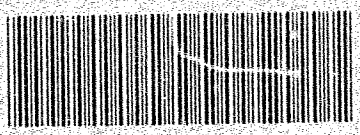
CONTAINS NO CBI

CYANAMID

American Cyanamid Company
One Cyanamid Plaza
Wayne, NJ 07470



88920002755

H. Michael D. Utidjian, M.
Corporate Medical Director

8EHQ-0592-4113
Init

May 19, 1992

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Att'n: Section 8(e) Coordinator (CAP Agreement)

RE: Study or Report Submitted Pursuant to the TSCA Section 8(e) Compliance Audit Program

Identification Number: 8ECAP-0041

Dear Sir/Madam:

American Cyanamid Company is submitting the attached study to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e) Compliance Audit Program (CAP Agreement) executed by American Cyanamid and EPA. This study does not involve effects observed in humans. These documents do not contain confidential business information.

The enclosed study provides information on a chemical mixture containing ~34% petroleum distillate hydrotreated light [CAS 64742-47-8] and water. This material is also known as CT-434-90N.

The title of the enclosed report is "Acute Toxicity of CT-434-90N to Daphnia magna," August 16, 1990.

Under the conditions of the study, this material has an acute aquatic 48-hour LC_{50} of less than 1 ppm.

In total, American Cyanamid is submitting three (3) copies of the enclosed report and this cover letter: an original and two (2) copies.

Further questions regarding this submission may be directed to Ms. Patricia A. Vernon, Associate Toxicologist at the address above or 201-831-2534.

Sincerely,

H. Michael D. Utidjian, M.D.
Corporate Medical Director

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

ID# 96514

COMPANY NO CBI

SPONSOR COMPANY

American Cyanamid Company
One Cyanamid Plaza
Wayne, New Jersey 07470

STUDY TITLE

Acute Toxicity of CT-434-90N to
Daphnia magna

DATA REQUIREMENT

U.S. EPA-TSCA 40 CFR, Part 797
Guideline 797.1300

AUTHORS

David Burgess
Biologist II

and

James Muckerman
Biological Technician III

STUDY COMPLETED ON

August 16, 1990

PERFORMING LABORATORY

Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Group
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

ABC LABORATORY PROJECT ID

Final Report #38874

Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205
(314) 474-8579

Prepared By:

James G. Muckerman — Date
Biological Technician III

David Burgess — 8-16-90 Date
Biologist II

Approved By:

Alan D. Forbis — 8/16/90 Date
Supervisor, Aquatic Toxicology

Tamara S. Driver — 8/16/90 Date
Quality Assurance Officer

Brenda Bunch Franklin — 8/16/90 Date
Manager, Aquatic Toxicology

:ekc

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INVERTEBRATE ACUTE TOXICITY COMPENDIUM

Subject: "Acute Toxicity of CT-434-90N to Daphnia magna", ABC Final Report Study Number 38874.

Sponsor: American Cyanamid Company

Test Facility: Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Group
P.O. Box 1097
Columbia, Missouri 65205 (314-474-8579)

Location of Original Raw Data and Final Report:

American Cyanamid Company
Toxicology and Product Safety Department
One Cyanamid Plaza
Wayne, New Jersey 07470

Test Material: CT-434-90N (LOT NO. S-16363-15B)

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

Dilution Water: 170 mg/L (hardness), 190 mg/L (alkalinity), 8.0 (pH), 360 µMhos/cm (conductivity)

Experimental Test Dates: Initiation - July 23, 1990
Termination - July 25, 1990

Length of Study: 48-hours

Results: 48-hour LC_{50} = 0.92 mg/L (95% C.I. 0.72 and 1.1 mg/L)
48-hour NOEL = 0.56 mg/L
48-hour dose-response slope = 4.8

Test Species: Daphnia magna

Source of Organisms: ABC Laboratories in-house culture.

Age of Organisms at Study Initiation: Neonates (≤24-hours old).

SUMMARY

The acute toxicity of CT-434-90N to Daphnia magna was assessed using the methods outlined in ABC Protocol 7806. Water quality parameters of temperature, dissolved oxygen and pH were measured at the beginning and at termination of the test and were within acceptable limits.

The results of the 48-hour static Daphnia magna toxicity study are summarized below. All reported values were based upon nominal concentrations of CT-434-90N.

Compound	48-Hour LC_{50} (95% C.I.)
CT-434-90N	0.92 mg/L (0.72 and 1.1 mg/L)

The no-effect level observed for CT-434-90N was considered to be 0.56 mg/L. The three mortalities in Replicate A of the 0.32 mg/L concentration were considered to be aberrant and not compound related.

INTRODUCTION

This definitive static bioassay was performed by the Aquatic Toxicology Group of Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri, for American Cyanamid Company, from July 23, 1990 to July 25, 1990. The purpose of this test was to determine the 24- and 48-hour LC_{50} levels for CT-434-90N to Daphnia magna. The study was performed using ABC Protocol #7806 as approved by American Cyanamid Company on March 30, 1990 and by ABC's study director on June 20, 1990.

METHODS AND MATERIALS

The procedures for static bioassay, as described in ABC Protocol 7806 were used in this experiment.

I. Test Organisms

The Daphnia magna used in the test were cultured at the ABC facilities in hard blended water. Hard blended water is a combination of well water and reverse-osmosis water blended to a hardness of 160-180 mg/L as ($CaCO_3$). The chemical characteristics of this water are presented in Table 1. The adult daphnids were fed algae (Selenastrum capricornutum and Ankistrodesmus falcatus) at least every three days prior to testing and supplemented with a suspension of Rangens® trout chow and Fleischmann's® active dry yeast. The original culture of daphnids at ABC Labs were identified to species using the taxonomic key presented by Pennak (1). A total of 140 neonates from ABC culture 90-AH, were used for the definitive bioassay.

II. Test Procedure

The static Daphnia magna bioassay was conducted in 250-ml glass beakers containing 200 ml of daphnid culture/test water at a depth of ~6.3 cm. All test vessels were covered with loose-fitting petri dish covers to minimize evaporation and prevent contamination during the study. The test solutions were not aerated during the study. These vessels were kept at 20 (±2.0)°C in a temperature controlled water bath. The lighting was maintained at 50-70 footcandles on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods.

A range finding experiment was conducted using ten Daphnia magna each in exposure concentrations of 1.0, 10 and 100 mg/L. After 48-hours, the 1.0, 10, and 100 mg/L concentrations were observed to have 90, 100, and 100-percent mortality. From these results, six concentrations of the test compound in duplicate replications with ten impartially assigned Daphnia magna (neonates less than 24 hours old) per beaker, were selected for the definitive bioassay. These concentrations were a logarithmic series ranging from 0.32 to

5.6 mg/L and included a dilution water control. All concentrations were observed once every 24 hours for mortality and abnormal effects such as surfacing and/or daphnids tending to the bottom of test chambers.

III. Test Compound

The CT-434-90N sample was received on June 13, 1990 as a white liquid and was stored at room temperature. The lot No. on the bottle was S163o3-15B and was assigned ABC Reference No. TS-4117. Sample stability confirmation was not investigated by ABC Labs. Sample purity was not specified by the study sponsor; therefore, test concentrations were prepared based on total product. The primary standard was prepared by weighing 0.0203 grams of CT-434-90N in a scintillation vial and transferring it to a 1000 ml volumetric flask. Five hundred sixty milliliters of the primary standard was transferred to a 2000 ml volumetric flask and brought to volume. All other dilutions were made from this concentration. Hard blended test water was used in the preparation of all working stock solutions. At 0 hour all test solutions were clear and remained clear for the duration of the 48 hour bioassay.

