



8EHQ-0393-8600

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American Cyanamid Company  
One Cyanamid Plaza  
Wayne, NJ 07470

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

March 18, 1993

Document Processing Center (T-790)  
Office of Toxic Substances  
U.S. Environmental Protection Agency  
401 M Street S.W.  
Washington, DC 20460



8EHQ-92-8600  
SP001 03/26/93

Attention: Section 8(e) Coordinator



89930000073

Dear Sir/Madam:

This shipment contains a follow-up report which is being submitted to the Environmental Protection Agency pursuant to the Toxic Substances Control Act (TSCA) Section 8(e). We are also terminating the confidentiality claim for this submission. Preliminary information was submitted to the agency, under section 8(e), on November 6, 1992; at that time a final report was not yet prepared. In accord with efforts to reduce paper consumption, we have provided the agency, here, with the Summary, Results and conclusions sections of the final report along with those tables which pertain to the information detailed in the earlier section 8(e) report.

If further information is required in the interim, please contact K.A. Traul Ph.D., Director of Regulatory Compliance at 609-799-0400 ext 2701 or 2347.

Sincerely,

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

93 MAR 26 AM 8:32

U.S. ENVIRONMENTAL PROTECTION AGENCY

50 pgs.





L A B O R A T O R I E S

Analytical Bio-Chemistry Laboratories, Inc

Contains NO CBI

Date Submitted: \_\_\_\_\_ MRID NO.: \_\_\_\_\_ VOLUME: \_\_\_\_\_

STUDY TITLE

Chronic Toxicity of AC 801,757 During the Complete Life-Cycle of *Daphnia magna* Under Flow-Through Test Conditions

DATA REQUIREMENT

U.S. EPA-FIFRA, 40 CFR, Section 158.145 Guideline 72-4 (b) and OECD Guidelines for Testing of Chemicals, Test No. 202

AUTHORS

Greg C. Blakemore, Principal Investigator  
ABC Laboratories, Inc., Columbia, Missouri

Luke Stuerman/Chemist II  
ABC Laboratories, Inc., Columbia, Missouri

Joseph D. Wisk, Ph.D., Study Director  
American Cyanamid Company, Princeton, New Jersey

REPORT SUBMITTED ON

February 25, 1993

PERFORMING LABORATORY

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

ABC LABORATORY PROJECT ID

Final Report #40145

AMERICAN CYANAMID STUDY NUMBER

#954-92-108

Page 1 of 139

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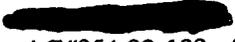
7200 E. ABC Lane ■ PO. Box 1097 ■ Columbia, MO 65205 U.S.A. ■ 314-474-8579 ■ Fax 314-443-9033

7200 E. ABC Lane ■ PO. Box 1097 ■ Columbia, MO 65205 U.S.A. ■ 314-474-8579 ■ Fax 314-443-9033

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AC#954-92-108, ABC LABS NO. 40145-2

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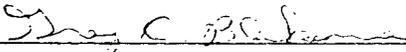
STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC Laboratories' Final Report #40145, American Cyanamid Company's Study #954-92-108, entitled "Chronic Toxicity of AC 801,757 During the Complete Life-Cycle of *Daphnia magna* Under Flow-Through Test Conditions" for American Cyanamid Company, Princeton, New Jersey.

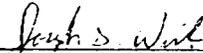
In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, and in accordance with American Cyanamid Company's intent that all studies sponsored by American Cyanamid Company follow good laboratory practices, ABC Laboratories' principal investigator for the above test herein confirms that the study was conducted in compliance with the Organization for Economic Cooperation and Development Good Laboratory Practice in the Testing of Chemicals and in compliance with the U.S. EPA Good Laboratory Practice Standards (40 CFR 160).

The stability of the test compound in the test system was not investigated. That was the responsibility of the study sponsor.

All original raw data were archived at American Cyanamid Company with the final report. A copy of the final report and the raw data report was retained in archives at ABC Laboratories. The sponsor is responsible for the retainment of the unused portion of the test material and its proper storage or disposal.

  
\_\_\_\_\_  
Greg C. Blakemore  
Principal Investigator, ABC Laboratories, Inc.

2/19/93  
Date

  
\_\_\_\_\_  
Joseph D. Wisk, Ph.D.  
Study Director, American Cyanamid Company

2/20/93  
Date

  
\_\_\_\_\_  
Sponsor

2/25/93  
Date

\_\_\_\_\_  
Submitter

\_\_\_\_\_  
Date

**QUALITY ASSURANCE STATEMENT**

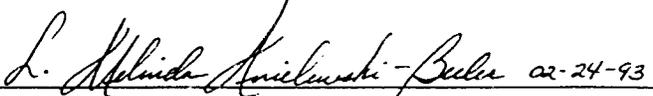
Quality Assurance Statement for ABC Laboratories' Study #40145R, American Cyanamid Company's Study #954-92-108, entitled, "Chronic Toxicity of AC 801,757 During the Complete Life-Cycle of *Daphnia magna* Under Flow-Through Test Conditions," for American Cyanamid Company, Princeton, New Jersey.

The report for AC 801,757 was reviewed by ABC's Quality Assurance Unit.

The following inspections were conducted on the AC 801,757 21-day toxicity test:

Date of Inspection	Phase Inspected	Date Reported To Principal Investigator	Date Reported To Study Director	Date Reported To ABC Management	Date Reported To American Cyanamid Management
07/10/92	Addition of Instar	07/10/92	07/14/92	08/12/92	07/21/92
07/20/92	Addition of Instar	07/20/92	07/29/92	N/A	08/07/92
07/29/92	Addition of Instar	07/29/92	07/29/92	08/12/92	08/07/92
08/10/92	Addition of Instar	08/10/92	08/17/92	09/15/92	08/24/92
08/17/92	Day 7 Water Chemistry	08/17/92	08/18/92	09/15/92	08/24/92
09/11/92	Addition of Daphnids	09/11/92	09/14/92	10/16/92	09/18/92
09/18/92	Day 7 Water Chemistry	09/18/92	09/24/92	10/16/92	09/30/92
09/23/92	Day 12 Instar Counts	09/23/92	09/24/92	10/16/92	09/30/92
10/02/92	Length Measurements	10/02/92	10/14/92	11/30/92	10/22/92
01/22/93	Draft Report and Raw Data	01/22/93	Not Applicable	01/22/93	Not Applicable
02/18/93	Final Report and Raw Data	02/18/93	Not Applicable	02/18/93	Not Applicable

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

  
 L. Melinda Anielewski-Beeler 02-24-93  
 Quality Assurance Officer I Date





American Cyanamid Company  
Agricultural Research Division  
Princeton, New Jersey 08543-0400

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Report Number:	ECO 92-108	Number of Pages:	139
Type of Report:	Final	Study Initiation Date:	92Feb7
Department:	Human and Environmental Safety	Expt. Start Date:	92March5
Group Number:	0954	Expt. Termination Date:	92Oct2
Type of Study:	Invertebrate life-cycle toxicity	Study Completion Date:	93Feb25
Study Number:	954-92-108	Report Issue Date:	93Feb25
CL Numbers:	AC 801,757	Approved By:	
Reported By:	J. J. Wisk G.C. Blakemore (ABC) L. Stuerman (ABC)		2/25/93
Work Done By:	G.C. Blakemore et al. (ABC)	C. L. McIntosh	Date

**TITLE:**

Chronic Toxicity of AC 801,757 During the Complete Life-Cycle of Daphnia magna  
Under Flow-Through Test Conditions.

**PURPOSE:**

The objective of this study was to determine the maximum acceptable toxicant concentration (MATC) of AC 801,757 during the complete life-cycle of Daphnia magna. The results from this study satisfy U.S. EPA data requirement 40 CFR Series 72-4 (b) and international regulatory requirements for OECD Test # 202, "Daphnia Reproduction Test".

**SUMMARY:**

A test was carried out at ABC Laboratories, Inc. in Columbia, Missouri, to evaluate the chronic toxicity of AC 801,757 during the complete life-cycle of Daphnia magna under flow-through test conditions. The criteria for effect were mortality, growth and reproduction of first generation Daphnia.

A no-treatment control, a vehicle blank and five concentrations of AC 801,757 were tested. A concentrated stock solution of AC 801,757 was prepared in dimethylformamide (DMF). A stock solution delivery system was used in conjunction with a proportional diluter to prepare and deliver the test solutions to the test vessels at a consistent flow rate. The following nominal concentrations were tested: 2.4, 4.8, 10, 20 and 40 µg of AC 801,757/L. The vehicle blank contained 0.05 mL of DMF/L, which represented the concentration of DMF in the highest test substance treatment level. The no-treatment control contained dilution water only. The dilution water was hard blended water which was prepared by blending ABC's well water with water that had been demineralized by reverse osmosis. The resulting blended water possessed a hardness of between 138 and 154 mg/L as CaCO<sub>3</sub>.

The definitive toxicity test was initiated with the addition of 10 Daphnia magna, less than 24 hours of age, to each treatment and control test vessel. The test vessels were 1-L glass beakers with notched drains that were covered with 50-mesh stainless steel. There were four replicate test chambers per treatment, providing a total of 40 Daphnia per each treatment and control. The test vessels were placed in a recirculating water bath that maintained the temperature of the test solutions between 20 and 21 °C during the test.

An HPLC method (M-2142) developed by American Cyanamid Company to determine the concentrations of AC 801,757 in water was validated by ABC Laboratories, Inc. (Study Number 954-92-107) prior to being used in this study (Appendix II). The validated method was used to determine the concentrations of AC 801,757 in the test solutions on test days (-)2, 0, 4, 7, 14, and 21. The mean measured concentrations during the 21-day exposure (excluding day(-)2) were: 2.4, 4.6, 8.8, 18, and 36 µg of AC 801,757/L. The mean measured concentrations ranged from 88 to 100% of the targeted nominal concentration. Based on the consistent measurements of AC 801,757 during the test, the test substance appeared to be stable in ABC's dilution water. The LOEC, NOEC and MATC values were based on the mean measured concentration of AC 801,757.

The number of live and dead Daphnia in each replicate were recorded daily and dead or immobile Daphnia were removed when observed. The number of offspring in each replicate was recorded three times per week. The average time to first brood was calculated. After 21 days of exposure, the number of young produced per adult Daphnia per reproduction day (the number of days that the daphnids were reproducing and the number of adult daphnids alive on each day) was calculated. After 21 days of exposure, the individual lengths and mean dry weights of remaining first generation Daphnia were determined.

