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06/26/87	07/26/94	FYI
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Chemical Category		
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CODING FORM FOR GLOBAL INDEXING

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HADLEY & McKENNA
ATTORNEYS AT LAW
1815 H STREET, N.W.
WASHINGTON, D. C. 20006

(202) 296-6300

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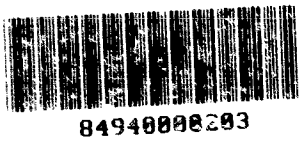

June 26, 1987

RECD
Jun 26 1987

Robert H. Brink
Executive Secretary
TSCA Interagency Testing Committee
TS-792
Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

Re: ITC Request for Information on Acrylonitrile

Dear Mr. Brink:

Pursuant to our several telephone conversations we are, as promised, enclosing herewith four studies which we believe will be of interest to you and the Committee. These studies are as follows:

1. Acute Toxicity of Acrylonitrile to Fathead Minnows (Pimephales promelas)

2. Acute Toxicity of Acrylonitrile to Rainbow Trout (Salmo gairdneri)

3. Acute Toxicity of Acrylonitrile to Daphnia magna

4. Early Life Stage Toxicity of Acrylonitrile to Fathead Minnows (Pimephales promelas) in a Flow-Through System

The foregoing data are the only pieces of information about the ecological effects of acrylonitrile we know at present to be in existence and not referenced in the Agency's Health Assessment Document for Acrylonitrile.

If we can be of any further assistance to you or the Committee with regard to acrylonitrile, please do not hesitate to let us know.

Very truly yours,

Joseph E. Hadley, Jr.

JEH:ry
Enclosures

ANALYTICAL BIO CHEMISTRY LABORATORIES INC
P O Box 1097 • Columbia MO 65205 • (314) 474-6579

MASTER FILE
Project No. AM80-539

Static Acute Bioassay Report
#25054

Submitted To:

Monsanto Industrial Chemicals Co., N2A
Attn: Ms. A. F. Warner
800 North Lindbergh Boulevard
St Louis, Missouri 63166

AM-80-539

Acute Toxicity of Acrylonitrile to
Fathead Minnows (Pimephales promelas)

June 20, 1980

Submitted By: Analytical BioChemistry Laboratories, Inc.
7200 East ABC Lane
P. O. Box 1097
Columbia, Missouri 65205

Prepared By:

Carl M. Thompson 6/24/80
Carl M. Thompson Date
Aquatic Supervisor

Jerry Griffen (CT) 6/24/80
Jerry Griffen Date
Biologist

Approved By:

James A Ault 6-25-80
James A. Ault Date
Quality Assurance Officer

Lyle D. Johnson 6-25-80
Lyle D. Johnson Date
Laboratory Manager

SUMMARY

The acute toxicity of Acrylonitrile to fathead minnows (Pimephales promelas) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1). Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.

As a quality check, the fathead minnows were challenged with a reference compound, Antimycin A. The observed 96 hour LC_{50} and 95% confidence limits (C.I.) were within the 95% confidence limits reported in the literature (2), indicating that the fish were in good condition.

The results of the four day static fish toxicity study using fathead minnows are summarized below. The 24 and 48 hour LC_{50} values were also determined and are given in Table 4.

Compound	96-hour LC_{50} (95% C.I.)
Acrylonitrile	15 mg/l (12-19 mg/l)
Antimycin A	0.000032 mg/l (0.000024-0.000042) mg/l

Also the results indicated a 96 hour, no observed effect concentration of 5.6 mg/l.

