FYI -0198-1326

Static Acute Bioassay Report
#25556

Submitted To:

Monsanto Chemical Company
Attn: Ms. A. F. Werner
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

FYI-98-001326

AB-80-541

[107-13-1]

Acute Toxicity of Acrylonitrile
to Daphnia magna

84980000028

June 12, 1980

ORIGINAL

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

Prepared By:

Alan D. Forbis — Date 6/13/80
Aquatic Biologist

Paul Boudreau — Date 6/13/80
Biologist

Approved By:

James A. Ault — Date 6/16/80
Quality Assurance Officer

Lyle D. Johnson — Date 6-16-80
Laboratory Manager

SUMMARY

The acute toxicity of Acrylonitrile to Daphnia magna was assessed using the methods outlined by the Commitee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen and pH were measured at the termination of the test and were within acceptable limits (4).

The results of the 48 hour static Daphnia magna toxicity study are summarized below.

Compound	48-hour LC_{50} (95% C.I.)
Acrylonitrile	22 (18-32) mg/l

The no effect level observed for Acrylonitrile was 3.2 mg/l after 48 hours.

INTRODUCTION

This definitive static bioassay was performed at the aquatic bioassay laboratory of Analytical BioChemistry Laboratories, Inc., Columbia, Missouri, for Monsanto Chemical Company, from June 7 to June 9, 1980. The purpose of this test was to determine the 24 and 48 hour LC_{50} levels for Acrylonitrile to Daphnia magna. The study was performed using ABC Protocol #7806.

METHODS AND MATERIALS

The procedures for static bioassay, as described in Standard Methods for Examination of Water and Wastewater (1) and Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (2), were used in this experiment. The Daphnia magna used in the test were cultured at the ABC facilities. The adult Daphnia were fed a suspension of trout chow and alfalfa (PR-11) daily until 24 hours prior to testing.

The static Daphnia bioassay was conducted in 250 ml glass beakers containing 200 ml of ABC well water with the chemical characteristics listed in Table 1. These vessels were kept at 20°C (±1). The photoperiod was controlled to give 16 hours daylight and 8 hours nightfall.

An initial range finding experiment was conducted using 10 Daphnia per concentration level. The range was found by beginning at 0.01 mg/l and increasing the amount of test material by a factor of 10 until a toxic level was found. Once this level had been determined, five concentrations in duplicate of the test compound with ten Daphnia (first instar less than 24 hours old) per beaker were selected for their respective bioassay. These concentrations were a logarithmic series ranging from 3.2 to 32 mg/l.

The Acrylonitrile standard was received on May 15, 1980, as a clear liquid and was stored at room temperature. Test concentrations were prepared based on total compound received. All standard weights and dilutions can be found in Appendix I. Deionized water was used in the preparation of all working stock solutions.

RESULTS

Table 2 presents the predicted LC_{50} values and 95% confidence intervals for Acrylonitrile. These values were obtained by employing a computerized LC_{50} program developed by Stephan et. al. (6).

Table 3 presents the mortality rate and water quality parameters measured during the test. There was an observed mortality at 3.6 mg/l after 48 hours of exposure, however, we feel that this is an aberrant mortality and is not necessarily compound related.

The study was conducted following the intent of the Good Laboratory Practice Regulations (5) and the final report was reviewed by Analytical BioChemistry Laboratories' Quality Assurance Unit. All original raw data was provided to Monsanto Chemical Company, with a copy retained at Analytical BioChemistry Laboratories.