The effect of the various treatments on survival, reproduction and growth of first generation Daphnia is summarized in the following table:

Treatment <sup>a</sup>	% Survival	Young/Adult/ Reproduction Day	Mean Dry Weights (mg)	Mean Lengths (mm)
no-treatment	97.5	8.79	1.00	4.47
vehicle	100	6.65	1.20	4.47
2.4 µg/L	97.5	6.23	1.22	4.51
4.6 µg/L	100	5.18	1.22	4.53
8.8 µg/L	94.9	4.95	1.25	4.53
18 µg/L	97.5	4.52	1.17	4.48
36 µg/L	92.5	1.38	0.84	4.36

<sup>a</sup>Concentrations in µg of AC 801,757/L.

After 21 days of exposure, there was no statistical difference in survival between the no-treatment controls and the vehicle blank. Therefore, survival in the treatment groups was compared with survival of the pooled controls. There was no statistical difference in survival between the treatment groups and the pooled controls when tested by analysis of variance (ANOVA). Therefore, the lowest level of observed effect (LOEC) and the no-observed effect concentration (NOEC) for effects on survival were > 36 µg of AC 801,757/L.



American Cyanamid Company  
Agricultural Research Division  
Princeton, New Jersey 08543-0400

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The average time to first brood was seven days in both control groups and the three lowest treatment levels (2.4, 4.6 and 8.8  $\mu\text{g}$  of AC 801,757/L). The average time to first brood in the 18 and 36  $\mu\text{g}$  of AC 801,757/L treatments were 9.3 and 14 days, respectively. The times to first brood in the two highest treatments were significantly longer than the pooled controls as determined by ANOVA and Dunnett's test. Therefore, the LOEC and NOEC values for this endpoint were 18 and 8.8  $\mu\text{g}$  of AC 801,757, respectively, and the MATC (the geometric mean of the LOEC and NOEC) was 13.4  $\mu\text{g}$  of AC 801,757/L.

After 21 days of exposure, the number of young/adult/reproduction day in the vehicle blank was significantly lower than the no-treatment controls when compared using a t-test. Therefore, the comparison of the number of young/adult/reproduction day was made between the treatment groups and the vehicle blank. As determined by ANOVA and Dunnett's test, the number of young/adult/reproduction day in all treatment levels except the lowest treatment (2.4  $\mu\text{g}$  of AC 801,757/L) was significantly lower than the vehicle blank. Therefore, the LOEC and NOEC values for this endpoint were 4.6 and 2.4  $\mu\text{g}$  of AC 801,757/L, respectively, and the MATC was 3.3  $\mu\text{g}$  of AC 801,757/L.

After 21 days of exposure, the average dry weights of the Daphnia in the vehicle blank were significantly greater than the average weights of the no-treatment controls as determined by a t-test. Therefore, the average weights of the treatment groups were compared with the average weights of the vehicle blank. Except for the highest treatment level (36  $\mu\text{g}$  of AC 801,757/L), the average weights of all treatments were statistically comparable to the vehicle blank, according to ANOVA and Dunnett's test. Therefore, the LOEC and NOEC values for this endpoint were 36 and 18  $\mu\text{g}$  of AC 801,757/L, respectively, and the MATC was 27  $\mu\text{g}$  of AC 801,757/L.

After 21 days of exposure, there was no statistical difference in the average length of Daphnia in the no-treatment controls and the vehicle blank, as determined by a t-test. Therefore, the average length of the Daphnia in the treatment groups was compared to the average length of the pooled controls. There was no statistical difference in the average length of Daphnia in any AC 801,757 treatment level in comparison to the pooled controls as determined by ANOVA. Therefore, the LOEC and NOEC values for this endpoint were  $> 36 \mu\text{g}$  of AC 801,757/L.

In summary, the most sensitive endpoint of AC 801,757 to Daphnia magna was reproduction (the number of young/adult/reproduction day). The LOEC, NOEC and MATC values based on this endpoint were 4.6, 2.4 and 3.3  $\mu\text{g}$  of AC 801,757/L, respectively.

0 0 0 9



QUALITY ASSURANCE STATEMENT

STUDY 954-92-108

REPORT NUMBER: ECO-92-108

The Quality Assurance Unit, Agricultural Research Division,  
American Cyanamid Company, conducted the following inspections/  
audits for this study.

Phase	Date Performed	Date Reported
PROTOCOL REVIEW	21-FEB-92	25-FEB-92
EXEC SUMM REPORT REVIEW	24-FEB-93	24-FEB-93

*Grace A. Drewicz /GAK*  
Grace A. Drewicz  
Quality Assurance Specialist  
25 Feb 1993  
Date

AC#954-92-108, ABC LABS NO. 40145-9

Performing Laboratory: ABC Laboratories, Inc.  
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P.O. Box 1097  
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Luke Stuerman 2/19/93  
Date  
Luke Stuerman  
Chemist II, ABC Laboratories

Joseph D. Wisk 2/25/93  
Date  
Joseph D. Wisk, Ph.D.  
Study Director, American Cyanamid Company

Approved by:

Alan D. Forbis 2/19/93  
Date  
Alan D. Forbis  
Manager, Aquatic Toxicology, ABC Laboratories, Inc.

L. Melinda Anielewski-Beeler 02-18-93  
Date  
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Quality Assurance Officer I

Tom Leak 2-18-93  
Date  
Tom Leak  
Manager, Aquatic Analytical, ABC Laboratories, Inc.

:jlv

SCIENTIFIC PERSONNEL SIGNATURE PAGE

The following personnel assisted with various phases of the study:

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Joseph D. Wisk	Research Toxicologist	<u>Joseph D. Wisk</u>	JW
Jennifer W. Blasberg	Biologist II	<u>Jennifer W. Blasberg</u>	JWB
Hugh Murrell	Biological Technician III	<u>Hugh Murrell (M)</u>	HM (M)
Scott J. Voney	Biologist I	<u>Scott J. Voney</u>	SSV
Steve L. Hicks	Biologist II	<u>Stephen L. Hicks</u>	SLH
Pamela E. Alt	Biologist I	<u>Pamela Alt</u>	PEA
Todd S. Butzlaff	Biological Technician III	<u>Todd S. Butzlaff (M)</u>	TBB (M)
James G. Muckerman	Biological Technician III	<u>James G. Muckerman</u>	JGM
Luke Stuermer	Chemist II	<u>Luke Stuermer</u>	LSM
Gerald Nothdurft	Laboratory Technician III	<u>Gerald A. Nothdurft</u>	GN
Dorothy England	Biologist II	<u>Dorothy England</u>	DE
Jane Bowman	Biologist III	<u>Jane Bowman</u>	JHB

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[REDACTED]  
AC#954-92-108, ABC LABS NO. 40145-15

SUMMARY AND TOXICITY COMPENDIUM

**Sponsor:** American Cyanamid Company

**Study Director:** Joseph Wisk, Ph.D.

**Location of Study:** ABC Laboratories, Inc.  
7200 E. ABC Lane  
Aquatic Toxicology Programs Division  
Columbia, Missouri 65205-1097

**Location of Original Raw Data and Final Report:**

American Cyanamid Company  
Agricultural Research Division  
P.C. Box 400  
Princeton, New Jersey 08543-0400

**Test Material:** AC 801,757

**Subject:** "Chronic Toxicity of AC 801,757 During the Complete Life-Cycle of *Daphnia magna* Under Flow-Through Test Conditions," ABC Laboratories' Study #40145, American Cyanamid Study #954-92-108

**Nominal Test Concentrations:** Control - 0.0 µg/L, vehicle blank (dimethylformamide) - 0.0 µg/L, 2.4, 4.8, 10, 20 and 40 µg/L

**Mean Measured Test Concentrations:** Control - (Not Detected), vehicle blank (dimethylformamide) - (Not Detected), 2.4, 4.6, 8.8, 18 and 36 µg/L

**Dilution Water:** 138 to 154 mg/L as CaCO<sub>3</sub> (hardness)  
150 to 172 mg/L as CaCO<sub>3</sub> (alkalinity)  
8.0 to 8.3 (pH)  
280 to 320 µMhos/cm (conductivity)

**Range Finding Test Dates:** March 5, 1992 to March 18, 1992

**Definitive Test Dates:** Initiation — September 11, 1992  
Termination — October 2, 1992

**Length of Definitive Test:** 21 Days

[REDACTED]  
AC#954-92-108, ABC LABS NO. 40145-16

Results: 21-Day  $EC_{50}$  =  $> 36 \mu\text{g/L}$  (95% Confidence Limits = could not be determined)  
21-Day NOEC =  $2.4 \mu\text{g/L}$   
21-Day LOEC =  $4.6 \mu\text{g/L}$   
21-Day MATC =  $3.3 \mu\text{g/L}$

Test Substance: AC 801,757, Lot #AC7196-23, 98.8% a.i., CAS #119168-77-3

Test Species: *Daphnia magna*

Source of Organisms: ABC Laboratories' in-house culture

Age of Organisms at Test Initiation: First-instar (<24 hours old)

## INTRODUCTION

American Cyanamid Company contracted the Aquatic Toxicology Programs division of ABC Laboratories, Inc., to conduct a five-concentration plus control and vehicle blank 21-day chronic life-cycle study with *Daphnia magna* exposed to AC 801,757. This definitive test was conducted from September 11, 1992, to October 2, 1992, to satisfy the data requirements of U.S. EPA-FIFRA, 40 CFR, Section 158.145 Guideline 72-4 (b) and OECD Guidelines for Testing of Chemicals, Test No. 202. The primary objective of the definitive test was to estimate the maximum acceptable toxicant concentration (MATC) limits for AC 801,757 using *Daphnia magna*, a sensitive, representative aquatic invertebrate.

The "MATC limits" (1) represents the no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The upper limit, or LOEC, is the lowest concentration that produces at least one statistically significant deleterious effect upon a measured parameter during the study (2). The lower limit, or NOEC, is the highest concentration that causes no statistically verifiable deleterious effect on any of the measured parameters. The point estimate of the MATC was determined as the geometric mean of the limits. In this study, these measured parameters refer particularly to daphnid survival, reproduction, and growth. A deleterious effect is one that is a statistically significant reduction ( $p \leq 0.05$ ) from the control for the parameter being measured. However, there may be some instances in which the statistically significant reduction is due to biological variability and not compound related. This report presents the results of the toxicity study and describes the technical approach used to meet the project objectives.

## METHODS AND MATERIALS

The biological methodology used for this study is patterned after procedures formulated by the American Society for Testing and Materials (1, 2), the U.S. Environmental Protection Agency (3, 4, 5, 6), and the Organization for Economic Cooperation and Development (7). This study was conducted following the procedures outlined in American Cyanamid Company's Protocol as approved by American Cyanamid Company's Study Director and Manager on February 7, 1992, and by ABC Laboratories' testing site representative on February 12, 1992.

### I. Test Organisms

Test specimens of *Daphnia magna* were obtained from an in-house 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 *Daphnia magna* strain indicated that CNFRL acquired their culture from the U.S. Fish and Wildlife Service Fish Control

Laboratory, LaCrosse, Wisconsin, in 1960; and they obtained their culture from Pennsylvania State University in 1954 (8).