IV. Statistics

Statistical analysis of the concentration vs. effect data (general mortality) was attempted by employing a computerized LC_{50} program developed by Stephan et at. (2). This program calculated the LC_{50} statistic and its 95-percent confidence limits using the binomial, the moving average and the probit tests. However, if no mortality occurred or if a dose response could not be demonstrated over a reasonable range (<37 to >63%), the LC_{50} and/or its 95 percent confidence limits could not be calculated. 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. The method of calculation selected for presentation in this report was that which gave the narrowest confidence limits for the LC_{50} (2, 3) although all three methods are valid. The 48 hour dose-response slope was calculated through linear regression.

RESULTS

Table 2 presents the individual mortality and behavioral observations. At 0-hour all organisms in the control and treated concentrations appeared normal and healthy. At the 24 hour observation, mortality and abnormal effects of surfacing, and daphnids tending to the bottom of the test chambers were observed in the 0.32, 1.0, and 5.6 mg/L test concentrations. At 48 hours, mortality and abnormal effects of daphnids tending to the bottoms of test vessels were observed in the 0.32, 1.0, 1.8, 3.2, and 5.6 mg/L test concentrations. All results were based on the nominal concentrations of 0.32, 0.56, 1.0, 1.8, 3.2, and 5.6 mg/L.

Table 3 presents LC_{50} data for the acute toxicity of CT-434-90N to Daphnia magna. The 24- and 48-hour LC_{50}'s were >5.6 and 0.92 mg/L, respectively. The 48-hour dose-response slope was 4.8. The 48-hour no-effect concentration was considered to be 0.56 mg/L, since there was no mortality or abnormal effect observed at this level. The 15% mortality observed in the 0.32 mg/L concentration was believed to be aberrant and not compound related. It was not believed to have effected the results of the study.

Table 4 presents the water quality parameters measured during the test. The dissolved oxygen concentrations ranged between 8.1 and 8.4 mg/L. These values represented 93 and 99-percent saturation at 20 and 21°C (corrected for local altitudinal pressure), respectively, and were considered adequate for testing. The pH values of the test solutions ranged from 8.0 to 8.4.

The study was conducted following the intent of the Good Laboratory Practice Regulations (4) and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data were provided to American Cyanamid Company with the final report and a copy retained at Analytical Bio-Chemistry Laboratories, Inc.

TABLE 1

Chemical Characteristics of Hard Blended Water[a] Used by ABC's Aquatic Toxicology Group

Monthly/Daily Screens[b]	
Hardness	170 mg/L as $CaCO_3$
Alkalinity	190 mg/L as $CaCO_3$
pH	8.0
Conductivity	360 µMHOS/cm
Total Organic Carbon	<1.0 ppm[c]
Suspended Solids	2.2 ppm[c]

Quarterly Screens[d]

Elements		Chlorinated Hydrocarbons	
Aluminum	<0.20 ppm	DDE	<0.05 ppb
Arsenic	1.2 ppb	DDD	<0.05 ppb
Boron	0.516 ppm	DDT	<0.05 ppb
Cadmium	<0.005 ppm	Dieldrin	<0.05 ppb
Chromium	<0.01 ppm	α-BHC	<0.01 ppb
Cobalt	<0.050 ppm	β-BHC	<0.01 ppb
Copper	<0.025 ppm	γ-BHC	<0.01 ppb
Fluoride	0.62 ppm	Δ-BHC	<0.01 ppb
Iron	<0.10 ppm	HCB	<0.01 ppb
Lead	1.4 ppb	Endrin	<0.05 ppb
Mercury	<0.0004 ppm	H.E.	<0.01 ppb
Nickel	<0.040 ppm	Mirex	<0.10 ppb
Silver	<1.0 ppb	Methoxychlor	<0.10 ppb
Zinc	0.111 ppm	Toxaphene	<1.0 ppb

Organophosphate Insecticides		PCB	
Vapona	<0.5 ppb	Total PCB's	<0.5 ppb
Thimet	<0.5 ppb		
Diazinon	<0.5 ppb		
Methyl Parathion	<0.5 ppb		
Ethyl Parathion	<0.5 ppb		
Ronnel	<0.5 ppb		
Malathion	<0.5 ppb		

Miscellaneous	
COD	<10 ppm
Unionized NH_3	0.010 ppm

[a] A blend of ABC well water and reverse osmosis (R.O.) water, all original data archived at ABC Laboratories.

[b] Represents the values measured during the testing period. Original data archived at ABC Laboratories.

[c] Represents an analysis from June, 1990.

[d] Represents the values obtained from the most recent screen of April, 1990.

TABLE 2

Individual Mortality and Behavioral Observations Obtained During the Acute Toxicity Test of CT-434-90N to Daphnia magna

Nominal Concentration mg/L (ppm)	Rep.	Number Placed in Test Vessel	24-Hour (Rep. A) Cum. Mort.	24-Hour (Rep. A) Observations[a]	48-Hour (Rep. B) Cum. Mort.	48-Hour (Rep. B) Observations[a]
Control	A	10	0	---	0	---
	B	10	0	---	0	---
0.32	A	10	3[b]	7N	3[b]	7N
	B	10	0	---	0	---
0.56	A	10	0	---	0	---
	B	10	0	---	0	---
1.0	A	10	0	---	5	3OB/ 2N
	B	10	1	9 N	10	---
1.8	A	10	0	---	8	1OB/ 1N
	B	10	0	---	10	---
3.2	A	10	0	---	10	---
	B	10	0	---	10	---
5.6	A	10	0	1 SUR/ 9 N	10	---
	B	10	0	1 OB/ 9 N	10	---

[a] Unless otherwise indicated, all daphnids were normal in appearance and behavior and all test solutions were clear without a precipitate.

[b] Mortality was considered to be aberrant and not compound related.

The following abbreviations were used for observations:

OB = On bottom; SUR = Surfacing; N = Normal.

TABLE 3

LC_{50} Values Obtained During the Acute Toxicity Test of CT-434-90N to *Daphnia magna*[a]

	LC_{50} (mg/L)	
Compound	24-Hours	48-Hours
CT-434-90N	>5.6[1]	0.92[2]
	[b]	(0.72 and 1.1)[c]

[a] Bioassay conducted at 20°C (±2.0).

[b] 95% confidence intervals could not be determined.

[c] 95% confidence limits.

The 48-hour no-effect concentration was considered to be 0.56 mg/L based on the lack of mortality and/or abnormal behavior at this concentration.

LC_{50} calculated using:

(1) Binomial Method
(2) Moving Average Method

TABLE 4

Water Quality Measurements During the Acute Toxicity Test of CT-434-90N to Daphnia magna

Nominal Concentration (mg/L)	Water Quality					
	0-Hour (Rep. A)			48-Hour (Rep. B)		
	Temp.[a] °C	D.O.[b] mg/L	pH[c]	Temp.[a] °C	D.O.[b] mg/L	pH[c]
Control	20	8.1	8.0	21	8.4	8.2
0.32	20	8.1	8.0	21	8.1	8.3
0.56	20	8.1	8.0	21	8.2	8.4
1.0	20	8.1	8.0	21	8.3	8.3
1.8	20	8.1	8.0	21	8.3	8.3
3.2	20	8.1	8.0	21	8.3	8.4
5.6	20	8.1	8.0	21	8.3	8.4

[a] Temperature - Digital thermometer.

