 <u>AA</u>	0	7 4	11	By <u>Man. Sword</u> date <u>6/14/94</u>

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 5-5-94
 Reviewed by: Man. Sword Date: 5/29/94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Tank/Aquarium No: 1 → LSB Source: Mt. Lassen Trout Farm
 Water Type: Hard Food Lot No.: 41447 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: <4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
4-21-94 AA	0	7 4	11	
4-22-94 AA	0	7 4	12	
4-23 LW	0	10	12	Removed 40 for Acclimation <u>Barrel A</u> Removed ~175 for Acclimation <u>Barrel FF</u>
4-24 LW	0	9	12	
4-25-94 AA	0	7 4	13	
4-26-94 AA	0	7 4	12	
4-27-94 AA	0	7 4	12	
4-28-94 RW	0	7	12	moved to LSB
4-29-94 LW	20	7 4	11	Returned fish pulled on 4-23-94 <u>WR</u> all 4-29-94
4-30-94 MCS	0	12 5	12	Removed ~30 for acclimation
5-1-94 MCS	0	1, 5	12	
5-2-94 LW	0	7 4	11	Removed ≈ 40 for acclimation <u>MOB</u>
5-3-94 LW	0	7 3	11	"This is an exact copy of The original document"
5-4-94 LW	0	7	11	

By Mura Award date 6/14/94

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 4-21-94
 Reviewed by: [Signature] Date: 5/7/94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Tank/Aquarium No: 1 Source: Mt. Lassen Trout Farms
 Water Type: Hard Food Lot No.: 41188 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: 4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
4-7-94 <u>II</u> ^h	0	7 3	11	
4-8-94 <u>II</u> ^h	0	7 4	11	
4-9-94 <u>DCS</u>	0	10	11	
4-10-94 <u>DCS</u>	0	10	11	
4-11-94 <u>II</u> ^h	0	7 3	11	
4-12-94 <u>II</u> ^h	4	7 4	11	Food Lot 41188 changed to 41447
4-13-94 <u>II</u> ^{AA}	0	7 4	11	
4-14-94 <u>II</u> ^{AA}	0	7 4	11	
4-15-94 <u>II</u> ^{AA}	0	7 4	11	
4-16-94 <u>JHB</u>	0	11:50	12	Removed ~35 for acclimation
4-17-94 <u>JHB</u>	0	11:44	12	"This is an exact copy of The original document"
4-18-94 <u>AA</u>	0	7 4	11	
4-19-94 <u>AA</u>	0	7 4	12	By <u>Karen Awood</u> date <u>6/14/94</u>
4-20-94 <u>II</u> ^h	0	7 4	11	Removed 100+ chance numbers & 3500 remain. Returned remaining fish from 4-16 acclimation JHB

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 4-7-94

Reviewed by: Mami Hoffman Date: 5-3-94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Tank/Aquarium No: 1 Source: M. Lassen Trout Farms
 Water Type: Hard Food Lot No.: 4118B 4144B
 Date Rec'd: 1-24-94 No. Rec'd: 500 Appx. No. Remaining: 4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
3-24-94 ^{SK} SA	0	7 4	11	Pulled ~150 fish for acclimation ① WR 3-24-94
3-25-94 ^h SA	0	7 4	11	Returned remaining fish pulled on 3-24-94 ① WR 3-25-94
3-26 ^h SA	0	9	11	① Entries made 3-29-94 WR
3-27 ^h SA	0	8	11	
3-28 ^{AA} SA	0	7 4	11	
3-29-94 ^{AA} SA	0	8 4	11	Reviewed Page 3/29/94 <u>Man Award</u>
3-30-94 ^{AA} SA	0	7 4	11	
3-31-94 ^{AA} SA	0	7 4	11	
4/1/94 MCS	0	9, 4	11	
4/2/94 MCS	0	10, 4	11	
4-3-94 MCS	0	11, 4	11	
4-4-94 ^{AA} SA	0	7 4	11	"This is an exact copy of The original document"
4-5-94 ^h SA	0	7 4	11	
4-6-94 ^h SA	0 ¹⁴	7 4	11	By <u>Man Award</u> date <u>6/14/94</u>

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 3-24-94
 Reviewed by: [Signature] Date: 4/9/94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Tank/Aquarium No: 1 Source: Mt. Lassen Trout Farm
 Water Type: Hard Food Lot No.: 4118F 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: 4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
3-10-94 AA	-	-	12	OEAA 3-10-94
3-11-94 JJ	-	-	12	
3-12-94 JCE	-	-	12	
3-13-94 JCE	-	-	11	
3-14 RJ	-	-	12	
3-15-94 JJ	-	-	12	
3-16-94 RJ	-	- 4	12	50% + swim-up
3-17-94 JJ	-	7	11	
3-18-94 JHB	4 -	7 + 4	12	① E-JJ 3-18-94 ② E-JHB 3-18-94
3-19 JHD	3	11 + 2	12	
3-20 JHB	1	11:30 + 3	13	
3-21-94 RJ	0 ⁺	7 3	12	"This is an exact copy of The original document" By <u>Mark Sword</u> date <u>6/14/94</u>
3-22-94 RJ	0	7 3	11	
3-23-94 JA	0	7 4	12	

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 3-10-94
 Reviewed by: Mark Sword Date: 3/29/94

FISH CULTURE RECORD

Species: Rainbow Trout, Eggs Lot #: 494
 Source: Mt. Lassen Trout Farm Water Type: Hard
 Incubator No./Tray No: A-68
 Date Received: 1-27-94 No. Received: 5000 Appx. No. Remaining: 4100

Date/ID	Mortality				Not Dist.	°C Temp.	Comments
	Nonviable	Deformed	Dead	Total Mort.			
2-24-94 ^h	—	100	25	125	—	8.4	
2-25 ^h	—	—	—	—	✓	8.0	Hatch Start
2-26 ^h	—	—	—	—	✓	8.2	25% Hatch
2-27 ^h	—	—	—	—	✓	8.0	50% Hatch
2-28 ^h	—	—	—	—	✓	8.5	90% Hatch
3-1 ^h	—	330	500	830	—	8.0	Hatch complete
3-2 ^h	—	—	—	—	✓	8.5	
3-3 ^h	—	—	—	—	✓	8.0	
3-4 ^h	—	18	—	18	—	8.0	
3-5 ^{mcs}	—	—	—	—	✓	8.2	
3-6 ^{mcs}	—	—	—	—	✓	8.2	
3-7 ^h	—	54 ⁺⁷⁶	—	54 ⁺⁷⁶	—	8.2	130 total mort
3-8 ^h	—	—	—	—	✓	7.8	increased temp slightly moved trays into tank 1 @ 10°C
3-9-94 ^h	—	—	—	—	✓	12	

This is an exact copy of the original document.
 BY Mark Anderson date 6/14/94

* Temperature taken with a mercury thermometer

Prepared by: Karen Wilkerson Date: 2-24-94

Reviewed by: W. Brothman Date: 3/12/94

FISH CULTURE RECORD

Species: Rainbow Trout, Eggs Lot #: 494
 Source: Mt. Lassen Trout Farm Water Type: Hard
 Incubator No./Tray No: A-6-8
 Date Received: 1-27-94 No. Received: 5000 Appx. No. Remaining: 4500

Date/ID	Mortality				Not Dist.	°C Temp.	Comments
	Nonviable	Deformed	Dead	Total Mort.			
2-10 94 <u>hw</u>	—	—	—	—	✓	12.2	
2-11 <u>hw</u>	—	—	—	—	✓	12.8	
2-12 <u>DCE</u>	—	—	—	—	✓	13.0	
2-13 <u>DCE</u>	—	—	—	—	✓	13.0	
2-14 <u>hw</u>	275	—	20 ⁺⁵	300	—	13.4	light-moderate eyed
2-15 <u>hw</u>	—	—	—	—	✓	13.2	
2-16 <u>hw</u>	—	—	+12	12	—	13.6	Decreased temp
2-17 <u>hw</u>	—	—	—	—	✓	11.6	Decreased temp
2-18 <u>hw</u>	—	—	—	—	✓	9.0	
① 2-19 <u>hw</u>	—	—	—	—	✓	8.0	①① RW 2-12-94
2-20 —	—	—	—	—	—	—	No obs taken No problems noted RW 2-22-94
2-21 <u>AA</u>	—	—	—	—	✓	8.3	
2-22 <u>hw</u>	—	—	65	65	—	8.7	
2-23 <u>hw</u>	—	—	—	—	✓	8.4	

**"This is an exact copy of
 The original document"**
 By Muriel David date 6/11/94

* Temperature taken with a mercury thermometer

Prepared by: [Signature] Date: 2-10-94
 Reviewed by: Muriel David Date: 3/5/94

FISH CULTURE RECORD

Species: Rainbow Trout, Eggs Lot #: 494
 Source: Mt. Lassen trout farm
 Date Received: 1-27-94 # Received: 5000 ~ # Remaining: N/A
 Incubator/Tray # A-6-8 Water Type: Hard

Date/ID	Mortality				°C Temp.	Comments
	Nonviable	Deformed	Dead	Total Mortality		
1-27-94 R	—	—	—	—	~10.5	Not Disturbed
1-28 R	—	—	—	—	11.0	Not Disturbed
1-29 R	—	—	—	—	11	Not Disturbed
1-30 R	—	—	16	16	10.4	↑ temp
1-31 R	—	—	—	—	12.0	
2-1 A	—	—	—	—	12.6	
2-2 A	—	—	—	—	12.4	
2-3 R	—	—	17	17	12.4	
2-4 R	—	—	—	—	12.6	Not Disturbed
2-5 ACS	—	—	—	—	12.5	"
2-6 ACS	—	—	—	—	12.5	"
2-7 R	—	—	24	24	12.1	
2-8 R	—	—	—	—	12.2	Not Disturbed
2-9 R	425	—	150	440	12.2	DE RW 2-9-94

This is an exact copy of
 the original document.
 BY Marc Sworel date 6/14/94

* Temperature taken with a mercury thermometer

Prepared by: [Signature] Date: 1-27-94
 Reviewed by: [Signature] Date: 3/5/94

CULTURE ROOM OBSERVATIONS/REMARKS FORM

Mt. Lassen
Trout

28125 Hwy 36E
Red Bluff, CA 96090
(916) 307-2722

S
P
R
I
N
G

ABC LABS
PO BOX 1097
7200 ABC Lane
Columbia, MO 65201

LIST ORDER NO.	DATE SHIPPED	SHIPPED VIA	TERMS
18643-RW	1/26	FED EXPRESS I	net 30 da

ORDERED	QTY	DESCRIPTION
	5000	GREEN EGGS & SPERM

Shipping
info for
lot 494
Rainbow trout
RW
1-27-94

MT. LASSEN TROUT FARM
SPAWN INFORMATION DATE: 1-26-94

SPECIES: Oncorhynchus mykiss
(formerly Salmo gairdneri)

SOURCE CODE: JC4
NUMBER OF EGGS: 5000 pooled
NUMBER OF FEMALES USED: 3
NUMBERS OF MALES USED: 3
(Normal or X-bearing)

PERSONNEL: Jerry Allen

This is an exact copy of
The original document

By Mrs. Swood date 2/14/94

Individual entries must be dated and initialed.

Prepared By: [Signature] Date: 1-27-94

Reviewed By: [Signature] Date: 3/5/94

RAINBOW TROUT EGG FERTILIZATION PROCEDURE

Arrival Date: 1-27-94

Acclimation Time: 3 1/2 hr

Source: mt Lassen Trout farm

Temp: 9.2°C

Temp: 30°C

ABC Lot Number: 494

Rainbow Trout Egg Fertilization Procedure

Eggs and milt were allowed to acclimate to desired temperature before fertilizing. Upon acclimation the unfertilized eggs were transferred to a dry plastic bowl and milt added. Isothermal dilution water was added until the eggs were well covered and the mixture gently stirred. After stirring the eggs were left undisturbed for approximately one minute. The excess water was poured off and the newly fertilized eggs were rinsed several times with dilution water to remove any excess milt and ovarian fluid. The eggs were then allowed to water harden for a period of at least one hour before transfer into an incubator or placement into test systems.

**"This is an exact copy of
The original document"**

By Ken Ford date 6/14/94

Fertilization Procedure

Conducted By: Tommy Strawn Date: 1-27-94

Reviewed By: [Signature] Date: 1-27-94

CYTEC

Order # 1738

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

ARC LABORATORIES

APR 26 1994

April 18, 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-529-94 Aquatic Toxicity Studies

Dear Dave:

You will receive, under separate cover, a material identified as CT-529-94. Please submit this material to the following tests at the following costs:

<u>Test</u>	<u>Protocol</u>	<u>Cost</u>	<u>Study #</u>
Acute Toxicity to Rainbow Trout.	7601	██████████	→ 41672
Acute Toxicity to <u>Daphnia Magna</u>	7806	██████████	→ 41673
Acute Toxicity to Green Algae	OECD-201	██████████	→ 41674

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

ABC LABS # 041672

pg 0053

Analytical Bio-Chemistry Laboratories, Inc.
Page 2
April 18, 1994

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-529-94 is an opaque liquid with an oily odor. This material is soluble in water but limited by viscosity. The following special handling conditions apply to this material: **CAUSES EYE AND SKIN IRRITATION** - Store at room temperature away from light and heat source. Avoid contact with iron, copper or aluminum equipment.

THE TOXICOLOGICAL PROPERTIES OF CT-529-94 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.

ABC LABS#041672

pg 0054

Analytical Bio-Chemistry Laboratories, Inc.
Page 3
April 18, 1994

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: APRIL 28, 1994

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.

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

pavABC



PROTOCOL ALTERATION NOTIFICATION

STUDY TITLE: "Static Acute Toxicity of CT-529-94 to Rainbow Trout (<i>Oncorhynchus mykiss</i>)"	
PROTOCOL NO.: 7601	ALTERATION NO.: 1
LABORATORY: ABC Laboratories, Inc.	LAB STUDY NO.: 41672
SPONSOR: Cytec Industries	EFFECTIVE DATE: June 14, 1994

AMENDMENTS:

1. Protocol Section: Test Specific Information - Testing Facility

The protocol lists the following as the testing facility:

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

Phone: (314) 474-8579
Fax: (314) 443-9089

In addition to ABC Laboratories, the following location is added to the protocol under testing facility:

Engineering Surveys and Services
1113 Fay Street
Columbia, Missouri 65201

Phone: (314) 449-2646

Reason: Engineering Surveys and Services conducts the total organic carbon (TOC) analyses of control water in support of this study. The TOC analyses are performed according to OECD GLP's.

Effect on Study: Identifies the site of the laboratory conducting the TOC analyses.

2. Protocol Section: 3.3 - Test Vessels

The protocol states that test temperature will be 15 ± 2 °C. The definitive test was performed at a range of 12 ± 2 °C.

Reason:

The temperature range was changed to maintain consistency between this study and a second static acute toxicity study of CT-529-94 to rainbow trout (*Oncorhynchus mykiss*), which was run concurrently with this study. The second study (ABC study #41679) was performed in dilution water amended with organic carbon (humic acid). The test temperature specified by the study protocol for the second study was 12 °C.

PROTOCOL ALTERATION NOTIFICATION

STUDY TITLE: "Static Acute Toxicity of CT-529-94 to Rainbow Trout
(*Oncorhynchus mykiss*)"

PROTOCOL NO.: 7601

ALTERATION NO.: 2

LABORATORY: ABC Laboratories, Inc.

LAB STUDY NO.: 41672

SPONSOR: Cytec Industries

EFFECTIVE DATE: June 15, 1994

DEVIATION:

1. Protocol Section: 4.6 - Test Procedure - Chemical and Physical

In addition to the water quality parameters listed in the protocol, total organic carbon (TOC) was measured in the 0 hour control dilution water.

Reason:

TOC was measured to maintain consistency between this study and a second static acute toxicity study of CT-529-94 to rainbow trout (*Oncorhynchus mykiss*), which was run concurrently with this study. The second study (ABC study #41679) was performed in dilution water amended with organic carbon (humic acid). TOC was measured in the clean water control and the humic acid control during that study.

Effect on Study:

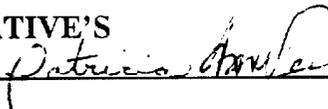
Provides TOC measurements in the 0 hour control water.

**STUDY DIRECTOR'S
SIGNATURE:**



DATE: 6/15/94

**SPONSOR REPRESENTATIVE'S
SIGNATURE**



DATE: 7/11/94



"Working for You"

PROTOCOL COVER SHEET

The protocol for ABC Laboratories study #41672 consists of copies of the following information:

ABC Laboratories Protocol: 7601

Study Authorization Letter Dated: April 18, 1994

Test Material: CT-529-94

Species: Rainbow Trout (*Oncorhynchus mykiss*)

Supplier: Mt. Lassen Trout Farms
Red Bluff, California

The proposed experimental start date is May 11, 1994

The proposed experimental completion date is May 15, 1994

Protocol Approval

ABC Laboratories' Study Director

Name (Signed): Marc C. Sword Date: 5/11/94

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

The above information has been collected and compiled into the protocol for study #41672. The signature date above constitutes the "Study Initiation Date".

DEFINITIVE CONCENTRATION NOTIFICATION

Test Material: CT-519-94 Protocol No.: 7601
5290
 Study Director: Marc Sword Study #: 41672

Firm: Cytoc Industries

Phone: (201) 357-3375

Sponsor's Study Representative: Patricia A. Vernon

Person Notified: Same (X) Other:

Date of Notification: 5/11/94

Notified by: Marc Sword

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

Vehicle: NA

<u>Test Material</u>	<u>ABC #</u>	<u>Test Species</u>	<u>Test Conc. (mg/L)</u>
<u>CT-519-94</u> <small>5290</small>	<u>41672</u>	<u>Rainbow Trout</u>	<u>control, 0.10, 0.13, 0.32, 0.56, and 1.0 mg/L</u>

DE mcs 5/11/94

Reviewed by: Dorothy England Date: 5/11/94
 Study Director: Marc Sword Date: 5/11/94



"Working for You"

RECEIVED

'94 MAR 4 AM 11 27

ABC LABS

ABC BLANKET PROTOCOL NO. 7601

(Revised February 22, 1994 for Cytec Industries Inc.)

Static Bioassay Procedure for Determining the Acute Toxicity
of Chemical Substances to Freshwater Fish

ABC Study Number _____

Test Substance _____

Test Organism _____

PROTOCOL APPROVAL

ABC Laboratories' Study Director

Name (signed) and Date: To be completed on the protocol cover sheet for each individual study

Name/Title (typed): To be completed on the protocol cover sheet for each individual study

Sponsor Representative

Name (signed): Patricia Ann Vernon Date: 2/28/94

Name/Title (typed): Patricia Ann Vernon, Associate Toxicologist

(Other sponsor-required signatures may be added below.)

TEST-SPECIFIC INFORMATION

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

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 substance. The sponsor also agrees to accept any and all of the test substance that remains unused at the end of testing and to assume responsibility for its proper disposal.

Testing Facility

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, MO 65202

Phone: (314) 474-8579
Fax: (314) 443-9089

Study Sponsor

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

Phone: (201) 357-3375
Fax #: (201) 357-3057

Test Substance(s) To be completed on the protocol cover sheet for each individual study
(name(s) used in report and correspondence)

Note: Written confirmation of percent purity along with specific activity and molecular weight, where applicable, must be provided.