Daphnids were cultured and tested in a temperature-controlled area at 20 ( $\pm 2$ ) °C. Lighting was provided by cool white fluorescent bulbs at an intensity of 67-68 foot-candles on a 16-hour daylight and 8-hour darkness photoperiod, with 30-minute dawn and dusk transition periods. The light reading records are on file at ABC Labs. The daphnids were maintained in 1.5-L glass containers. Daphnids were cultured in hard blended water (Table I). 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*. Along with the algae, the daphnids were fed a supplement consisting of trout chow, supplied by Zeigler Brothers, Inc., Gardners, Pennsylvania, and Fleishmann's active dry yeast (*Saccharomyces* sp.). This trout chow was analyzed for possible contaminants prior to its use. The data for these analyses are on file at ABC Laboratories, Inc. Only first-instar daphnids (<24 hours old) were selected for testing. Detailed logs of the cultures used to obtain test daphnids were maintained and are included in the raw data.

## II. Test System

A half-liter proportional diluter system described by Mount and Brungs (9), with a Hamilton® Micro Lab 420 syringe dispenser, was used to intermittently introduce dilution water and AC 801,757 into the test chambers. The system (Figure 1) contained seven sets of four replicate 1-L test chambers, designated as control, vehicle blank, and level #1 through level #5. Flow-splitting boxes were used to thoroughly mix and divide each AC 801,757 concentration for delivery to the test chambers. To minimize turbulence, the influent water was introduced into the test chambers via 14-gauge hypodermic needles. One-liter glass beakers, labeled A, B, C, and D, were used as test chambers (10). These chambers had notched drains that were covered with 50-mesh stainless steel screens to prevent the test daphnids from escaping. The test daphnids were placed in each of the quadruplicate chambers. The dilution water used in this study was a hard blended water prepared to a total hardness between 138-154 mg/L as CaCO<sub>3</sub> (11). The specific water hardness was obtained by blending ABC Laboratories' well water with reverse osmosis water. Water quality parameters of temperature, hardness, dissolved oxygen, pH, alkalinity, and conductivity were measured daily (Monday-Friday) for the duration of the definitive test. Table I contains water quality values of the dilution water obtained from the most recent chemical screening for these factors. Records of all water quality values are maintained at ABC Laboratories, Inc. The dilution water was delivered to each test chamber at an

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average rate of 4.1 mL/min, an amount which was sufficient to replace the 1-L test volume 5.9 times in a 24-hour period. The test chambers were immersed in a temperature-controlled water bath held at 20 ( $\pm$  2 °C). Temperature was recorded continuously with a Rustrak® Ranger™ Data Logger. Lighting for the test system was provided by cool white fluorescent bulbs and consisted of 16-hour daylight, 8-hour darkness, and 30-minute dusk and dawn transition periods. Light intensity ranged from 55 to 58 foot-candles at the water surface during the conduct of the study.

The proportional diluter system was calibrated prior to testing by checking volume deliveries from the diluter mixing cell to obtain a dilution factor used for stock preparations. The diluter system was set to provide test levels at ~50% dilutions of each other. The system was checked twice daily throughout the study to insure that it was working properly.

### III. Test Compound

#### A. Compound Receipt, Physical Description, and Solutions Preparation

CL 801,757 (lot #AC 7196-23) was received on September 13, 1991, from American Cyanamid in good condition, and was assigned ABC reference #PS-5165. The sample was observed to be a white powder and was stored in the refrigerator (0-5 °C). Sample purity was specified to be 98.8%. A 1.70 mg/mL primary standard solution was prepared in acetone on August 10, 1992. Subsequent dilutions were prepared in high performance liquid chromatographic (HPLC) grade methanol:ABC reagent water (50:50) for use as chromatographic standards, and a dilution was prepared in dimethylformamide (DMF) for use as a fortification solution. This sample was also used to prepare the stock solution for the preliminary test performed from March 5, 1992, to March 18, 1992. Correspondence with the sponsor confirmed that the names AC 801,757 and CL 801,757 are synonymous.

A second AC 801,757 sample (lot #AC-7196-23) was received on February 13, 1992, from American Cyanamid Company in good condition and was assigned ABC reference #PS-5505. The sample was observed to be off-white crystals and was stored in a refrigerator (0-5 °C). Sample purity was specified at 98.8%. This sample was used to prepare the stock solutions for the definitive test performed from September 11, 1992, to October 2, 1992.

The two AC 801,757 diluter stock solutions were prepared on August 28, 1992, and September 15, 1992, respectively, by weighing 0.0407 g and 0.0409 g of the AC 801,757 test material (PS-5505) into a 100-mL volumetric flask. The volumetric flask was brought to volume with dimethylformamide (DMF). The resulting diluter stock solutions had a final concentration of 400 mg/L. There was no visible precipitate in the diluter stock solutions. The diluter stock was placed in a 10-mL graduated culture tube for use by the diluter system, while the remaining stock was stored in the refrigerator when not in use. A 0.1 mL aliquot of the diluter stock was injected into the mixing box containing 1000 mL of hard blended water. Since DMF was used to prepare the diluter stock solution, the vehicle blank received an aliquot of DMF (0.05 mL/L) which was equivalent to the amount received by the highest test concentration (level 5).

#### IV. Analytical Measurements of AC 801,757 in Test Water

##### A. Method of Analysis

The measured concentrations of AC 801,757 in test dilution water were determined at test days -2, 0, 4, 7, 14, and 21 of the toxicity test by means of HPLC from samples collected by ABC Laboratories' personnel. Control and AC 801,757 fortified samples were also determined at each sample period. The analysis of the test water samples for AC 801,757 was accomplished based on a test method validated prior to the initiation of the definitive test as the AC 801,757 method validation study (ABC Study #40143, American Cyanamid #954-92-107). The method is described below:

Using an inverted 100-mL pipet, 250 mL of test system water were measured in a glass graduated cylinder, and transferred to a 500-mL flat bottom flask. The quality control samples were prepared. Each sample received 50 mL of UV grade acetonitrile (ACN) and was filtered using a water aspirator through an 11.0-cm Whatman GF/A filter paper in a plastic Büchner funnel into a second 500-mL flat bottom flask. The first flasks and filtering apparatus were rinsed with deionized (DI) water into the second flask.

A Waters Sep Pak® cartridge (500 mg, C18) was activated with 10 mL ACN, followed by 10 mL ACN:DI water (40:60) using two 10-cc glass syringes (Multifit B-D, Luer-Lok). Two 1-mL Eppendorf pipet tips were attached to opposite ends of an approximately 2-ft section of

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3/8 in. i.d. X 9/16 in. o.d. polyvinylchloride tubing. The cartridge was attached to one of the tips, and the second tip was passed through a hole in a silicone stopper. The stopper was placed in a glass adaptor that was attached to an empty 500-mL flat bottom flask.

The activated cartridge was placed into the filtered water. The water was passed through the cartridge using a vacuum pump. The flask was rinsed twice with approximately 5 mL DI water, and the vacuum was allowed to continue briefly to partially dry the cartridge. The cartridge was removed from the tip and attached to the syringe used for activation with ACN:DI water. The cartridge was rinsed with 5 mL ACN:DI water (40:60) and 5 mL air. The cartridge was attached to the syringe used for rinsing with ACN, then eluted with 4 mL ACN and 6 mL air.

The ACN was evaporated just to dryness under a gentle nitrogen stream, and the residue was resuspended in an appropriate volume of HPLC grade methanol:water (50:50) such that the concentration of the extract was contained within the range of calibration standards.

The chromatographic data were collected and stored utilizing a DEC Microvax equipped with VAX<sup>®</sup> Multichrom<sup>®</sup> software. This data system is described in Appendix II B. Operating parameters for the HPLC were as follows:

Instrument: Waters 510 HPLC pump  
Varian 2050 UV detector  
Shimadzu SCL-6A system controller  
Shimadzu SIL-6A auto sampler

Column: Supelcosil LC-8-DB, 5  $\mu$ m, 15 cm, 4.6 mm i.d. with a Supelco LC-8-DB guard column

Mobile Phase: HPLC grade MeOH:ABC reagent water (68:32)

Flow Rate: 2.0 mL/min

Detector Wavelength: 240 nm

Injection Volume: 1.5  $\mu$ L

B. Calculations

Calculations of AC 801,757 concentrations were performed using an external standard analysis function of a DEC Microvax using VAX® Multichrom® software.

Concentrations of AC 801,757 residues in the samples were determined directly from the standard curve by the following equation:

$$\frac{\left( \begin{array}{l} \mu\text{g/L AC 801,757} \\ \text{equivalents from} \\ \text{standard curve} \end{array} \right) \left( \begin{array}{l} \text{Volume for} \\ \text{analysis in mL} \end{array} \right)}{\text{(sample volume in mL)}} = \mu\text{g/L AC 801,757}$$

Example calculation for AC 801,757; Day 4, Level 1:

Peak area = 25398

Standard curve:  $Y = 41.2259 x - 851.382$

where y is peak area units and  
x is concentration in  $\mu\text{g/L AC 801,757}$

$\mu\text{g/L}$  equivalents from standard curve: 636.721  $\mu\text{g/L AC 801,757}$ .

$$\frac{(636.721 \mu\text{g/L}) (1 \text{ mL})}{(250 \text{ mL})} = 2.55 \mu\text{g/L AC 801,757}$$

$$\% \text{ Nominal of Level 1: } \frac{2.55 \mu\text{g/L}}{2.4 \mu\text{g/L}} \times 100 = 106\%$$

The protocol specifies that the measured concentrations should be within the range of 80 to 120% of the nominal concentrations. During the test there were 2 recoveries that were out of this range. The measured concentrations for level 4 (20  $\mu\text{g/L}$  nominal) were 123% of nominal on day 4 and 78% of nominal on day 21. All other measurements were within the protocol specified range. Due to the consistent recoveries throughout the test, and the fact that the results were based on the mean measured concentrations of the test substance, the Study Director and the Principal Investigator agreed that the deviation would not compromise the results of the test.

The recovery of the day-0, low-level QC spike sample (nominal concentration of 1.59  $\mu\text{g/L}$ ) was 76% of the nominal concentration and the recovery of the day 4 mid-level QC spike sample (nominal concentration of 10.2  $\mu\text{g/L}$ ), was 130% of the nominal concentration. Both of these recoveries are outside the 80 to 120% recovery specified in the protocol. However, the recoveries from the other 2 QC spike samples were within the range of acceptable recoveries. Therefore, the low recovery from the low-level spike on day 0 and the high recovery of the day 4 mid-level spike, did not affect the validity of the sample analyses. In the scientific judgement of the Study Director and the Principal Investigator, this deviation did not affect the test results.