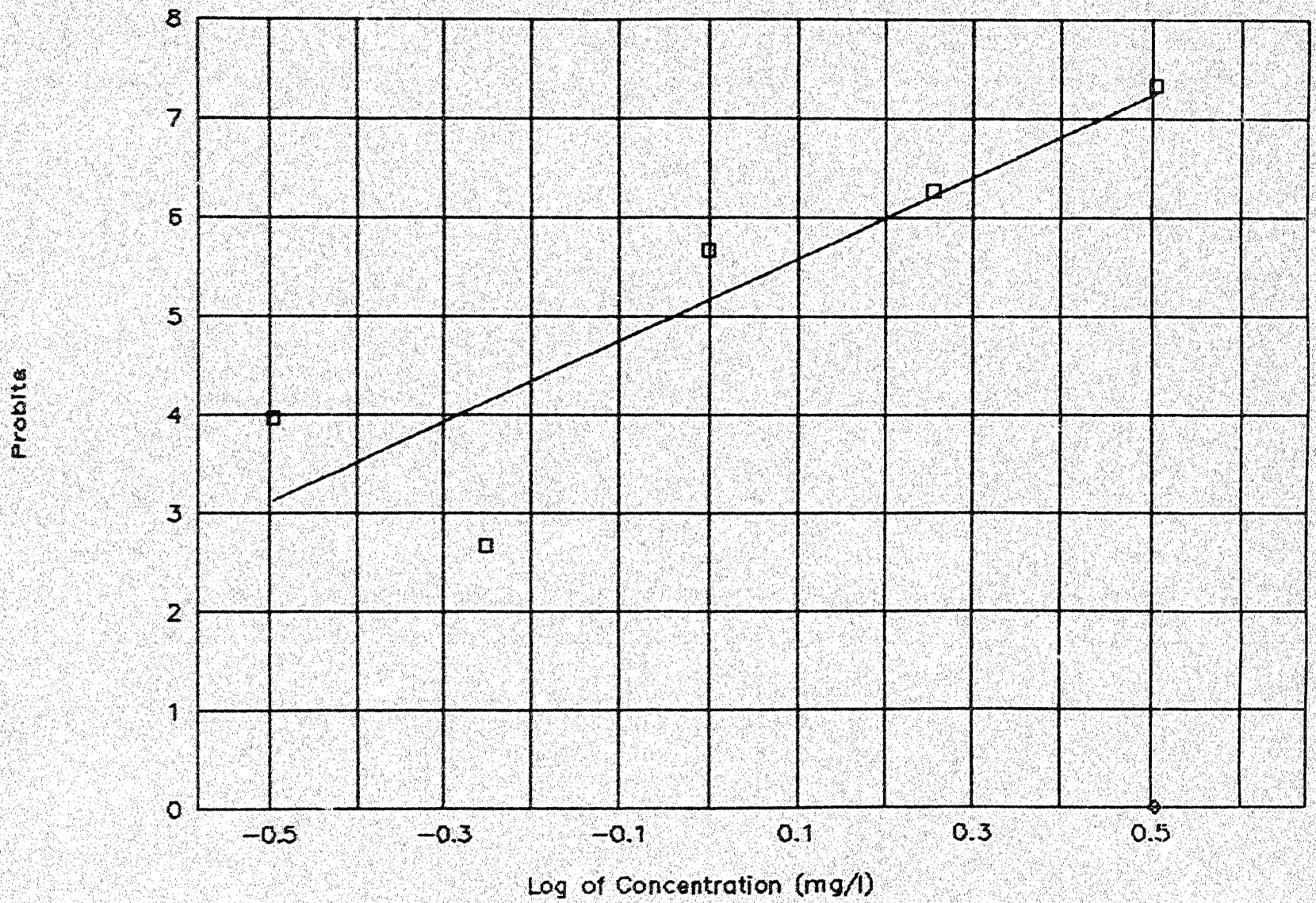
[b] Dissolved oxygen concentrations - YSI 54 ARC Dissolved Oxygen Meter.

[c] pH - Corning 140 pH/mV meter with Sensorex S200C electrode.

Note: Dissolved oxygen saturation at the test temperatures of 20 and 21°C are 8.7 and 8.5 mg/L, respectively (corrected for altitudinal pressure at ABC Labs).

FIGURE 1

The 48-Hour Dose-Response Line for CT-434-90N



LITERATURE CITED

(1) Pennak, R. W. 1978. Freshwater Invertebrates of the United States. John Wiley & Sons, New York, 2nd ed. 803 p.

(2) 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.

(3) 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.

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

(b) U. S. Food and Drug Administration 1987. Nonclinic Laboratory Studies. Good Laboratory Practice Regulations (21 CFR, Part 58). Federal Register, Volume 52 No. 172: 33779-33782.

(c) 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.

QUALITY ASSURANCE STATEMENT

Quality Assurance Statement for ABC final report #38874 entitled, "Acute Toxicity of CT-434-90N to Daphnia magna", for American Cyanamid Company; Wayne, New Jersey.

In accordance with ABC Laboratories' intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for CT-434-90N was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit.

A procedure audit of 0-hour water chemistries was performed on July 23, 1990. Results were reported to the study director that same day, and not yet reported to management. A final inspection of this report and all data and records on August 10, 1990, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by Analytical Bio-Chemistry Laboratories, Inc.

Tamara S. Driver 8/16/90

Tamara S. Driver Date
Quality Assurance Officer

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC final report #38874 entitled, "Acute Toxicity of CT-434-90N to Daphnia magna", for American Cyanamid Company, Wayne, New Jersey.

In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, 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; Toxic Substances Control Act (40 CFR 792), Organization for Economic Cooperation and Development (Annex 2) and U.S. Food and Drug Administration (21 CFR, Part 58). Analytical confirmation of test concentrations was not investigated by ABC Labs.

All original raw data were provided to American Cyanamid with the final report and a copy retained at Analytical Bio-Chemistry Laboratories, Inc.

David Burgess 8-16-90

David Burgess
ABC Study Director

Date

APPENDICES - RAW DATA

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

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

C - Error in calculations

E - Entry error

S - Spelling error

E - Recording error

F - Form change

T - Transcription error

APPENDIX I - BIOLOGICAL RAW DATA

Project Personnel
A. Preliminary Raw Data
B. Definitive Raw Data

Project Personnel

PROJECT PERSONNEL

TEST COMPOUND: CT 434-90N	Lab Form No.: 611 (12/7/89)
ABC STUDY NO: 38874	Analytical Bio-Chemistry Labs 7200 ABC Lane, P.O. Box 1097 Columbia, Missouri 65205
ABC STUDY DIRECTOR: D. Burgess	

THE FOLLOWING PERSONNEL ASSISTED WITH VARIOUS PHASES OF THIS STUDY:

	NAME	TITLE	INITIALS
1.	[illegible]	Biological Technician III	JG[illegible]
2.	David Burgess	BIOLOGIST II	DB
3.	[illegible]	Biologist I	GCB
4.	Scott J Vaney	Biological Technician I	SJV
5.	[illegible]	[illegible] I	JWB
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			
21.			