INTRODUCTION

This static bioassay was performed at the aquatic bioassay laboratory of Analytical BioChemistry Laboratories, Inc., Columbia, Missouri, for Monsanto Industrial Chemicals Co., from June 16 to June 20, 1980, as authorized in a letter from Monsanto Chemicals Co. on May 2, 1980 (Appendix I). The purpose of this test was to determine the 24, 48 and 96 hour LC_{50} levels for Acrylonitrile to fathead minnows (Pimephales promelas). A preliminary range-finding study was conducted from June 11 to June 13, 1980, to determine the concentration range for the definitive bioassay. The study was performed following the procedures outlined in ABC Protocol Number 7601 (Appendix I), as approved by Ms A. F. Werner, Monsanto Chemicals Co., on June 16, 1980.

METHODS AND MATERIALS

The procedures for static bioassay, as described in Standard Methods for Examination of Water and Wastewater (3) and Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1), were used in this experiment. The fathead minnows used in the test were obtained from Fattig Fish Hatchery in Brady, Nebraska. The fish were identified to species using the taxonomic keys developed by Eddy (4). All test fish were held in culture tanks on a 16 hour daylight photoperiod and observed for at least fourteen days prior to testing. Fish culture techniques used were basically those described by Brauhn et. al. (5). A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in Appendix I. During this period, the fish received a standard commercial fish food (Rangen's) daily until 48 hours prior to testing at which time feeding was discontinued. The fathead minnows used for this experiment had a mean weight of 0.21 g and a mean standard length of 24.8 mm. Weight and length measurements were made on the control group of fish at the termination of the test and are included in Appendix I.

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconsituted water composed of the following compounds in the amounts stated per liter of deionized water:

48 mg $NaHCO_3$
30 mg $CaSO_4$
30 mg $MgSO_4$
2 mg KCl

The water parameters of this dilution water were, dissolved oxygen: 9.0 mg/l; pH: 7.0; total hardness: 45 mg/l $CaCO_3$; and total alkalinity: 35 mg/l $CaCO_3$. The well water source from which this dilution water was prepared had the characteristics shown in Table 1.

These vessels were kept in a water bath at 22°C (±1.0). The test fish were acclimated to the dilution water and test temperature and held without food 48 hours prior to testing.

A 48 hour range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 5.6 and 56 mg/l. Based on the results of preliminary testing, five concentrations of the test compound, ranging in a logarithmic series from 5.6 to 56 mg/l, with ten fish per concentration were selected for definitive bioassay. The fish were added to the test chambers by random assignment within 30 minutes after addition of toxicant aliquots.

The Acrylonitrile standard was received on May 15, 1980, in good condition. The sample upon receipt was observed to be a clear liquid and was stored at room temperature (20°C). Sample purity was ~99%. Test concentrations were prepared based on the total compound. The test concentrations were obtained by transferring appropriate aliquots of a working solution directly into the test chambers. The working solution was prepared in deionized water. All standard weights and dilution values are listed in Appendix I.

RESULTS

Table 2 presents the predicted LC_{50} values and 95% confidence intervals for Acrylonitrile and the reference test against Antimycin A, a piscicide. These values were obtained by employing a computerized LC_{50} program developed by Stephan et. al. (10). Mortality rates, test concentrations and water quality data are presented in Table 3.

The dissolved oxygen concentration which stayed between 40 and 100% saturation was considered adequate for testing. The pH values remained consistent with the control throughout the study. The ammonia concentrations were below the toxic limit (7).

The study was conducted following the intent of the Good Laboratory Practice Regulations (8) and the final report was reviewed by Analytical BioChemistry Laboratories' Quality Assurance Unit. All original raw data was provided to Monsanto Industrial Chemicals Co., 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	0.0157 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 and Antimycin A to Fathead Minnows (Pimephales promelas)*

Compound	LC_{50} in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
Acrylonitrile	42 (32-56)**	22 (10-32)	15 (12-19)
Antimycin A***	0.000050 (0.000024-0.000075)	0.000037 (0.000024-0.000075)	0.000032 (0.000024-0.000042)

*Bioassay was conducted at 22°C (±1.0), mean weight and length, 0.21 g and 24.8 mm.