#### V. Test Procedure — Biological

##### A. System Calibration, Initiation, and Randomization

The proportional diluter system was calibrated before testing by taking the average delivery volumes of the diluter mixing cell and using this value to calculate the dilution factor. The dilution factor was used to calculate the concentration of AC 801,757 stock to be prepared. Volume deliveries to individual replicate chambers were collected and measured before study initiation, which confirmed that volume deliveries were within  $\pm 10\%$  of the mean for each level.

The AC 801,757 test solutions were allowed to flow through the test system for approximately 7 days before test initiation to equilibrate the test system. The test was designated as experimentally initiated when all *Daphnia magna* were distributed to the test chambers. The daphnids were distributed to each replicate test chamber by random assignment of 10 first-instar (<24 hours old) from ABC Laboratories' lot #92-J. The adults from this culture were 25 days old with many prior broods. Forty daphnids were exposed to each test level. The loading rate for each test level was ~1 daphnid per 100 mL of dilution water.

The test daphnids were randomly placed in the test chambers by means of a computer-generated randomization table. This was accomplished by placing 10 first-instar daphnids into scintillation vials labeled #1 through #28. The test daphnids were then transferred to the replicate test chambers according to the randomization table that assigned each numbered vial to a specific test replicate. The exposure was initiated on September 11, 1992, and changeovers (removal of instar) were performed on Monday, Wednesday, and Friday throughout the definitive test.

B. Feeding, Maintenance, and Observations

The test daphnids were uniformly fed an equal volume of an algal suspension (*Selenastrum capricornutum*/*Ankistrodesmus falcatus*) per test chamber, which provided at least  $4 \times 10^8$  cells/L. ABC Laboratories' mass algal cultures were concentrated by centrifugation. A cell count by hemacytometer was made and from this count, the density of concentrate was determined for each mass algae culture harvested. Daphnids were fed twice daily with 1 mL per test chamber of a 2.5 mg/mL suspension of trout chow and yeast, which gave a final suspended solids concentration of 5 mg/L.

Survival, abnormal effects, and observance of first brood of the organisms were recorded every day throughout the definitive test. Reproductive success was measured by counting and discarding the offspring produced in each concentration every Monday, Wednesday, and Friday for the duration of the definitive test. Separating neonates from adults was accomplished by gently removing adult daphnids from each chamber by means of a smooth glass pipet and pouring the remaining water and young daphnids through a 50-mesh stainless steel screen. The test water was collected in a 1-L beaker. The young collected on the screen were placed in sample jars for counting before being discarded. The strained water and adult daphnids were returned to their respective chambers and replaced in the test system. The test beakers were cleaned every changeover day with a nylon-bristled brush and rinsed with ABC Laboratories' well water. Diluter operation observations were documented twice each day during the definitive test.

C. Termination

After test termination on day 21 of exposure, the surviving adults were removed from the test chambers and isolated on a numbered glass slide. The length of each adult daphnid was measured from the apex of the helmet to the base of the posterior spine, as illustrated in Figure 2, with a binocular dissecting microscope (Olympus VM 200M) and a calibrated eyepiece micrometer. Each observation was recorded and used for subsequent statistical analysis. After length measurements were complete, all of the daphnids from each individual replicate were placed in a preweighed foil pan. The daphnids were dried for approximately 48 hours at 60 °C. After drying, the daphnids were allowed to cool for a few minutes on the counter before being weighed. Mean daphnid weights were determined and used for statistical analysis.

VI. Test Procedure — Chemical and Physical

Temperature, dissolved oxygen, and pH were measured on days 0, 4, 7, 14, and 21 in alternating duplicate replicates (test chambers A and C on days 0, 7, and 21 and test chambers B and D on days 4 and 14) of the control, low, middle, and high test concentrations. Temperature of the water bath was recorded continuously with a thermal data logger (Rustrak® Ranger™, Gulton Industries). Temperatures of the test solutions were measured using a digital thermometer. Dissolved oxygen levels were determined with a YSI Model 54 ARC dissolved oxygen meter, while the pH values were measured with a Fisher 13-620-287 electrode and Corning Model 140 pH/mV meter. Light intensity was measured with a Licor Model LI-189 light meter at 0 hour and on days 4, 7, 14, and 21 of the study.

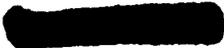
Water quality parameters of temperature, hardness, dissolved oxygen, pH, alkalinity, and conductivity were measured on the dilution water daily. Temperature, dissolved oxygen, and pH were measured with the instrumentation as described above. Alkalinity and hardness were measured using a titrimetric method adopted from standard methods (12). Conductivity was measured using a YSI Model 33 (S-C-T) meter.

VII. Statistical Analysis

Four test chambers were grouped and assigned to a treatment concentration. This arrangement provided a nested experimental design. Experimental units, the parts on which observations or measurements were made, were the individual daphnids (i.e., survival, adult length) or the replicated chamber (i.e., number surviving, total young/adult/reproductive day or time in days to first brood). Survival data were analyzed using frequency analysis that compared the test concentration to the control. This analysis was coupled with a one-tailed Fisher's exact test and the chi-square statistic to determine whether the test concentration exhibited a response significantly less than the control.

Reproductive data consisted of two parameters indicative of reproductive success. These parameters were time to first brood and young/adult/reproduction days. Total young/adult/reproduction day for each replicate was calculated in the following manner:

$$\frac{\text{Total Number of Young Produced}}{\text{Total Number of Adult Reproduction Days}}$$



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The total number of adult reproduction days during the 21-day test was based on the number of days daphnids were reproducing and the number of adult daphnids alive on each day. The number of reproduction days (normally 13-15) was counted from the day first neonate production was observed to the last day of the study. The value for adult reproduction days was calculated by summing the number of adult daphnids alive in each replicate for each reproduction day.

Example: Test Level Replicate: 1C

First Brood = Day 7

15 Reproduction Days

10 Adults x 15 Days = 150

150 Reproduction Days

$$\frac{1016 \text{ Young}}{150 \text{ Reproduction Days}} = 6.77 \text{ Young/Adult/Reproduction Day}$$

Reproduction data were analyzed by a one-way analysis of variance (ANOVA) to compare the experimental design similar to that described by McClave et al. (13). A Dunnett's one-tailed multiple means comparison procedure (14) was used to determine those exposure levels exhibiting responses significantly different than that of the control. When a t-test between the control and vehicle blank indicated no significant difference, all controls were pooled for that measured parameter. If the result was a rejection of equality, then only the vehicle blank was used to compare to the treatment levels.

Adult daphnid length and weight data were assessed by a one-way analysis of variance (ANOVA) procedure. A Dunnett's one-tailed multiple means comparison procedure was used to determine those test concentrations exhibiting responses significantly less than that of the control. Control and vehicle blank responses were pooled if a t-test indicated no significant difference. A Shapiro-Wilk normality test was computed within each test concentration to assess departures from a normal distribution. When the normality test indicated a deviation from strict normality, the data for each concentration were examined for indications of central tendency. Homogeneity of variance was assessed by examining the correlation between the mean and both the variance and the standard deviation. In cases where the assumptions for an ANOVA hold entirely or even approximately, the ANOVA is generally the more efficient statistical test for detecting departures from the null hypothesis (15).



All statistical analyses were conducted using PC DOS SAS/STAT Release 6.03 (16), with conclusions of statistical significance based on a 95 % confidence level ( $p \leq 0.05$ ).

The 21-day  $EC_{50}$  was calculated by employing a computerized  $EC_{50}$  program developed by Stephan et al. (17). This program calculated the  $EC_{50}$  statistic and its 95 % confidence limits by means of the binomial, moving average, and probit procedures. Three different methods of analyzing data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The calculation method selected for presentation in this report was that which gave the narrowest confidence interval for the  $EC_{50}$  (17, 18), although all three models are valid.

## RESULTS

### I. Preliminary Test

A 13-day flow-through preliminary test was conducted from March 5, 1992, to March 18, 1992, at the following nominal concentrations: 6.0, 12, 25, 50, and 100  $\mu\text{g/L}$ . Four test chambers containing ten daphnids per test concentration were exposed to AC 801,757. After 13 days, there was 30% mortality in the high test concentration of 100  $\mu\text{g/L}$ . The control, vehicle blank, and nominal test concentrations of 6.0, 12, 25, and 50  $\mu\text{g/L}$  had 0, 2.5, 0, 0, 0, and 2.5% mortality, respectively. All the surviving daphnids in the test concentration of 100  $\mu\text{g/L}$  were small after 13 days of exposure. All the other daphnids appeared normal except for 1 daphnid in test concentration 25  $\mu\text{g/L}$ , which appeared smaller. The control, vehicle blank, and nominal test concentrations of 6.0 and 12  $\mu\text{g/L}$  produced instar on day 7 of the test. Replicates A and B for test concentration 25  $\mu\text{g/L}$  produced instar on day 7, while replicates C and D produced instar on day 8 of the test. Replicates A and D for test concentration 50  $\mu\text{g/L}$  produced instar on day 9, while the remaining replicates B and C and all of level 5 (100  $\mu\text{g/L}$ ) didn't produce instar during the 13 day test. The control, vehicle blank, and test concentrations of 6.0, 12, 25, 50, and 100  $\mu\text{g/L}$  had mean total instar production of 541, 577, 449, 492, 293, 6, and 0, respectively.

### II. Definitive Test

The analytical method used in this study was validated in the AC 801,757 method validation study (ABC Study #40143, Appendix III). Fortification concentrations for the method validation ranged from 0.798 to 79.0  $\mu\text{g/L}$  and yielded a mean ( $\pm$ SD) recovery of 95% ( $\pm$  9.0%). The results of the method validation were presented to American Cyanamid Company in ABC report #40143.

Prior to the successful definitive test, there were four unsuccessful attempts. All these definitive tests were conducted at the nominal concentrations of 2.4, 4.8, 10, 20, and 40  $\mu\text{g/L}$ . The reasons for these unsuccessful tests are listed below. The raw data for these tests will be kept with the final report in the archives of American Cyanamid Company.

The first unsuccessful definitive attempt was conducted for 21 days from April 27, 1992, to May 17, 1992, using  $^{14}\text{C}$ -AC 801,757, as specified in the original protocol. This test was considered unsuccessful due to excessive and erratic mortality in all the test levels and controls. This excessive mortality occurred from day 17 of the test on and was believed to be due to contamination in the test water. After this test was terminated, it was decided to run the definitive test using non-radiolabeled AC 801,757 instead of  $^{14}\text{C}$  test material (see protocol amendment #3, Appendix III). This was decided upon because of the limited amount of  $^{14}\text{C}$  test material and because these test levels, based on cold material, could easily be detected analytically by the validated method.

The second unsuccessful definitive attempt was conducted for 5 days from July 10, 1992, to July 15, 1992. This test was terminated due to excessive mortality in test replicates vehicle blank A and level 1 C and the general health of the *Daphnia*. It is believed that the mortality was due to health of the *Daphnia* used to initiate this test.