Analytical Confirmation (Please check the appropriate statement.)

Analytical confirmation will be performed by ABC Laboratories, Inc.

Sponsor requests no analytical confirmation.

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

Test Concentrations Definitive test concentrations will be specified in a notification form or protocol amendment.

Test Organism

Species: To be completed on the protocol cover sheet for each individual study

Supplier: To be completed on the protocol cover sheet for each individual study

Experimental Dates

Proposed starting date: To be completed on the protocol cover sheet for each individual study

Proposed termination date: To be completed on the protocol cover sheet for each individual study

GLP Compliance (Check the most appropriate):

EPA-FIFRA _____; EPA-TSCA _____; FDA _____; OECD X; Other _____

1.0 INTRODUCTION

Aquatic toxicity tests have been used extensively in the assessment of the environmental effects of chemical substances. Indeed, aquatic bioassays are required by federal laws such as the Toxic Substances Control Act (1), FIFRA (2), and the Clean Water Act of 1977 (3). With the testing guidelines for these laws in mind, as well as FDA's Good Laboratory Practice Regulations (4) which complement them, ABC Laboratories, Inc. has prepared the following protocol. The static bioassay method presented here was patterned after procedures that were formulated by the U.S. Environmental Protection Agency (5), American Public Health Association (6), and the American Society for Testing and Materials (7).

2.0 OBJECTIVES

The primary objective of the toxicity test described herein is to evaluate the acute toxicity of a chemical substance to freshwater fish under static conditions. This is achieved by determining LC_{50} levels of the toxicant during a 96-hour exposure period. An LC_{50} is the approximate concentration of the test substance that produces 50 percent mortality of test organisms after prescribed intervals. The method is designed to yield LC_{50} values following 24, 48, and 96 hours of exposure.

3.0 RANGE-FINDING STUDY

3.1 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 Laboratories may perform range-finding tests at the discretion of the study director and study sponsor. The information derived from this preliminary test will be used to set concentration levels for the definitive bioassay described in the Definitive Study section.

3.2 Test Organism. The fish species to be used should be selected by the study sponsor from the list of recommended species in Table I. The most common species used for toxicity testing includes bluegill (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus mykiss*), and fathead minnow (*Pimephales promelas*). Fish ranging in size from actively-feeding fry to 5.0 g will be used as test organisms. The fish used in a test will be approximately the same size and the standard length of the largest fish will be no more than twice that of the shortest fish. Length and weight will not be measured on fish used for definitive testing. The fish will be obtained from an established commercial hatchery or in-house cultures. The particular source to be used is dependent upon the seasonal availability of test organism and will be listed in the test-specific information on the protocol cover sheet, along with the species selected by the study sponsor.

The test organism lot will be from the same year class and same source and will be cultured following the techniques described by Brauhn and Schoettger (8). All test organism will be held on a 16-hour daylight photoperiod with a 30-minute transition period and observed for at least 14 days prior to testing. A daily record of fish observations during the holding period, along with any prophylactic and therapeutic disease treatments, will be kept and included with the final report. During the holding period, the fish will receive a standard commercial fish food, which may be supplemented by live brine shrimp nauplii (*Artemia* sp.) and/or other aquatic invertebrates. The fish food lot will be analyzed for contaminants prior to use. The food used during the culture period as well as the source of the food will be indicated in the report.

- 3.3 Test Vessels. The range-finding test will be conducted in five gallon widemouth glass jars containing 15 L of test solution and five fish per concentration. The mouth of the jars will be covered with a polystyrene lid or with aluminum foil to keep out foreign objects. These test vessels will be immersed in a water bath with temperatures maintained within $\pm 1^\circ\text{C}$ of the desired test temperature (Table I). For temperature control in the water bath, thermostatically controlled heating elements will be used for warmwater tests ($23 \pm 2^\circ\text{C}$) and refrigeration units for coldwater bioassays ($15 \pm 2^\circ\text{C}$). The dilution water used will be well water prepared to a total hardness of approximately 130 to 160 mg/L (as CaCO_3). The specific water hardness will be achieved by blending naturally hard well water with well water that has been demineralized by reverse osmosis. The dilution water used in the study will not contain any contaminant in amounts that adversely affect the test organism. Therefore, ABC test waters will be screened at least two times each year for organochlorine pesticides, organophosphate pesticides, chemical oxygen demand (COD), unionized ammonia and inorganic trace elements. Monthly measurements on well water and the hard blended water will be made for total organic carbon and suspended solids. In addition measurements of hardness, alkalinity, dissolved oxygen, conductivity, and pH will be made on these waters at least three times a week. Water quality values obtained from periodic screening of blended dilution water for selected chemical/physical factors will be included in the report. The test system was selected to meet the requirements of the U.S. EPA.
- 3.4 Test Substance. Specific information regarding the test material is to be supplied by the sponsor and should be sent to the attention of ABC Laboratories' sample accountability officer with the test material. Such information should include, but not be limited to, name of the test material, lot or batch number, physical description, solubility in water and organic solvents, vapor pressure, stability, purity, available toxicity information and handling precautions. The assay of purity (e.g. % active ingredient) and impurities of the test substance, and

retainment of such data, is the responsibility of the study sponsor. Of particular importance as pre-study 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 PRIOR TO OR WITH THE SAME SHIPMENT.** The characterization, stability and solubility studies will be the responsibility of the sponsor. The test concentrations will be prepared on a weight/volume basis unless otherwise specified. A record of all sample weights and dilutions will be kept, checked by a second party, and furnished in the final report. Under certain experimental conditions, the sponsor may elect to confirm test concentrations by approved analytical techniques. Factors such as limited aqueous solubility and compound volatility may require analysis at 0 and 96 hours. If analytical confirmation is desired, the study sponsor must submit an appropriate analytical method to ABC Laboratories. If analytical confirmation of test concentrations is not conducted as required by Good Laboratory Practices (GLP's), a disclaimer statement will be included in the GLP compliance statement in the report.

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

- 3.5.1 Test organism will be acclimated gradually to the test temperature and dilution water for at least 48 hours prior to testing, during which time they will be held without food. If mortality of the test lot in acclimation exceeds 3 percent in the 48 hours prior to testing, the fish will not be used.
- 3.5.2 If performed, the range-finding test will be initiated by exposing groups of five fish to one or more toxicant concentrations usually spaced apart by a factor of 10. No preliminary test will be performed if the sponsor requests a specific concentration regime. ABC Laboratories will test up to a maximum of 1,000 mg/L in the definitive test, unless otherwise requested by the study sponsor. The test organisms will be placed in the test chambers in an impartial manner within 30 minutes after solution preparations. Impartial distribution will be achieved by sequentially adding one or two fish to each test chamber until all test chambers have their complement of test organisms. The initial toxicant concentration regime used will be 1, 10, and 100, and 1000 mg/L, unless otherwise requested by the study sponsor.
- 3.5.3 The test chambers will be observed for mortality and/or abnormal behavioral (sublethal) effects every 24 hours. Dead individuals, if any, will be removed at each observation and a record maintained of mortality

and abnormal behavior. Dependent upon 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. For example, if the 24-hour exposure results in total mortality, new solutions will be prepared below the lowest initial concentration until no mortality or partial mortality is reached. In the converse situation, if no mortality is observed after 24 hours, new concentrations may be added above the highest initial level until mortality is noted. However, if no mortality is observed at preliminary test levels in the range of 100-1000 mg/L, additional concentrations may not be added. In this manner, a bracket is formed for the toxic range of the compound.

- 3.5.4 The preliminary test will be conducted for a period of at least 24 hours with the exact duration dependent upon the results of the initial concentrations tested.
- 3.5.5 Results of the range-finding study may be used to set the concentration range of the definitive study described in the Definitive Study section. At least five toxicant concentrations have proven to be adequate in assessing the aquatic toxicity of most compounds.

4.0 DEFINITIVE STUDY

- 4.1 General. Following the preliminary range-finding study discussed in the Range-Finding Study section, the definitive test will be conducted by the procedures described below.
- 4.2 Test Organisms. Aspects concerning the acquisition, culture, and acclimation of test organisms will be the same as discussed in the Test Organism section.
- 4.3 Test Vessels. For all definitive testing, the test vessel size will conform to the maximum loading limitation of 0.8 grams of fish per liter of solution for coldwater bioassays and 0.5 grams of fish per liter of solution for warmwater bioassays (5). One of the following types of glass test chambers will be used: (a) 5 gallon glass jars containing 15 L of solution, (b) aquaria containing 30 L of solution, or (c) aquaria containing 75 L of solution. ABC Laboratories will select the type of chamber to be used. In most instances, the test chambers used will be 5 gallon glass jars containing 15 L of solution, which is a recommended test vessel for static bioassays (6). The jar will be covered with a polystyrene lid or a piece

of aluminum foil to keep out foreign objects. The water bath system described in the Test Vessels section will be used to control test temperatures. The test dilution water will be the same as discussed in the Test Vessels section.

- 4.4 Test Substance. Specific information regarding the test substance is to be supplied by the sponsor. The remaining test sample will be sent back to the study sponsor upon approval of the final report.
- 4.5 Test Procedure – Biological. The basic test procedure for the definitive bioassay will be as follows:

4.5.1 The test organisms will be gradually acclimated to the test temperature and dilution water for at least 48 hours prior to testing, during which time they will be held without food.

4.5.2 The definitive test will be initiated by exposing groups of 10 organisms to at least 5 toxicant concentrations and a dilution water control. The test concentrations used will be based upon the results of the range-finding test and will fall within a geometric or logarithmic series in which the ratio between consecutive concentrations is between 1.5 and 2.0. As an alternate, the definitive test will be conducted with triplicate test chambers at 100 or 1000 mg/L or at the water solubility limits of the test substance and triplicate dilution water control chambers. The definitive test concentrations will be selected after discussing preliminary results with the study sponsor. Additional test concentrations may be added during the course of the test, if necessary, to ensure a suitable dose response for calculation of LC_{50} values. The concentration range used must yield at least one concentration with a mortality of $\leq 30\%$ and at least one concentration with a mortality of $\geq 65\%$. The above mentioned mortality response is not necessary in instances of maximum exposure testing at the water solubility limit of the test substance. This mortality response is also not necessary at a concentration of 100 mg/L or greater where the test is designed to confirm no toxicity (or minimal toxic response, $< 50\%$) at these levels:

If a vehicle (organic solvent) is used in the preparation of test solutions, an additional vehicle blank chamber will be used which will receive an aliquot of the vehicle equivalent to the highest amount used in the test chambers. The concentration of vehicle in

be placed in the test chambers in an impartial manner within 30 minutes after solution preparations, unless special, lengthy solution preparation (such as stirring overnight) is required. If special preparation is needed the method of preparation will be documented. Impartial distribution will be as described in the Test Procedure section.

ABC Laboratories will test up to a maximum of 1,000 mg/L in the definitive test. This may be above the water solubility limit of the compound and ABC Laboratories will report and observed signs of solubility problems, such as precipitation or surface films. It should be noted that regulatory agencies may require a repeat of a study in which the water solubility limit was exceeded and analytical confirmation of test solutions has not been performed. In this event, ABC Laboratories will retest the material if requested by the study sponsor at an additional charge which includes analytical confirmation costs.

- 4.5.3 As an alternate test design, duplicate test chambers per concentration containing 5 fish each will be used to reduce biomass loading density when using test organisms near the upper size limit of that recommended.
- 4.5.4 The test chambers will be observed for mortality and/or abnormal behavioral (sublethal) effects every 24 hours (± 1 hour from time of test initiation). If more than 10% of the fish die in the control population, then the test will be deemed unacceptable and the study will be terminated. Dead individuals will be removed at each observation and a record maintained of mortality and observed abnormal behavior for each concentration tested.
- 4.5.5 Length and weight measurements will be made on either a representative group of the test organisms prior to testing or on the control or vehicle blank group at test termination.
- 4.6 Test Procedure – Chemical and Physical. Water quality parameters of temperature, dissolved oxygen and pH will be monitored throughout the test. Measurements of these parameters will be made at 0, 24, 48, 72, and 96 hours of the test in all control, vehicle blank (if necessary), and test concentration chambers. Additional measurements may also be made at the discretion of the study director. Dissolved oxygen values in exposure solutions will be judged to have been 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.

In the event that the definitive test is conducted with triplicate test chambers at 100 or 1000 mg/L or at the water solubility limit of the test substance, the temperature, dissolved oxygen and pH will be measured in all test levels and controls at 0, 24, 48, 72, and 96 hours of the test.

If, at any point in the study during routine water quality analyses, the control dissolved oxygen level falls below 40% saturation, ABC Laboratories' study director will deem the test unacceptable and terminate it. ABC Laboratories recommends that the test solutions not be artificially aerated or pH-adjusted since these practices are felt questionable by regulatory agencies. If the study sponsor anticipates that the compound may create impacts on water quality and desires to adjust test solutions in some manner, this must be authorized in writing before test initiation. In the event that aeration or pH-adjustment is desired by the sponsor, ABC recommends that the concentrations of test substance be measured by an appropriate analytical method to determine if these procedures have affected nominal exposure levels.

- 4.7 Analysis of Results. The results of the definitive study will be statistically analyzed for 24, 48, 72, and 96 hour LC₅₀ values and their corresponding 95 percent confidence limits, if possible. These values will be determined by an LC₅₀ computer program (9, 10). The slope of the dose-response line will be calculated for the 96-hour data, if possible. Depending upon the mortality data, the slope may be calculated by least squares regression analysis in the above mentioned computer calculations (log of concentration vs. probit transformation of percent mortality).

5.0 DATA MAINTENANCE/REPORTING

- 5.1 Records to be Maintained. Records to be maintained will include, but not be limited to, compound receipts; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC); material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until finalization of the study. Upon completion of the project, all raw data specifically for this study will be submitted to the sponsor as part of the final report.

- 5.2 Report. A final report containing all original raw data and/or certified copies of certain raw data records will be submitted to the sponsor. A copy of the report and associated raw data will be kept on file in ABC Laboratories' archives. The final report will include, but not be limited to, the following:
- 5.2.1 Study dates, name, and address of test facility.
 - 5.2.2 Objectives and test procedures as stated in approved protocol.
 - 5.2.3 A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
 - 5.2.4 Description of test substance (date of receipt, storage conditions, purity, physical characteristics, and method of preparing stock and/or test solutions).
 - 5.2.5 Description of methods used during the study.
 - 5.2.6 Description of test organisms (source, culture techniques, etc.).
 - 5.2.7 Summary of all data and a statement of the conclusions drawn from any data analyses, if appropriate.
 - 5.2.8 Location of raw data.
 - 5.2.9 List and signatures of study personnel.
 - 5.2.10 GLP compliance statement by study director and a statement by ABC Laboratories' Quality Assurance Unit.
 - 5.2.11 An appendix or separate raw data report will contain the original raw data or certified copies of raw data, letter of test authorization (if available), protocol alterations, and the approved protocol.

6.0 DEFINITIVE TEST CONCENTRATION NOTIFICATION

After discussion with a representative of the study sponsor, definitive test concentrations will be specified in a notification form. This form will be signed and dated by the study director and will be attached to the protocol before the start of the definitive study. If after discussion with a sponsor representative it is determined that the definitive test concentrations are to be changed, the new concentrations will be specified in another

notification form. The study director will sign and date the new form and will document on the form the reason for the change in definitive test concentrations.

7.0 PROTOCOL ALTERATIONS

The study director, upon approval of the sponsor representative, may make other alterations to this protocol. Proposed alterations, except for those which involve the test concentrations (see Definitive Test Concentration Notification above), will take the form of a written Protocol Alteration describing the alteration, the reason for the alteration, and the effect on the study, if any. All alterations will be signed and dated by both the study director and the sponsor representative. The signed Protocol Alteration Notification form will be maintained with the protocol.

Should a significant problem develop while the study is in progress, the study director will notify the sponsor representative as soon as practical to discuss the problem and any corrective actions taken. Upon verbal authorization from the sponsor representative, the study director will proceed with any further actions deemed appropriate. If the sponsor representative cannot be reached, the study director will proceed with the appropriate modifications and will notify the sponsor representative as soon as possible.

In the event of protocol deviations, an attempt will be made to notify the sponsor representative within a reasonable period of time. A written description of the deviation(s) will be submitted on a Protocol Alteration Notification form to the sponsor representative. All deviations will be signed and dated by both the study director and the sponsor representative.

8.0 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 guidelines listed in this protocol. The results of these inspections will be reported to the study director and ABC management. The draft and final reports will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures used were followed. A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the study director and management.

9.0 GLP COMPLIANCE

To be in compliance with the Federal Good Laboratory Practice regulations, the report of the investigation conducted utilizing this protocol will contain a statement that the study was conducted in accordance with one of the following GLP's.

1. U.S. F.D.A. Good Laboratory Practice Regulations (21 CFR 58).
2. U.S. E.P.A. Good Laboratory Practice Standards; Toxic Substances Control (40 CFR 792).
3. U.S. E.P.A. Good Laboratory Practice Standards; Pesticide Programs (40 CFR 160).
4. OECD Principles of Good Laboratory Practice; Annex 2 C(81) 30(Final).
5. Any state or local GLP which may apply.

The specific GLP regulations, for which this study specific protocol and resultant study must be in compliance with, is to be designated by the sponsor's representative on page 4 of this protocol.

TABLE I
RECOMMENDED SPECIES AND TEST TEMPERATURES¹

<u>Recommended Species</u>	<u>Recommended Test Temperature (°C)²</u>
Rainbow Trout (<i>Oncorhynchus mykiss</i>)	12
Brook Trout (<i>Salvelinus fontinalis</i>)	12
Bluegill (<i>Lepomis macrochirus</i>)	22
Fathead Minnow (<i>Pimephales promelas</i>)	22
Channel Catfish (<i>Ictalurus punctatus</i>)	22
Carp (<i>Cyprinus caprio</i>) ³	22

¹ Adapted from (5).

² OECD test temperatures are 15 and 23°C

³ Recommended for compounds used in Japan.

10/0 REFERENCES

- (1) U.S. Congress. 1976. Toxic Substances Control Act. Public Law 94-469. *Federal Register*, October 11, 1976. 2003-2051.
- (2) U.S. Environmental Protection Agency. 1978. Registration of pesticides in the United States, proposed guidelines. *Federal Register*, July 10, 1978:29696-29741.
- (3) U.S. Congress. 1977. Clean Water Act of 1977. Public Law 95-217. *Federal Register*, December 27, 1977: 1566-1611.
- (4) (a) U.S. Food and Drug Administration. 1987. Nonclinical Laboratory Studies, Good Laboratory Practice Regulations (21 CFR, Part 58). *Federal Register*, Vol. 52, No. 172:33779-33782.
- (b) U.S. Environmental Protection Agency. 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). *Federal Register*, Vol. 54, No. 158: 34067-34074.
- (c) U.S. Environmental Protection Agency. 1989. Toxic Substances Control Act; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). *Federal Register*, Vol. 54; No. 158:34043-34050.
- (d) Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C(81) 30 (Final):7-28.
- (5) 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. 61 p.
- (6) American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, D.C. 1134 p.
- (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. 25 p.