A. Preliminary Raw Data

DAPHNIA ACUTE TOXICITY BIOASSAY

☐ Definitive ☑ Preliminary

Test Material: CT-434-90 A1

Study No.: 38874 Protocol: 7806

ABC Study Director: D. Burgess

Lab Form No.: 67A (7/10/89)

Analytical Bio-Chemistry Labs
7200 ABC Lane, P.O. Box 1097
Columbia, Missouri 65205

Test Species: [ ] Daphnia magna [ ] _____ (Lot # 90-AF)

Dilution Water: [ ] Recon. [ ] Well [✓] Blended Water Bath # 2

No. Neonates/Vessel: 10 Test Soln. Prepared on: 7-19-90

Time: 11:30 a.m. Date initiated: 7-19-90 Date Terminated: 7-21-90

Test Conc. mg/l	Mortality and Behavioral Observations								
	0-Hour			24-Hour			48-Hour		
	Dead	Cum. Mort	obs.	Dead	Cum. Mort	obs.	Dead	Cum. Mort	obs.
Control	0	0	10N CLR	0	0	10N CLR	0	0	10N CLR
1	0	0	10N CLR	1	1	2OB 7N "	8	9	10B "
10	0	0	10N CLR P	0	0	6OB 4N "	10	10	- "
100	0	0	10N CLD P	10	10	— CLD P	—	10	- CLD
Observer Date Time	JGM 7-19-90 11:30 AM			JGM 7-20-90 12:30 pm			GCB 7-21-90 11:10 AM		

Remarks: N = Normal, SUR = Surfacing, C = Clumping, ERR=Erratic Movement, TR = Trailing _____, CT = Coated with extraneous material, Q = Quiescent; OB = On Bottom, CLR = Clear, P = Precipitate, CLD = (Cloudy) colored solution, SF = Surface Film

Prepared By: _____ Date: 7-19-90

Checked By: _____ Date: 8-3-90

COMPOUND PREPARATIONS: ☑ Preliminary ☐ Definitive

Test Material: CT-434 90N

Study No.: 38874 Lot No.: S-16363-15B

Protocol No. 7806 Purity: ——— %

Lab Form No.: 410 (8/7/89)

Analytical Bio-Chemistry Labs
7200 ABC Lane, P.O. Box 1097
Columbia, Missouri 65205

Prep. of Primary Standard/ Weighed by: JGM Date: 7-19-90

Nominal Target Weight 0.1000 g
Actual Gross Weight 0.1003 g
Tare Weight 0.0000 g
Net Weight 0.1003 g

Dil. Vol. and Solvent 1000 Hard Blended H_2O ml
Concentration 0.1 mg/ml

Balance checked with class S weights:

Adjusted Net Weight Corrected for Purity ——— g

0.1000 g (class S) + 0.0000 g (tare) = 0.1001 g (final wt)

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

Conc. of Parent Sol.	Aliq. Vol. (ml)	Dilution Vol. (ml)	Dilution Solvent	Final Concentration

Prep. of Test Conc./Transferred by: JGM Time: 11:25am Date: 7-19-90

Concentration of Standard (mg/ml)	Aliquot Volume (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
0.1	10	1.0	1
0.1	100	1.0	10
~~0.1~~ ①	~~200~~ ①	~~1.0~~ ①	~~20~~ ①
0.1	200	0.2	100

Remarks: Balance: ☑ Sartorious R 300 S; ABC Material #1905-475
☐ Other:

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

Control(s), Description: Hard Blended H_2O

① E 7-19-90 JGM

Form Prepared By: [signature] Date: 7-19-90

Checked By: Scott J Vaney Date: 8-6-90

Study Director: [signature] Date: 8-6-90

ABC LABS# 038874

B. Definitive Raw Data

WATER QUALITY

Test Material: CT-434-90N Study No.: 38874

Protocol No.: 7806 ABC Study Director: D. Burgess

Lab Form No.: 411 (6/7/90)

Analytical Bio-Chemistry Labs
7200 ABC Lane, P.O. Box 1097
Columbia, Missouri 65205

Concen. mg/l	0 Hrs. Obs. 7-23-90 1030am JCA				48 Hrs. Obs. 7-25-90 JCA				Hrs. Obs.				Hrs. Obs.			
	Temp. [a] °C	D.O. [b] mg/l	[c] pH	Rep	Temp. °C	D.O. mg/l	pH	Rep	Temp. °C	D.O. mg/l	pH		Temp. °C	D.O. mg/l	pH	
Control	20	8.1	8.0	A	21	8.4	8.2	B								
0.32	20	8.1	8.0	A	21	8.1	8.3	B								
0.56	20	8.1	8.0	A	21	8.2	8.4	B								
1.0	20	8.1	8.0	A	21	8.3	8.3	B								
1.8	20	8.1	8.0	A	21	8.3	8.3	B								
3.2	20	8.1	8.0	A	21	8.3	8.4	B								
5.6	20	8.1	8.0	A	21	8.3	8.4	B								

[a] Digital Thermometer

[b] YSI Model 54 ARC Dissolved Oxygen Meter, ABC Material #1905-485.

[c] Corning 140 pH/mV meter, ABC Material #1714-175 and Sensorex S200C Electrode.

Remarks:

Checked By: Scott J Carey Date: 8-6-90 Study Director: David Burgess Date: 8-6-90

COMPOUND PREPARATIONS: ☐ Preliminary ☑ Definitive

Test Material: CT-434-90N

Study No.: 38874 Lot No.: S-16363-15B

Protocol No. 7806 Purity: —— %

Lab Form No.: 410 (8/7/89)

Analytical Bio-Chemistry Labs
7200 ABC Lane, P.O. Box 1097
Columbia, Missouri 65205

Prep. of Primary Standard/ Weighed by: JGM Date: 7-23-90

Nominal Target Weight 0.0200 g
Actual Gross Weight 0.0203 g
Tare Weight 0.0000 g
Net Weight 0.0203 g

Dil. Vol. and Solvent 1000 Hard Blended ml
Concentration 0.02 mg/ml

Balance checked with class S weights:

Adjusted Net Weight ——
Corrected for Purity ——— g

0.0200 g (class S) + 0.0200 g (tare) = 0.0202 g (final wt)

Prep. of Working Standard/ Transferred by: JGM Date: 7-23-90

Conc. of Parent Sol.	Aliq. Vol. (ml)	Dilution Vol. (ml)	Dilution Solvent	Final Concentration
0.0200 mg/ml	560	2000	Hard Blended H2O 2000	0.0056 mg/ml

Prep. of Test Conc./Transferred by: JGM Time: 10:05 AM Date: 7-23-90

Concentration of Standard (mg/ml)	Aliquot Volume (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
0.0056	57	1.0	0.32
0.0056	100	1.0	0.56
0.0056	179	1.0	1.0
0.0056	321	1.0	1.8
0.0056	571	1.0	3.2
0.0056	200	0.2	5.6

Remarks: Balance: ☑ Sartorious R 300 S: ABC Material #1905-475
☐ Other:

Each Conc.: Duplicate Replicate 250 ml test vessels w/ 200 ml test vol.

Control(s), Description: Hard Blended H_2O

① E 7-23-90 JGM

Form Prepared By: [signature] Date: 7-23-90

Checked By: Scott A Vaney Date: 8-6-90

[signature] Date: 8-6-90

ABC LABS # 038874 pg 0028

LC_{50} Calculations	
ABC Study No. : 38874 Compound Name : CT 434-90N Species : DAPHNIA MAGNA Exposure Period: 24 HOURS Study Director : d. bURGESS	Analytical Bio-Chemistry Labs 7200 East ABC Lane P.O. Box 1097 Columbia, Missouri 65205 07-19-1990 Page # 1

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

CONC. PPM	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5.6	20	0	0	LESS THAN 0.001
3.2	20	0	0	LESS THAN 0.001
1.8	20	0	0	LESS THAN 0.001
1	20	1	5	2.002714205001754D-03
.56	20	0	0	LESS THAN 0.001
.32	20	3	15	.1288412585886468