The third unsuccessful definitive attempt was conducted for 5 days from July 29, 1992, to August 4, 1992. This test was terminated due to low availability of algae to feed the test organisms. The daphnids for this test appeared normal and the health of the culture used to initiate this test was excellent.

The fourth, and last, unsuccessful definitive attempt was conducted for 16 days from August 10, 1992, to August 26, 1992. This test was terminated due to low instar production for the vehicle blank and all test levels. The control had normal instar production during the test. Since the vehicle blank replicates didn't produce instar along with the test levels, it is believed that the low instar production was due to contaminated solvent (DMF).

The successful definitive test was initiated on September 11, 1992, and was successfully conducted for 21 days before it was terminated on October 2, 1992.

Fortification concentrations (Table II) were 1.59, 10.2, and 47.6  $\mu\text{g/L}$  and yielded average recoveries of 1.38, 10.9, and 45.6  $\mu\text{g/L}$ , respectively. These average fortification concentration recoveries represented 87, 107, and 96% of the nominal concentrations, respectively.

Results of the determination of AC 801,757 in the test system are presented in Table III. The mean measured concentrations of AC 801,757 were 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$ , which represent 100, 96, 88, 90, and 90%, respectively, of the nominal test concentrations. The mean measured concentration of the diluter stock solution was 424 mg/L, which represented 106% of the nominal concentration of 400 mg/L. The test material appeared to be stable in ABC dilution water based on the consistent measurements of AC 801,757 during the test.

The control ( $97.5 \pm 5.0\%$ ) and vehicle blank ( $100 \pm 0.0\%$ ) survival rates were not significantly different and were therefore pooled ( $98.8 \pm 3.5\%$ ). Survival of *Daphnia magna* after the 21-day exposure to AC 801,757 was not significantly affected at any of the mean measured test concentrations when compared to the pooled controls. The mean measured concentrations of 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$  had mean percent survivals of  $97.5 \pm 5.0$ ,  $100 \pm 0.0$ ,  $94.9 \pm 5.8$ ,  $97.5 \pm 5.0$ , and  $92.5 \pm 5.0\%$ , respectively (Table IV). A day 21  $\text{EC}_{50}$  based on mortality was  $>36 \mu\text{g/L}$ , as calculated by the binomial method.

The lengths of adult *Daphnia magna* in the control ( $4.47 \pm 0.10 \text{ mm}$ ) and vehicle blank ( $4.47 \pm 0.10 \text{ mm}$ ) groups were not significantly different and therefore were pooled ( $4.47 \pm 0.09 \text{ mm}$ ). When compared to the pooled controls, there was not a significant difference in adult length for any of the mean measured concentrations (Table IV). The mean measured concentrations of 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$  had mean daphnid lengths of  $4.51 \pm 0.06$ ,  $4.53 \pm 0.09$ ,  $4.53 \pm 0.06$ ,  $4.48 \pm 0.06$ , and  $4.36 \pm 0.05 \text{ mm}$ , respectively.

Time to first brood in the control ( $7.0 \pm 0.0 \text{ days}$ ) and vehicle blank ( $7.0 \pm 0.0 \text{ days}$ ) groups were the same and were therefore pooled ( $7.0 \pm 0.0 \text{ days}$ ). There was a significant difference in days to first brood for the mean measured test concentrations of 18 and 36  $\mu\text{g/L}$  when compared to the pooled controls (Table V). The mean measured test concentrations of 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$  had days to first brood of  $7.0 \pm 0.0$ ,  $7.0 \pm 0.0$ ,  $7.0 \pm 0.0$ ,  $9.3 \pm 1.5$ , and  $14 \pm 0.0 \text{ days}$ .

Young/adult/reproduction day (YAD) values for the control ( $8.79 \pm 0.33 \text{ YAD}$ ) and vehicle blank ( $6.65 \pm 0.14 \text{ YAD}$ ) were not pooled for statistical analysis since there was a significant difference between them. Therefore, the test levels were compared to the vehicle blank for YAD analysis. Between test days 14 and 17, reproduction in the vehicle blank and all treatment levels became very sporadic. On day 17, the DMF and the stock solution in the test system were replaced by new DMF and new stock, and the injector lines were rinsed with fresh DMF. The instar production in the treatment levels and in the vehicle blank

from day 17 on was consistent with instar production in these treatments prior to day 14. The reason for the sporadic reproduction between days 14 and 17 is unknown. Young/adult/reproduction day was significantly affected when compared with the vehicle blank at the mean measured test concentrations of 4.6, 8.8, 18, and 36  $\mu\text{g/L}$  (Table V). The mean measured concentrations of 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$  had mean young/adult/reproduction days of  $6.23 \pm 0.43$ ,  $5.18 \pm 0.62$ ,  $4.95 \pm 0.69$ ,  $4.52 \pm 0.52$ , and  $1.38 \pm 0.57$ , respectively. The average total number of young for control, vehicle blank, and the mean measured test concentrations of 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$  were 1293, 997, 928, 777, 720, 565, and 105, respectively. All young produced during the test appeared normal.

The mean weights of adult *Daphnia magna* in the control ( $1.00 \pm 0.14$  mg) and vehicle blank ( $1.20 \pm 0.08$  mg) groups were significantly different and therefore were not pooled. Therefore, the test levels were compared to the vehicle blank for the weight analysis. When compared to the vehicle blank, there was a significant difference in adult weights for the mean measured concentration of 36  $\mu\text{g/L}$  (Table VI). The mean measured concentrations of 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$  had mean daphnid weights of  $1.22 \pm 0.08$ ,  $1.22 \pm 0.06$ ,  $1.25 \pm 0.04$ ,  $1.17 \pm 0.03$ , and  $0.84 \pm 0.10$  mg, respectively.

Based on the statistical analysis of survival, adult mean length, adult mean weight, days to first brood, and young/adult/reproduction day from this 21-day *Daphnia magna* chronic toxicity test, MATC limits were determined to be 2.4  $\mu\text{g/L}$  (NOEC) and 4.6  $\mu\text{g/L}$  (LOEC). The geometric mean of the MATC limits is 3.3  $\mu\text{g/L}$ . The day 21  $\text{EC}_{50}$  was calculated to be  $> 36$   $\mu\text{g/L}$ .

Individual mortality and behavioral observations for days 0, 7, 14, and 21 are included in Table VII.

Water quality parameters of temperature, dissolved oxygen, and pH were measured in the control, low, medium, and high test concentrations at 0, 4, 7, 14, and 21 days with the results presented in Table VIII. Temperature, which was recorded continuously and checked daily, ranged from 18.2-20.5  $^{\circ}\text{C}$  throughout the test. The dissolved oxygen concentrations ranged from 6.4 to 8.1 mg/L, representing 74 and 95% saturation at 20 and 21  $^{\circ}\text{C}$ , respectively. These values were considered adequate for testing (4). The pH values of the treatment levels were consistent with the control throughout the definitive test, ranging from 8.1 to 8.4.

The study was conducted following Good Laboratory Practice regulations (19) and the final report was reviewed by ABC Laboratories' Quality Assurance Unit.

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All original raw data were archived at American Cyanamid Company with the final report. A copy of the final report and the raw data report were retained at ABC Laboratories, Inc.

### CONCLUSION

The 21-day flow-through toxicity test of AC 801,757 to *Daphnia magna* was conducted from September 11, 1992, to October 2, 1992. Forty daphnids were exposed to each test concentration, hard blended water control, and vehicle blank. The nominal concentrations of AC 801,757 were 2.4, 8, 10, 20, and 40  $\mu\text{g/L}$ . Analytical measurements of AC 801,757 were made from samples collected at -2, 0, 4, 7, 14, and 21 days of the study. The measured concentrations (excluding day -2 samples) averaged 2.4, 4.6, 8.8, 18, and 36  $\mu\text{g/L}$ , respectively. No precipitates were observed during the test and all control and test chambers were clear during the test. Water chemistry measurements of dissolved oxygen, pH, and temperature were consistent throughout the term of the experiment and remained in acceptable ranges throughout the test.

Adult daphnid length and survival were not significantly affected at any of the AC 801,757 test concentrations. Adult daphnid mean weight was significantly affected at the mean measured concentration of 36  $\mu\text{g/L}$ . Days to first brood was significantly affected at the mean measured concentrations of 18 and 36  $\mu\text{g/L}$ . Young/Adult/Reproduction days was significantly affected at the mean measured concentrations of 4.6, 8.8, 18, and 36  $\mu\text{g/L}$ . A day 21  $\text{EC}_{50}$  was estimated at greater than the mean measured concentration of 36  $\mu\text{g/L}$ . The MATC limits for AC 801,757 are estimated to be the mean measured concentrations of 2.4  $\mu\text{g/L}$  (NOEC) and 4.6  $\mu\text{g/L}$  (LOEC). The point estimate MATC value (defined as the geometric mean of the NOEC and LOEC) is 3.3  $\mu\text{g/L}$  AC 801,757.

### STUDY INTEGRITY

Nothing occurred during the conduct of this study that affected the integrity of the study.

TABLE I

**Chemical Characteristics of Hard Blended Water<sup>a</sup> Used by  
ABC Laboratories' Aquatic Toxicology Programs Division**

Monthly/Weekly Screens <sup>b</sup>			
Hardness	138-154 mg/L as CaCO <sub>3</sub>	Total Organic Carbon:	<1.0-1.33 ppm <sup>c</sup>
Alkalinity	150-172 mg/L as CaCO <sub>3</sub>	Suspended Solids	2.9 ppm <sup>c</sup>
pH	8.0-8.3		
Conductivity	280-320 $\mu$ Mhos/cm		
Quarterly Screens <sup>d</sup>			
Elements		Chlorinated Hydrocarbons	
Aluminum	<0.20 mg/L	DDE	<0.25 $\mu$ g/L
Arsenic	0.0085 mg/L	DDD	<0.25 $\mu$ g/L
Boron	0.302 mg/L	DDT	<0.25 $\mu$ g/L
Cadmium	<0.005 mg/L	Dicodrin	<0.25 $\mu$ g/L
Chromium	<0.01 mg/L	$\alpha$ -BHC	<0.05 $\mu$ g/L
Cobalt	<0.05 mg/L	$\beta$ -BHC	<0.05 $\mu$ g/L
Copper	<0.025 mg/L	$\gamma$ -BHC	<0.05 $\mu$ g/L
Fluoride	0.537 mg/L	$\Delta$ -BHC	<0.05 $\mu$ g/L
Iron	<0.10 mg/L	HCB	<0.05 $\mu$ g/L
Lead	0.005 mg/L	Endrin	<0.25 $\mu$ g/L
Mercury	<0.002 mg/L	H.E.	<0.05 $\mu$ g/L
Nickel	<0.04 mg/L	Mirex	<0.50 $\mu$ g/L
Selenium	<0.002 mg/L	Methoxychlor	<0.50 $\mu$ g/L
Silver	<0.01 mg/L	Toxaphene	<5.0 $\mu$ g/L
Zinc	<0.02 mg/L		
Organophosphate Insecticides		PCB	
Vapona	<2.50 $\mu$ g/L	Total PCBs	<2.50 $\mu$ g/L
Diazinon	<2.50 $\mu$ g/L		
Methyl Parathion	<2.50 $\mu$ g/L	Miscellaneous	
Ethyl Parathion	<2.50 $\mu$ g/L	COD	<10.0 mg/L
Ronnel	<2.50 $\mu$ g/L	Un-ionized Ammonia	Not Detected
Malathion	<2.50 $\mu$ g/L	Chlorine	Not Detected
Thimet <sup>e</sup>	<0.50 $\mu$ g/L		

<sup>a</sup> A blend of ABC Laboratories' well water and reverse osmosis (R.O.) water. All data supporting these values are on file at ABC Laboratories, Inc.