-
- (8) Brauhn, J. L. and R. A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975. 45 p.
 - (9) 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.
 - (10) Stephan, C. E. 1977. Methods for calculating an LC_{50} . In: Aquatic Toxicology and Hazard Evaluation. Mayer, F. L. and J. L. Hamelink, eds. American Society for Testing and Materials. ASTM STP 634. 315 p.



SPONSOR

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

RECEIVED

JUL 21 1994

P. A. VERNON

STUDY TITLE

Static Acute Toxicity of CT-529-94
to Rainbow Trout (*Oncorhynchus mykiss*)
in Dilution Water Amended with 20 mg/L Humic Acid

DATA REQUIREMENT

U.S. EPA-TSCA, 40 CFR, Part 797
Guideline 797.1400
OECD Guideline 203

AUTHORS

Marc C. Sword
Biologist II/Study Director

REPORT SUBMITTED ON

July 15, 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 #41679

Page 1 of 87

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed Study #41679, "Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*) in Dilution Water Amended with 20 mg/L Humic Acid," for Cytec Industries, West Paterson, New Jersey.

The following inspections/audits were conducted on this study.

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Reported to Study Director</u>	<u>Date Reported to Management</u>
05-16-94	Weigh and Measure Fish	05-16-94	05-16-94
05-23-94	TOC Analysis 0-Hour at ES&S Facility	05-23-94	05-23-94
06-20-94	Draft Report	06-20-94	06-24-94
07-13-94	Final Report	07-13-94	07-14-94

The undersigned conducted the draft and final report audits. The audits indicate the report is an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.



Greg Veltri
Quality Assurance Officer II

7/14/94
Date

SIGNATURE PAGE

Submitted by: ABC Laboratories, Inc.
/ Environmental Toxicology
7200 East ABC Lane
Columbia, Missouri 65202

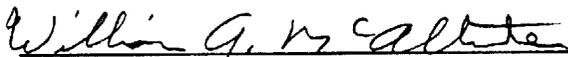
Prepared by:



Marc C. Sword
Biologist II, Study Director

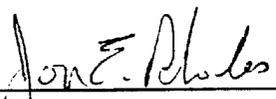
7-15-94
Date

Approved by:



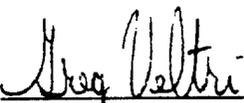
William A. McAllister
Manager, Environmental Toxicology

7-14-94
Date



Jon E. Rhodes
Team Leader, Environmental Toxicology

7-14-94
Date



Greg Veltri
Quality Assurance Officer II

7/14/94
Date

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FISH ACUTE TOXICITY COMPENDIUM

Subject: "Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*) in Dilution Water Amended with 20 mg/L Humic Acid," ABC Laboratories' Study Number 41679

Sponsor: Cytec Industries

Testing Facility: ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
Columbia, Missouri 65202 (314-474-8579)

Location of Original Raw Data and Final Report:

Cytec Industries
West Paterson, New Jersey

Test Substance: CT-529-94

Nominal Test Concentrations: Control, Humic Acid Control, 5.6, 10, 18, 32, 56, and 100 mg/L

Dilution Water: 140 mg/L as CaCO₃, (total hardness)
152 mg/L as CaCO₃, (total alkalinity)
8.2 (pH)
340 μ Mhos/cm (conductivity)

Experimental Test Dates: Start - May 12, 1994
Termination - May 16, 1994

Length of Study: 96 Hours

Results: 96-Hour LC₅₀ = 42 mg/L (95% C.I. = 32 to 56 mg/L)

96-Hour NOEC = 5.6 mg/L

96-Hour Slope of the Dose-Response Line = 8.0

Test Species: Rainbow Trout (*Oncorhynchus mykiss*)

Source of Organisms: Mt. Lassen Trout Farm
Red Bluff, California

Mean \pm SD Wet Weight: 0.48 \pm 0.11 g

Mean \pm SD Standard Length: 35 \pm 3 mm

Standard Length Range: 30 to 39 mm

SUMMARY

The acute toxicity of CT-529-94 to rainbow trout (*Oncorhynchus mykiss*) in dilution water amended with 20 mg/L humic acid was assessed using the methods outlined by the protocol for this study. Water quality parameters of temperature, dissolved oxygen, and pH were measured throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following duplicate nominal concentrations of CT-529-94: 5.6, 10, 18, 32, 56, and 100 mg/L, with concurrent control and humic acid control chambers. Twenty fish, with a mean \pm SD wet weight of 0.48 ± 0.11 g, a mean \pm SD standard length of 35 ± 3 mm, and a standard length range of 30 to 39 mm were exposed to each test concentration and control.

At 0 hour, the control solutions were clear. All other chambers were dark brown in appearance, with the high concentrations appearing murky.

At 24 hours and during the remainder of the test the control chambers were clear with no precipitate; the humic acid control chambers were clear, brown in color, with no precipitate; the 5.6 and 10 mg/L chambers were clear, brown in color, with a small amount of brown precipitate; and the 18 mg/L chambers were slightly cloudy with brown precipitate on bottom of the test chamber and floating in the water.

At 24 hours the 32 mg/L chambers were slightly cloudy with a large amount of brown precipitate on the bottom of the test chamber and floating in the water. At 48 hours and for the remainder of the test the 32 mg/L chambers clear with a large amount of brown precipitate on bottom of the test chamber and floating/suspended in the water.

At 24 and 48 hours the 56 mg/L chambers were cloudy/milky brown with small amounts of precipitate and brown surface scum. At 72 hours the 56 mg/L chambers appeared the same, except they were less cloudy than previously observed. At 96 hours the 56 mg/L chambers were clear with large amounts of precipitate on bottom and floating/suspended in the water.

At 24 hours the 100 mg/L chambers were cloudy/milky brown with small amounts of precipitate and brown surface scum. These observations remained the same for the rest of the test, except that white surface scum was noted in the 100 mg/L chambers at 48 and 72 hours in both replicates and at 96 hours in the B replicate.

The results of the four day static rainbow trout toxicity test of CT-529-94 in dilution water amended with humic acid are summarized as follows. The fish in the control, humic acid control, and 5.6 mg/L test concentration were normal throughout the test except for one mortality in the control B replicate at 48 hours. Abnormal effects of mortality, labored respiration, fish on bottom of the test chamber, and/or dark discoloration were observed during the 96-hour exposure period in the 10, 18, 32, 56, and 100 mg/L test concentrations. The 96-hour LC_{50} was 42 mg/L with 95% confidence limits of 32 and 56 mg/L. The slope of the 96-hour dose-response line was 8.0. The 96-hour no-observed effect concentration was 5.6 mg/L, which was based on the lack of mortality or abnormal effects at this concentration.

INTRODUCTION

This static acute toxicity test was performed by the Environmental Toxicology division of ABC Laboratories, Inc., Columbia, Missouri, for Cytec Industries. The test was conducted from May 12, 1994, to May 16, 1994, as authorized in a letter of April 27, 1994. The purpose of this study was to determine the 24-, 48-, 72-, and 96-hour LC₅₀ values for CT-529-94 to rainbow trout (*Oncorhynchus mykiss*) in dilution water amended with 20 mg/L humic acid. Range-finding tests were conducted from April 27, 1994, to May 9, 1994, to determine the concentration range for the definitive test. The study was conducted following the procedures outlined in ABC Protocol No. 8901-PMN as approved by a representative of Cytec Industries on April 27, 1994 and by the study director on May 5, 1994.

METHODS AND MATERIALS

The procedures for static bioassays, as described in *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians* (3) and *Standard Methods for the Examination of Water and Wastewater* (4), were used in this experiment.

I. Test Fish

The 160 rainbow trout (lot #494) used in the test were obtained from Mt. Lassen Trout Farm in Red Bluff, California. The rainbow trout were received as green eggs and milt and were fertilized on January 27, 1994, at ABC Laboratories. After hatching, the fish were reared and maintained in hard blended water and were fed newly hatched brine shrimp (*Artemia* sp.) and/or a commercially available fish food (salmon starter) daily. The fish food was received from Zeigler Bros. Inc. in Gardners, PA, and the brine shrimp was received from Aquarium Products in Glen Burnie, Maryland. All fish food was analyzed for contaminants and the results of those analyses are on file at ABC Laboratories.

The fish were held in ABC hard blended water at the approximate test temperature for approximately 10 weeks post hatch before the test. All test fish were held on a 16-hour daylight photoperiod with a 30-minute transition period and were observed for at least fourteen days prior to testing. Fish culture techniques were basically those described by Brauhn and Schoettger (5). A daily record of fish observations during the holding period, along with any prophylactic or therapeutic disease treatments, was maintained.

A subplot of fish was acclimated to the test temperature approximately 72 hours before the test. The fish were not fed during the acclimation and test periods. Weight and length measurements were made on the control group of fish at the termination of the test. The rainbow trout (*Oncorhynchus mykiss*) used as the control group for this experiment had a mean \pm SD wet weight of 0.48 ± 0.11 g, a mean \pm SD standard length of 35 ± 3 mm, and a standard length range of 30 to 39 mm. This gave a test chamber loading biomass of 0.32 g/L for the definitive study. The control group was representative of all the fish used in the definitive study.

II. Test Substance

The CT-529-94 sample (batch #31) was received from Cytec Industries on April 21, 1995, in good condition and was assigned ABC reference #TS-7238. The sample upon receipt was observed to be a viscous milky liquid and was stored at room temperature. Sample purity was not specified. The expiration date was given as February, 1995. All test concentrations were based on the total substance, i.e. not corrected for sample purity. All standard weights and dilution values were recorded.

The definitive test concentrations were obtained as follows. Test chambers were filled with dilution water and allowed to come to test temperature. Direct weights of the test substance were weighed into 600 mL beakers. The beakers were labeled to match each respective test chamber. Approximately 500 mL of dilution water was removed from each CT-529-94 exposure chamber and transferred to each respective beaker. The beakers were placed on stir plates and stirred with a teflon coated stir bar until the test substance went into solution. The solutions from the beakers were quantitatively transferred to each respective test chamber at 0-hour and the test solutions were stirred with a glass rod.

Just before solutions in the beakers were added to the test chambers, 0.300 g of humic acid were added directly to each test chamber (except for the control chambers) for a humic acid concentration of 20 mg/L.

III. Test Water

The static toxicity test was conducted in five gallon glass vessels containing 15 liters of hard blended water. This dilution water was prepared by blending naturally hard well water with well water that had been demineralized by reverse osmosis. This blended water was prepared to yield a total hardness of 130-160 mg/L as CaCO_3 . The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.1 mg/L and pH 8.2. Table I provides results of the most recent screening for selected water chemical/physical parameters and contaminants in blended dilution water. The test vessels were kept in a waterbath at $12 (\pm 1)^\circ\text{C}$. Waterbath temperature was continuously recorded using a datalogger and thermistor probe.

The dilution water was amended with humic acid (organic carbon) at a concentration of 20 mg/L. The humic acid (lot #01816HH) was received from the Aldrich Chemical Company on March 13, 1992. The humic acid was observed to be a black, crystalline powder and was stored at room temperature.

IV. Biological Test Procedure

Range-finding tests were conducted to determine the concentration range for the definitive test. The test concentrations were set at 0.10, 1.0, 10, and 100 mg/L for the first test, 3.2 and 32 mg/L for the second test, and 100 and 320 mg/L for the third test.

Based on the results of preliminary testing, 6 concentrations of the test substance, ranging in a logarithmic series from 5.6 to 100 mg/L, were selected for the definitive toxicity test. Dilution water control and humic acid control chambers were also included. There were duplicate replicates of each control and test concentration. Ten fish were added to each control and test chamber in an impartial manner within 30 minutes after addition of test substance for a total of 20 fish per concentration or control. All test organisms were observed once every 24 hours for mortality and abnormal (sublethal) effects. Dead individuals, if any, were removed from the test chambers after each 24-hour observation. The test was on a 16-hour daylight photoperiod with a 30-minute transition period. Light intensity measured over the waterbath during the test averaged 63 footcandles (678 lux). Light measurement data are kept on file at ABC Laboratories.

Temperature ($^{\circ}\text{C}$), dissolved oxygen (mg/L), and pH were measured at 0, 24, 48, 72, and 96 hours of testing in all test chambers. Total organic carbon (TOC) values were measured in triplicate on composited dilution water control A and dilution water control B samples collected at 0 hour. Just before fish addition, approximately 100 mL of water from each control replicate was collected from mid-chamber level with a 100-mL volumetric pipet. The two 100-mL aliquots were composited in an amber bottle and pH was adjusted to <2.0 with sulfuric acid. A backup sample was collected in the same manner on each sample day. The primary samples were delivered to Engineering Surveys and Services in Columbia, Missouri, for TOC analysis. The backup samples were retained in a refrigerator at ABC Laboratories. TOC was analyzed on the humic acid control water at 0 hour also. Samples were collected, composited, and analyzed in the same manner as described above for the control water.

V. Biological Data Analysis

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC_{50} program developed by Stephan et al. (6). If possible, this program calculated the LC_{50} statistic and its 95 percent confidence limits using the binomial, moving average, and probit tests. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<37 to $>63\%$), an LC_{50} and/or its 95 percent confidence limits could not be calculated. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC_{50} (6, 7) although all three models are valid. The dose-response line was calculated using least squares regression analysis of percent mortality transformed to probits versus log concentration. All calculations were based on the nominal concentrations of CT-529-94.

RESULTS AND DISCUSSION

The definitive test was successfully completed on May 16, 1994. An examination of the fish culture and acclimation records for this test indicated that the fish were in good condition for testing.

Water quality data are presented in Table II. The dissolved oxygen concentrations ranged from 6.3 to 9.4 mg/L during the test. These values represented 62 and 91% saturation at 13 and 12 °C, respectively, and were considered adequate for testing (3), therefore, the test chambers were not aerated during testing. The pH values ranged from 7.7 to 8.7. Temperature measured with a mercury thermometer ranged from 12.1 to 12.9 °C. Temperature measured continuously in the waterbath during the test was within the protocol specified temperature range.

Total organic carbon (TOC) measured in triplicate on control water collected at 0 hour before fish were added to the test chambers was 2.62, 2.62, and 2.7 mg/L (\bar{x} = 2.7 mg/L). TOC measured in triplicate on humic acid control water collected at 0 hour before fish were added to the test chambers was 14.5, 14.0, and 13.9 mg/L (\bar{x} = 14.1 mg/L).

At 0 hour, the control solutions were clear. All other chambers were dark brown in appearance, with the high concentrations appearing murky.

At 24 hours and during the remainder of the test the control chambers were clear with no precipitate; the humic acid control chambers were clear, brown in color, with no precipitate; the 5.6 and 10 mg/L chambers were clear, brown in color, with a small amount of brown precipitate; and the 18 mg/L chambers were slightly cloudy with brown precipitate on bottom of the test chamber and floating in the water.

At 24 hours the 32 mg/L chambers were slightly cloudy with a large amount of brown precipitate on the bottom of the test chamber and floating in the water. At 48 hours and for the remainder of the test the 32 mg/L chambers clear with a large amount of brown precipitate on bottom of the test chamber and floating/suspended in the water.

At 24 and 48 hours the 56 mg/L chambers were cloudy/milky brown with small amounts of precipitate and brown surface scum. At 72 hours the 56 mg/L chambers appeared the same, except they were less cloudy than previously observed. At 96 hours the 56 mg/L chambers were clear with large amounts of precipitate on bottom and floating/suspended in the water.

At 24 hours the 100 mg/L chambers were cloudy/milky brown with small amounts of precipitate and brown surface scum. These observations remained the same for the rest of the test, except that white surface scum was noted in the 100 mg/L chambers at 48 and 72 hours in both replicates and at 96 hours in the B replicate.

The 56 and 100 mg/L test concentrations both elicited 100% mortality after 24 hours of exposure. No mortality was observed in the control, humic acid control, 5.6, 10, or 32 mg/L test concentrations during the test, except for one mortality in the control B replicate at 48 hours. The 18 mg/L test concentration elicited 0% mortality at 24, 48, and 72 hours, and 5% mortality at 96 hours. Mortality and abnormal/sublethal effects data are presented in Table III.

The abnormal effects of mortality, labored respiration, fish on bottom of the test chamber, and/or dark discoloration were observed in the 10, 18, 32, 56, and 100 mg/L test concentrations during the 96-hour exposure period. All fish in the control, humic acid control, and 5.6 mg/L test concentrations were normal throughout the exposure period, except for the single control B replicate mortality at 48 hours.

The results of the 96-hour static toxicity test with rainbow trout (*Oncorhynchus mykiss*) exposed to CT-529-94 in dilution water amended with 20 mg/L humic acid are presented in Table IV. The 24-, 48-, 72-, and 96-hour LC₅₀ values for CT-529-94 were all 42 mg/L. The no-observed effect concentration (NOEC) was 5.6 mg/L based on the lack of mortality or abnormal effects at this concentration. The slope of the 96-hour dose-response line for rainbow trout exposed to CT-529-94 in dilution water amended with 20 mg/L humic acid was 8.0 as calculated by least squares regression analysis of percent mortality transformed to probits versus log concentration (Figure 1).