**95% confidence interval.

***Antimycin A standard obtained from Sigma Chemical Company, Type III, crystalline, Lot 125C-0152.

TABLE 3

Mortality Rates and Water Quality Measurements During the Acute Toxicity Test of Acrylonitrile to Fathead Minnows (Pimephales promelas)

mg/l Concentration	Percent Mortality Hours			Water Quality											
				0 hours				48 hours				96 hours			
	24	48	96	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	0	0	0	21	9.0	6.6	<0.10	21	7.5	6.6	---	21	4.5	7.0	0.20
5.6	0	0	0	21	8.9	6.6	<0.10	21	7.6	6.6	---	21	6.0	7.1	0.32
10	0	0	10												
18	0	20	70					21	7.9	6.6	---	21	6.5	6.9	0.26
32	0	100	100												
56	100	100	100	21	8.9	6.6	<0.10								

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

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

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

LITERATURE CITED

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

(2) Berger, B. L., R. E. Lennon and J. W. Hogan. 1969. Laboratory Studies on Antimycin A as a fish toxicant. U. S. Department of Interior, Investigations in Fish Control No. 26. 21 p.

(3) American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed. Washington, DC. 1193 p.

(4) Eddy, Samuel. 1969. The Freshwater Fishes. 2nd ed. W. C. Brown Company, Dubuque, IA. 286 p.

(5) Brauhn, J. L. and R. A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May, 1975. 45 p.

(6) Litchfield, J. T., Jr. and F. Wilcoxon. 1949. A Simplified Method of Evaluating Dose-Effect Experiments. Jour. Pharm. Exp. Ther. 96:99113.

(7) National Academy of Sciences. 1971. Water Quality Criteria, 1972. U. S. Department of Commerce, PB-236 199. 592 p.

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

(9) 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.

(10) 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.

Quality Assurance Statement for final report #25554 entitled, "Acute Toxicity of Acrylonitrile to Fathead Minnows (Pimephales promelas)," for Ms. A. F. Werner, Monsanto Industrial Chemicals Co., 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 an acceptable form by a member of our Quality Assurance Unit. An inspection of the daily mortality rate of the test organisms prior to the initiation of the study indicated they were in good health and should not bias the observed mortality in the study. A final inspection of all data and records on June 23, 1980, indicated 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 6-24-80

James A. Ault Date
Quality Assurance Officer

APPENDIX I

RAW DATA

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant Acrylonitrile Test Species Fathead Minnows (Lot #2490) Study No. 255-5-4
Date Initiated 6/16/80 Time 2:30pm Date Terminated 6/20/80
Dilution Water Recon H_2O No./Vessel 10 Vessel Size 1.5 L

Test Conc. mg/l (ppm)	MORTALITY AND BEHAVIORAL OBSERVATIONS									
	24 hr.		48 hr.		72 hr.		96 hr.			
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	0	N	0	N	0	N	0	N		
5.6	0	N	0	N	0	N	0	N		
10.0	0	N	0	N	1	9N	1	9N		
18.0	0	N	2	6SUR	4	(1)6 2LOE	7	3LOE		
32.0	0	2 LOE 8 SUR	10		10		10			
56.0	10		10		10		10			
Observer Date	[illegible] 6/17		[illegible] 6/18		[illegible] 6/19		CT 6/20			

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

(1) entry error CT 6/20/80

Prepared By: [illegible] Checked By: Carlton Thompson

Test Acrylonitrile 96 hr Static

Species Fathead Minnows (Lot # 2451)

Prepared By

Checked By

Water Quality

Concentration mg/l	6/16 Day 0 Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	6/18 Day 2 Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Day 4 CJ 20 Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Day Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l
Control	21	5.0	6.6	<.1	21	7.5	6.6	—	21	4.5	7.0	0.20				
5.6	21	5.9	6.6	<.1	21	7.6	6.6	—	21	6.0	7.1	0.32				
10.0																
18.0					21	7.9	6.6	—	21	6.5	6.9	0.26				
32.0																
56.0	21	5.9	6.6	<.1			~~6.6~~*									

*entry error 6/18

*Dissolved oxygen concentrations - Dissolved Oxygen Probe (YSI Dissolved Oxygen System 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 671 pH and mV meter.