<sup>b</sup> Represents the values measured during the testing period

<sup>c</sup> Represents the values obtained from the most recent screen of September 1992

<sup>d</sup> Represents the values obtained from the most recent screen of July 1992

<sup>e</sup> Represents the values obtained from the most recent screen of January 1992; this value not available from July 1992

TABLE II  
 Fortifications Levels and Recoveries of the AC 801,757 Quality Control Samples  
 During the 21-Day Flow-Through Toxicity Test with *Daphnia magna*

Study Day	Low Spike (1.59 µg/L)	Percent of Nominal	Mid Spike (10.2 µg/L)	Percent of Nominal	High Spike (47.6 µg/L)	Percent of Nominal
-2	1.27	80	9.57	94	47.9	101
0	1.21	76*	10.3	101	48.1	101
4	1.42	89	13.9	136*	48.1	101
7	1.53	96	10.2	100	45.0	95
14	1.28	81	11.2	110	42.0	88
21	1.48	93	8.88	87	44.8	94
Mean	1.38		10.9		45.6	
S.D.	0.135		1.87		2.57	
Percent of Nominal	87		107		96	

Note: Day -2 concentrations not included in mean calculations.  
 \* Protocol deviation from suspected spike preparation error.

TABLE III

Measured Concentrations of AC 801,757 During the 21-Day Flow-Through Toxicity Test with *Daphnia magna*

Study Day	Control	Vehicle Blank	Level 1	Percent of	Level 2	Percent of	Level 3	Percent of
			(2.4 µg/L)	Nominal	(4.8 µg/L)	Nominal	(10 µg/L)	Nominal
-2	N/D*	N/D*	2.49	104	4.80	100	8.85	89
0	N/D*	N/D*	2.50	104	4.74	99	8.31	83
4	N/D*	N/D*	2.55	106	5.08	106	9.18	92
7	N/D*	N/D*	2.61	109	4.26	89	9.53	95
14	N/D*	N/D*	2.25	94	4.23	88	8.51	85
21	N/D*	N/D*	2.16	90	4.51	94	8.48	85
Mean	---	---	2.4		4.6		8.8	
SD	---	---	0.20		0.36		0.53	
Percent of Nominal	---	---	100		96		88	

Note: Day -2 concentrations not included in mean calculation  
 \* N/D = not detected, limit of detection was 0.380 µg/L.

TABLE III (continued)  
 Measured Concentrations of AC 801,757 During the 21-Day  
 Flow-Through Toxicity Test with *Daphnia magna*

Study Day	Level 4 (20 µg/L)	Percent of Nominal	Level 5 (40 µg/L)	Percent of Nominal	Diluter Stock (400 mg/L)	Percent of Nominal
-2	16.2	81	37.1	93	346	87
0	16.5	83	31.8	80	470	118
4	24.5	123	35.2	88	442	111
7	17.5	86	35.6	89	414	104
14	17.7	89	40.0	100	384	96
21	15.5	78	36.5	91	409	102
Mean	18		36		424	
SD	3.6		2.9		33.0	
Percent of Nominal	90		90		106	

Note: Day -2 concentrations not included in mean calculation  
 \* N/D = Not detected

TABLE IV

Percent Survival and Adult Length of *Daphnia magna* Exposed to  
AC 801,757 for 21 Days During the Chronic Toxicity Test

Chamber ID (Nominal Conc.)	Mean Measured Conc. (µg/L)	Rep.	Day 21 Survival <sup>a</sup>			Day 21 Adult Daphnid Length <sup>b</sup>	
			Initial Number Instar	Adult Surv.	Percent Surv.	Mean Length (mm)	Mean + SD (mm)
Control	N/A	A	10	10	100		4.56
		B	10	10	100	97.5 ± 5.0	4.46
		C	10	9	90		4.55
		D	10	10	100		4.34
Vehicle Blank	N/A	A	10	10	100		4.38
		B	10	10	100	100 ± 0.0	4.58
		C	10	10	100		4.39
		D	10	10	100		4.52
Pooled Controls <sup>c</sup>					98.8 ± 3.5	4.47 ± 0.09	
Level 1 (2.4 µg/L)	2.4	A	10	10	100		4.52
		B	10	9	90	97.5 ± 5.0	4.52
		C	10	10	100		4.43
		D	10	10	100		4.59
Level 2 (4.8 µg/L)	4.6	A	10	10	100		4.64
		B	10	10	100	100 ± 0.0	4.43
		C	10	10	100		4.49
		D	10	10	100		4.57
Level 3 (10 µg/L)	8.8	A	9 <sup>d</sup>	9	100		4.47
		B	10	9	90	94.9 ± 5.8	4.59
		C	10	10	100		4.58
		D	10	9	90		4.50
Level 4 (20 µg/L)	18	A	10	9	90		4.40
		B	10	10	100	97.5 ± 5.0	4.50
		C	10	10	100		4.55
		D	10	10	100		4.47
Level 5 (40 µg/L)	36	A	10	10	100		4.40
		B	10	9	90	92.5 ± 5.0	4.41
		C	10	9	90		4.30
		D	10	9	90		4.34

<sup>a</sup> Data were subjected to frequency analysis coupled with a one-tailed Fisher's exact test.

<sup>b</sup> Data were subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple means comparison test.

<sup>c</sup> Control and vehicle blank were compared by one-tailed Fisher's exact test or t-test. If significantly different, comparison was made with vehicle blank; otherwise, controls were pooled.

<sup>d</sup> There was an accidental mortality during day 10 changeover and therefore, won't be included in the statistical analysis.

TABLE V

**Young/Adult/Reproduction Days and Time to First Brood of *Daphnia magna*  
Exposed to AC 801,757 for 21 Days During the Chronic Toxicity Test**

Chamber ID (Nominal Conc.)	Mean Measured Conc. ( $\mu\text{g/L}$ )	Rep.	Day 21 Reproduction <sup>a</sup>			Time to First Brood <sup>a</sup>		
			Total Young	Adult Reprod. Days	Young/ Adult/ Reprod. Days	Mean $\pm$ SD	Days	Mean $\pm$ SD (days)
Control	N/A	A	1291	150	8.61		7	
		B	1291	150	8.61	8.79 $\pm$ 0.33	7	7.0 $\pm$ 0.0
		C	1292	139	9.29		7	
		D	1298	150	8.65		7	
Vehicle Blank	N/A	A	1020	150	6.80		7	
		B	981	150	6.54	6.65 $\pm$ 0.14	7	7.0 $\pm$ 0.0
		C	977	150	6.51		7	
		D	1010	150	6.73		7	
Pooled Controls <sup>b</sup>						Not Pooled	7.0 $\pm$ 0.0	
Level 1 (2.4 $\mu\text{g/L}$ )	2.4	A	858	150	5.72		7	
		B	898	145	6.19	6.23 $\pm$ 0.43	7	7.0 $\pm$ 0.0
		C	1016	150	6.77		7	
		D	938	150	6.25		7	
Level 2 (4.8 $\mu\text{g/L}$ )	4.6	A	888	150	5.92		7	
		B	746	150	4.97	5.18 $\pm$ 0.62*	7	7.0 $\pm$ 0.0
		C	669	150	4.46		7	
		D	803	150	5.35		7	
Level 3 (10 $\mu\text{g/L}$ )	8.8	A	630	138	4.57		7	
		B	617	148	4.17	4.95 $\pm$ 0.69*	7	7.0 $\pm$ 0.0
		C	828	150	5.52		7	
		D	805	145	5.55		7	
Level 4 (20 $\mu\text{g/L}$ )	18	A	518	115	4.50		10	
		B	580	150	3.87	4.52 $\pm$ 0.52*	7	9.3 $\pm$ 1.5*
		C	545	120	4.54		10	
		D	618	120	5.15		10	
Level 5 (40 $\mu\text{g/L}$ )	36	A	141	80	1.76		14	
		B	134	75	1.79	1.38 $\pm$ 0.57*	14	14 $\pm$ 0.0*
		C	105	75	1.40		14	
		D	41	72	0.57		14	

\* Denotes values significantly different ( $P \leq 0.05$ ) from the vehicle blank.

• Denotes values significantly different ( $P \leq 0.05$ ) from the pooled controls.

<sup>a</sup> Data were subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple means comparison test.

<sup>b</sup> Control and vehicle blank were compared by t-test. If significantly different, comparison was made with vehicle blank otherwise controls were pooled.

TABLE VI

**Adult Weights of *Daphnia magna* Exposed to  
AC 801,757 for 21 Days During the Flow-Through Toxicity Test**

Chamber ID (Nominal Conc.)	Mean Measured Conc. ( $\mu\text{g/L}$ )	Rep.	Initial Number Instar	Adult Surv.	Total Weight (mg)	Average daphnid <sup>a</sup> Weight (mg)	Mean $\pm$ SD (mg)
Control	N/A	A	10	10	9.30	0.93	
		B	10	10	12.0	1.20	1.00 $\pm$ 0.14
		C	10	9	8.10	0.90	
		D	10	10	9.60	0.96	
Vehicle Blank	N/A	A	10	10	11.3	1.13	
		B	10	10	12.3	1.23	1.20 $\pm$ 0.08
		C	10	10	11.5	1.15	
		D	10	10	13.0	1.30	
Pooled Controls <sup>b</sup>							Not Pooled
Level 1 (2.4 $\mu\text{g/L}$ )	2.4	A	10	10	12.9	1.29	
		B	10	9	10.3	1.14	1.22 $\pm$ 0.08
		C	10	10	11.6	1.16	
		D	10	10	13.0	1.30	
Level 2 (4.8 $\mu\text{g/L}$ )	4.6	A	10	10	12.8	1.28	
		B	10	10	12.1	1.21	1.22 $\pm$ 0.06
		C	10	10	11.5	1.15	
		D	10	10	12.4	1.24	
Level 3 (10 $\mu\text{g/L}$ )	8.8	A	10	9 <sup>c</sup>	11.0	1.22	
		B	10	9	10.8	1.20	1.25 $\pm$ 0.04
		C	10	10	12.6	1.26	
		D	10	9	11.7	1.30	
Level 4 (20 $\mu\text{g/L}$ )	18	A	10	9	10.0	1.11	1.17 $\pm$ 0.08
		B	10	10	11.1	1.11	
		C	10	10	12.7	1.27	
		D	10	10	11.8	1.18	
Level 5 (40 $\mu\text{g/L}$ )	36	A	10	10	9.00	0.90	0.84 $\pm$ 0.10 <sup>*</sup>
		B	10	9	8.50	0.94	
		C	10	9	6.70	0.74	
		D	10	9	7.00	0.78	

\* Denotes values significantly different ( $P \leq 0.05$ ) from the vehicle blank

<sup>a</sup> Data were subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple means comparison test.