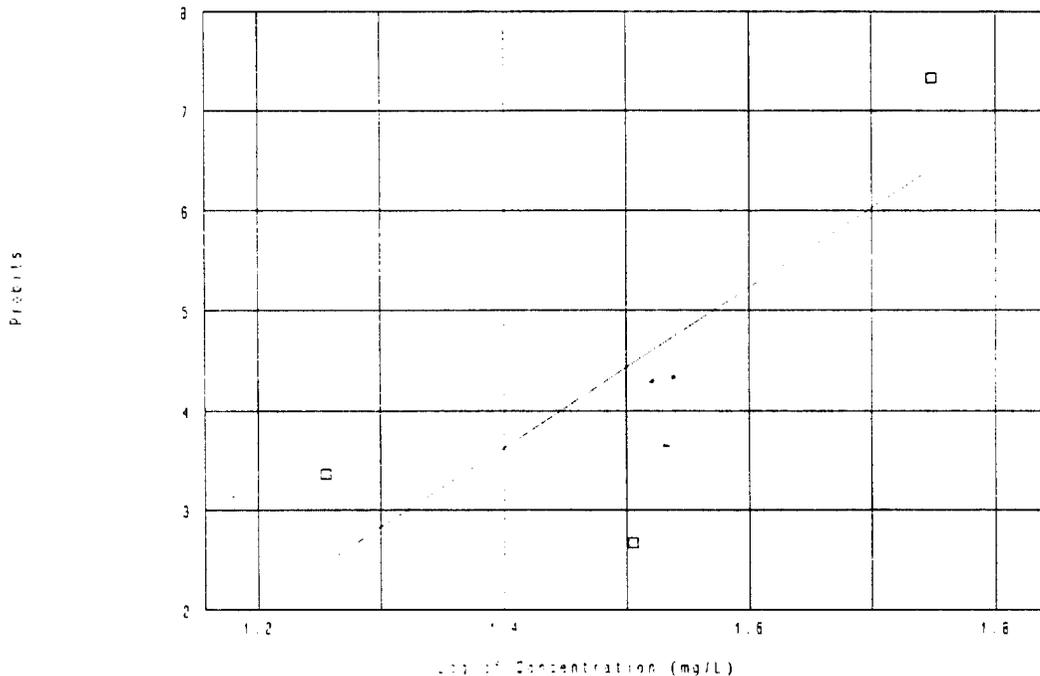


Figure 1: The 96-Hour Dose-Response Line for Rainbow Trout (*Oncorhynchus mykiss*) Exposed to CT-529-94 in Dilution Water Amended with 20 mg/L Humic Acid

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

TABLE I

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

Dilution Water Screen ^a		Monthly Screens (mg/L) ^b	
Hardness	140 mg/L (as CaCO ₃)	Total Organic Carbon	< 1.0 mg/L
Alkalinity	152 mg/L (as CaCO ₃)	Suspended Solids	0.2 mg/L
pH	8.2		
Conductivity	340 μ Mhos/cm		
Biyearly Screens ^c			
Elements (mg/L)		Chlorinated Hydrocarbons (μ g/L)	
Aluminum	< 0.20	DDE	< 0.01
Arsenic	< 0.01	DDD	< 0.01
Boron	0.246	DDT	< 0.01
Cadmium	< 0.0005	Dieldrin	< 0.01
Chromium	< 0.001	Endrin	< 0.01
Cobalt	< 0.050	α -BHC	< 0.01
Copper	< 0.020	β -BHC	< 0.01
Fluoride	0.8	γ -BHC-Lindane	< 0.01
Iron	< 0.10	Δ -BHC	< 0.01
Lead	< 0.003	Heptachlor	< 0.01
Mercury	0.00024	Heptachlor Epoxide	< 0.01
Nickel	< 0.05	Aldrin	< 0.01
Selenium	< 0.005	Chlordane	< 0.3
Silver	< 0.0005	Methoxychlor	< 0.05
Zinc	< 0.04	Toxaphene	< 4.0
		Endosulfan Sulfate	< 0.03
		Endosulfan I	< 0.01
		Endosulfan II	< 0.01
		HCB	< 0.01
		Mirex	< 0.01
		Telodrin	< 0.01
		PCBs	< 1.0
Organophosphate (μ g/L)		Miscellaneous (mg/L)	
Diazinon	< 0.1	Chemical Oxygen Demand	< 10
Methyl Parathion	< 0.02	Un-Ionized Ammonia	< 0.1
Ethyl Parathion	< 0.02	Nitrite N	< 0.02
Malathion	< 0.05	Nitrate N	0.16
Ronnel	< 0.01	Total Phosphorus	< 0.05
Ethion	< 0.02	Chlorine Residual	< 0.02
Trithion	< 0.05		
Vapona	< 2.0		

^a Represents the values measured on dilution water used for the test

^b Represents the values obtained from the screens of May 1994

^c Represents the values obtained from the screen of January 1994

Note: Results of the chemical analyses supporting these data are on file at ABC Laboratories.

TABLE II

Water Quality Measurements During the Static Acute Toxicity Test
of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)
in Dilution Water Amended with 20 mg/L Humic Acid

Nominal Test Concentration (mg/L)	Water Quality											
	0 Hour				24 Hours				48 Hours			
	Temp. ^a °C	DO ^b mg/L	pH ^c	Temp. °C	DO mg/L	pH	Temp. °C	DO mg/L	pH	Temp. °C	DO mg/L	pH
Control A/B	12.6/12.9	9.1/9.1	8.2/8.2	12.3/12.2	8.4/8.3	8.0/8.1	12.7/12.7	7.9/7.9	7.9/7.9	12.7/12.7	7.9/7.9	7.9/7.9
Humic Acid Control A/B	12.5/12.1	9.2/9.1	8.2/8.7	12.2/12.2	8.3/8.2	8.0/8.0	12.7/12.7	7.9/7.9	7.9/7.9	12.7/12.7	7.9/7.9	7.9/7.9
5.6 A/B	12.1/12.2	9.1/9.0	8.6/8.5	12.1/12.1	8.0/8.0	8.1/8.1	12.7/12.6	7.5/7.7	7.9/8.0	12.7/12.6	7.5/7.7	7.9/8.0
10 A/B	12.2/12.4	8.9/8.9	8.5/8.6	12.1/12.2	8.3/8.4	8.2/8.2	12.7/12.7	8.1/8.0	8.1/8.1	12.7/12.7	8.1/8.0	8.1/8.1
18 A/B	12.6/12.6	8.8/8.9	8.5/8.5	12.2/12.1	8.2/7.9	8.1/8.1	12.7/12.7	7.7/7.3	8.0/7.9	12.7/12.7	7.7/7.3	8.0/7.9
32 A/B	12.5/12.5	9.2/9.1	8.4/8.4	12.2/12.2	8.2/8.5	8.1/8.1	12.7/12.8	7.6/7.4	7.9/7.9	12.7/12.8	7.6/7.4	7.9/7.9
56 A/B	12.5/12.6	9.2/9.2	8.4/8.4	12.1/12.1	9.3/9.2	8.3/8.3	12.8/12.9	9.1/9.1	8.3/8.3	12.8/12.9	9.1/9.1	8.3/8.3
100 A/B	12.9/12.8	9.4/9.2	8.4/8.3	12.2/12.2	9.3/9.4	8.3/8.3	12.8/12.8	9.1/9.2	8.3/8.3	12.8/12.8	9.1/9.2	8.3/8.3

^a Temperature measured with a mercury thermometer

^b Dissolved oxygen concentrations - YSI Dissolved Oxygen System Model 54 ARC

^c pH - Beckman Model Φ 34 pH meter with a Beckman Model 39841 probe

NOTES: Dissolved oxygen saturation values corrected for altitude at the test temperatures of 12 and 13°C are 10.3 and 10.1 mg/L, respectively. Total organic carbon (TOC) measured in triplicate on control water collected at 0 hour before fish were added to the test chambers was 2.62, 2.62, and 2.82 mg/L (\bar{x} = 2.69 mg/L). Total organic carbon (TOC) measured in triplicate on humic acid control water collected at 0 hour before fish were added to the test chambers was 14.5, 14.0, and 13.9 mg/L (\bar{x} = 14.1 mg/L).

TABLE II (continued)

Water Quality Measurements During the Static Acute Toxicity Test
of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)
in Dilution Water Amended with 20 mg/L Humic Acid

Nominal Test Conc. (mg/L)	Water Quality					
	72 Hours			96 Hours		
	Temp. ^a °C	DO ^b mg/L	pH ^c	Temp. °C	DO mg/L	pH
Control A/B	12.4/12.4	7.7/7.9	7.7/7.7	12.7/12.8	7.6/7.5	7.8/7.8
Humic Acid Control A/B	12.4/12.4	7.8/7.7	7.7/7.7	12.8/12.7	7.3/7.1	7.7/7.7
5.6 A/B	12.3/12.4	7.2/7.6	7.8/7.9	12.8/12.9	6.8/7.0	7.7/7.8
10 A/B	12.4/12.6	7.9/7.8	8.0/8.0	12.8/12.8	7.2/7.1	7.9/7.9
18 A/B	12.6/12.6	7.7/7.3	7.9/7.8	12.7/12.7	7.1/7.0	7.8/7.8
32 A/B	12.6/12.6	7.3/6.8	7.8/7.8	12.7/12.8	7.1/6.3	7.7/7.8
56 A/B	12.4/12.4	8.2/7.8	8.2/8.2	12.7/12.7	6.9/6.7	8.1/8.1
100 A/B	12.4/12.5	9.0/9.1	8.3/8.3	12.6/12.8	8.3/8.3	8.3/8.3

^a Temperature measured with a mercury thermometer

^b Dissolved oxygen concentrations – YSI Dissolved Oxygen System Model 54 ARC

^c pH – Beckman Model Φ 34 pH meter with a Beckman Model 39841 probe

NOTE: Dissolved oxygen saturation values corrected for altitude at the test temperatures of 12 and 13°C are 10.3 and 10.1 mg/L, respectively.

TABLE III

Mortality and Behavioral Observations During the Static Acute Toxicity Test of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*) in Dilution Water Amended with 20 mg/L Humic Acid

Nominal Test Conc. (mg/L)	Number Placed in Test	24 Hours		48 Hours		72 Hours		96 Hours	
		Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations
Control	20	0	20 N	1	19 N	1	19 N	1	19 N
Humic Acid Control	20	0	20 N	0	20 N	0	20 N	0	20 N
5.6	20	0	20 N	0	20 N	0	20 N	0	20 N
10	20	0	20 N	0	20 N	0	1 DK; 19 N	0	1 DK; 19 N
18	20	0	20 N	0	3 LR; 17 N	0	3 LR; 17 N	1	1 DK; 1 LR; 17 N
32	20	0	8 LR; 12 OB/LR	0	6 LR; 14 N	0	7 LR; 13 N	0	7 DK/LR; 13 N
56	20	20	---	20	---	20	---	20	---
100	20	20	---	20	---	20	---	20	---

Key to Observations: N = Normal; LR = Labored Respiration; OB = Fish on Bottom of Test Chamber; DK = Dark Discoloration

TABLE IV

Static Acute Toxicity of CT-529-94 to Rainbow Trout (*Oncorhynchus mykiss*)
in Dilution Water Amended with 20 mg/L Humic Acid

Test Substance	LC ₅₀ in mg/L			
	24-Hour	48-Hour	72-Hour	96-Hour
CT-529-94 ^a	42 ^b	42 ^b	42 ^b	42 ^b
	(32 and 56) ^c			

N = 20 fish per concentration

^a Toxicity test as conducted at $12 \pm 1^\circ\text{C}$, mean \pm SD fish wet weight and standard length, 0.48 ± 0.11 g and 35 ± 3 mm, standard length range 30 to 39 mm.

^b The LC₅₀ was calculated using the binomial method.

^c 95% confidence limits.

The 96-hour no-observed effect concentration was 5.6 mg/L, based on the lack of mortality or abnormal effects at this concentration.

REFERENCES

- (1) U.S. Environmental Protection Agency. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). *Federal Register*.
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RAW DATA APPENDICES

ABC Laboratories Study # 41679

Static Acute Toxicity of CT-529-94
to Rainbow Trout (*Oncorhynchus mykiss*)
in Dilution Water Amended with 20 mg/L Humic Acid

NOTE: Some of the records that appear in this raw data appendix 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 acclimation records, organism culture logs, and blended water chemical screen results.

Some of the following abbreviations may have been used in the raw data:

E - Entry Error

R - Recording Error

S - Spelling Error

T - Transcription Error

F - Form Change

C - Calculation 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

Jane Bowman
Biologist III

Dorothy England
Biologist II

Marvin D. Hoffman
Biologist I

Warren Railton
Laboratory Technician II

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

**"This is an exact copy of
The original document"**

NAME	SIGNATURE	INITIAL	DATE
William J. Adams	<i>William J. Adams</i>	WJA	2/9/94
William A. McAllister	<i>William A. McAllister</i>	WAM	2-4-94
Alan D. Forbis	<i>Alan D. Forbis</i>	AF	2-4-94
Jon E. Rhodes	<i>Jon E. Rhodes</i>	JR	2-4-94
James B. Bussard	<i>James B. Bussard</i>	JBB	2-9-94
Tom Leak	<i>Tom Leak</i>	TL	2-4-94
Charles E. Jameson	<i>Charles E. Jameson</i>	CEJ	2-4-94
Timothy J. Madsen	<i>Timothy J. Madsen</i>	TJM	02-04-94
Stephen L. Hicks	<i>Stephen L. Hicks</i>	SLH	2-7-94
Scott J. Voney	<i>Scott J. Voney</i>	SSV	2-7-94
Paul Cohle	<i>Paul Cohle</i>	PC	2-4-94
Doug Gledhill	<i>Doug Gledhill</i>	DG	2-7-94
Hugh Murrell	<i>Hugh Murrell</i>	HMM	2-4-94
Ryan Warbritton	<i>Ryan Warbritton</i>	RW	2-4-94
Amy Adams	<i>Amy Adams</i>	AA	2-8-94
Robert Pezold	<i>Robert Pezold</i>	RGP	2-4-94
Marc C. Sword	<i>Marc C. Sword</i>	MCS	2/4/94
Tammy Strawn	<i>Tammy Strawn</i>	TS	2-7-94
Dorothy C. England	<i>Dorothy C. England</i>	DCE	2/7/94
Warren Railton	<i>Warren Railton</i>	WR	2-4-94
Jane H. Bowman	<i>Jane Bowman</i>	JHB	2-4-94
Janelle L. Downing	<i>Janelle L. Downing</i>	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	<i>Laurie L. Roesel</i>	LAR	2-7-94
Michelle A. Muckerman	<i>Michelle A. Muckerman</i>	MM	2/7/94
Jamie L. Veltri	<i>Jamie L. Veltri</i>	JLV	2/7/94
John Bucksath	<i>John Bucksath</i>	JDB	2/7/94
Kathryn Konering	<i>Kathryn Konering</i>	KK	2-8-94
Bret Hurshman	<i>Bret A. Hurshman</i>	BAH	2-8-94
Luke Stuerman	<i>Luke Stuerman</i>	LMS	2/8/94
Gerald A. Nothdurft	<i>Gerald A. Nothdurft</i>	GAN	2-7-1994
Yuan Yang	<i>Yuan Yang</i>	YY	2-7-94
Jianping Liu	<i>Jianping Liu</i>	JL	2/8/94
Karen March	<i>Karen March</i>	KM	2-8-94
David Burgess	<i>David Burgess</i>	DB	2-8-94
Debbie Jameson	<i>Debbie Jameson</i>	dj	2-7-94
Anita M. Klick	<i>Anita M. Klick</i>	AMK	2-9-94
Donna S. Hoek	<i>Donna S. Hoek</i>	DST	2/7/94
John Ingersoll	<i>John Ingersoll</i>	J	2-8-94
Edward Harper	<i>Edward Harper</i>	EH	2-8-94
D. Abram	<i>D. Abram</i>	DA	5-3-94
Christopher J. Pope	<i>Christopher J. Pope</i>	CJP	5-24-94
Marvin D. Hoffman	<i>Marvin D. Hoffman</i>	MH MDH	6-15-94
"This is an exact copy of The original document"			

By Laurie Roesel date 6-22-94

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.

**SIGNATURE & INITIAL IDENTIFICATION
ENGINEERING SURVEYS AND SERVICES**

NAME & TITLE	SIGNATURE	INITIAL	DATE
Chris Segafredo Director of Environmental Services	<i>Chris Segafredo</i>	<i>CS</i>	30 Dec 93
Linda Adams, Laboratory Manager	<i>Linda L. Adams</i>	<i>LA</i>	30 Dec 93
Rebecca Ann Bergfield, Chemist	<i>Rebecca Bergfield</i>	<i>RB</i>	30 DEC 93
Tina M. Tucholski, Chemist	<i>Tina M. Tucholski</i>	<i>TMT</i>	30 Dec 93
<p>"This is an exact copy of The original document"</p> <p>By <u><i>Ann Wood</i></u> date <u><i>6-22-94</i></u></p>			

Note: This list includes personnel of Engineering Surveys and Services Testing Laboratories located at 1113 Fay Street in Columbia, Missouri, who provided analyses in support of various studies performed by the Environmental Toxicology division of ABC Laboratories. This list of personnel is not study specific. Primary project personnel will be identified separately for each project.

MORTALITY AND BEHAVIORAL OBSERVATIONS - FISH

Test Material: CT-529-94 w 20mg/L HA Protocol No.: 8701 PMP Definitive
 Study Director: MARC Sward Study #: 41679 Preliminary

Test Species: *L. macrochirus* *O. mykiss* *P. promelas*
 No./Vessel: 10 Dil. Water: Blended Fish Lot #: 494 Fish Fed: NO
 Acclimation Mortality: 0 Waterbath: 1 Date Initiated: 5-12-94
 Compound Added: 3:23 PM Fish Added: 3:42 PM Date Terminated: 5-16-94

Test Conc. mg/L (ppm)	24 Hours		48 Hours			72 Hours			96 Hours		
	Dead	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.
Control A	0	10N	0	0	10N	0	0	10N	0	0	10N
Humic Acid Control	0	10N	0	0	10N	0	0	10N	0	0	10N
5.6A	0	10N	0	0	10N	0	0	10N	0	0	10N
10A	0	10N	0	0	10N	0	0	10N	0	0	10N
18A	0	10N	0	0	2LR 8N	0	0	2LR 8N	0	0	10K 1LA 8N
32A	0	7LR, 0QB 3LR	0	0	3LR 7N	0	0	4LR 6N	0	0	4DK, LR 6N
56A	10	---	0	10	---	0	10	---	0	10	---
100A	10	---	0	10	---	0	10	---	0	10	---

Observer Date/Time: MDH 5-12-94 3:46 JHB 5-14-94 3:55 PM JHB 5-15-94 4:15 PM MDH 5-16-94 3:30

REMARKS: N = Normal; LOE = Loss of Equilibrium; Q = Quiescent; SUR = Surfacing; ES = Erratic Swimming; DK = Dark Discoloration; OB = On Bottom; LR = Labored Respiration

QA Procedure Audit
 (1) E MDH 5-13-94 (2) E MDH 5-16-94 (3) E MDH 6-3-94

Prepared by: Maria Hoffman Date: 5-12-94
 Reviewed by: Marc Sward Date: 5-26-94
 Approved by: Marc Sward Date: 5-26-94

MORTALITY AND BEHAVIORAL OBSERVATIONS - FISH

Test Material: CT 52-94 w/20mg/L HA Protocol No.: 8901 PMN Definitive
 Study Director: MARC Sward Study #: 41679 Preliminary
 Test Species: *L. macrochirus* *O. mykiss* *P. promelas*
 No./Vessel: 10 Dil. Water: Blended Fish Lot #: 494 Fish Fed: NO
 Acclimation Mortality: 0 Waterbath: 1 Date Initiated: 5-12-94
 Compound Added: 3:23 pm Fish Added: 3:42 pm Date Terminated: 5-16-94

Test Conc. mg/L (ppm)	24 Hours		48 Hours			72 Hours			96 Hours		
	Dead	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.
Control B	0	10N	1	1	9N	0	1	9N	0	1	9N
Humic Acid Control B	0	10N	0	0	10N	0	0	10N	0	0	10N
5.6 B	0	10N	0	0	10N	0	0	10N	0	0	10N
10 B	0	10N	0	0	10N	0	0	10N	0	0	10N
18 B	0	10N	0	0	1LR 9N	0	0	1LR 9N	0	0	10N
32 B	0	5LR, OB 5LR	0	0	3LR 7N	0	0	3LR 7N	0	0	3DK, LR 7N
56 B	10	—	0	10	—	0	10	—	0	10	—
100 B	10	—	0	10	—	0	10	—	0	10	—

Observer Date/Time: Marianne Hoffmann 5/13/94 3:45 JHB 5-14-94 3:55pm JHB 5-15-94 4:15pm MDH 5-16-94 3:30pm

REMARKS: N = Normal; LOE = Loss of Equilibrium; Q = Quiescent; SUR = Surfacing; ES = Erratic Swimming; DK = Dark Discoloration; OB = On Bottom; LR = Labored Respiration

QA Procedure Audit

Prepared by: Marianne Hoffmann Date: 5-12-94
 Reviewed by: Marc Sward Date: 5-26-94
 Approved by: Marc Sward Date: 5-26-94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 w 20mg/L HA Protocol No. 8901 PMV

Study Director: Marc Sword Study #: 41679

5-12-94 MDH - 500 ml beakers were used to weigh out test material into. They were labeled with; Study #, Compound, Test level and Concentration. After Test Compound was added to the beakers A Teflon Coated Stir bar + 250 ml of test water from each^① of the 15 L Test systems were added to the beakers and placed on Stir plates until solution went in. The beakers were dumped into the matching test Jar and the beaker was rinsed with a 25 ml DI H₂O.