AT A CONFIDENCE LEVEL OF 95 PERCENT THE BINOMIAL TEST SHOWS THAT THE LC_{50} IS ABOVE 5.6 PPM

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

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

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

ITER	G	H	CHI-SQUARE	PROBABILITY
5	1.079267109131455	1	2.612015242997157	.6246961277560806

PREPARED BY: [signature] DATE: 7-25-90

REVIEWED BY: David Burgess DATE: 8-3-90

LC_{50} Calculations

ABC Study No. : 38874 Compound Name : CT 434-90N Species : DAPHNIA MAGNA Exposure Period: 24 HOURS Study Director : d. BURGESS	Analytical Bio-Chemistry Labs 7200 East ABC Lane P.O. Box 1097 Columbia, Missouri 65205 07-19-1990 Page # 2

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

SLOPE = -1.95972494704957
95 PERCENT CONFIDENCE LIMITS =-3.995639574648597 AND 7.618968054945657D-02

LC_{50} = 8.301174640655518D-02 PPM
95 PERCENT CONFIDENCE LIMITS = 0 AND .2273848503828049

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

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

PREPARED BY: [signature] DATE: 7-25-90

REVIEWED BY: [signature] DATE: 8-3-90

QE DA 8-3-90

LC_{50} Calculations

ABC Study No. : 38874	Analytical Bio-Chemistry Labs
Compound Name : CT 434-90N	7200 East ABC Lane
Species : DAPHNIA MAGNA	P.O. Box 1097
Exposure Period: 48 HOURS	Columbia, Missouri 65205
Study Director : d. BURGESS	07-19-1990 Page # 1

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

CONC. PPM	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5.6	20	20	100	LESS THAN 0.001
3.2	20	20	100	LESS THAN 0.001
1.8	20	18	90	2.0122513178204460-02
1	20	15	75	2.069471366706704
.56	20	0	0	LESS THAN 0.001
.32	20	3	15	.12884175858864680

THE BINOMIAL TEST SHOWS THAT .56 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 LC_{50} OF .8555777668952942 PPM IS OBTAINED BY
NONLINEAR INTERPOLATION BETWEEN .56 AND 1

---RESULTS CALUCLATED USING THE MOVING AVERAGE METHOD----

SPAN	G	LC_{50}, PPM	95 PERCENT CONFIDENCE LIMITS LOWER	UPPER
4	8.221322605095894D-02	.9189212918281555	.7231279611587524	1.149512052536011
3	.1465737738594657	.9997659325599671	.7808855772018433	1.413874626159668
2	7.602779839147154D-02	.9366573691368103	.8046048879623413	1.078349590301514

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

PREPARED BY: [signature] DATE: 7-25-90

REVIEWED BY: [signature] DATE: 8-3-90

LC_{50} Calculations	
ABC Study No. : 38874 Compound Name : CT 434-90N Species : DAPHNIA MAGNA Exposure Period: 48 HOURS Study Director : d. bURGESS	Analytical Bio-Chemistry Labs 7200 East ABC Lane P.O. Box 1097 Columbia, Missouri 65205 07-19-1990 Page # 2

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

ITER	G	H	CHI-SQUARE	PROBABILITY
4	.6314329287603394	3.16718612820249 6	12.66874451280998	1.301283603500994D-02

BECAUSE THE PROBABILITY IS LESS THAN 0.05, RESULTS
CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD
NOT BE USED.
SLOPE = 4.047235059764201
95 PERCENT CONFIDENCE LIMITS = .8311906166850682 AND 7.263279502843334

LC_{50} = .8282613158226013 PPM
95 PERCENT CONFIDENCE LIMITS = .3628925383090973 AND 1.741986155509949

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

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

PREPARED BY: [signature] DATE: 7-25-90

REVIEWED BY: David Burgess DATE: 8-3-90

DOSE-RESPONSE CALCULATIONS

Test Material: CT 434-20N

ABC Study Number: 38824

Protocol Number: 7906

Study Director: D Burgess

Data at 48 Hours of Exposure.

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

$$Y = m X + b$$

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

Actual Conc.	Log Conc.	Percent Mort.	Probits	Calc. Probits
3.2	0.505	100.0	7.33	7.26
1.8	0.255	90.0	6.28	6.23
1	0.000	75.0	5.67	5.17
0.56	-0.252	0.0	2.67	4.13
0.32	-0.495	15.0	3.96	3.12

Regression Output:

Constant	5.17060
Std Err of Y Est	1.01410
R Squared	0.77741
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	4.14040
Std Err of Coef.	1.27908

Y = 4.140 X + (5.171)

Dose Response Slope = 4.14

Correlation Coefficient (r) = 0.88

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: [signature] Date: 8-13-90

Reviewed By: Jennifer L. Blankenship Date: 8-13-90

ENVIRONMENTAL CHAMBER

mber No.: 2

Lab Form No.: 737 (5/23/90)

Analytical Bio-Chemistry Labs
7200 ABC Lane, P.O. Box 1097
Columbia, Missouri 65205

te/I.D.	Light Reading PAR[a]	Light Reading LUX[b]	Footcandles (LUX X 0.0929)	Temp °C[c]	Comments
-90/SLH	6.9	575	53	20	
2-90/DF	7.5	625	58	20	
90/DF	6.9	575	53	20	
H-90/DF	7.5	625	58	20	
5-90/DF	7.2	600	56	20	
8-90/DF	6.9	575	53	20	
9-90/TW	7.5	625	58	20	
20-90/DF	6.9	575	53	20	
21-90/DF	7.2	600	56	20	
22-90/DF	7.5	625	58	20	
24-90/TW	7.5	625	58	20	
25-90/DF	7.2	600	56	20	
26-90/DF	6.9	575	53	20	
27-90/DF	7.5	625	58	20	
28-90/SLH	6.6	550	51	20	
29-90/SLH	7.2	600	56	20	
1-90/GLO	6.8	567	53	21	
2-90/DF	6.9	575	53	20	
3-90/DF	6.9	575	53	20	
4-90/TW	7.2	600	56	20	
5-90/DF	6.9	575	53	20	

[a] PAR = LI-COR Model LI-185B Quantum/Radiometer/Photometer (ABC Material #1905-160) and LI-COR Model LI-190SB Quantum Sensor (Serial #Q 7517). If PAR sensor is used then the following equation is used to determine LUX value:

$$\text{LUX} = \frac{\text{PAR Reading}}{\text{I2 (Correction factor for flouresecent lighting)}} \times 1000$$

[b] LUX = LI-COR Model LI-185B Quantum/Radiometer/Photometer (ABC Material #1905-160) and LI-COR LI-210SB Photometric sensor (Serial #PH 4391).

[c] Mercury Thermometer.

Checked By: [signature] Date: 7-6-90

DE 6-25-90 DF DE 6-27-90 DF

Page No: 001

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By [signature] — date 7-31-90

ENVIRONMENTAL CHAMBER

Chamber No.: 2

Lab Form No.: 737 (5/23/90)

Analytical Bio-Chemistry Labs
7200 ABC Lane, P.O. Box 1097
Columbia, Missouri 65205

Date/I.D.	Light Reading PAR[a]	Light Reading LUX[b]	Footcandles (LUX X 0.0929)	Temp[c] °C	Comments
7-6-90/DF	6.6	550	51	20	
7-7-90/AF	6.9	575	53	20	
7-8-90/AF	6.6	550	51	20	
7-9-90/SLH	6.6	550	51	20	
7-10-90/SLH	6.9	575	53	20	
7-11-90/DF	6.9	575	53	20	
7-12-90/SLH	6.9	575	53	20	
7-13-90/DF	6.9	575	53	20	
7-15-90/SLH	6.9	575	53	20	
7-16-90/DF	6.6	550	51	20	
7-17-90/DF	6.9	575	53	20	
7-18-90/SLH	6.3	525	49	20	
7-19-90/DF	@ 6.3 ~~7.5~~	@ 525 ~~625~~	@ 49 ~~58~~	20	
7-20-90/DF	7.5	625	58	20	
7-21-90/DF	8.1	675	63	20	
7-24-90/DF	8.1	675	63	20	
7-26-90/SLH	7.2	600	56	20	
7-27-90/SLH	7.8	650	60	20	
7-29-90/DF	7.8	650	60	20	
7-30-90/DF	6.9	575	53	20	
7-31-90/JGM	6.8	567	53	20	

[a]PAR = LI-COR Model LI-185B Quantum/Radiometer/Photometer (ABC Material #1905-160) and LI-COR Model LI-190SB Quantum Sensor (Serial #Q 7517). If PAR sensor is used then the following equation is used to determine LUX value:

$$LUX = \frac{\text{PAR Reading}}{12 \text{ (Correction factor for flourescent lighting)}} \times 1000$$

[b]LUX = LI-COR Model LI-185B Quantum/Radiometer/Photometer (ABC Material #1905-160) and LI-COR LI-210SB Photometric sensor (Serial #PH 4391).