2

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Retest

Toxicant Acrylonitrile Test Species Fathead Minnows (Lot #2460) Study No. 25554

Date Initiated 6/11/80 Time 11:30 am Date Terminated 6/13/80

Dilution Water Recon H₂O No./Vessel 5 Vessel Size 15Q

Test Conc. mg/l (ppm)	MORTALITY AND BEHAVIORAL OBSERVATIONS									
	24 hr.		48 hr.		72 hr.		96 hr.			
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control										
~~5.6-5.6~~ [1]	0	N	0	N						
~~5.6-5.6~~ [2]	5		5							
Observer	~~JJ~~	~~JJ~~	~~JJ~~	~~JJ~~						
Date	6/12	6/12	6/13	6/13						

[1] entry error CT 6/20/80

Remarks:

Prepared By: JJ Checked By: Carl T. Hopper

c 42

LC_{50} COMPUTER ANALYSIS DATA SHEET
ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile

Test Species: Fathead Minnow

Lab No.: 25554 Exposure Period: 72 hrs

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)
56	10	10	100	.09765625
32	10	10	100	.09765625
18	10	4	40	37.6953125
10	10	1	10	1.07421875
5.6	10	0	0	.09765625

THE BINOMIAL TEST SHOWS THAT 10 AND 32 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 98.828125 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 19.362344350767

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

SPAN	G	LC50	95 PERCENT CONFIDENCE	
4	.112835602303	16.812848165756	12.667550702863	21.910067920965
3	.113736458066988	16.881936021608	13.556883077578	22.299667787999
2	.167754058524444	17.76211238945	14.137515085873	22.224027538081

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

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.285607052663999	1	.672962343539

SLOPE = 6.586246140383
95 PERCENT CONFIDENCE LIMITS = 3.0664102051327 AND 10.106082075633

LC50 = 17.615878394453
95 PERCENT CONFIDENCE LIMITS = 13.748934957456 AND 22.491787098372

LC1 = 7.8095166889502
95 PERCENT CONFIDENCE LIMITS = 2.9123434861296 AND 10.889664431172

Method Reported:

☐ Binomial ☐ Moving Average ☑ Probit

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

LC_{50} analysis performed by a computerized LC_{50} program developed by Stephan, C.E., A. Busch, K. 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: 6/20/80 Checked by: [signature] Date: 6/20/80

: 42

LC_{50} COMPUTER ANALYSIS DATA SHEET
ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile
Test Species: Fathead Minnow
Lab No.: 25594 Exposure Period: 96 hrs

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)
56	10	10	100	.09765625
32	10	10	100	.09765625
18	10	7	70	17.1875
10	10	1	10	1.07421875
5.6	10	0	0	.09765625

THE BINOMIAL TEST SHOWS THAT 10 AND 32 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 98.828125 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 14.982140628984

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

SPAN	G	LC50	95 PERCENT CONFIDENCE	
4	.113587226927 15	14.80760760357	10.867441066466	19.173474270347
3	.113394351781 81	14.85614789299 8	11.889759040701	19.053095058816
2	.167754058524 44	15.2073126195	11.566059642333	18.704479101486
1	.509802071631 54	14.982140656604	11.959681753271	23.612258459138

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

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.328578485718 22	1	.990482822627

SLOPE = 7.5910366339507
95 PERCENT CONFIDENCE LIMITS = 3.23972037778 AND 11.942352890121

LC50 = 15.045765493716
95 PERCENT CONFIDENCE LIMITS = 11.862533759327 AND 19.051217061874

LC1 = 7.4284158015014
95 PERCENT CONFIDENCE LIMITS = 2.7463639998756 AND 10.053097706863

****

ethod Reported:

☐ Binomial ☐ Moving Average ☐ Probit

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

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 lculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, -publication manuscript, August 1978.

pared By

: 42

LC_{50} COMPUTER ANALYSIS DATA SHEET
ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile
Test Species: Fathead Minnow
Lab No.: 25554 Exposure Period: 48 hrs

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)
56	10	10	100	.09765625
32	10	10	100	.09765625
18	10	2	20	5.46875
10	10	0	0	.09765625
5.6	10	0	0	.09765625

THE BINOMIAL TEST SHOWS THAT 10 AND 32 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 99.8046875 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 21.573533697768

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
****

ethod Reported:

☑ Binomial ☐ Moving Average ☐ Probit

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

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 culating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, -publication manuscript, August 1978.

42

LC_{50} COMPUTER ANALYSIS DATA SHEET
ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile
Test Species: Fathead Minnow
Lab No.: 25554 Exposure Period: 24hrs

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)
56	10	10	100	.09765625
32	10	0	0	.09765625
18	10	0	0	.09765625
10	10	0	0	.09765625
5.6	10	0	0	.09765625

THE BINOMIAL TEST SHOWS THAT 32 AND 56 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 99.8046875 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 42.332027348798

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

****

ethod Reported:

☑ Binomial ☐ Moving Average ☐ Probit

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

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 iculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, e-publication manuscript, August 1978.

epared By: Cathy Thayer Date: 6/20/80 Checked by: Mark Tobe Date: 6/20/80

7

ANALYTICAL BIOCHEMISTRY LABS - AQUATIC BIOASSAY LAB

COMPOUND PREPARATIONS

Compound Acrylonitrile Lot No. _____ Purity _____ % Lab No. 25555-9

Preparation of Concentrated Working Standard

Date 6/11/80 Chemist [signature]

Final Gross Weight 1.510 g Dilution Volume 100 (DI. H_2O) ml

Tare Weight 0.00 g Concentration 15 mg/ml

Net Weight 1.510 g Balance calibrated with class S weights:

Adj. Net Weight 1.510 g* 1.00 g (class S) + 0.00 g (tare) = 1.00 g (final wt.)

Preparation of Test Concentrations

Test Prelim FHM Date 6/11/80 Chemist [signature]

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	-	-	-	-
1.	15	5.6	15	5.6
2.	15	5.6	15	5.6
3.				
4.				
5.				
6.				
7.				

Preparation of Concentrated Working Standard

Date 6/16/80 Chemist [signature]

Final Gross Weight 3.00 g Dilution Volume 200 (DI H_2O) ml

Tare Weight 0.00 g Concentration 15 mg/ml

Net Weight 3.00 g Balance calibrated with class S weights:

Adj. Net Weight 3.00 g* 3.00 g (class S) + 0.00 g (tare) = 3.00 g (final wt.)

Preparation of Test Concentrations

Test Definitive FHM Date 6/16/80 Chemist [signature]

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	-	-	15	-
1.	15	5.6	15	5.6
2.	15	10.0	15	10.0
3.	15	18.0	15	18.0
4.	15	32.0	15	32.0
5.	15	56.0	15	56.0
6.				
7.				

Remarks: _____

Prepared By: [signature] Checked By: [signature]

*corrected for purity of primary standard.