The Humic Acid was weighed into scint vial labeled to correspond with Test jars. And rinsed with D.I. H₂O.

The Test solutions were stirred with a glass rod prior to fish addition

All Test solutions had A dark appearance except the control. The higher levels had a murky^② milky look.

- 5-13-94 MDH Control - clear no precip or surface film A+B
- A+B Humic Control - Clear + Brown no precipitate
- A+B 5.6 mg/L - Clear, Brown + small amount of Brown precipitate
- A+B 10 mg/L - Clear Brown with Brown precipitate
- A+B 18 mg/L - Slightly Cloudy with Brown precipitate on bottom and floating
- A+B 32 mg/L - Slightly Cloudy with Large amount of Brown precipitate on Bottom + floating

① w MDH 579-94 ② s acc 6/15/94

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword Date: 5-26-94

Study Director: Marc Sword Date: 5-26-94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 w/ 20mg/L HA Protocol No. 8901 PMK

Study Director: Marc Sword Study #: 41679

5/13/94
MOH A+B 56 mg/L - milky, Cloudy brown with small amounts of precipitates, brown surface scum.
A+B 100mg/L - Same Observation AS For the 56mg/L solution

5-14-94
JHB solution observations on control, 5.6 and 10 mg/L solutions were the same as on 5-13-94.
The humic acid control were clear brown with small amount of brown precipitate
the 18 mg/L solution were the same as on 5-13-94.
the 32 mg/L solution were nearly colorless with tinge of brown with large amount of brown precipitate on bottom and some floating precipitate
the 56 and 100 mg/L solution were the same as on 5-13-94 except the 100 mg/L solution has a large amount of white scum on the surface.

5-15-94
JHB same solution observations as on 5-14-94 except the 56 mg/L solution were less cloudy than earlier.

5-16-94
MOH Control A+B, Clear, No precipitate
Humic Acid Control A+B - Same as observation as 5-13
5.6 mg/L A+B - Same observation as on 5-13
10 mg/L A+B - Same observation as on 5-13
18 mg/L A+B - Same observation as on 5-13
32 mg/L A+B - Clear, heavy precipitate on bottom, small ^{FLECKS} in suspension
56 mg/L A+B - Clear, heavy precipitate on bottom, floating precipitate + some in suspension
100 mg/L A - The same as on 5-13
B - White floating precipitate & the same as on 5-13

① E JHB 5-14-94

② S mcs 6/15/94

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword Date: 5/26/94

Study Director: Marc Sword Date: 5/26/94

WATER QUALITY

Test Material: CT-529-94 w/20mg/L Ht Protocol No.: 8901 PMN
 Study Director: MARC SWORD Study #: 41679

Concentration (mg/L)	MDH 0 Day 5/12/94				MDH 5/13/94 Day 1				5-14-94 Day 2						
	Temp °C	DO mg/L	pH ^f	Hard. ^d	Total Alk. ^e	Temp °C	DO mg/L	pH ^c	Hard. ^d	Total Alk. ^e	Temp °C	DO mg/L	pH ^c	Hard. ^d	Total Alk. ^e
Control A	12.6	9.1	8.6	8.2		12.3	8.7	8.0			12.7	7.9	7.9		
Humic Acid Control	12.5	9.2	8.2			12.2	8.3	8.0			12.7	7.9	7.9		
5.6 A	12.1	9.1	8.6			12.1	8.0	8.1			12.7	7.5	7.9		
10 A	12.0	8.9	8.5			12.1	8.3	8.2			12.7	8.1	8.1		
18 A	12.6	8.8	8.5			12.2	8.2	8.1			12.7	7.7	8.0		
32 A	12.5	9.2	8.4			12.2	8.2	8.1			12.7	7.6	7.9		
56 A	12.5	9.2	8.4			12.1	9.3	8.3			12.8	9.1	8.3		
100 A	12.9	9.4	8.4			12.2	9.3	8.3			12.8	9.1	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 CaCO₃) analyzed using titrimetric method adopted from Standard Methods
 e Other:

Remarks: (1) Inform of addl 5-16-94 MDH 5/16/94 (2) conductivity, m/s 6-15-94
 Prepared by: Marc D. Hoffmann Date: 5-12-94
 Reviewed by: Marc Sword Date: 5-26-94

WATER QUALITY

Test Material: CT 589-94 w/20 mg/L HA Protocol No.: 8901 PMN
 Study Director: MARC Sward Study #: 41679

Concentration (mg/L)	Day 0				Day 1				Day 2				
	Temp °C	DO mg/L	pH ^c	Hard. d	Temp °C	DO mg/L	pH ^c	Hard. d	Temp °C	DO mg/L	pH ^c	Hard. d	Total Alk. d
Control B	12.9	9.1	8.2		12.1	8.3	8.1		12.7	7.9	7.9		
Humic Acid Control B	12.1	9.1	8.7		12.2	8.2	8.0		12.7	7.9	7.9		
56 B	12.2	9.0	8.5		12.1	8.0	8.1		12.7	7.7	8.0		
10 B	12.4	8.9	8.6		12.2	8.4	8.2		12.7	8.0	8.1		
18 B	12.6	8.9	8.5		12.1	7.9	8.1		12.7	7.3	7.9		
32 B	12.5	9.1	8.7		12.2	8.5	8.1		12.8	7.4	7.9		
56 B	12.6	9.2	8.9		12.1	9.2	8.3		12.9	9.1	8.3		
100 B	12.8	9.2	8.3		12.2	9.4	8.3		12.8	9.2	8.0		

- 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 CaCO₃) analyzed using titrimetric method adopted from Standard Methods
- e Other:

Remarks: OE 1105-14-94
 Prepared by: Marc Sward
 Reviewed by: Marc Sward

Date: 5-12-94
 Date: 5-26-94

WATER QUALITY

Protocol No.: 8901-PMN
 Study #: 41628

Test Material: CT-529-94 w/20 mg/L HA
 Study Director: *Marc Leonard*

Concentration (mg/L)	5-15-94				5-16-94				Day 9 MDH						
	Temp °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e	Temp °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e	Temp °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e
Control A	12.4	7.7	7.7			12.7	7.6	7.8							
Min. acid Control A	12.4	7.8	7.7			12.8	7.3	7.7							
5.6 A	12.3	7.2	7.8			12.5	6.8	7.7							
10 A	12.4	7.9	8.0			12.8	7.2	7.9							
18 A	12.6	7.7	7.9			12.7	7.1	7.8							
32 A	12.6	7.3	7.8			12.7	7.1	7.7							
56 A	12.7	8.2	8.2			12.7	6.9	8.1							
100 A	12.4	9.0	8.3			12.6	8.3	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 CaCO₃) analyzed using titrimetric method adopted from Standard Methods
- e Other:

Remarks:

Prepared by: *Jane Brown* Date: 5-15-94
 Reviewed by: *Marc Leonard* Date: 5-26-94

WATER QUALITY

Test Material: CT-529-94 w/20mg/L HA
 Study Director: Marie Scott

Protocol No.: 8901-PMN
 Study #: 41679

Concentration (mg/L)	Day 3 8/10					Day 4 MD1A					Day 5-16-94				
	Temp ^a °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e	Temp ^a °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e	Temp ^a °C	DO ^b mg/L	pH ^c	Hard. ^d	Total Alk. ^e
Control B	12.4	7.9	7.7			12.8	7.5	7.8			12.8	7.5	7.8		
Alumic Acid Control B	12.4	7.7	7.7			12.7	7.1	7.7			12.7	7.1	7.7		
5.6 B	12.4	7.6	7.9			12.9	7.0	7.8			12.9	7.0	7.8		
10 B	12.6	7.8	8.0			12.8	7.1	7.9			12.8	7.1	7.9		
8 B	12.6	7.3	7.8			12.7	7.0	7.8			12.7	7.0	7.8		
32 B	12.6	6.8	7.8			12.8	6.3	7.8			12.8	6.3	7.8		
56 B	12.4	7.8	8.2			12.7	6.7	8.1			12.7	6.7	8.1		
100 B	12.5	9.1	8.3			12.8	8.3	8.3			12.8	8.3	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 634 pH meter, ABC material code 163-640 used with a Beckman Model 39841 probe
- ^d Hardness and total alkalinity (mg/L as CaCO₃) analyzed using titrimetric method adopted from Standard Methods
- ^e Other:

Remarks:

Prepared by: Jane Boone Date: 5-15-94
 Reviewed by: Ken Shepard Date: 5-26-94

Continuous Temperature Graph

Test Material: CT-529-94 w/ 20 mg/L HA

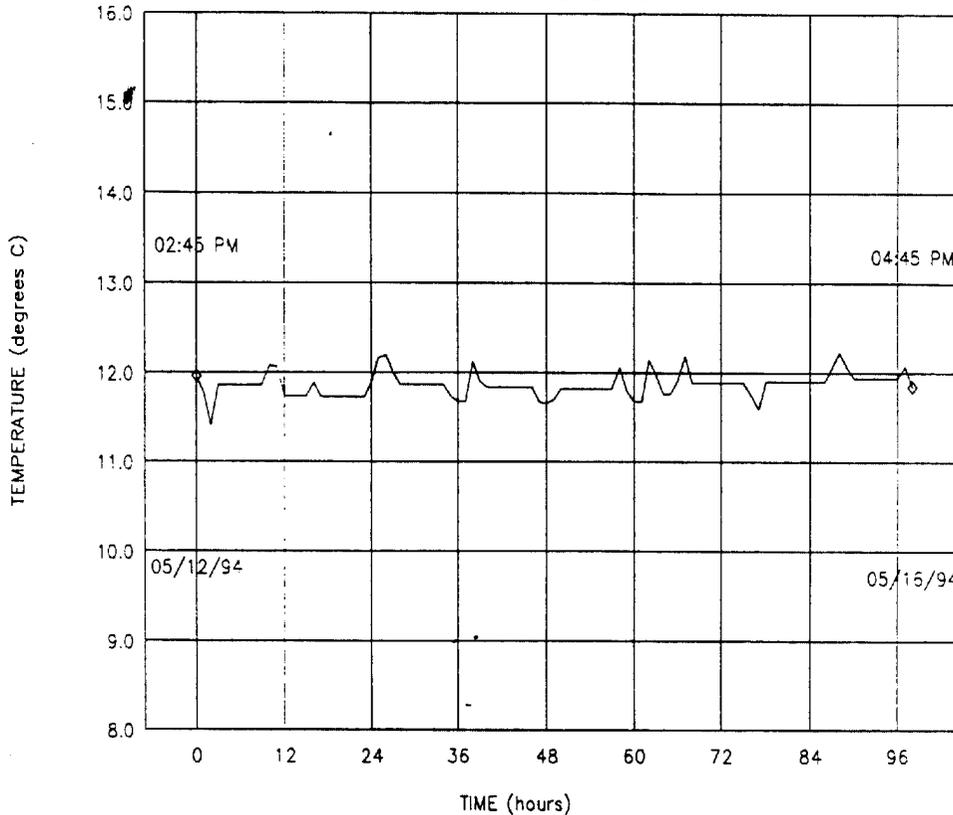
ABC Study Number: 41679

Protocol Number: 8901-PAN

Lab Form No: AQ9 (4/15/91)
ABC Laboratories
P.O.Box 1097, 7200 East ABC Lane
Columbia, Missouri 65205

Data Logger
Material Cont. No.: 1905-425

Waterbath No. 1
DEFINITIVE TEMPERATURE RECORDING



Comments: _____

DD-SCE 6/16/94

Prepared By: Ken Sword Date: 6/14/94

Reviewed By: Dorothy England Date: 6/16/94

Approved By: Ken Sword Date: 6/22/94

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST SOURCE WATER

WATER SOURCE: WAM Warm Hard Blended

TEMPERATURE: 24.0 °C

DO: 8.2 mg/L

pH: 8.2

ALKALINITY:* 152 mg/L

HARDNESS:* 140 mg/L

CONDUCTIVITY: 340 μmhos/cm

OTHER: —

INSTRUMENTS:

TEMPERATURE Mercury Digital

METER TYPE	BRAND	MODEL	MATERIAL CODE
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DISSOLVED

OXYGEN <input checked="" type="checkbox"/>	YSI	54ARC	1905-730
<input type="checkbox"/>	_____	_____	_____

pH Meter: <input checked="" type="checkbox"/>	BECKMAN	Φ34	163-640
Probe: <input type="checkbox"/>	BECKMAN	39841	-----
<input type="checkbox"/>	_____	_____	_____

CONDUCTIVITY: <input checked="" type="checkbox"/>	YSI	33	1905-520
<input type="checkbox"/>	_____	_____	_____

* Total alkalinity and hardness measured using a titrimetric method adapted from Standard Methods (mg/L as CaCO₃)

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By: John Sword date: 6/14/94

Analysis by: [Signature] Date: 5-12-94

Reviewed by: Jane Bowman Date: 5-17-94

SAMPLE COLLECTION FOR TOC ANALYSIS

Test Material: CT-529-94 w/20 mg/L HA Protocol No.: 8901-PMN

Study Director: MARC Sward Study #: 41679

Date/ID: 5-12-94 MDH

For each control or test level sampled, approximately 100 mL of dilution water were drawn from mid-chamber level of each replicate with a volumetric pipette and composited into an amber bottle for a total volume of approximately 200 mL. A second composite sample was collected in the same manner to retain as a back-up sample for each control or test level sampled. The samples were collected at 0 hour before fish addition. Samples were then prepared for total organic carbon (TOC) analysis by adding sulfuric acid until the pH was <2.0. The back-up sample(s) were then placed into a refrigerator at approximately 3°C for storage and the primary sample(s) were delivered to Engineering Surveys and Services Testing Laboratory in Columbia, MO, for TOC analysis.

Test Levels Sampled:

- Control
- Humic Acid Control
- Vehicle Blank
- Level 1
- Level 2
- Level 3
- Level 4
- Level 5

Sulfuric Acid

Lot #: 8592-1

Supplier: Fisher Scientific

pH meter: Beckman Model ϕ 34 with Beckman Model 39841 pH probe
ABC Material Control #163-640

Refrigerator ABC Material Control #166-12

Notes:

Prepared by: Maria Hoffman Date: 5-12-94

Reviewed by: Marc Sward Date: 5-26-94

**ENGINEERING SURVEYS AND SERVICES
TESTING LABORATORY**

1113 Fay Street • Columbia, Missouri 65201 • (314) 449-2646
802 El Dorado Drive • Jefferson City, Missouri 65101 • (314) 636-3303

ABC LABORATORIES
JUN 01 1994

Date 26 May 1994
Lab No. 4614

Project: ABC Laboratories - 103678G
Location: Columbia, Missouri **Date Received:** 16 May 1994
Sample No./
Description: 903 / Study 41672, Control, 0 hour, 5/11/94
904 / Study 41679, Control, 0 hour, 5/12/94
905 / Study 41679, Humic Acid Control, 0 hour, 5/12/94
906 / Study 41484, Control, Day 0, 5/13/94

TEST RESULTS

PARAMETER	METHOD	SAMPLE NUMBER				DETECTION LIMIT
		903	904	905	906	
Total Organic Carbon, mg/l	5310 B	ND	2.62	14.5	ND	1.00
Total Organic Carbon, mg/l	5310 B	ND	2.62	14.0	ND	1.00
Total Organic Carbon, mg/l	5310 B	ND	2.86	13.9	ND	1.00

Note: Sample secured and delivered to lab by others.

Note: Test performed in accordance with accepted GLP procedure.

ND = None Detected

**"This is an exact copy of #
The original document"**

P; Man Anual date 6/13/94

Method number from Standard Methods for the Examination of Water & Wastewater, current edition, unless otherwise noted.

1 Sword

CC:

ENGINEERING SURVEYS AND SERVICES
BY:

Chris L. Segarredo
Chris L. Segarredo

pg 0036

REC 1468704 1679

PREPARATION OF TEST CONCENTRATIONS (weight/volume)

Test Material: CT-529-94 w/20mg/L HA Protocol No.: 8901-PMN

Study Director: MARC Sward Study #: 41679

Purity: NA % (Batch/Lot No.: 3) ID No.: TS 7738

Sample Number	Target Weight (g)	Tare Weight (g)	Net Weight (g)	Adj. Net Weight* (g)	Dilution Volume (L)	Final Conc. (mg/L)
1	0.000	0.000	0.000	—	15	Control
2	0.000	0.000	0.000	—	15	Control
3	0.000	0.000	0.000	—	15	HA Control
4	0.000	0.000	0.000	—	15	HA Control
5	0.084 ¹	0.000	0.084	—	15	A 5.6
6	0.084	0.000	0.084	—	15	B 5.6
7	0.150	0.000	0.150	—	15	A 10
8	0.150	0.000	0.150	—	15	B 10
9	0.270	0.000	0.270	—	15	A 18
10	0.270	0.000	0.270 ²	—	15	B 18
11	0.480	0.000	0.480 ³	—	15	A 32
12	0.480	0.000	0.480 ³	—	15	B 32
13	0.840	0.000	0.840 ³	—	15	A 56
14	0.840	0.000	0.8399 ⁴	—	15	B 56

1.5 mL of N/A added to each test weight before addition to test chamber

1.5 mL of N/A added to vehicle blank test chamber

- * Corrected for purity or active ingredient of test compound
- Based on total product, i.e., not corrected for purity or active ingredient of test compound.

Balance Calibration: $\frac{0.1000}{0.200} \text{ g} + \frac{0.0000}{0.000} \text{ g} = \frac{0.1000}{0.200} \text{ g}$
Sartorius (Class C wt) (Tare) (Final wt) 1905-475

Balance used: Mettler PM460 ABC material control #: 1905-1020

Sample weight was stirred into the dilution volume with a glass stir rod or stainless steel paddle.