[c]Mercury Thermometer.

Checked By: [signature] Date: 7-31-90

Page No. 002

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By [signature] date 7-31-90

pg 0035

ABC LABS # 038874

INVERTEBRATE CULTURE RECORD

Species: Daphnia magna

Lot No.: 90-AH Date Initiated: 7-6-90

Culture Volume: 1500 ml

Number of instar used to initiate culture: 20 (*)

ABC Form No.: 396 (4/17/90)

Analytical Bio-Chemistry Labs
7200 East ABC Lane
Columbia, Missouri 65202

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By [signature] date 7-31-90

Date	Number Adult Alive	Approx. Instar Present	Volume* Algae Concen. Fed (ml)	** Algae Concen. Cells/ml	Algae Lot #	Study ID and Number of instar used	Obs. By
7-6	20	—	0.3/0.2	5.7x10^8 / 7.8x10^8	90-B16/90-A16	—	DF
7-8	20	10	"	"	90-B16/90-A16	—	AF
7-9	—	—	0.3/0.2	5.7x10^8/7.8x10^8	90-B16/90-A16	—	SLH
7-10	—	—	0.3/0.2	5.7x10^8/7.8x10^8	90-B16/90-A16	—	SLH
7-11	20	30	0.3/0.2	5.7x10^8/9.7x10^8	90-B16/90-C16	—	DF
7-12	—	—	—	—	—	28883-25 instar composited with 90-AF	JB
7-12	17	50	0.2/0.4	9.7x10^8/3.8x10^8	90-C16/90-D16	—	SLH
7-13	—	—	0.3/0.2	5.7x10^8/9.7x10^8	90-B16/90-C16	—	DF
7-14	—	—	—	—	—	—	JB
7-15	15	100	0.2/0.4	9.7x10^8/3.8x10^8	90-C16/90-D16	—	SLH
7-16	—	—	0.2/0.4	9.7x10^8/3.8x10^8	90-C16/90-D16	—	DF
7-17	15	175	1.0/0.2	1.6x10^8/8.7x10^8	90-A17/90-E16	—	DF
7-18	—	—	—	—	—	—	SLH
7-19	13	100	0.8/0.2	1.6x10^8/8.7x10^8	90-A17/90-E16	—	DF
7-20	—	—	0.2/0.6	8.7x10^8/2.4x10^8	90-E16/90-B17	—	DF
7-21	—	—	—	—	—	—	DF
7-22	@ 10	@ 150	0.2/0.6	7.2x10^8/2.4x10^8	90-B18/90-B17	—	DF
7-23	—	—	—	—	—	140 used for 38874	JG
7-23	—	—	0.2/0.6	7.2x10^8/2.4x10^8	90-B18/90-B17	—	DF
7-24	—	—	—	—	terminated	—	DF

Remarks: (*) adults taken from daphnia magna mass culture 7-6-90 DF @ E 7-22-90 DF

*Culture also fed 1.5 ml of a 5.0 mg/ml suspension of Trout chow and yeast for a final solids concentration of 5.0 mg/l.

**Concentration of algae concentrate.

Prepared by: [signature] Date: 7-6-90

Reviewed by: [signature] Date: 7-31-90

ABC LABS# 038874 PG 0036

COMPOUND RECEIPT

Lab Form No. 352 AF 38F

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.
P.O. Box 1097 • Columbia, MO 65205
Shipping Address: 7200 East ABC Lane, Columbia, MO 65202
(314) 474-5579 • Answer Back (ABCLAB UD)
FAX (314) 443-8033

Compound CT-434-90N

ABC SUPPLIED INFORMATION

Date Received: 6-13-90 Logged In By: JH ABC Ref # TS-4117

Storage: ROOM TEMP Total Weight: 230.50 g

Physical Description: WHITE LIQUID

Remarks:

SUPPLIER PROVIDED INFORMATION

Firm: AMERICAN CYANAMID

Address: BOX 60

City/State/Zip STAMFORD, CT 06904 Phone: 203-321-2200

Batch/Lot No. S-16363-15B CAS # N/A Purity: N/A

Amount Declared: 100 g Expiration Date: 3-30-91

Storage Instructions N/A

Other

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The original document"

By [signature] date 7-30-90

CHEMICAL/PHYSICAL MEASUREMENTS OF TEST-SOURCE WATER	Lab Form No.: 509H (5/30/90) Analytical Bio-Chemistry Labs 7200 ABC Lane, P.O. Box 1097 Columbia, Missouri 65202
Water Source: AQ3-Hard Blended Water Preparation Date: 7-23-90	Alkalinity[a]: 190 mg/l Hardness[a]: 170 mg/l pH[b]: 8.0 Temperature[c]: ~~19~~ 22 °C Conductivity[d]: 360 µMhos/cm D.O.[e]: 8.2 mg/l

Comments:

"This is an exact copy of
The original document"

By [signature] date 7-31-90

QE 7-23-90 DO

[a]Total alkalinity and hardness using a titrimetric method adapted from Standard Methods (mg/l as CaCO).

[b]pH probe (Sensorex Model S200C) used with a Corning Model 140 pH and mV meter, ABC Material Control 1714-175.

[c]digital thermometer.

[d]Conductivity using a Salinity-Conductivity-Temperature (S-C-T) Meter, ABC Material Control #1905-530.

[e]Dissolved oxygen probe (YSI Dissolved Oxygen System Model 54 ARC) ABC Material Control #1905-485.

Analysis By: [signature] Date: 7-23-90

Reviewed By: [signature] Date: 7-30-90

APPENDIX II - CORRESPONDENCE AND PROTOCOL

DEFINITIVE CONCENTRATION NOTIFICATION

Firm: American Cyanamid Company	Lab Form No.: 186
Address: one Cyanamid Plaza	Analytical Bio-Chemistry Labs
City/State/Zip: Wayne N.J. 07470	Aquatic Toxicology Division
Phone: (201) 831-2534	7200 ABC Lane, P.O. Box 1097
	Columbia, MO 65202

Sponsor's Study Director: C.A. Vernon

Person notified: same (✓) Other:

Date of Notification: 7-23-90

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

Test Material	ABC #	Test Species	Test Conc. (mg/l)
CT-434-90 N	38874	Daphnia magna	
			0.32
			0.56
			1.0
			1.8
			3.2
			5.6

Notified By: [signature] Date: 7-23-90

ABC LABS# 038874 Pg 0040

American Cyanamid Company
Chemicals Group
Toxicology and Product Safety Department
One Cyanamid Plaza
Wayne, NJ 07470
Fax 201-831-2821

RECEIVED
'90 MAY 25 AM 10 23
A B C LABS

May 22, 1990

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

Attention: Carl Thompson, Vice President
Aquatic Toxicology/Field Studies

Reference: CT-434-90N Aquatic Toxicity Studies

Dear Carl:

You will receive under separate cover a material identified as CT-434-90N. Please submit this material to the following test at the following cost:

Test	Protocol	Cost
Acute Toxicity to Daphnia Magna	7806	$900.00

RANGE FINDING SHOULD BEGIN WITH 10-100 MG/L.