C930.007

MASTER FILE
Project No. AB-80-542

JOHN D. WALKER

...TICAL BIOCHEMISTRY LABORATORIES INC
P.O. Box ... • Columbia, MO 65205 • 314/474-6579

Early Life Stage Final Report
#25673

Submitted To:

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

AB-80-542

Early Life Stage Toxicity of Acrylonitrile to Fathead Minnows (Pimephales promelas) in a Flow-Through System

December 16, 1980

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 — 12/16/86 Date
Aquatic Biologist

Paul A. Boudreau — 12/16/86 Date
Biologist

Walter Cranor — 12-16-86 Date
Metabolism Supervisor

Larry Franklin — 12-16-86 Date
Chemist

Approved By:

James A. Ault — 12/16/86 Date
Quality Assurance Officer

Lyle D. Johnson — 12-16-86 Date
Laboratory Manager

/gsl

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SUMMARY

A flow-through 30 day early life stage toxicity study of Acrylonitrile (AN) to fathead minnows (Pimephales promelas) was conducted to estimate the Maximum Acceptable Toxicant Concentration (MATC) limits. A one-liter proportional diluter system was used to maintain constant test concentrations. Exposure concentrations of Acrylonitrile were determined through the use of gas chromatographic methods. The mean measured levels of Acrylonitrile were 0.34 mg/l, 0.44 mg/l, 0.86 mg/l, 1.8 mg/l and 3.6 mg/l.

The survival of fathead minnow fry was significantly reduced in the highest levels (7.6 mg/l, 1.8 mg/l and 0.86 mg/l) of exposure to Acrylonitrile. Growth of the fry, as measured by standard length and weight, was the most sensitive measured parameter in the Acrylonitrile study. At all concentrations ≥0.44 mg/l, there was a significant decrease in growth of the fathead minnows after 30 days of exposure. At the 0.34 mg/l concentration, one of two duplicate exposures showed a significant effect upon growth; therefore a "no effect" concentration could not be established. Based on this data, it is our opinion that the upper limit of the MATC for a 30 day exposure of Acrylonitrile to fathead minnows is 0.34 mg/l.

INTRODUCTION

Monsanto Chemical Company contracted the Aquatic Toxicology Division of Analytical BioChemistry Laboratories, Inc., to conduct a dynamic 30 day early life stage (egg-fry) bioassay with fathead minnows (Pimephales promelas) exposed to Acrylonitrile. The primary objective of the study was to estimate Maximum Acceptable Toxicant Concentration (MATC) limits for Acrylonitrile using what is believed to be the most critical and sensitive life stage (embryo to the juvenile stage) of the fathead minnows, (1, 2). This was achieved by measuring the effects of Acrylonitrile on hatchability, survival, growth, behavior and morphological changes of the embryoes and fry. This is determined by comparison between the control and exposure concentrations. The study was authorized by A. F. Werner, Monsanto Chemical Company, in a letter dated June 3, 1980 and was conducted from October 6 to November 10, 1980.

The term Maximum Acceptable Toxicant Concentration (MATC) (3) as used in this report is the concentration limit of which the lower value is the concentration that causes no observable deleterious effect for any of the parameters measured during the study. The upper value is the concentration that produces at least one deleterious effect of the measured parameters (4). A deleterious effect is one that is a statistically significant (P=0.05) reduction from the control for the parameter being measured.

METHODS AND MATERIALS

The biological methods used for this early life stage study with fathead minnow are basically those described in: Recommended Bioassay Procedure for Egg and Fry Stages of Fresh Water Fish (5) and ASTM Standard Practice for Conducting Toxicity Tests on the Early Life Stages of Fishes (4). The study was conducted following the methods outlined in ABC Protocol No. 7809, as approved by A. F. Werner, Monsanto Chemical Company.

I. Test Fish

The fathead minnow eggs used for the initiation of the test were obtained from the U.S.D.I. Columbia National Fisheries Research Laboratory in Columbia, Missouri. These eggs were less than 18 hours old and from adults that had been identified to species using the taxonomic keys developed by Eddy (6). The fathead minnow stock culture as well as the test system was maintained at 25°C (±2°C) with a 16 hour daylight photoperiod. During holding, acclimation and test periods, the fish received a mixed diet of live newly hatched brine shrimp nauplii (Artemia salina) and ground commercial fish food (Rangen's®) at least twice daily ad libitum.