TABLE 1

Chemical Characteristics of Well Water at ABC's Aquatic Bioassay Laboratory

Parameter	Concentration
Dissolved Oxygen	9.3 ppm
pH	8.2
Hardness ($CaCO_3$)	255 ppm
Alkalinity ($CaCO_3$)	368 ppm
Conductivity	50 µmhos/cm
Total Ammonia (NH_3)	<0.05 ppm
NO_3-N	0.15 ppm
Ortho-Phosphate	0.10 ppm
Aluminum	<0.01 ppm
Arsenic	<0.001 ppm
Cadmium	<0.001 ppm
Chromium	0.001 ppm
Cobalt	<0.001 ppm
Copper	<0.01 ppm
Iron	0.012 ppm
Lead	0.009 ppm
Mercury	<0.0001 ppm
Nickel	0.015 ppm
Zinc	<0.01 ppm
Measured organophosphorus pesticides	a
Measured organochlorine pesticides plus PCB's	a

[a] See appendix for individual analyses.

TABLE 2

The Acute Toxicity of Acrylonitrile to Daphnia magna

Compound	LC_{50} (mg/l) 24 hours	48 hours
Acrylonitrile	---	22 (18-32)*

*95% confidence interval (6).

NOTE: The LC_{50} values presented above were the results of a computerized program (6) performing the following statistical tests: binomial, moving average and probit tests. The results from the moving average or probit tests will be used when there are two or more partial mortalities. If there is not more than one partial mortality, the results from the binomial test will be recorded.

TABLE 3

Mortality Rates and Water Quality Measurements During the Acute Toxicity of Acrylonitrile to Daphnia magna

mg/l Concentration	Percent Mortality Hours		Water Quality							
			0 hours				48 hours			
	24 hr	48 hr	Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l
Control	0	0	20	8.3	7.8	---	19	8.6	8.1	---
2	0	0					19	8.6	8.0	---
6	0	5								
10.0	0	0					19	8.5	8.0	---
18.0	0	20								
32.0	25	100					19	8.6	8.1	---

*Dissolved oxygen concentrations - Dissolved Oxygen System (YSI Model 54).

**pH - pH Probe (Orion Model 91-06) used with an Extech Model 671 pH and mV meter.

***Ammonia concentrations - Ammonia Probe (Extech Model 8002-8) used with an Extech Model 6... pH and mV meter.

LITERATURE CITED

(1) American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed., New York.

(2) Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Stephan, C. E., Chairman. 1975. Committee on Methods for Toxicity Tests with Aquatic Organisms. U.S. EPA, Ecol. Res. Ser. 660/3-75009.

(3) Calculated employing the technique of Litchfield, J. T., Jr. and Wilcoxon, F. A Simplified Method of Evaluating Dose-Effect Experiments. J. Pharm. & Exp. Ther. 96,99 (1949).

(4) U. S. Environmental Protection Agency. Water Quality Criteria. 1971. Prepared by National Academy of Sciences.

(5) Food and Drug Administration. Regulations for Good Laboratory Practice in Non-Clinical Animal Studies. 21 CFR Part 58.

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

Quality Assurance Statement for final report #25556 entitled, "Acute Toxicity of Acrylonitrile to Daphnia magna," for Ms. A. F. Werner, Monsanto Chemical Company, St. Louis, Missouri.

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 Acrylonitrile was conducted and found to be in acceptable form by a member of our Quality Assurance Unit. A final inspection of all data and records on June 12, 1980, indicating that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

Should you have any questions relating to the information provided in this statement or the function of our Quality Assurance Unit, please contact me at your convenience.

James A. Ault 6/12/80

James A. Ault Date

Quality Assurance Officer

APPENDIX I

RAW DATA

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant [illegible] Test Species Daphnia magna (Lot #) Study No. 25556
Date Initiated 6/7/80 Time 10:30am Date Terminated 6/9/80
Dilution Water [illegible] No./Vessel 10 Vessel Size 250 (200) ml

MORTALITY AND BEHAVIORAL OBSERVATIONS

Test Conc. mg/l (ppm)		24 hr. Dead	24 hr. Obs.	48 hr. Dead	48 hr. Obs.	72 hr. Dead	72 hr. Obs.	96 hr. Dead	96 hr. Obs.	Dead	Obs.
Control	a	0	N	0	N						
	b	0	N	0	N						
3.2	a	0	N	0	N						
	b	0	N	0	N						
5.6	a	0	N	1	N						
	b	0	N	0	N						
10	a	0	N	0	N						
	b	0	N	0	N						
18	a	0	N	3	N						
	b	0	N	1	N						
32	a	2	N	10							
	b	3	N	10							
Observer		PB		PB							
Date		6/8		6/9							