<sup>b</sup> Control and vehicle blank were compared by one-tailed Fisher's exact test or t-test. If significantly different, comparison was made with vehicle blank; other wise, controls were pooled.

<sup>c</sup> Accidental mortality during day 10 changeover

TABLE VII  
Individual Mortality and Behavioral Observations During the 21-Day  
Flow-Through Toxicity Test of *Daphnia magna* Exposed to AC 801,757

Nominal Concentration (µg/L)	Rep.	Number Placed in Test Vessel	Day 0		Day 7		Day 14		Day 21	
			Cum. Mort.	Obs. <sup>a</sup>						
Control	A	10	0	...	0	...	0	...	0	...
	B	10	0	...	0	...	0	...	0	...
	C	10	0	...	0	...	1	9N	1	9N
	D	10	0	...	0	...	0	...	0	...
Vehicle Blank	A	10	0	...	0	...	0	...	0	...
	B	10	0	...	0	...	0	...	0	...
	C	10	0	...	0	...	0	...	0	...
	D	10	0	...	0	...	0	...	0	...
2.4 (2.4) <sup>b</sup>	A	10	0	...	0	...	0	...	0	...
	B	10	0	...	0	...	0	...	0	...
	C	10	0	...	0	...	0	...	0	...
	D	10	0	...	0	...	0	...	0	...
4.8 (4.6) <sup>b</sup>	A	10	0	...	0	...	0	...	0	...
	B	10	0	...	0	...	0	...	0	...
	C	10	0	...	0	...	0	...	0	...
	D	10	0	...	0	...	0	...	0	...
10 (8.8) <sup>b</sup>	A	10	0	...	0	...	0	...	0	...
	B	10	0	...	0	...	0	...	0	...
	C	10	0	...	0	...	0	...	0	...
	D	10	0	...	0	...	0	...	0	...
20 (18) <sup>b</sup>	A	10	0	...	0	...	0	...	0	...
	B	10	0	...	0	...	0	...	0	...
	C	10	0	...	0	...	0	...	0	...
	D	10	0	...	0	...	0	...	0	...
40 (36) <sup>b</sup>	A	10	0	...	0	...	0	...	0	...
	B	10	0	...	0	...	0	...	0	...
	C	10	0	...	0	...	0	...	0	...
	D	10	0	...	0	...	0	...	0	...

<sup>a</sup> Unless otherwise indicated, the test water was clear and free of precipitate and all daphnids were normal in appearance and behavior. The following abbreviations were used for observations: N = Normal  
<sup>b</sup> Mean measured concentration  
<sup>c</sup> Accidental mortality during day 10 changeover.

TABLE VIII

Water Quality Measurements During the 21-Day Flow-Through Toxicity Test of *Daphnia magna* Exposed to AC 801,757

Study Day	Rep.	Control		Level 1 (2.4 µg/L) <sup>a</sup>		Level 3 (10 µg/L) <sup>a</sup>		Level 5 (40 µg/L) <sup>a</sup>	
		Temp. <sup>b</sup> °C	D.O. <sup>c</sup> (mg/L)	Temp. <sup>b</sup> °C	D.O. <sup>c</sup> (mg/L)	Temp. <sup>b</sup> °C	D.O. <sup>c</sup> (mg/L)	Temp. <sup>b</sup> °C	D.O. <sup>c</sup> (mg/L)
0	A	20	7.9	20	8.0	20	8.0	20	8.0
	C	20	7.9	20	8.0	20	8.0	20	8.0
4	B	21	7.9	21	7.9	21	7.9	21	8.0
	D	21	7.9	21	7.9	21	7.9	21	8.1
7	A	20	7.8	20	6.4	20	6.5	20	7.0
	C	20	7.8	20	6.4	20	6.5	20	7.0
14	B	20	8.1	20	7.5	20	7.8	20	8.0
	D	20	8.1	20	7.5	20	7.8	20	8.0
21	A	20	7.7	20	7.7	20	7.9	20	7.8
	C	20	7.3	20	7.7	20	7.9	20	7.8

<sup>a</sup> Nominal Concentrations

<sup>b</sup> Temperature--Mercury Thermometer

<sup>c</sup> Dissolved oxygen concentrations--dissolved oxygen meter (YSI Model 54 ARC), ABC material control #1905-485

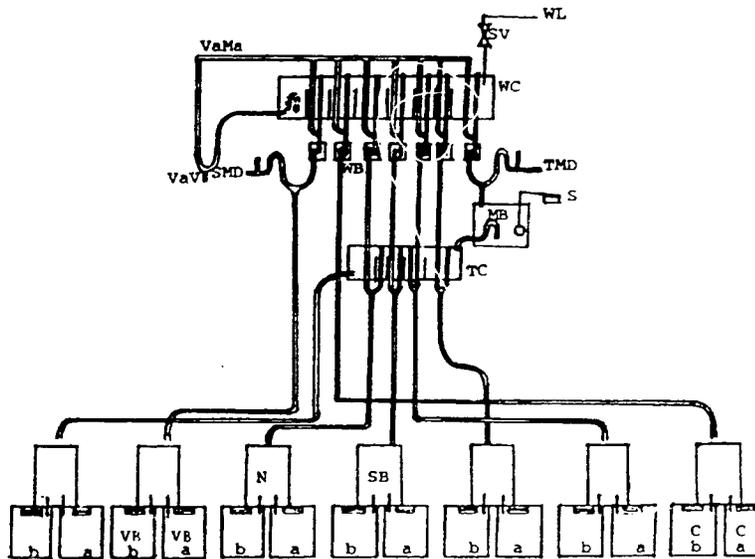
<sup>d</sup> pH Electrode (Fisher 13-620-287) used with a Corning Model 140 pH/mV meter, ABC material #1714-175

Note: Dissolved oxygen saturation at the test temperatures of 20 and 21 °C are 8.7 and 8.5 mg/L, respectively. These values have been corrected for altitudinal pressure at ABC Laboratories.

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FIGURE I

Semi-Schematic Drawing of 7-Concentration *Daphnia magna* Diluter System



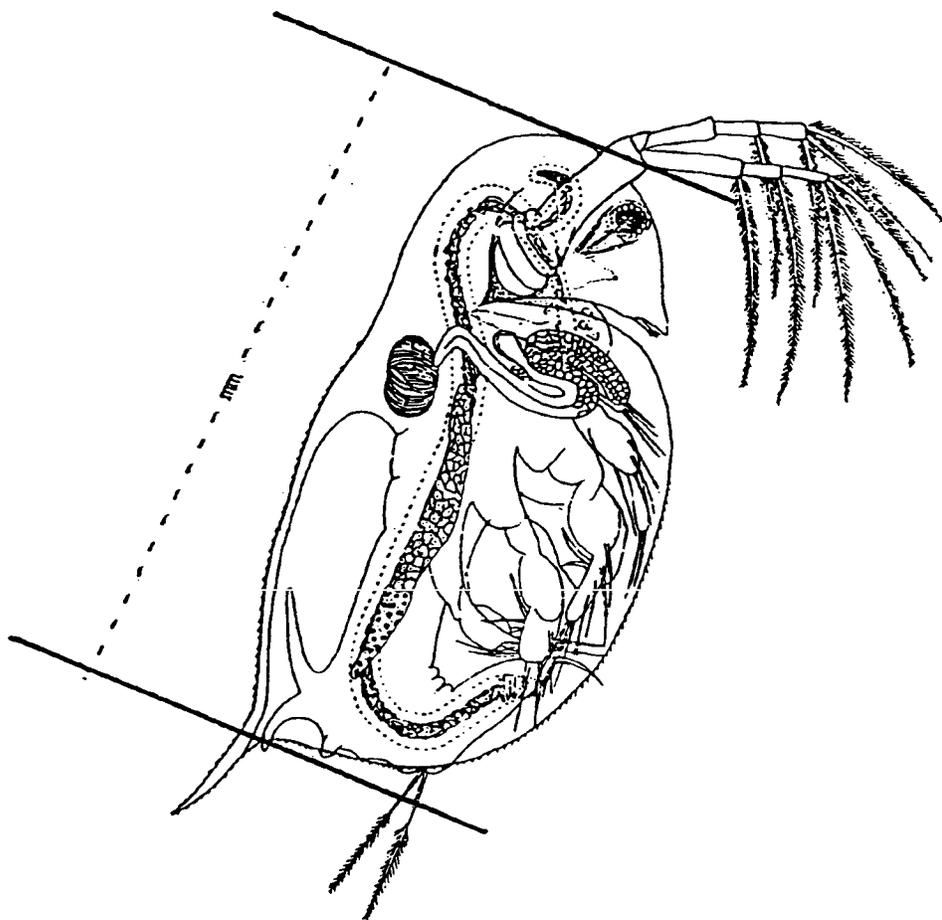
LEGEND

C	= Control	TC	= Toxicant Cell
MB	= Mixing Box	TMD	= Toxicant Metering Device
N	= Needle Used for Splitting	VaMa	= Vacuum Manifold
S	= Micro-Switch	VaV	= Vacuum Venturi
SB	= Splitting Box	WB	= Water Block
VB	= Vehicle Blank	WC	= Water Cell
SMD	= Solvent Metering Device	WL	= Water Line
SV	= Solenoid Valve	a & b	= Represents two of the four chambers per concentration used in the diluter system

AC#954-92-108, ABC LABS NO. 40145-42

FIGURE 2

*Daphnia magna* Length Measurement from the Apex of the Helmet to the Base of the Posterior Carapace Spine



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AC#954-92-108, ABC LABS NO. 40145-45