Comments: ¹ Changed balance because of error message Sartorius ABC #1905-475

² w/ MDH 5-12-94 4865
³ Balance calibration for Sartorius MDH 5 17-94

Note: After error message, a heavy class C weight was used to check calibration, 1.0000 g = 0.9999 g, MCS TARE 5-12-94

Weighed by: Maria Hoffman Date: 5-12-94

Reviewed by: Marc Sward Date: 5-26-94

Study Director: Marc Sward Date: 5-26-94

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

^① 9
CT 524-94

ABC SUPPLIED INFORMATION

Date Received: 04/21/94 Logged In By: RMF ABC Ref # TS-7238

Storage: Room Temp Total Weight: 436.9 g

Physical Description: Cloudy liquid

Remarks: _____

SUPPLIER PROVIDED INFORMATION

Firm: Cytec

Address: 2715 Miller Road

City/State/Zip Kalamazoo MI 49001 Phone: 616/349-6627

Batch/Lot No. 31 CAS # N/A Purity: N/A

Amount Declared: 250 ml Expiration Date: 2/95

Storage Instructions N/A

Other _____

① Compound mislabeled by Cytec. Should be CT 529-94 as per
Patty Vernon on 4/26/94
RMF

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The original document"**

By Paul Arnold date 4/14/94

PREPARATION OF TEST CONCENTRATIONS (weight/volume)						
Test Material: <u>CT-259[ⓐ] 94-529-94[ⓑ]</u>			Protocol No.: <u>8901-PMN</u>			
Study Director: <u>MARC Sward</u>			Study #: <u>411679</u>			
Purity: <u>N/A</u> %		Batch/Lot No.: <u>N/A</u>		ID No.: <u>NA</u>		
Sample Number	Target Weight (g)	Tare Weight (g)	Net Weight (g)	Adj. Net Weight* (g)	Dilution Volume (L)	Final Conc. (mg/L) [ⓐ]
1	0.300	0.000	0.300	—	15	20
2	0.300	0.000	0.300	—	15	20
3	0.300	0.000	0.300	—	15	20
4	0.300	0.000	0.301	—	15	20
5	0.300	0.000	0.301	—	15	20
6	0.300	0.000	0.300	—	15	20
7	0.300	0.000	0.301	—	15	20
8	0.300	0.000	0.301	—	15	20
9	0.300	0.000	0.300	—	15	20
10	0.300	0.000	0.300	—	15	20
11	0.300	0.000	0.302	—	15	20
12	0.300	0.000	0.300	—	15	20
13	0.300	0.000	0.301	—	15	20
14	0.300	0.000	0.300	—	15	20

1.5 mL of N/A added to each test weight before addition to test chamber

1.5 mL of N/A added to vehicle blank test chamber

* Corrected for purity or active ingredient of test compound
 Based on total product, i.e., not corrected for purity or active ingredient of test compound.

Balance Calibration: $\frac{0.200}{\text{(Class C wt)}} \text{ g} + \frac{0.000}{\text{(Tare)}} \text{ g} = \frac{0.200}{\text{(Final wt)}} \text{ g}$

Balance used: Mettler PM460 ABC material control #: 1905-1020

Sample weight was stirred into the dilution volume with a glass stir rod or stainless steel paddle.

Comments:
[ⓐ] E MDH 5-12-94
[ⓑ] Weights of Humic Acid (Aldrich lot # 01816HH) MDH 5-12-94 [ⓐ] E MRS 5-26-94

Weighed by: Maria Hoffman Date: 5-12-94
 Reviewed by: Marc Sward Date: 5-26-94
 Study Director: Marc Sward Date: 5-26-94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: ^{Receipt of} Humic Acid, Sodium Salt Protocol No. Various

Study Director: Various Study #: Various

4/4/94 The Humic Acid, Sodium salt, Tech. was received from Aldrich Chemical Company on 3/13/92. The catalog number was H#1,675-2, and the lot Number was 01816HH. The amount received was 1 kg. The humic acid is a black-crystalline powder and is stored at room temperature.

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By Tom Swast date 6/5/94

① E mes 4/4/94

② OF mes 3/4/94

NOTE: Individual entries must be dated and initialed.

Reviewed by: Tom Swast Date: 4/4/94

① Prepared
Reviewed by: Jane Boorman Date: 4-4-94
Study Director:

STATIC/FLOW THROUGH TEST ORGANISM MEASUREMENTS

Test Material: CT-529-94 w/20mg/L HA
 Study No.: 41679 Protocol No.: 8901 PMN
 ABC Study Director: MARC SWORD

Lab Form No. 539 (1/17/90)
 Analytical Bio-Chemistry Labs
 7200 ABC Lane, P.O. Box 1097
 Columbia, Missouri 65202

Test Species: O. mykiss Lot No.: 494
 Source: Mt. Lassen Trout Farm
 Date Measured: 5-16-94 By: M. Sword Group Measured: Control
 Balance Used: Mettler PM 460 ABC Material Control No.: 1905-10-20

A ⁰ Number	B ⁰	A ⁰ Standard Length (mm)		A ⁰ Weight (g)	
		A ⁰	B ⁰	A ⁰	B ⁰
1	11	32	36	0.411	0.520
2	12	36	35	0.374	0.498
3	13	32	34	0.355	0.440
4	14	39	30	0.607	0.307
5	15	33	32	0.398	0.357
6	16	37	35	0.555	0.502
7	17	36	38	0.522	0.552
8	18	39	38	0.732	0.623
9	19	35	36	0.534	0.519
10	20	32	— ^②	0.316	— ^③
Mean (±Standard Deviation)		35 ± 1 ^③ 2.8 ^③	35 ± 3 2.6 ^③	48 0.48 ± 0.11 1.3 ^③	48 1.0 ^③

Biomass loading rate at any point in-time:

Total weight of fish: $\frac{9.122 \text{ g} \text{ (3)} + 0.430 \text{ g} \text{ (7)}}{30 \text{ l of water}} = 0.32 \text{ g/l}$
 (9.122g + 0.430g)^⑦

Biomass Loading Rate for the Flow-Through System: Total weight of fish: _____ g ÷ _____ l/day on the average = _____ g/l/day

Remarks: ② The B replicate only had 9 fish remaining, mcs 5/17/94
 ④ Σ weight of 19 fish + mean wt = 9.602g, or Σ wt for 20 fish, mcs 6/14/94
 O'F MOH 5-16-94 ③ c mcs 6/14/94

Calculations By: Marie Hoffman / Marc Sword Date: 5-18-94 / 6-14-94
 Prepared By: Marie Hoffman Date: 5-16-94
 Checked By: Elizabeth S. ... Date: 6/16/94
 Study Director: Marc Sword Date: 6/22/94

LC₅₀ Calculations

ABC Study No. : 41679
 Compound Name : CT-529-94 with 20 mg/L HA
 Species : Rainbow Trout
 Exposure Period: 24 & 48 & 72 Hours
 Study Director : Marc Sword

Analytical Bio-Chemistry Labs
 7200 East ABC Lane
 P.O. Box 1897
 Columbia, Missouri 65205
 05-19-1994

—RESULTS CALCULATED USING THE BINOMIAL METHOD—

CONC. mg/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	20	20	100	LESS THAN 0.001
56	20	20	100	LESS THAN 0.001
32	20	0	0	LESS THAN 0.001
18	20	0	0	LESS THAN 0.001
10	20	0	0	LESS THAN 0.001
5.6	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT 32 AND 56 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 LC₅₀ OF 42.33200836181641 mg/L IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN 32 AND 56

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE OR PROBIT METHOD CAN GIVE STATISTICALLY SOUND RESULTS.

Method Reported: Binomial Moving Average Probit

Note: Method selected is that which gives the narrowest confidence limits for LC₅₀.

PREPARED BY: Marc Sword

DATE: 5/19/94

REVIEWED BY: Jane Bowman

DATE: 5 26 94

LC₅₀ Calculations

ABC Study No. : 41679	Analytical Bio-Chemistry Labs
Compound Name : CT-529-94 with 20 ug/L HA	7200 East ABC Lane
Species : Rainbow Trout	P.O. Box 1097
Exposure Period: 96 Hours	Columbia, Missouri 65205
Study Director : Marc Sword	05-19-1994

Page # 1

—RESULTS CALCULATED USING THE BINOMIAL METHOD—

CONC. ug/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	20	20	100	LESS THAN 0.001
56	20	20	100	LESS THAN 0.001
32	20	0	0	LESS THAN 0.001
18	20	1	5	2.002714205001754D-03
10	20	0	0	LESS THAN 0.001
5.6	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT 32 AND 56 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 LC₅₀ OF 42.33200836181641 ug/L IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN 32 AND 56

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE OR PROBIT METHOD CAN GIVE STATISTICALLY SOUND RESULTS.

Method Reported: Binomial Moving Average Probit

Note: Method selected is that which gives the narrowest confidence limits for LC₅₀.

PREPARED BY: Marc Sword

DATE: 5/19/94

REVIEWED BY: Jane Brown

DATE: 5-26-94

DOSE-RESPONSE CALCULATIONS

Test Material: LT-529-94 w/20 mg/L HAABC Study Number: 41679Protocol Number: 8901-PMNStudy Director: Mark SwordData at 96 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
56	1.748	100.0	7.33	6.42
32	1.505	0.0	2.67	4.47
18	1.255	5.0	3.36	2.47

Regression Output:

Constant	-7.5747
Std Err of Y Est	2.20654
R Squared	0.61513
No. of Observations	3
Degrees of Freedom	1

X Coefficient(s)	8.00340
Std Err of Coef.	6.33055

$$Y = 8.003 X + (-7.575)$$

Dose Response Slope = 8.00

Correlation Coefficient (r) = 0.78

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.

Data Entered By: Mark SwordDate: 6/14/94Reviewed By: Dorothy EnglandDate: 6/16/94

MORTALITY AND BEHAVIORAL OBSERVATIONS - FISH

Test Material: CT-524-94 w/200% HA Protocol No.: 8901-PMM Definitive
 Study Director: Marc Swartz Study #: 41677 Preliminary

Test Species: *L. macrochirus* *O. mykiss* *P. promelas*
 No./Vessel: 5 Dil. Water: Blended Fish Lot #: 494 Fish Fed: NO
 Acclimation Mortality: 0 Waterbath: 1 Date Initiated: 4/27/94
 Compound Added: 5:05 P.M. Fish Added: 5:10 P.M. Date Terminated: 5/1/94

Test Conc. mg/L (ppm)	24 Hours		48 Hours			72 Hours			96 Hours		
	Dead	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.	Dead	Cum. Mort	Obs.
0.10	0	5N				0	0	5N	0	0	5N
1.0	0	5N				0	0	5N	0	0	5N
10	0	5N				0	0	2 DK, 1 OB, 2 Q, 2N	0	0	2 DK, 1 OB, 2 Q, 2N
100	5	-				0	5	-	0	5	-
Observer	WR		①			MCS			MCS		
Date/Time	4-28-94/3:45 PM					4/30/94 3:56 PM			5/1/94 4:20 PM		

REMARKS: N = Normal; LOE = Loss of Equilibrium; Q = Quiescent; SUR = Surfacing;
 ES = Erratic Swimming; DK = Dark Discoloration; OB = On Bottom; LR = Labored Respiration

QA Procedure Audit ① No observations performed on 4/29/94 MCS 4/30/94 E.E. MCS 5-1-94

Prepared by: Wesley Radtke Date: 4-27-94
 Reviewed by: Norothy England Date: 5/4/94
 Approved by: Marc Swartz Date: 5/6/94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 w/20mg/L HA Protocol No. 8901-PAN

Study Director: Marc Sward Study #: 41679

4-27-94 WR The 10 and 100 mg/L solutions are cloudy
The 1.0 and 0.10 mg/L solutions are clear
All solutions are brown in color from the
Humic Acid

4-28-94 WR All solutions are less cloudy than on
4-27-94 but otherwise appear the same

4-30-94
MCS 100 mg/L solution has been discarded ^{due} ~~due~~ to
100% mat. 0.10, 1.0, and 10 mg/L solutions
are clear/Brown, some brown precipitate.
No observations recorded 4/29/94

5-1-94
MCS Same obs as 4/30/94

① 0 mg/L 5/1/94

NOTE: Individual entries must be dated and initialed.

Reviewed by: [Signature] Date: 5/4/94

Study Director: [Signature] Date: 5/6/94

PREPARATION OF TEST CONCENTRATIONS (weight/volume)

Test Material: CT-529-94 w/2-15/L HA Protocol No.: 8701-PAN
 Study Director: Marc Sward Study #: 41679

Purity: 100 % Batch/Lot No.: 31 ID No.: TS-7238

Sample Number	Target Weight (g)	Tare Weight (g)	Net Weight (g)	Adj. Net Weight* (g)	Dilution Volume (L)	Final Conc. (mg/L)
1	0.0015	0.0000	0.0015	—	15	1.0
2	0.015	0.000	0.015	—	15	10
3	0.150	0.000	0.150	—	15	100
4	1.500	0.000	1.500	—	15	1000
5						
6	0.200	0.000	0.200	—	15	20
7	0.300	0.000	0.300	—	15	20
8	0.300	0.000	0.300	—	15	20
9				—	15	20
10						
11						
12						
13						
14						

1.5 mL of acetone added to each test weight before addition to test chamber

1.5 mL of HA added to vehicle blank test chamber

- * Corrected for purity or active ingredient of test compound
- Based on total product, i.e., not corrected for purity or active ingredient of test compound.

Balance Calibration: $\frac{1.000 \text{ g}}{\text{(Class C wt)}} + \frac{0.000 \text{ g}}{\text{(Tare)}} = \frac{0.999 \text{ g}}{\text{(Final wt)}}$

Balance used: Mettler PM 460 ABC material control #: 1905-1020

Sample weight was stirred into the dilution volume with a glass stir rod or stainless steel paddle.

Comments: DE w/4-27-94 These are weights of Humic Acid w/4-27-94

Balance used: Sartorius R300's Mat. Cont. #1661-43 w/4-27-94

Note: Humic Acid is from Aldrich Chemical Co lot no. C1816 HH w/4-28-94

Weighed by: Warren Kasper Date: 4-27-94
 Reviewed by: Walter J. Englund Date: 5/4/94
 Study Director: Marc Sward Date: 5/6/94

PREPARATION OF TEST CONCENTRATIONS (weight/volume)

Test Material: CT-529-94 w/20 mg/L HA Protocol No.: 8901-PMJ
 Study Director: Marc Sword Study #: 41679

Purity: N/A % Batch/Lot No.: 31 ID No.: TS-7238

Sample Number	Target Weight (g)	Tare Weight (g)	Net Weight (g)	Adj. Net Weight* (g)	Dilution Volume (L)	Final Conc. (mg/L)
1	0.048	0.000	0.048	—	15	3.2
2	0.48	0.000	0.481	—	15	32
3				—		
4	0.300	0.000	0.299	—	15	20 [Ⓟ]
5	0.300 [Ⓟ]	0.000	0.300	—	15	20 [Ⓟ]
6						
7						
8						
9						
10						
11						
12						
13						
14						

1.5 mL of N/A added to each test weight before addition to test chamber

1.5 mL of N/A added to vehicle blank test chamber

- * Corrected for purity or active ingredient of test compound
- Based on total product, i.e., not corrected for purity or active ingredient of test compound.

Balance Calibration: $\frac{0.500}{\text{(Class C wt)}} \text{ g} + \frac{0.000}{\text{(Tare)}} \text{ g} = \frac{0.500}{\text{(Final wt)}} \text{ g}$

Balance used: Mettler PM460 ABC material control #: 1905-1020

Sample weight was stirred into the dilution volume with a glass stir rod or stainless steel paddle.

Comments: Ⓟ Humic acid wts MH 55-94

Weighed by: Marc Sword Date: 5-5-94
 Reviewed by: Marc Sword Date: 5/6/94
 Study Director: Marc Sword Date: 5/6/94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 w/o mg/L HA Protocol No. 8901 PMN

Study Director: MARC SWORD Study #: 41679

5-5-94 MDH Added 5 Fish to the 2 Test Concentrations (3.2 + 32) with the Humic Acid The Test Solutions appear very dark and cloudy. In the low concentration it appears that all of the test compound is in solution. The high level (32 mg/L) seems to have small white flecks floating in it.

5-6-94 MDH Test solutions appear the same as at 0 Hour except for a small amount of precipitation in the low level (3.2 mg/L). The high level (32 mg/L) had a large amount of precipitate formed on the bottom.

5/7/94
DCS The 3.2 mg/L jar is brown colored with some ppt.
The 32 mg/L jar is brown + somewhat cloudy with lots of brown ppt.

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword Date: 5/26/94

Study Director: Marc Sword Date: 5/26/94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT 529-94 w/20 mg/L HA Protocol No. 8901-PMU

Study Director: MARC SWORD Study #: 41679

5-6-94 MSH After solutions were stirred they appeared cloudy. the low level had some foam & the high level had some flakes floating.

fish were added at 2:30 pm, one at a time low level then high level until 5 fish were in each Test level.

5-7-94 DCE Both 100 and 320 mg/L jars are cloudy ^{+ brown} with a surface scum having a whitish color.

Page 2

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword Date: 5/26/94

Study Director: Marc Sword Date: 5/26/94

OBSERVATIONS AND/OR REMARKS FORM

Test Material: CT-529-94 W/20 mg/L HA Protocol No. 8901-PMNStudy Director: MARC Sword Study #: 41679

5-6-94 MOH Took 2 Scintillation vials, labeled them (Humic Acid, and the date) placed them in the balance tared them individually. Humic Acid, Aldrich lot # 01816HH. Used a metal Spatula to weigh out Vial 1 = .304g Vial 2 = 0.304

Weighing out test Compound:

2, 500 ml beaker were tared on the balance.

Beaker 1 + Com. = ~~Beaker 2~~ . The

Beakers were labeled with Study Number, Date Test Compound AND Concentration. A Pipet & Bulb were used to transfer Compound to the beakers. Beaker 1 = 1.502 Beaker 2 = 4.804

A Graduate Cylinder with 300 ml of DI water was poured into the beakers and a teflon Stir bar was also added.

PAAFilm was placed over the tops of the beakers and they were placed on a stir plate for 20 minutes.

Poured Humic Acid into the test vessels and rinsed the scint vials with approximately 15mls of DI Water.

The took beaker of test solution and poured them into the tests systems. rinsed the beakers with 20mls of test water and poured into test systems. Stirred test system with glass rod starting with low → high levels

DE MOH 5/6/94

Page 1

NOTE: Individual entries must be dated and initialed.

Reviewed by: Marc Sword

Date: 5/26/94

Study Director: Marc Sword

Date: 5/26/94

PREPARATION OF TEST CONCENTRATIONS (weight/volume)						
Test Material: <u>CT-529-94 w/20 mg/L HA</u>				Protocol No.: <u>8901-PMN</u>		
Study Director: <u>MARC Sward</u>				Study #: <u>41679</u>		
Purity: <u>N/A</u> % (Batch)/Lot No.: <u>31</u>				ID No.: <u>TS-7238</u>		
Sample Number	Target Weight (g)	Tare Weight (g)	Net Weight (g)	Adj. Net Weight* (g)	Dilution Volume (L)	Final Conc. (mg/L)
1	1.500	0.000	1.502	—	15	100
2	4.800	0.000	4.804	—	15	320
3						
4	0.300	0.000	0.304	—	15	20 [Ⓢ]
5	0.300	0.000	0.304	—	15	20 [Ⓢ]
6						
7						
8						
9						
10						
11						
12						
13						
14						

1.5 mL of N/A added to each test weight before addition to test chamber

1.5 mL of N/A added to vehicle blank test chamber

* Corrected for purity or active ingredient of test compound
 Based on total product, i.e., not corrected for purity or active ingredient of test compound.

Balance Calibration: 0.200 g + 0.000 g = 0.201 g
 (Class C wt) (Tare) (Final wt)

Balance used: Mettler PM 460 ABC material control #: 1905-1020

Sample weight was stirred into the dilution volume with a glass stir rod or stainless steel paddle.