Remarks: N= Normal, LOE= Loss of Equilibrium, SF= Surfacing

Recorded by: [illegible] Checked By: [illegible]

Test Daphnid

Species Daphnia magna

Prepared By [signature]

Checked By [signature]

Water Quality

Concentration mg/l	0 hour				48 hours				96 hours							
	Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l
Control	20	8.3	7.8	—	19	8.6	8.1									
3.2					19	8.6	8.0									
5.6																
10					19	8.5	8.0									
18																
32					19	8.6	8.1									

*Dissolved oxygen concentrations - Dissolved Oxygen ~~Probe (Extech Model 8012) used with an Extech Model 671 pH and mV meter~~ SYSTEM (YSI MODEL # 54A).

**pH - pH Probe (Fisher Model 13-639-108) used with an Extech Model 671 pH and mV meter.

***Ammonia concentrations - Ammonia Probe (Extech Model 8002-8) used with an Extech Model 671 pH and mV meter.

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant: Acrylonitrile Test Species: Daphnia magna (Lot #) Study No. 25556

Date Initiated: 5/18/80 Time: 4:00pm Date Terminated: 5/20/80

Dilution Water: well water No. Vessel: 10 Vessel Size: .25? (.28)

MORTALITY AND BEHAVIORAL OBSERVATIONS

Test Conc. mg/l (ppm)	24 hr.		48 hr.		72 hr.		96 hr.			
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
~~control~~										
0.01	0		0							
0.10	0		0							
1.00	0		0							
ADDED 5/20/80 10.00	0		5							
Observer	PB		PB							
Date	5/19		5/20							

Remarks:

Performed By: Paul Boudreau Checked By: Edna E. Foster

ANALYTICAL BIOCHEMISTRY LABS - AQUATIC BIOASSAY LAB

COMPOUND PREPARATION

Compound Azinphosmethyl Lot No. _____ Purity _____ % Lab No. _____

Preparation of Concentrated Working Standard

Date 5/12/80 Chemist Paul Bowman

Final Gross Weight 2.100 g Dilution Volume 50 ml

Tare Weight 2.000 g Concentration 2 mg/ml

Net Weight 0.100 g Balance calibrated with class S weights

Adj. Net Weight _____ g* 2.000 g (class S) + 0.000 g (tare) = 2.000 g (final wt.)

Preparation of Test Concentrations

Test Azinphosmethyl Problem Date 5/13/80 Chemist Paul Bowman

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	2.0	0.001	.2	0.010
1.	2.0	0.010	.2	0.100
2.	2.0	0.100	.2	1.000
3.	2.0	1.000	.2	10.000
4.				
5.				
6.				
7.				

ADD 4.0 5/20/80

Preparation of Concentrated Working Standard

Date 6/2/80 Chemist Paul Bowman

Final Gross Weight 2.200 g Dilution Volume 10 ml

Tare Weight 0.000 g Concentration 2 mg/ml

Net Weight 2.200 g Balance calibrated with class S weights

Adj. Net Weight _____ g* 2.000 g (class S) + 0.200 g (tare) = 2.200 g (final wt.)

Preparation of Test Concentrations

Test Azinphosmethyl Dynamic Date 6/2/80 Chemist Paul Bowman

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	—	—	.2	—
1.	20.0	0.032	.2	3.2
2.	20.0	0.056	.2	5.6
3.	20.0	0.100	.2	10.0
4.	20.0	0.180	.2	18.0
5.	20.0	0.320	.2	32.0
6.				
7.				

Remarks: _____

Prepared By: Paul Bowman Checked By: _____

*corrected for purity of primary standard.

ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile

Test Species: Daphnia magna

Lab No.: 25556 Exposure Period: 24 hr

Laboratory:
Analytical BioChemistry Labs
Aquatic Toxicology Division
7200 ABC Lane, P.O. Box 1097
Columbia, MO 65205

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
32	20	5	25	2.069473266616
18	20	0	0	9.536743164062SE-05
10	20	0	0	9.536743164062SE-05
5.6	20	0	0	9.536743164062SE-05
3.2	20	0	0	9.536743164062SE-05

THIS DATA SET DOES NOT MEET THE CRITERIA ESTABLISHED BY THE COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS BECAUSE NO PERCENT DEAD IS GREATER THAN 65 PERCENT.

NEITHER THE BINOMIAL TEST NOR THE MOVING AVERAGE METHOD CAN GIVE ANY RESULTS FOR THIS DATA SET. EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50 PERCENT OR THE LOWEST KILLED MORE THAN 50. IF THE PROBIT SLOPE IS NEGATIVE, ENTER DATA AGAIN USING NUMBER ALIVE INSTEAD OF NUMBER DEAD.

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

****

Which Test(s):

☐ Binomial ☐ Moving Average ☐ Probit

Use Method Determined to be that which gives the narrowest confidence limits for the LC50.

The analysis performed by a computerized LC50 program developed by Stephan, C.E. A. Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for [illegible] Environmental Protection Agency, Duluth, Minnesota. [illegible] August 1978.

Entered By: [signature] Date: 6/12/80 Checked by: [signature] Date: 6/12/80

bc 42

LC_{50} COMPUTER ANALYSIS DATA SHEET
ACUTE TOXICITY BIOASSAY

Test Material: Amphotericin B
Test Species: Daphnia magna
Lab No.: 25556 Exposure Period: 48hr

Laboratory:
Analytical BioChemistry Lab.
Aquatic Toxicology Division
7200 ABC Lane, P.O. Box 109
Columbia, MO 65205

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
32	20	20	100	9.536743164062SE-05
18	20	4	20	.590896606445 31
10	20	0	0	9.536743164062SE-05
5.6	20	1	5	2.002716064453 1E-03
3.2	20	0	0	9.536743164062SE-05

THE BINOMIAL TEST SHOWS THAT 18 AND 32 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.409008026125 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 21.525727365273

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS SET OF DATA BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

--------RESULTS CALCULATES USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
10	8.388441730751 3	20.555245712239	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

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

SLOPE = 5.6301693281422
95 PERCENT CONFIDENCE LIMITS = -10.67638127680 1 AND 21.936719933085

LC50 = 20.118468815381
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC1 = 7.7682494315428
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINIT

****

Method Reported:

☑ Binomial ☐ Moving Average ☐ Probit

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

LC_{50} analysis performed by a computerized LC_{50} program developed by Stephan, C.E., 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.

Prepared By: [signature] Date: 6/12/80 Checked by: [signature] Date: 6/13/80

ANALYTICAL BIO CHEMISTRY LABORATORIES, INC
P.O. Box 1097 • Columbia, MO 65205 • (314) 474-8579

February 27, 1979

ORGANOPHOSPHATE SCREEN

ANALYSIS RESULTS FOR:

Aquatic Toxicology Department
ABC Labs
P.O. Box 1097
Columbia, MO 65205

Lab No.:	23168-1	23168-2
Customer ID.:	Fish Food	Well Water
	parts per billion	Nanograms/liter
DDVP:	250	< 40
Diazinon:	<10	< 20
Disyston:	<10	< 20
Methyl Parathion:	< 30	< 80
Malathion:	< 30	< 110
Ethyl Parathion:	< 30	< 80

Gary Brockhart
Gary L. Brockhart
Residue Supervisor

< indicates minimum detectable amount.