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

The test material is a white, opaque emulsion which is soluble in water. The following special handling conditions apply to this material: Store at room temperature away form direct sunlight. Avoid skin contact. No MSDS is available on this product.

Please inform me of your receipt of this material. My office number is (201) 831-2534.

Please have all draft and final reports, invoices, and test material information sent to my attention. The Cyanamid Representative to whom all technical questions regarding this project should be addressed is P. A. Vernon.

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,

AMERICAN CYANAMID COMPANY

By: Patricia Ann Vernon Ph.D
Name: Patricia Ann Vernon
Title: Toxicology Program Coordinator

ACCEPTED: _____, 1990

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.

By: _____
Name:
Title:

pavABC

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

August 16, 1990

American Cyanamid Company
Attn: Ms. Patricia A. Vernon
Toxicology Program Coordinator
One Cyanamid Plaza
Wayne, New Jersey 07470

RE: "Acute Toxicity of CT 434-90N to Daphnia magna", ABC Study Number 38874.

Dear Patti:

During the conduct of the above cited study, a deviation from the study protocol was noted. To be in conformance with our laboratory policy, we need your signed acknowledgment for this matter. It should be noted that, in our opinion, the deviation listed did not compromise the results of this study.

The protocol deviation and reason for it was:

1) **Protocol Deviation:** Section 4.2

Light reading fell below 50 footcandles.

Reason: Old light bulbs needed replacement.

Please sign both copies of this letter, return one copy to ABC Laboratories for inclusion in the final report. The other copy is for your files.

If you have any questions or need additional information, please give me a call (314-474-8579).

Sincerely,

David Burgess
Biologist II/Study Director

ACKNOWLEDGED BY:

Patricia A Vernon 8/20/90

Ms. Patricia A. Vernon Date
American Cyanamid Company

DB/bsb

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

PROTOCOL COVER SHEET

The protocol for study CT-434-90N consists of copies of the following information:

ABC Generic Protocol 7806

Study Authorization Letter dated May 22, 1990

The proposed experimental start date is June 1990

The proposed experimental completion date is July 1990

The above information has been collected and complied into the protocol for study 38874. The signature date below constitutes the "Study Initiation Date".

David Burgen 6-20-90

ABC Study Director (Date)

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC
P.O. Box 1097 • Columbia, MO 65205
Shipping Address: 7200 East ABC Lane, Columbia, MO 65202
(314) 474-8579 • Telex 821 814 • Answer Back (ABCLAB UD)
Fax (314) 443-9033

RECEIVED
'90 APR 4 AM 8 39
ABC LABS

ABC PROTOCOL NO. 7806

(Revised April 4, 1989)

STATIC BIOASSAY PROCEDURE FOR DETERMINING THE ACUTE TOXICITY OF CHEMICAL SUBSTANCES TO DAPHNIA MAGNA

ABC Study Number _____

Test Material _____

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 Regulation (4) which complement them, Analytical Bio-Chemistry 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, 8).

2.0 OBJECTIVES

The primary objective of the toxicity test described herein is to evaluate the acute toxicity of a chemical substance to Daphnia magna under static conditions. This is achieved by determining LC_{50} levels of the toxicant during a 48-hour exposure period. An LC_{50} is the approximate concentration of the test material that produces 50 percent mortality of test daphnids after prescribed intervals. Mortality as used in this protocol is defined as the cessation of movement. The method is designed to yield LC_{50} values following 24 and 48 hours of exposure.

3.0 TESTING FACILITY

The study will be conducted by the Aquatic Toxicology Division of Analytical Bio-Chemistry Laboratories, Inc., 7200 East ABC Lane, P. O. Box 1097, Columbia, Missouri 65205, 314-474-8579.

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 this preliminary test will be used to set concentration levels for the definitive bioassay described in section 5.0.

4.2 Test Daphnids. The test lot of Daphnia magna will be obtained from an in-house culture. The taxonomic key presented by Pennak (9) will be used to identify to species the test daphnids. All test daphnids will be held in a controlled temperature area at 20 ±2°C. The lighting will be 50-70 footcandles on a 16 hour daylight photoperiod. During the holding period, they will be fed algae (Selenastrum capricornutum) supplemented with a suspension of commercial fish food and/or cereal leaves and yeast at least every 3 days. First-instar daphnids (<24 hours old) will be selected for testing and will not be fed during the test.

4.3 Test 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 ±2°C. The dilution water used in toxicity testing at ABC Laboratories will be ABC well water prepared to a total hardness of between 160 to 180 mg/l (as $CaCO_3$). 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 (RO). Water quality values obtained from periodic screening of ABC well water for selected chemical/physical factors will be included in the report.

4.4 Test Material. A letter of authorization for the study should be sent with the test material or previous to its shipment and should contain the following chemical/physical properties of the compound if available: name of test material, code number, physical description, purity, stability, suggested storage conditions, water and organic solvent solubility, available toxicity information, and handling precautions. 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, volatility and aquaria aeration may require analysis at 0 and 48 hours. 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 included in the report.

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

4.5.1 Test daphnids will be selected from a sub-culture of first-instar (<24 hours old) individuals.

4.5.2 The range-finding test will be initiated by exposing groups of five to ten daphnids to at least one or more toxicant concentrations, usually spaced by a factor of 10. The test daphnids will be placed in the test chambers by impartial assignment within 30 minutes after solution preparations. The initial toxicant concentrations most often used are 1, 10 and 100 mg/l. Numerous static tests by ABC have shown that a significant percentage of the compounds tested have aquatic toxicities which fall within this range. ABC will test up to a maximum of 1,000 mg/l in both the preliminary and definitive tests, unless otherwise requested by the study sponsor.

4.5.3 After 24 hours of exposure, the test chambers will be observed for mortality and adverse behavioral effects. A record will be maintained of mortality and abnormal behavior at each

observation. Dependent upon this observation, additional tes concentrations may be added at levels above or below the initia concentrations. This procedure will be followed until a toxi range is determined. For example, if the 24-hour exposure result in total mortality, new solutions will be prepared at a factor o 10 below the lowest initial concentration until no mortality o partial mortality is reached. In the converse situation, if n mortality is observed after 24 hours, new solutions will be adde at concentrations spaced by a factor of 10 above the highes initial level until mortality is noted. In this manner, a bracke is formed for the toxic range of the compound.

4.5.4 The preliminary test will be conducted for a period o 24 to 48 hours - the exact duration dependent upon the results o the initial concentrations tested. In most cases, a preliminar test for 48 hours at 3 toxicant concentrations is sufficient t determine the toxic range.

4.5.5 Results of the range-finding study will be used to se the concentration range of the definitive study described i section 5.0. At least five toxicant concentrations have proven t be adequate in assessing the aquatic toxicity of most compounds.

5.0 DEFINITIVE STUDY

5.1 General. Following the preliminary range-finding stud discussed in section 4.0, the definitive test will be conducted b the procedures described below. Test-specific information regard ing the sponsor, test material, proposed study dates, stud personnel and study approvals will be included in section 8.0 a the time of protocol approval.

5.2 Test Daphnids. Aspects concerning the culture an acclimation of test daphnids will be the same as discussed i section 4.2.