Comments:
 ① Humic Acid wts MH 5-6-94
 ② w mth 5-6-94

Weighed by: <u>Marc Hoffman</u>	Date: <u>5-6-94</u>
Reviewed by: <u>Marc Sward</u>	Date: <u>5/26/94</u>
Study Director: <u>Marc Sward</u>	Date: <u>5/26/94</u>

FISH ACCLIMATION RECORD

Species: Rainbow Trout Species Lot #: 494
 Acclimation Tank #: *Both DP # Removed from Culture ≈260
 Culture Tank/Aquarium (Living Stream) Section #: LSB 1,2+3
 Date Removed: 5-9-94 Date Returned: 5-13-94
 Dilution Water Type: Hard Blended Well Salt

Acclimation Time (Hr)	Date	Obs.	Acclimation Temp.° (°C)	Acclimation Mortality	Number Used	Study Number
0	5/9/94	MDH	13°C	----	----	----
24	5/10/94	MDH	13°C	0		
48	5/11/94	MDH	13°C	0	60	41672
72	5/12/94	MDH	13°C	0	160	41679
96	5-13-94	MDH	13°C	0		

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 By Kurt Axel date 6/14/94

Comments:
 -
 -

* Temperature measured using a mercury/digital thermometer
 Remarks: Note all fish held without food during acclimation
 Prepared by: Main Hoffman Date: 5-9-94
 Reviewed by: Duss Award Date: 6-2-94

FISH ACCLIMATION RECORD

Species: Rainbow trout Species Lot #: 494
 Acclimation Tank #: FF # Removed from Culture ~175
 Culture (Tank/Aquarium/Living Stream) Section #: Tank 1
 Date Removed: 4-23-94 Date Returned: 4-29-94
 Dilution Water Type: Hard Blended Well Salt

Acclimation Time (Hr)	Date	Obs.	Acclimation Temp. ^a (°C)	Acclimation Mortality	Number Used	Study Number
0	4-23-94	KJ	-12	----	----	----
24	4-24	KJ	12	0		
48	4-25	JHB	15 13	0		
72	4-26	WR	13	0	15	41587
96	4-27	JHB	13	0	15 20	41672
					15 20	41679

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The original document"

By M. Sworel date 6/14/94

Comments: ① E JHB 4-25 94
 ② E WR 4-27-94

^a Temperature measured using mercury/digital thermometer
 Remarks: Note - all fish held without food during acclimation

Prepared by: [Signature] Date: 4-23-94
 Reviewed by: M. Sworel Date: 5/2/94

FISH ACCLIMATION RECORD

Species: Rainbow Trout Species Lot #: 494
 Acclimation Tank #: GG # Removed from Culture ≈ 40
 Culture Tank/Aquarium/Living Stream Section #: B
 Date Removed: 5-2-94 Date Returned: 5-6-94
 Dilution Water Type: Hard Blended Well Salt

Acclimation Time (Hr)	Date	Obs.	Acclimation Temp. ^a (°C)	Acclimation Mortality	Number Used	Study Number
0	5-2-94	MDH	11	----	----	----
24	5-3-94	MDH	12	0		
48	5-4-94	MDH	12	0		
72	5-5-94	MDH	12	0	10	41679
96	5-6-94	MDH	12	0	10	41679

**"This is an exact copy of
 The original document"**
 P: Brentwood 6-14-94
 6/14/94

Comments: OD mcs 6-14-94

^a Temperature measured using a mercury/digital thermometer
 Remarks: Note - all fish held without food during acclimation
 Prepared by: Main Hoffman Date: 5-2-94
 Reviewed by: Kurt Awood Date: 6-2-94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Aquarium No: LSB Source: Mt. Lassen Trout Farm
 Water Type: Hard Food Lot No.: 41447 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: <3800
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
5-5-94 L	0	7 3	11	Returned fish pulled on 4-30-94 that were not used for studies. MDH 5-5-94
5-6-94 AA	0	7 4	11	Returned those removed on 5-2 MCS
5-7-94 DCE	1	10	11	
5-8-94 DCE	0	10	11	
5-9 L	1	9 4	11	Removed 260 Fish for Acclimation MDH
5-10-94 JL	0	7 4	11	
5-11-94 L	0	7 4	11	
5-12-94 L	0	7 4	11	
5-13-94 L	0	7 4	11	Returned acclimation fish pulled on 5-9-94 not used for study
5-14-94 JHB	1	11:45 AM + 8 PM	11	
5-15-94 JHB	0	11:30 AM	11	
5-16-94 JL	0	7 5	12	
5-17-94 AA	0	7 4	10	
5-18-94 AA	0	7 4	11	

**This is an exact copy of
The original document**

By handwritten date 6/14/94

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 5-5-94
 Reviewed by: Ken Sword Date: 5/29/94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Tank/Aquarium No: 1 → LSB Source: Mt. Lassen Trout Farm
 Water Type: Hard Food Lot No.: 41447 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: <4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort. %	Feeding Schedule	°C Temp.	Treatment and/or Comments
4-21-94 AA	0	7 4	11	
4-22-94 AA	0	7 4	12	
4-23 LW	0	10	12	Removed 40 for Acclimation <u>Barrel A</u> Removed ~175 for Acclimation <u>Barrel FF</u>
4-24 LW	0	9	12	
4-25-94 AA	0	7 4	13	
4-26-94 II AA	0	7 4	12	
4-27-94 AA	0	7 4	12	
4-28-94 RW	0	7	12	moved to LSB
4-29-94 LW	20	7 4	11	Returned fish pulled on 4-23-94 <u>WR</u> all <u>4-29-94</u>
4-30-94 MCS	0	12 5	12	Removed ~30 for acclimation
5-1-94 MCS	0	1 5	12	
5-2-94 LW	0	7 4	11	Removed ≈ 40 for acclimation <u>MOB</u>
5-3-94 LW	0	7 3	11	"This is an exact copy of The original document"
5-4-94 II	0	7	11	

By Mark Award date 6-14-94

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 4-21-94
 Reviewed by: [Signature] Date: 5/7/94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Aquarium No: 1 Source: Mt. Lassen Trout Farms
 Water Type: Hard Food Lot No.: 41188 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: 4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
4-7-94 <u>II</u> ^W	0	7 3	11	
4-8-94 <u>II</u> ^W	0	7 4	11	
4-9-94 <u>DCI</u>	0	10	11	
4-10-94 <u>DCI</u>	0	10	11	
4-11-94 <u>II</u> ^W	0	7 3	11	
4-12-94 <u>II</u> ^W	4	7 4	11	Food lot 41188 changed to 41447
4-13-94 <u>II</u> ^{AA}	0	7 4	11	
4-14-94 <u>II</u> ^{AA}	0	7 4	11	
4-15-94 <u>II</u> ^{AA}	0	7 4	11	
4-16-94 <u>JHB</u>	0	11:50	12	Removed ~35 for acclimation
4-17-94 <u>JHB</u>	0	11:4	12	"This is an exact copy of The original document"
4-18-94 <u>AA</u>	0	7 4	11	By <u>M. Hoffmann</u> date <u>6-14-94</u>
4-19-94 <u>AA</u>	0	7 4	12	
4-20-94 <u>II</u> ^W	0	7 4	11	Removed 100+ tank numbers 2350s remain. Returned remaining fish from 4-16 acclimation JHB

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 4-7-94

Reviewed by: Mami Hoffmann Date: 5-3-94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Tank/Aquarium No: 1 Source: M. Lasser Trout Farms
 Water Type: Hard Food Lot No.: 41198 41443
 Date Rec'd: 1-24-94 No. Rec'd: 500 Appx. No. Remaining: 4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
3-24-94 ^{JK} SJ	0	7 4	11	Pulled ~150 fish for acclimation ① WR 3-24-94
3-25-94 AJ	0	7 4	11	Returned remaining fish pulled on 3-24-94 ① WR 3-25-94
3-26 h	0	9	11	① Entries made 3-29-94 WR
3-27 h	0	8	11	
3-28 AJ AA	0	7 4	11	
3-29-94 AA	0	8 4	11	Reviewed Page 3/29/94 man sword
3-30-94 AA	0	7 4	11	
3-31-94 AA	0	7 4	11	
4/1/94 MCS	0	9, 4	11	
4/2/94 MCS	0	10, 4	11	
4-3-94 MCS	0	11, 4	11	
4-4-94 AJ A	0	7 4	11	"This is an exact copy of The original document"
4-5-94 AJ h	0	7 4	11	
4-6-94 h	0 ⁺	7 4	11	① <u>man sword</u> date <u>6-14-94</u>

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 3-24-94
 Reviewed by: [Signature] Date: 4/9/94

FISH CULTURE RECORD

Species: Rainbow Trout Lot #: 494
 Tank/Aquarium No: 1 Source: Mt. Lassen Trout Farm
 Water Type: Hard Food Lot No.: 41188 41448
 Date Rec'd: 1-24-94 No. Rec'd: 5000 Appx. No. Remaining: 4100
 Lights On: 6:00 a.m. Lights Off: 10:00 p.m. Transition: 30 min

Date/ID	Mort.	Feeding Schedule	°C Temp.	Treatment and/or Comments
3-6-94 ^{AA} II ①	-	-	12	DEA 3-10-94
3-11-94 II	-	-	12	
3-12-94 ^{DC} II	-	-	12	
3-13-94 ^{DC} II	-	-	11	
3-14 R	-	-	12	
3-15-94 II	-	-	12	
3-16-94 ^R II	-	- 4	12	50% + swimming up
3-17-94 II	-	7	11	
3-18-94 ^{JHB} II	4 -	② 7 + 4	12 ^②	② E. II. 3-18-94 ② E JHB 3-18-94
3-19 JHD	3	11 + 2	12	
3-20 JHB	1	11:30 + 3	13	"This is an exact copy of The original document"
3-21-94 ^R II	0 ⁺	7 3	12	
3-22-94 ^R II	0	7 3	11	By <u>Ann Sword</u> date <u>6-14-94</u>
3-23-94 ^{IA} II	0	7 4	12	

Note: Temperature taken with a mercury thermometer.

Prepared by: [Signature] Date: 3-10-94
 Reviewed by: Ann Sword Date: 3/29/94

FISH CULTURE RECORD

Species: Rainbow Trout, Eggs Lot #: 494
 Source: Mt. Lassen Trout Farm Water Type: Hard
 Incubator No./Tray No: A-68
 Date Received: 1-27-94 No. Received: 5000 Appx. No. Remaining: 4100

Date/ID	Mortality				Not Dist.	°C Temp.	Comments
	Nonviable	Deformed	Dead	Total Mort.			
2-24-94 ^{hw}	—	100	25	125	—	8.4	
2-25 ^{hw}	—	—	—	—	✓	8.0	Hatch Start
2-26 ^{hw}	—	—	—	—	✓	8.2	25% Hatch
2-27 ^{hw}	—	—	—	—	✓	8.0	50% Hatch
2-28 ^{hw}	—	—	—	—	✓	8.5	90% Hatch
3-1 ^{hw}	—	330	500	830	—	8.0	Hatch complete
3-2 ^{hw}	—	—	—	—	✓	8.5	
3-3 ^{hw}	—	—	—	—	✓	8.0	
3-4 ^{hw}	—	18	—	18	—	8.0	
3-5 ^{mcs}	—	—	—	—	✓	8.2	
3-6 ^{mcs}	—	—	—	—	✓	8.2	
3-7 ^{hw}	—	54 ⁺⁷⁶	—	54 ⁺⁷⁶	—	8.2	130 total mort
3-8 ^{hw}	—	—	—	—	✓	7.8	increased temp slightly moved trays into tank 1 ~10°C
3-9-94 ^{hw}	—	—	—	—	✓	12	

This is an exact copy of
 The original document.
 By Kenneth date 6-14-99

* Temperature taken with a mercury thermometer

Prepared by: Kenneth Date: 2-24-94

Reviewed by: Kenneth England Date: 3/12/94

FISH CULTURE RECORD

Species: Rainbow Trout, Eggs Lot #: 494
 Source: Mt. Lassen Trout Farm Water Type: Hard
 Incubator No./Tray No: A-6-8
 Date Received: 1-27-94 No. Received: 5000 Appx. No. Remaining: 4500

Date/ID	Mortality				Not Dist.	°C Temp.	Comments
	Nonviable	Deformed	Dead	Total Mort.			
2-10 ⁹⁴ R ₂	—	—	—	—	✓	12.2	This is an exact copy of The original document BY <u>[Signature]</u> date <u>6-14-94</u>
2-11 R ₁	—	—	—	—	✓	12.8	
2-12 DCE	—	—	—	—	✓	13.0	
2-13 DCE	—	—	—	—	✓	13.0	
2-14 R ₂	275	—	20 ⁺⁵	300	—	13.4	light-moderate eye
2-15 R ₁	—	—	—	—	✓	13.2	
2-16 R ₂	—	—	+12	12	—	13.4	Decreased temp
2-17 R ₁	—	—	—	—	✓	11.6	Decreased temp
2-18 R ₁	—	—	—	—	✓	9.0	
2-19 ^① R ₁	—	—	—	—	✓	8.0	① R ₂ 2-12-94
2-20 —	—	—	—	—	—	—	No OBS taken No problems noted R ₂ 2-22-94
2-21 ^{AA} R ₁	—	—	—	—	✓	8.3	
2-22 R ₁	—	—	65	65	—	8.7	
2-23 R ₁	—	—	—	—	✓	8.4	

* Temperature taken with a mercury thermometer
 Prepared by: [Signature] Date: 2-10-94
 Reviewed by: [Signature] Date: 3/5/94

FISH CULTURE RECORD

Species: Rainbow Trout, Eggs Lot #: 494
 Source: Mt. Lassen trout farm
 Date Received: 1-27-94 # Received: 5000 ~ # Remaining: N/A
 Incubator/Tray # A-6-8 Water Type: Hard

Date/ID	Mortality				°C Temp.	Comments
	Nonviable	Deformed	Dead	Total Mortality		
1-27-94 R	-	-	-	-	~10.5	Not Disturbed
1-28 R	-	-	-	-	11.0	Not Disturbed
1-29 R	-	-	-	-	11	Not Disturbed
1-30 R	-	-	16	16	10.4	↑ temp
1-31 R	-	-	-	-	12.0	This is an exact copy of the original document BY <u>M. Swore</u> data 6-14-94
2-1 A	-	-	-	-	12.6	
2-2 A	-	-	-	-	12.4	
2-3 R	-	-	17	17	12.4	
2-4 R	-	-	-	-	12.6	
2-5 ACS	-	-	-	-	12.5	"
2-6 ACS	-	-	-	-	12.5	"
2-7 R	-	-	24	24	12.1	
2-8 R	-	-	-	-	12.2	Not Disturbed
2-9 R	425	-	15 ⁰	440	12.2	DE RW 2-9-94

* Temperature taken with a mercury thermometer

Prepared by: [Signature] Date: 1-27-94
 Reviewed by: [Signature] Date: 3/5/94

CULTURE ROOM OBSERVATIONS/REMARKS FORM

Mt. Lassen
Trout

28125 Hwy 36E
Red Bluff, CA 96080
(916) 271-2777

S O U R C E

ABC LABS
PO BOX 1097
7200 E. ABC Lane
Columbia, MO 65201

CLIENT ORDER NO. 18643-RW	DATE SHIPPED 1/26	SHIPPED VIA PFD EXPRESS I	TERMS net 30 da
QUANTITY ORDERED	BOX	QUANTITY SHIPPED	DESCRIPTION
		5000	GREEN EGGS & SPERM

"This is an exact copy of
The original document"

By *Mrs. Snow* date 2-14-94

Shipping
info for
lot 494
Rainbow trout
RW
1-27-94

MT. LASSEN TROUT FARM
SPAWN INFORMATION DATE: 1-26-94

SPECIES: Oncorhynchus mykiss
(formerly Salmo gairdneri)

SOURCE CODE: JC4

NUMBER OF EGGS: 5000 pooled

NUMBER OF FEMALES USED: 3

NUMBERS OF MALES USED: 3

(Normal or X-bearing)

PERSONNEL: *Jerry Alby*

Individual entries must be dated and initialed.

Prepared By: *Mrs. Habita* Date: 1-27-94

Reviewed By: *Mrs. Snow* Date: 3/5/94

RAINBOW TROUT EGG FERTILIZATION PROCEDURE

Arrival Date: 1-27-94

Acclimation Time: 3 1/2 hr

Source: Mt Lassen Trout farm

Temp: 9.2°C

Temp: 30°C

ABC Lot Number: 494

Rainbow Trout Egg Fertilization Procedure

Eggs and milt were allowed to acclimate to desired temperature before fertilizing. Upon acclimation the unfertilized eggs were transferred to a dry plastic bowl and milt added. Isothermal dilution water was added until the eggs were well covered and the mixture gently stirred. After stirring the eggs were left undisturbed for approximately one minute. The excess water was poured off and the newly fertilized eggs were rinsed several times with dilution water to remove any excess milt and ovarian fluid. The eggs were then allowed to water harden for a period of at least one hour before transfer into an incubator or placement into test systems.

**"This is an exact copy of
The original document"**

By Ken Oswald date 6-14-94

Fertilization Procedure

Conducted By: Lanny Strawn Date: 1-27-94

Reviewed By: [Signature] Date: 1-27-94

CYTEC

Order ~~1738~~ 1738

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

ABC LABORATORIES

APR 28 1994

April 27, 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-529-94 Aquatic Toxicity Studies

Dear Dave:

You will receive, under separate cover, a material identified as CT-529-94. Please submit this material to the following test at the following cost:

<u>Test</u>	<u>Protocol</u>	<u>Cost</u>
Acute Toxicity to Rainbow Trout in Dilution Water Amended with Organic Carbon	8901-PMN	 → 41679

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

ABC LABS#G 41679

pg 0069

Analytical Bio-Chemistry Laboratories, Inc.
Page 2
April 27, 1994

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

The test material, CT-529-94 is an opaque liquid with an oily odor. This material is soluble in water but limited by viscosity. The following special handling conditions apply to this material: CAUSES EYE AND SKIN IRRITATION - Store at room temperature away from light and heat source. Avoid contact with iron, copper or aluminum equipment.

THE TOXICOLOGICAL PROPERTIES OF CT-529-94 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.

-

Analytical Bio-Chemistry Laboratories, Inc.
Page 3
April 27, 1994

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 INC.

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

ACCEPTED: APRIL 29, 1994

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.

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

pavABC

DEFINITIVE CONCENTRATION NOTIFICATION

Test Material: CT-549-94 Protocol No.: 8101-PMN
529

Study Director: Marc Sword Study #: 41679

Firm: Cytac Industries

Phone: (201) 357-3375

Sponsor's Study Representative: Patricia A. Vernon

Person Notified: Same (X) Other:

Date of Notification: 5/11/94

Notified by: Marc Sword

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

Vehicle: N/A

<u>Test Material</u>	<u>ABC #</u>	<u>Test Species</u>	<u>Test Conc. (mg/L)</u>
① CT-549-94, <small>529</small> 20 mg/L Humic Acid Amended Dilution Water	41679	Rainbow Trout	Control, Humic Acid Control, 5.6, 10, 18, 32, 56, and 100 mg/L

① EMCS 5/11/94

Reviewed by: Dorothy England Date: 5/11/94

Study Director: Marc Sword Date: 5/11/94



"Working for You"

ABC LABORATORIES

MAY 4 1994

ABC PROTOCOL NO. 8901-PMN

(Revised April 18, 1994)

Static Bioassay Procedure for Determining the Acute Toxicity
of Chemical Substances to Freshwater Fish in Dilution
Water Amended with Organic Carbon

ABC Study # 41679

Test Substance CT-259-94

Test Species Rainbow Trout

PROTOCOL APPROVAL

ABC Laboratories' Study Director

Name (signed): Marc Sword Date: 5/5/94

Name/Title (typed): Marc Sword, Biologist II

Sponsor Representative

Name (signed): Patricia Ann Werner Date: 4/27/94

Name/Title (typed): ASSOCIATE TOXICOLOGIST

(Other sponsor-required signatures may be added below.)