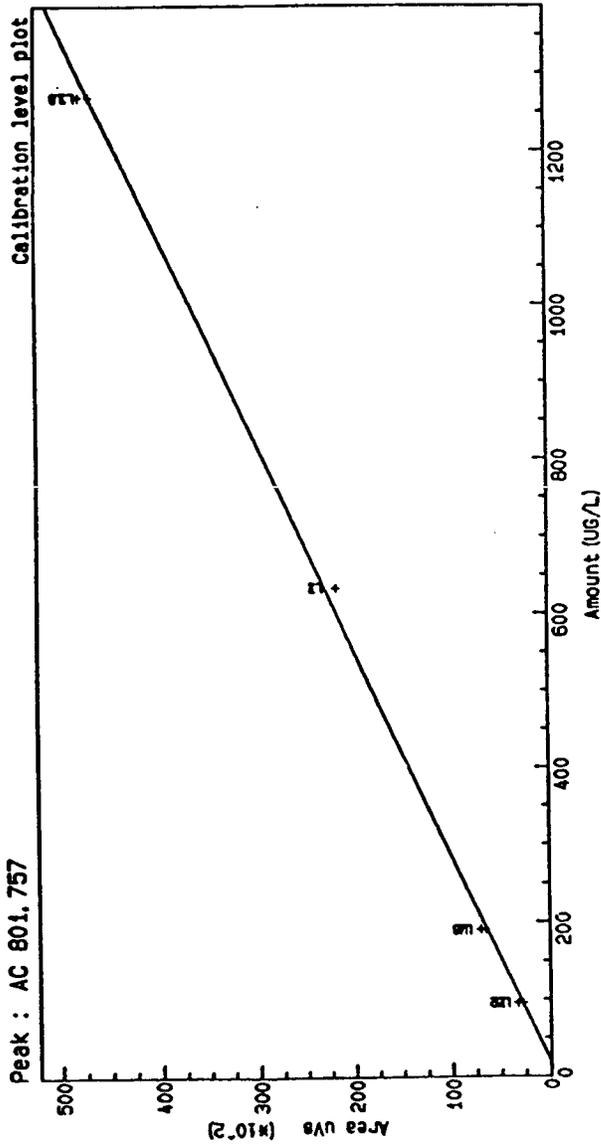
APPENDIX I  
REPRESENTATIVE CHROMATOGRAMS

ABC Laboratories, Inc. Multichrom ver 1.8-2

Calibration Name : 20 40145039.

40145039

Peak : AC 801.757



Constant :  $-7.129571E+2$   
1st degree :  $3.79833E+1$

Curve fit : Linear  
Correlation coefficient : 0.99905  
Standard error :  $8.74346E+2$

Reported on 21-SEP-1992 at 08:41

AC#954-92-108, ABC LABS NO. 40145-46

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The original document"

By                      date 1/4/92

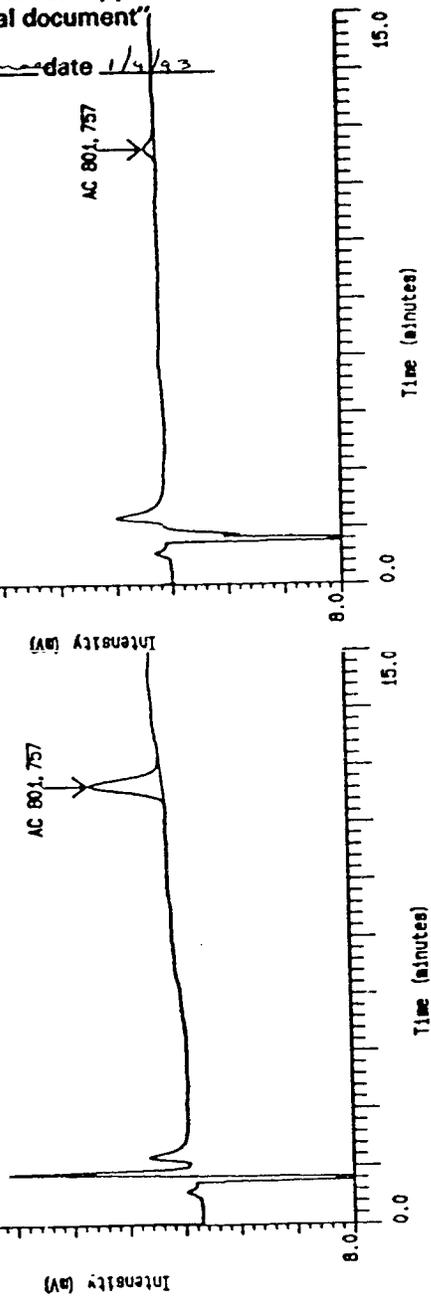
ABC Laboratories, Inc.

MCPD ver 2.3  
Date of Report: 21 SEP 92 at 08:43:57

M100 [DEPROJECT] 20 40145039, 1.1  
AC 801,757 STD 533 U6/L  
Acquired on 18-Sep-1992 at 16:57  
Peak Name RT (mins) Area U6/L  
AC 801,757 11.48 21812 602.529

M100 [DEPROJECT] 20 40145039, 2.1  
AC 801,757 STD 95.0 U6/L  
Acquired on 18-Sep-1992 at 17:14  
Peak Name RT (mins) Area U6/L  
AC 801,757 11.43 3368 109.178

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The original document"  
By [Signature] date 1/4/93



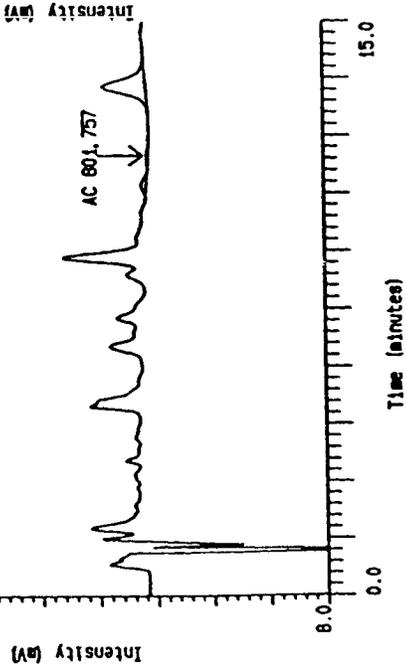
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ABC Laboratories, Inc. HCPP ver 2.3  
Date of Report: 21 SEP 92 at 08:43:57

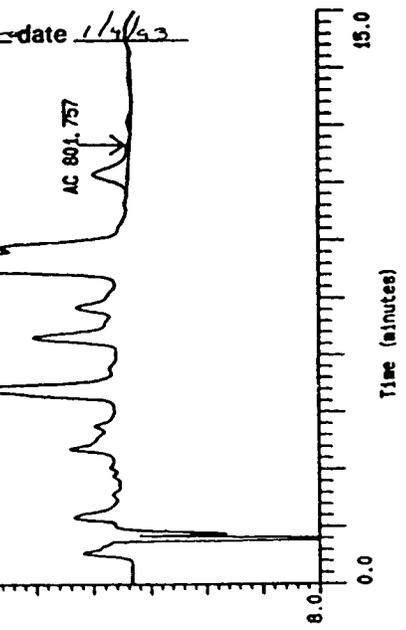
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The original document"

By *D. J. Blahut* date *1/14/93*

MC00 [DEPROJECT] 20 40145039. 3. 1  
40145.196 DAY 7 CONTROL  
Acquired on 18-Sep-1992 at 17:31  
Peak Name RT (mins) Area U6/L  
AC 801.757 11.48 539 0.134



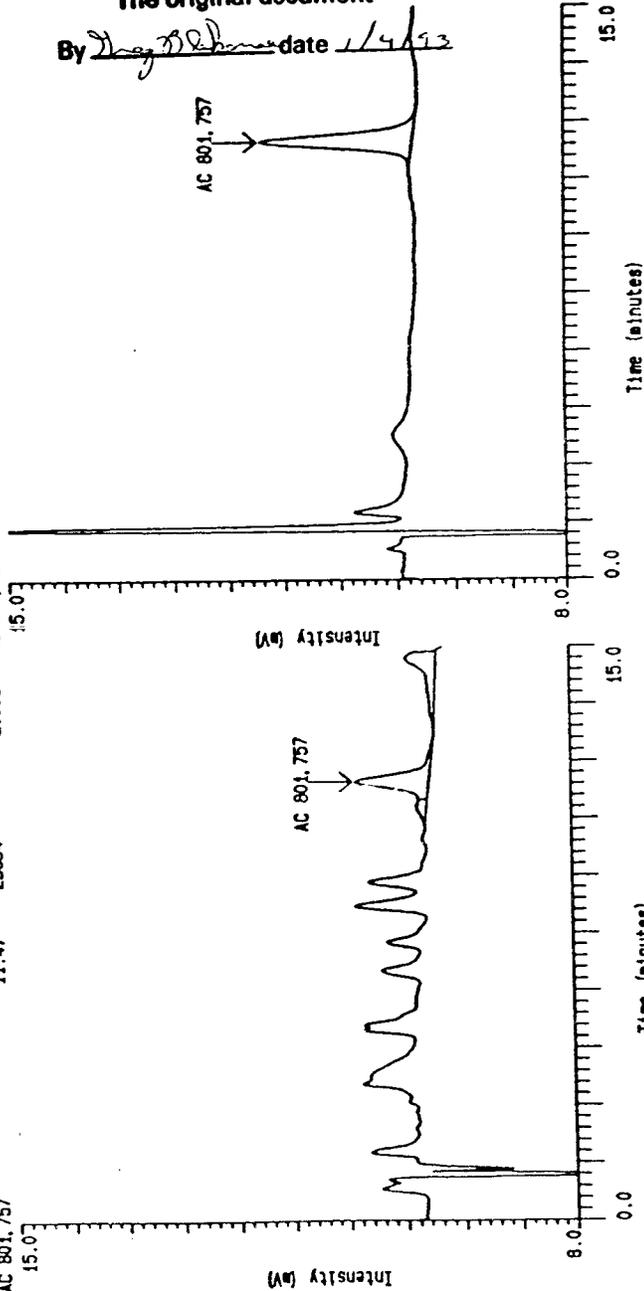
MC00 [DEPROJECT] 20 40145039. 4. 1  
40145.197 DAY 7 VEHICLE BLANK  
Acquired on 18-Sep-1992 at 17:48  
Peak Name RT (mins) Area U6/L  
AC 801.757 0.00 0 0



ABC Laboratories, Inc. HCPP ver 2.3  
Date of Report: 21 SEP 92 at 08:43:57

MC00 [DEPROJECT] 20 40145039.5.1  
40145.196 DAY 7 LEVEL 1  
Acquired on 18-Sep-1992 at 18:05  
Peak Name RT (mins) Area US/L  
AC 801.757 11.47 2363A 2.605

MC10 [DEPROJECT] 20 40145039.6.1  
AC 801.757 STD 1270 US/ML  
Acquired on 18-Sep-1992 at 18:22  
Peak Name RT (mins) Area US/L  
AC 801.757 11.48 46748 1269.557



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