5.3 Test System. The test system for the definitive stud will be the same as outlined in section 4.3.

5.4 Test Material. Specific information regarding the tes material is to be supplied by the sponsor. The remaining tes material will be sent back to the study sponsor upon approval o the final report.

5.5 Test Procedure-Biological. The basic test procedure fo the definitive bioassay will be as follows:

5.5.1 Test daphnids will be selected from a sub-culture o first instar (<24 hours old) individuals.

5.5.2 The definitive test will be initiated by exposing th test daphnids to at least 5 toxicant concentrations and a dilutio water control. The test will be conducted using duplicate beaker

per concentration, with 10 daphnids per beaker. The test concentrations used will be based upon the results of the range-finding test and will be selected from a logarithmic series or a geometric series in which the ratio between consecutive concentrations is between 1.5 and 2.0. The definitive test concentrations will be selected after discussing preliminary results with study sponsor. If a solvent is used in the preparation of test solutions, the control chamber will receive an aliquot of the solvent equivalent to the highest amount used in the test chambers but will not exceed 0.1 ml/liter. The test organisms will be placed in the test chambers by impartial assignment within 30 minutes after solution preparations.

ABC 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 will report any 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 will retest the material if requested by the study sponsor at an additional charge which includes analytical confirmation costs.

5.5.3 As alternate test designs, single or triplicate test chambers containing 10 daphnids each may be used, if so authorized by the sponsor.

5.5.4 The test chambers will be observed for mortality and adverse behavioral effects every 24 hours. A record will be maintained for mortality and abnormal behavior for each concentration tested.

5.6 Test Procedure - Chemical and Physical. The temperature, dissolved oxygen concentration and pH will be measured at least in the control, high, medium and low toxicant concentrations at the beginning and termination of the study (5).

5.7 Analysis of Results. The results of the definitive study will be statistically analyzed for 24 and 48 hour LC_{50} values and their corresponding 95 percent confidence limits. These values will be determined by an LC_{50} computer program (10, 11).

5.8 Report. One copy of the report will be submitted. The report will contain all original raw data. Additional copies will be provided, if requested by the sponsor, at a copy rate of $50 per copy. The report will include the following:

5.8.1 Study dates, name and address of test facility.

5.8.2 Objectives and test methods.

5.8.3 Reference to the statistical methods used for data analysis.

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

5.8.5 Description of test design.

5.8.6 Summary of the data analysis, mortality observations and test water quality.

5.8.7 Location of raw data.

5.8.8 List and signatures of study personnel.

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

5.8.10 The report appendix will contain the original raw data for mortality observations and water quality, letter of test authorization, letters of authorized protocol changes, and a copy of the approved protocol.

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

6.0 PROTOCOL CHANGES

In the event that modifications of this protocol are deemed necessary, a written statement of any changes and reason(s) proposed by the study sponsor or ABC will be submitted to the other party. All agreed changes will be expressed in writing, signed and dated by the sponsor's study director. The signed changes will be appended to the protocol and included with the final report. This protocol is subject to revision when felt necessary to comply with changes in federal regulations and state of the art methodology. The protocol for a specific study will not be changed while the study is in progress without discussion with the study sponsor.

7.0 SPONSOR AUTHORIZATIONS DURING THE STUDY

Should a problem develop while the study is in progress, ABC will notify the sponsor's study director within 24 hours. Theproblem and suggested test modifications will be discussed by telephone. ABC will proceed with the changes felt necessary upon the verbal authorization of the sponsor's study director. A letter for written authorization will then be submitted by ABC to the sponsor's study director and handled in the same manner discussed in section 6.0.

8.0 TEST-SPECIFIC INFORMATION

8.1.1 General. The following items will be addressed for each static bioassay. This information is necessary to be in compliance with Good Laboratory Practice Regulations (4). Sections 8.2, 8.3, 8.7 and 8.9 are to be completed by the study sponsor. Sections 8.4, 8.5 and 8.6 will be completed by ABC.

8.1.2 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 regulation, for which this study specific protocol and resultant study must be in compliance with, is to be designated by the study sponsor in section 8.7 of this protocol.

8.1.3 PR Notice 86-5 Compliance. To be in compliance with U.S. EPA PR Notice 86-5: Standard format for data submitted under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and certain provisions of the Federal Food, Drug and Cosmetic Act (FFDCA) (12), the report of the investigation conducted utilizing this protocol must contain either a confidentiality or a non-confidentiality claim. Should you claim non-confidentiality of the report and raw data, as defined by the criteria of FIFRA §10(d)(1)(A), (B) or (C), a NO DATA CONFIDENTIALITY CLAIM is attached and must be signed and dated for inclusion in the study report as page 2. If you claim that the study report or portions of it include confidential business information as defined by the criteria of FIFRA §10(d)(1)(A), (B) or (C) then you must provide ABC Laboratories with a statement of data confidentiality for inclusion in the study report as page 2.

8.2 Study Sponsor:

8.2.1 Company American Cyanamid

8.2.2 Address One Cyanamid Plaza

Wayne, New Jersey 07470

8.2.3 Sponsor's Representative

Patti Vernon	Toxicology Program Coordinator
Name	Title

8.3 Test Material:

Name _____

8.4 Test Concentrations: To be determined after preliminary testing and consultation with the study sponsor.

8.4.1 Sponsor's Study Director notified of preliminary results: Date _____ ABC Biologist _____

8.4.2 Definitive test concentrations agreed upon:

_____.

8.5 Study Dates:

8.5.1 Proposed starting date of definitive study _____

8.5.2 Proposed completion date of definitive study _____

8.6 ABC Study Personnel:

8.6.1 Study Director

Dave Burgess	Biologist II
Name	Title

8.6.2 ABC's Supervisor

James P. Swigert, Ph.D.	Supervisor, Aquatic Toxicology
Name	Title

8.7 GLP Compliance (check the most appropriate):

EPA-FIFRA ___; EPA-TSCA ___; FDA ___; OECD ___; Other _____.

8.8 Protocol Approvals. The following is to be signed by the appropriate study personnel:

8.8.1 Sponsor's Representative

Name	Title	Date
Patricia Ann Lenon	Toxicology Program Coordinator	3/30/90

8.8.2 ABC's Study Director

Name	Title	Date
	Biologist II	

8.8.3 ABC's Group Leader

Name	Title	Date
	Group Leader, Aquatic Toxicology	

8.9 Authorization to return the test material and sample:

8.9.1 Sponsor's authorized agent to receive the test material and sample.

Name _____ Title _____

8.9.2 Shipping Address

Company _____

Street Address _____

City _____ State _____ Zip Code _____

Phone Number _____

8.9.3 Hazardous Shipment Information

Proper Shipping Name _____

Class of Restricted Article (e.g. flammable, poison, radioactive, etc.) _____

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. 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 Adminstration. 1978. Nonclinical Laboratory Studies, Good Laboratory Practice Regulations (21 CFR, Part 58). Federal Register, Vol. 43, No. 247: 59986-60025.

(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; 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) American Society for Testing and Materials. 1981. Proposed standard practice for conducting renewal life cycle toxicity tests with the Daphnid, Daphnia magna. Draft 8, ASTM Committee E-35.21. 29 p.

(9) Pennak, R. W. 1978. Fresh-Water Invertebrates of the United States, 2nd Ed. 365 p.

(10) Stephan, C. 1977. Methods for calculating an LC_{50}, p. 65-84. In F. L. Mayer and J. L. Hamelick (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.

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

(12) U.S. Environmental Protection Agency. 1986. PR Notice 86-5. Standard format for data submitted under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and certain provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA). U.S. E.P.A., O.P.T.S., 1986. 17 p.

CERTIFICATE OF AUTHENTICITY

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