TEST-SPECIFIC INFORMATION

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

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 substance. The sponsor also agrees to accept any and all of the test substance that remains unused at the end of testing and to assume responsibility for its proper disposal.

Testing Facility

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Phone: (314) 474-8579
Fax: (314) 443-9089

Study Sponsor

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

Phone: (201) 357-3375
Fax: (201) 357-3057

Sponsor Identification Number Check here if not applicable.

Number: _____

Test Substance(s) ⁼ CT-529-94
(name(s) used in report and correspondence)

Note: Written confirmation of percent purity along with specific activity and molecular weight, where applicable, must be provided.

Purity Correction (Please check the appropriate statement.)

Test concentrations will be based on purity.

Test concentrations will be based on total product.

Analytical Confirmation (Please check the appropriate statement.)

Analytical confirmation will be performed by ABC Laboratories, Inc.

Sponsor requests no analytical confirmation.

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

Concentration of Humic Acid

5 mg/L 10 mg/L 15 mg/L 20 mg/L

Special Instructions and/or Comments Check here if not applicable.

Test Concentrations Definitive test concentrations will be specified in a notification form or protocol alteration.

Test Organism (to be completed by testing facility)

Species: Rainbow Trout (Oncorhynchus mykiss)

Supplier: Mount Lassen Trout Farm, Red Bluff, CA

Experimental Dates (to be completed by testing facility)

Proposed starting date: May, 1994

Proposed termination date: May, 1994

1.0 INTRODUCTION

Aquatic toxicity tests have been used extensively to assess the environmental effects of chemical substances. ~~Indeed~~, 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 the Premanufacture Notification (PMN) process of TSCA, 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) which complement them, ABC Laboratories, Inc. (ABC) has prepared the following protocol to assist PMN submitters with 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, 11).

For compounds expected to be unstable under the static test conditions outlined in this protocol, consideration should be given to running the test under dynamic (flow-through) test conditions.

2.0 OBJECTIVE

The primary objective of the toxicity test described herein is to evaluate the acute toxicity of a chemical substance to freshwater fish using dilution water with various levels of organic carbon. This is achieved by determining LC₅₀ levels of the toxicant using organic carbon-amended dilution water during a 96-hour exposure period. An LC₅₀ is the approximate concentration of the test substance that produces 50% mortality of test fish after prescribed intervals. The method is designed to yield LC₅₀ values following 24, 48, 72, and 96 hours of exposure.

3.0 RANGE-FINDING STUDY

3.1 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 fish. The information derived from this preliminary test will be used to set concentration levels for the definitive bioassay described in Section 5.0.

- 3.2 Test System (Organism). The most common freshwater species used for aquatic toxicity testing includes bluegill (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus mykiss*), and fathead minnows (*Pimephales promelas*). Fish ranging in size from actively feeding fry to 5.0 g will be used as test organisms. The fish used in a test will be approximately the same size with the standard length of the largest fish being no more than twice that of the shortest fish. The fish will be obtained from an established commercial hatchery or in-house cultures. The particular source to be used is dependent upon the seasonal availability of test fish and will be listed in the test-specific information section on page 4, along with the species selected by the study sponsor or required by the EPA.

The test fish lot will be from the same year class and will be identified to species, if necessary, using the taxonomic keys developed by Eddy (12) and cultured following the techniques described by Brauhn et al. (13). All test fish will be held on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A record of fish observations during the holding period, along with any prophylactic and therapeutic disease treatments, will be kept and included with the final report. During the holding period, the fish will receive a standard commercial fish food, which may be supplemented by brine shrimp nauplii (*Artemia* sp.) and/or other aquatic invertebrates. The fish food will be analyzed for contaminants prior to use. The test lot will be held without food and acclimated gradually to the test temperature and dilution water for at least 48 hours prior to testing. The acclimation period will be initiated by transferring a subplot of fish from the culture system to a 50:50 mixture of culture water:test dilution water. After 24 hours, this water will be replaced with 100% dilution water, and daily replacements of dilution water will be made thereafter for a total acclimation period not to exceed 96 hours. If mortality of the test lot exceeds 3% in the 48 hours previous to testing, the fish will not be used.

- 3.3 Exposure System. The range-finding test will be conducted in 1-L glass beakers or 5-gallon widemouth glass jars containing 500 mL or 15 L of test solution, respectively, and five fish per concentration. The jars will be covered to keep out foreign objects. Each test vessel will be unambiguously labeled as to test compound, concentration, and study number. These test vessels will be immersed in a water bath with temperatures maintained within ± 1 °C of the desired test temperature. For temperature control in the water bath, thermostatically controlled heating elements will be used for warm water tests (22 ± 1 °C) and refrigeration units for cold water bioassays (12 ± 1 °C).

The dilution water used in aquatic toxicity testing at ABC Laboratories will be ABC well water prepared to a total hardness of between approximately 130 to 160 mg/L as CaCO₃. The specific water hardness will be achieved by blending

naturally hard ABC well water with ABC well water that has been demineralized by reverse osmosis. Data from periodic screening of dilution water for selected contaminants are archived at ABC Laboratories. The final report will contain results of the most recent analysis. This test design was selected to meet the requirements of the U.S. EPA.

To meet the objective of the study, ABC dilution water will be fortified with a natural occurring organic carbon. ABC will use humic acid as the organic carbon. The level of humic acid will be specified at the time of protocol approval.

3.4 Test Substance. A letter of authorization for the study should be sent with the test substance or before its shipment and should contain the following chemical/physical properties of the compound if available: name of test substance, 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: solubility in water and organic solvents, vapor pressure, hydrolysis rate and photolysis rate. **A Material Safety Data Sheet (MSDS) must be sent before or with sample shipment.**

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

3.5.1 Test fish will be acclimated gradually (Section 3.2) to the test temperature and dilution water for at least 48 hours prior to testing, during which time they will be held without food.

3.5.2 The range-finding test will be initiated by exposing groups of five fish to two or three toxicant concentrations, usually spaced apart by a factor of 10. If acute toxicity information is available from previous studies or if the sponsor indicates that the material is basically nontoxic only one preliminary test concentration may be used before the definitive test (e.g., 100 mg/L) to confirm this information. In certain cases where the toxic range is known or the sponsor requests a certain range of concentrations, preliminary testing will not be performed. The test fish will be placed in the test chambers in an impartial manner within 30 minutes after solution preparations. This will be achieved by sequentially adding one to two fish to each chamber until all chambers contain their complement of test fish. The initial toxicant concentrations most often used are 1.0, 10, and 100 mg/L. Numerous static tests by ABC have shown that a significant percentage of the compounds tested have aquatic toxicities that fall within this range.

- 3.5.3 After 24 hours of exposure, the test chambers will be observed for mortality and/or abnormal behavioral (sublethal) effects. Dead fish, if any, will be removed at each observation and a record will be maintained of mortality and abnormal behavior. 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. For example, if the 24-hour exposure results in total mortality, new solutions will be prepared below the lowest initial concentration until no mortality or partial mortality is reached. In the converse situation, if no mortality is observed after 24 hours, new concentrations will be added above the highest initial level until mortality is noted. Range-finding concentrations will usually be conducted to a maximum of 100 mg/L unless higher concentrations are necessary to determine the definitive concentration range or if the study sponsor so requests.
- 3.5.4 The preliminary test will be conducted for a period of 24 to 96 hours with the exact duration dependent upon the results of the initial concentrations tested.

4.0 DEFINITIVE STUDY

- 4.1 General. Following the preliminary range-finding study discussed in Section 3.0, the definitive test will be conducted by the procedures described below. Test-specific information regarding the sponsor, test substance, test fish, proposed study dates, study personnel, and study approvals will be included on pages 2 and 3 at the time of protocol approval.
- 4.2 Test System (Organisms). Aspects concerning the acquisition, culture, and acclimation of test fish will be the same as discussed in Section 3.2.
- 4.3 Exposure System. Two replicate test chambers will be used for each control and test solution. For all definitive testing, the test vessel size will conform to the maximum loading limitation of 0.5 g of fish per liter of solution (8). One of the following types of glass test chambers will be used: (a) 1- or 2-L glass beakers containing at least 1000 mL of solution, (b) 5-gallon glass jars containing 15 L of solution, (c) aquaria containing 30 L of solution, or (d) aquaria containing 75 L of solution. ABC will select the type of chamber to be used. In most instances, the test chambers used will be 5-gallon glass jars containing 15 L of solution, which is a recommended test vessel for static bioassays (8). The 5-gallon glass beakers or jars will be covered to keep foreign objects out. Each test vessel will be unambiguously labeled as to test compound, concentration, and study number. The water bath system described in Section 3.3 will be used to

control test temperatures. The test dilution water will be the same as discussed in Section 3.3.

- 4.4 Test Substance. The sponsor will supply specific information regarding the test substance as detailed in Section 3.4. A Material Safety Data Sheet should be submitted to ABC before or with the sample shipment. The remaining test sample will be sent back to the study sponsor upon approval of the final report. Characterization, stability and solubility studies will be the sponsor's responsibility unless otherwise contracted to ABC Laboratories, Inc.

The test concentrations will be prepared on a weight/volume nominal basis unless otherwise specified. Test concentrations will be corrected for percent active ingredient, if the study sponsor so requests. Otherwise, concentrations will be based on the total formulated product. A record of all sample weights and dilutions will be kept, checked by a second party, and furnished in the final report. The test substance will be returned to the study sponsor upon study completion. Archival of a retention will therefore be the sponsor's responsibility.

Under certain experimental conditions (Section 5.7), the sponsor may be required to confirm test concentrations by approved analytical techniques. Factors such as limited aqueous solubility, rapid hydrolysis/photolysis rates, and compound volatility may require analysis at 0 and 96 hours. If the EPA requires analytical confirmation, the study sponsor must submit an appropriate analytical method to ABC prior to testing for review and quotation, or ABC may be contracted to develop methodology. If the study sponsor elects not to analytically verify the test article concentrations, it should be noted that the study may be deemed in nonconformance with the Good Laboratory Practice (GLP) guidelines (6). If analytical confirmation is performed, the validated method and sampling schedule will be amended to the protocol.

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

- 4.5.1 The test fish will be gradually acclimated to the test temperature and dilution water for at least 48 hours prior to testing as discussed in Section 3.2, during which time they will be held without food.
- 4.5.2 Test vessels will be filled with dilution water and placed in the appropriate warm or cold water bath. Once the dilution water has reached the proper test temperature, the exposure solutions will be prepared at the study director's discretion.

- 4.5.3 Exposure solutions may be prepared by adding the test substance directly to the dilution water or by adding an aliquot of a working standard to the chambers of the test substance dissolved in a vehicle (organic solvent).
- 4.5.4 If a vehicle other than water is used to prepare test solutions, a vehicle blank chamber will be used that will receive an aliquot of the vehicle equivalent to the highest amount used in the test chambers. The concentration of vehicle in any test chamber will not exceed 0.1 mL/L. The vehicle system used will be one of the following: acetone, ethanol, methanol, dimethylformamide, or triethylene glycol.
- 4.5.5 Ten fish will be placed into each replicate test chamber (20 per concentration in two replicates) within 30 minutes after solution preparations. Placement will be by impartial assignment of fish, one or two at a time to a test chamber, until all test chambers have a full complement of 10 fish.

Normally the test fish are added to the test chambers within 30 minutes after solution preparation. However, in certain instances when there is a lengthy or complex solution preparation (such as stirring overnight), the test solutions will be prepared and then placed in the water bath to reach the proper test temperature. The fish will be added after the exposure solutions have reached the proper temperature. All test fish will then be added within a 30-minute period.

- 4.5.6 As an alternate test design, more replicate test chambers per concentration containing an appropriate number of fish will be used to reduce biomass loading when using test fish near the upper size limit of that recommended.
- 4.5.7 The test chambers will be observed for mortality and/or abnormal behavioral (sublethal) effects every 24 hours (± 1 hour from time of test initiation). If more than 10% of the fish died in the dilution water control or in the humic acid control, the test will be deemed unacceptable and the study will be terminated. Dead fish, if any, will be removed at each observation and a record will be maintained of mortality and observed abnormal behavior for each test concentration. The test concentration range used must yield at least one concentration with mortality $\leq 35\%$ and at least one concentration with a mortality of $\geq 65\%$ (8), except in instances of testing nontoxic materials at their water solubility limit or at a maximum of 1000 mg/L where the test is designed to confirm no toxicity (or minimal toxic response, $< 50\%$) at these levels.

4.5.8 Length and weight measurements will be made on the control or vehicle blank group of fish at test termination.

4.6 Test Procedure – Chemical and Physical.

4.6.1 Water quality parameters of temperature, dissolved oxygen, and pH will be monitored throughout the test. Measurements of these parameters will be made at 0, 48, and 96 hours of testing in all control and test chambers.

Just before the fish are added, the control and humic acid control chambers will be sampled for TOC (total organic carbon) analysis. Approximately 100 mL will be collected from each dilution water control chamber, the two 100-mL samples will be composited, and the composite will be analyzed in triplicate for TOC. Approximately 100 mL will be collected from each humic acid control, the two 100-mL samples will be composited, and the composite will be analyzed in triplicate for TOC. Backup samples may be collected and retained at ABC Laboratories.

4.6.2 If at any point during routine water quality analyses the control dissolved oxygen level falls below 40% saturation, 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 the regulatory agencies believe these practices are questionable. If the study sponsor anticipates that his compound may create impacts on water quality and he desires to adjust the test solutions in some manner, he must authorize this in writing before test initiation. If the sponsor desires aeration or pH-adjustment, ABC recommends that the concentrations of test substance be measured by an appropriate analytical method to determine if these procedures have affected nominal exposure levels.

4.6.3 Under certain experimental conditions the sponsor may elect to confirm exposure concentrations of the test substance by analytical techniques. Factors such as limited aqueous solubility, rapid hydrolysis/photolysis rates, and compound volatility may make analytical confirmation desirable. It will be the study sponsor's responsibility to determine whether EPA requires analytical confirmation of test solutions. If so, the study sponsor must submit to ABC Laboratories an appropriate analytical method for measuring the test substance. If one does not exist, ABC will aid the sponsor in developing a method at an appropriate cost.

4.7 Analysis of Results.

- 4.7.1 The results of the definitive study will be statistically analyzed for 24-, 48-, 72-, and 96-hour LC₅₀ values and their corresponding 95% confidence limits. These values will be determined by an LC₅₀ computer program developed by Stephan et al. (14).
- 4.7.2 The slope of the dose response line will be calculated for each observation period. The slope will be calculated by the computer program mentioned above or by least-squares regression analysis (log of concentration vs. probit transformation of percent mortality).

5.0 DATA MAINTENANCE/REPORTING

- 5.1 Records to be Maintained. Records to be maintained will include, but not be limited to, compound receipts; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC); material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until finalization of the study. Upon completion of the project, all raw data specifically for this study will be submitted to the sponsor as part of the final report.
- 5.2 Report. A final report containing all original raw data and/or certified copies of certain raw data records will be submitted to the sponsor. A copy of the report and associated raw data will be kept on file in ABC Laboratories' archives. The final report will include, but not be limited to, the following:
- 5.2.1 Study dates, name, and address of test facility.
- 5.2.2 Objectives and test procedures as stated in approved protocol.
- 5.2.3 A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
- 5.2.4 Description of test substance (date of receipt, storage conditions, purity, physical characteristics, and method of preparing stock and/or test solutions).
- 5.2.5 Description of methods used during the study.
- 5.2.6 Description of test organisms (source, culture techniques, etc.).

- 5.2.7 Summary of all the data and a statement of the conclusions drawn from any data analyses, if appropriate.
- 5.2.8 Location of raw data.
- 5.2.9 List and signatures of study personnel.
- 5.2.10 GLP compliance statement by study director and a statement by ABC Laboratories' Quality Assurance Unit.
- 5.2.11 An appendix or separate raw data report will contain the original raw data or certified copies of raw data, letter of test authorization (if available), protocol alterations, the approved protocol, and the analytical method appendix (if analytical confirmation is performed by ABC Laboratories).

6.0 PROTOCOL ALTERATIONS

The study director, upon approval of the sponsor representative, may make other changes to this protocol. All changes will take the form of a written protocol alteration describing the change, the reason for the change, and the effect on the study, if any. All alterations will be signed and dated by both the study director and the sponsor representative. The signed Protocol Alteration Notification form will be maintained with the protocol.

Should a significant problem develop while the study is in progress, the study director will notify the sponsor representative as soon as practical to discuss the problem and any corrective actions taken. Upon verbal authorization from the sponsor representative, the study director will proceed with any further actions deemed appropriate. If the sponsor representative cannot be reached, the study director will proceed with the appropriate modifications and will notify the sponsor representative as soon as possible.

In the event of protocol deviations, an attempt will be made to notify the sponsor representative within a reasonable period of time. A written description of the deviation(s) will be submitted on a Protocol Alteration Notification form to the sponsor representative. All deviations will be signed and dated by both the study director and the sponsor representative.

7.0 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 guidelines listed in this protocol. The results of these inspections will be reported to the study director and ABC

management. The draft and final reports will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures used were followed. A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the study director and management.

8.0 GLP COMPLIANCE

This study will be conducted in accordance with U.S. EPA Good Laboratory Practice Standards; Toxic Substance Control (40 CFR 792). The report will contain a statement attesting to that fact.

9.0 REFERENCES

- (1) U.S. Congress. 1976. Toxic Substances Control Act. Public Law 94-469. *Federal Register*, October 11, 1976. 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. 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 7796, 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), 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. 25 p.
- (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. 61 p.

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- (9) U.S. Environmental Protection Agency. 1985. Toxic Substances Control Act Test Guidelines; Final Rules, Fish Acute Toxicity Test. *Federal Register*, Vol. 50 (No. 188), September 27, 1985, 797.1400.
 - (10) U.S. Environmental Protection Agency. 1982. Environmental Effects Test Guidelines, Fish Acute Toxicity Test. EPA 560/6-82-002, EG-9, August 1982.
 - (11) U.S. Environmental Protection Agency. Fish Acute Toxicity Mitigated by Dissolved Organic Carbon. Unpublished Proposed Guideline § 795.115. 18 p.
 - (12) Eddy, Samuel. 1969. *The Freshwater Fishes*. 2nd ed. W.C. Brown Company, Dubuque, IA. 286 p.
 - (13) Brauhn, J.L. and R.A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975. 45 p.
 - (14) Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A Computer Program for Calculating an LC₅₀. U.S. Environmental Protection Agency, Duluth, MN, Pre-publication Manuscript, August 1978.