ANALYTICAL BIO CHEMISTRY LABORATORIES INC
P O Box 1097 • Columbia Mo 65201 • (314) 474 8579

March 31, 1980

NONIONIC CHLORINATED HYDROCARBON RESIDUE ANALYSIS

FOR: ABC Aquatic Toxacology

Lab No.	25107	25126-1*	25126-2*
Customer I.D.	Well Water	Recon Water	DI Water
	2/6/80	2/8/80	2/8/80
Compound	Nanograms/liter		
BHC	<0.4	0.4	<0.4
αBHC	<0.4	0.43	<0.4
βBHC	<0.4	<0.4	<0.4
γBHC	<0.4	<0.4	<0.4
σBHC	<0.4	<0.4	<0.4
Chlordane		7.3	6.8
Heptachlor	<0.4	<0.4	<0.4
Heptachlor Epoxide	<0.8	---	<0.8
αChlordane	7.7	7.3	6.8
γ Chlordane	<0.8	<0.8	<0.8
Trans Chlordane	<2	<2	<2
Dieldrin + Aldrin	<1	<1	<1
Aldrin	<0.4	<0.4	<0.4
Dieldrin	<1	<1	<1
DDT + Metabolites	<2	<2	<2
o,p'-DDE	<1	<1	<1
p,p'-DDE	<1	<1	<1
o,p'-DDT	<2	<2	<2
p,p'-DDD	<2	<2	<2
p,p'-DDT	<2	<2	<2
Endrin	<2	<2	<2
Heptachlor	<0.4	<0.4	0.66
Heptachlor Epoxide	<0.8	2.3	<0.8
Lindane	<0.4	<0.4	<0.4
Methoxychlor	<8	**	**
Toxaphene	<50	<50	<50
PCB	<30	<30	<30
Aroclor 1242	<30	<30	<30
Aroclor 1016	<30	<30	<30
Aroclor 1254	<30	<30	<30
Aroclor 1260	<30	<30	<30
HCB	0.37	0.76	0.73
Mirex	<4	<4	<4

<indicates less than, if present at all.
** Very strong response observed at retention time corresponding to diethyl hexylphthalate.
* Occlu by DEHP response.

Gary Brockhart
Residue Supervisor

Chronic Mesocosm Culture 4

Date	# Dead	# Alive	# Instar	# Young Adult / Day	Study ID	Comments	Initials
6/1/80	–	43	–	–	—	fed & changed	AE
6/6/80	–	43	–	–	–	fed + changed	
6/7/80	–	43	496	H.5	25556 – 25561 DW	fed + changed	
6/9/80	–	42	–	–	–	fed + changed	
6/11/80	–	42	–	–		fed + changed	AE

Monsanto

MONSANTO INDUSTRIAL CHEMICALS CO.
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166
Phone: (314) 694-1000

May 2, 1980

Mr. Alan Forbis
Analytical Biochemistry Laboratories
P. O. Box 1097
Columbia, MO 65205

Dear Alan:

I would like to authorize the studies outlined on Page 1 of your proposal B216 for acrylonitrile. This will include 96 hour acute statics on bluegill and rainbow trout (Protocol #7601), a 48 hour acute static using daphnids (Protocol #7806) and a time independent flow-through study using bluegill (Protocol #7807).

A "cold" sample of acrylonitrile will be shipped directly to you from our Texas facility and should arrive shortly. The ^{14}C-acrylonitrile will be shipped to you from here after characterization; hopefully this will I be in the next two weeks.

Please forward your cost estimates for equipping the flow-through chambers to minimize and contain any volatiles.

If you have any questions, don't hesitate to contact me.

Sincerely,

Fran Werner

A. F. Werner

AFW/bh

cc: J. D. Wilson - Monsanto

5/5/80

ABC PROTOCOL NO. 7806

(Revised May 22, 1980)

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

ABC Study Number 25556

Test Material Acrylonitrile

1.0 INTRODUCTION

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

2.0 OBJECTIVES

The primary objective of the toxicity test described herein is to evaluate the acute toxicity of a chemical substance to 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. 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 BioChemistry Laboratories, Inc., 7200 East ABC Lane, P. O. Box 1097, Columbia, Missouri 65205.

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. All test daphnids will be held in a controlled temperature area on a 16-hour daylight photoperiod at the same temperature used for testing (20 ±2°C). During the holding period, they will receive a standard PR-II food preparation (8). 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 on a bench top in a controlled temperature area with temperatures maintained at 20 ±2°C. The dilution water

used will be from a deep well source with chemical and physical characteristics shown in Table 2.

4.4 Test Material. Specific information regarding the test material is to be supplied by the sponsor and will be addressed at the time of protocol approval in section 8.3. 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.

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 three widely spaced toxicant concentrations, usually spaced by a factor of 10. The test daphnids will be placed in the test chambers by stratified random 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.

4.5.3 After 24 hours of exposure, the test chambers will be observed for mortality and/or adverse behavioral effects. A record will be maintained of mortality and abnormal behavior at each observation. Dependent upon this observation, additional test concentrations may be added at levels above or below the initial concentrations. This procedure will be followed until a toxic range is determined. For example, if the 24 hour exposure results in total mortality, new solutions will be prepared at a factor of 10 below the lowest initial concentration until no mortality or partial mortality is reached. In the converse situation, if no mortality is observed after 24 hours, new solutions will be added at concentrations spaced by a factor of 10 above the highest initial level until mortality is noted. In this manner, a bracket is formed for the toxic range of the compound.

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

4.5.5 Results of the range-finding study will be used to set the concentration range of the definitive study described in section 5.0. At least five toxicant concentrations selected from the geometric scale presented in Table 1, which fall within the preliminary test range, have proven to be adequate in assessing most compounds.

5.0 DEFINITIVE STUDY

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

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

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

5.4 Test Material. Specific information regarding the test material is to be supplied by the sponsor and will be addressed in section 8.3 at the time of protocol approval.

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

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

5.5.2 The definitive test will be initiated by exposing the test daphnids to at least 5 toxicant concentrations and a dilution water control. The test will be conducted using duplicate beakers 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 one of the logarithmic series presented in Table 1. The exact concentrations to be used will be addressed in section 8.4 at the time of protocol approval. 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. The test organisms will be placed in the test chambers by stratified random assignment within 30 minutes after solution preparations.

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/or adverse behavioral effects every 24 hours. Dead individuals will be removed at each observation and a record maintained of mortality and abnormal behavior for each concentration tested.

5.6 Test Procedure-Chemical and Physical. The dissolved oxygen concentration must be measured in the control at the beginning of the test and every 48 hours thereafter to the end of the test in the control and the high, medium and low toxicant concentrations. The pH should be measured at least once in the control

and the high, medium and low toxicant concentrations (5). If at any point in the study dissolved oxygen levels are observed to be below or approaching 40 percent saturation, ABC will contact the study director for authorization to artificially aerate the test chambers with compressed air for the duration of the study. The authorization procedure used will be that described in section 7.0.

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 developed by Stephan et. al. (11) or by manual methods described by Litchfield-Wilcoxon (9) and Stephan (10).

5.8 Report. A final report of the definitive study will be submitted to the study sponsor and will include the following. A draft of the final report will be submitted for sponsor review if so requested at an additional charge.

5.8.1 Study dates of both preliminary and definitive phases.

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 [illegible]ion of raw data.

5.8.8 List and signatures of study personnel.

5.8.9 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.

7.0 SPONSOR AUTHORIZATIONS DURING THE STUDY

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

8.0 TEST-SPECIFIC INFORMATION

8.1 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 and 8.3 are to be completed by the study sponsor. Sections 8.4, 8.5 and 8.6 will be completed by ABC.

8.2 Study Sponsor:

8.2.1 Company Monsanto Chemical Company

8.2.2 Address 800 North Lindbergh Boulevard
St. Louis, MO 63166

8.2.3 Study Director (Coordinator)

A. F. Werner — Name
Study Director — Title

8.3 Test Material:

8.3.1 Name Acrylontrile

8.3.2 Code Number —

8.3.3 Physical Description Clear liquid

8.3.4 Purity Confidential

8.3.5 Stability ~6 months if kept in the dark

8.3.6 Recommended Solvent H_2O

8.3.7 Water Solubility 73%

8.3.8 Handling Precautions gloves. Avoid breathing vapors
Use in well ventilated area

8.4 Test Concentrations:

8.4.1 Definitive Concentrations 3.2, 5.6, 10.0, 18.0, 32.0 mg/l

8.5 Study Dates:

8.5.1 Proposed starting date of definitive study 6/1/80

8.5.2 Proposed completion date of definitive study 6/15/80

8.6 ABC Study Personnel:

8.6.1 Study Director

Alan D. Forbis	Aquatic Supervisor
Name	Title

8.6.2 Principal Investigator

Paul Boudreau	Biologist
Name	Title

8.6.3 Quality Assurance Officer

James A. Ault	Quality Assurance Officer
Name	Title

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

8.7.1 Sponsor's Study Director

AF Werner	Environmental Biologist	5/30/80
Name	Title	Date

8.7.2 ABC's Study Director

Alan D Forbis	Aquatic Supervisor	5/30/80
Name	Title	Date

8.7.3 ABC's Laboratory Director

E Johnson	Lab Director	5/30/80
Name	Title	Date

TABLE 1: Guide to selection of experimental concentrations based on progressive bisection of intervals on logarithmic scale.

Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
10.0	10.0	10.0	10.0	10.0
				8.7
			7.5	7.5
				6.5
		5.6	5.6	5.6
				4.9
			4.2	4.2
				3.7
3.2	3.2	3.2	3.2	3.2
				2.8
			2.4	2.4
				2.1
		1.8	1.8	1.8
				1.55
			1.35	1.35
				1.15
1.0	1.0	1.0	1.0	1.0

TABLE 2: Chemical characteristics of well water at ABC's Aquatic Bioassay Laboratory.

Parameters	Concentration
Dissolved Oxygen	9.3 ppm
pH	8.2
Hardness ($CaCO_3$)	255 ppm
Alkalinity ($CaCO_3$)	368 ppm
Conductivity	50 µmhos/cm
Total Ammonia (NH_3)	<0.05 ppm
NO_3-N	0.15 ppm
Ortho-Phosphate	0.10 ppm
Aluminum	<0.01 ppm
Arsenic	<0.001 ppm
Cadmium	<0.001 ppm
Chromium	0.001 ppm
Cobalt	<0.001 ppm
Copper	<0.01 ppm
Iron	0.012 ppm
Lead	0.009 ppm
Mercury	<0.0001 ppm
Nickel	0.0157 ppm
Zinc	<0.01 ppm
Measured organophosphorus pesticides	[a]
Measured organochlorine pesticides plus PCB's	[a]

[a]Individual analyses for these parameters will be included in the final report of the study.

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) Food and Drug Administration. 1978. Regulations for Good Laboratory Practice. Federal Register 43(247), December 22, 1978: 59986-60025.

(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. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed. Washington, D.C. 1193 p.

(7) American Society for Testing and Materials. 1978. Proposed standard practices for conducting basic acute toxicity tests with fish, macroinvertebrates and amphibians. Draft No. 7, April 27, 1978, ASTM Committee E-35.21. 54 p.

(8) American Society for Testing and Materials. 1978. Proposed standard practice for conducting renewal life cyle toxicity tests with the Daphnid, Daphnia magna. Draft No. 4, August, 1978, ASTM Committee E-35.21. 29 p.

(9) Litchfield, J. T., Jr. and F. Wilcoxon. 1949. A Simplified Method of Evaluating Dose-Effect Experiments. Jour. Pharm. Exp. Ther. 96:99-113.

(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.

ORIGINAL

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 8 (Month) 27 (Day) 98 (Year) Barbara Smith Camera Operator

Place Syracuse (City) New York (State)

AMTEK corp