II. Test System

A one-liter proportional diluter system described by Mount and Brungs (7), with the modifications of McAllister et. al (8), was

used for the intermittent introduction of Acrylonitrile to duplicate control and exposure aquaria. Clear 4 mil polyethylene curtains supported by a wooden framework completely enclosed the diluter system. Fumes of Acrylonitrile were vented from under the enclosure to the outside of the laboratory building by means of an exhaust fan. Flow-splitting chambers divided each of the five Acrylonitrile concentrations and one control four ways into duplicate test aquaria with replicate growth chambers in each test aquarium (Figure 2). The glass aquaria measured 35 X 30 X 30 cm with a water depth of 24 cm. Each test aquaria was divided by a glass partition to provide space for two growth chambers which measured 23 X 15 X 30 cm and had stainless steel screening (40 mesh) attached to one end (Figure 1-B). Aerated well water (Table 1) was delivered to the glass aquaria at an average rate of 100 ml/minute/aquarium, an amount which was sufficient to replace the 25 liter test volume at least 5.5 times in a 24 hour period. The test aquaria were immersed in a circulating water bath held at 25°C (±2°C) by electronically controlled submersible heating elements.

The fathead minnow eggs were incubated in cups suspended in the treatment and control water (Figure 1-A). Egg cups were made from 4.5 cm OD glass tubing with stainless steel screening (40 mesh) attached to the bottom with silicon sealer. To insure exchange of water, the egg cups were oscillated in the test solution and/or water by means of a rocker arm apparatus driven by a 4 r.p.m. electric motor (9).

III. Test Compound

The Acrylonitrile standard was received on May 15, 1980, in good condition. The sample upon receipt was observed to be a clear liquid and was stored in the dark at 4°C. The stock solutions were prepared as needed on a weight:volume basis by dissolving in deionized water and were delivered to the diluter from a Mariotte bottle enclosed in aluminum foil. During the study, a new stock solution of Acrylonitrile was prepared every two to four days.

IV. Statistical Analyses

The design of the early life stage study was a complete block design. Measured parameters in the duplicate exposure chambers were analyzed at least once during the study, using two-way analysis of variance with an interaction model to determine if significant differences existed between duplicates (4). If the analysis showed no significant differences (P=0.05) or interactions, duplicate data were pooled. The pooled growth data was subjected to one-way analyses of variance. If analysis showed a significant difference, duplicate data was not pooled and considered as two distinct data sets. When treatment effects were indicated following a significant F-test of the means, a multiple means comparison test, Fisher's protected Least Significant Difference (LSD), was used. Treatment effects on percent survival data were transformed using the following equation (10):

$$\text{Angle} = 2 \arcsin \sqrt{\frac{X}{N}}$$

where, N = number of organisms tested
X = number of organisms hatched or alive.

This was followed by analysis of variance and a least significant difference test. Those concentrations exhibiting decreased survivability were not used in any further ANOVA programs so as not to bias the results. All differences we considered significant at P=0.05 (95% confidence level).

V. Test Procedure - Biological

Before initiating the biological portion of the study, the test solution was allowed to flow through the test aquaria for a 24 hour equilibration period. The test concentration was confirmed by gas chromatographic analysis before introducing the eggs. Days for analytical confirmation (water residue days) were numbered from 0 to 35 beginning with the initiation of the study.

The biological phase of the study was initiated on October 10, 1980, by distributing 50 randomly selected fathead minnow eggs into each of two incubator cups per duplicate exposure auqaria i.e. 200 eggs per concentration. Egg mortality, as discerned by a distinct change in coloration, was recorded daily and dead eggs were removed to prevent fungal growth. When hatching commenced the approximate percent of embryos hatching in each incubation cup was recorded daily until hatching was completed. The date of complete hatch was designated as "day 0" for growth sampling periods (growth days). The eggs were exposed to Acrylonitrile for 5 days before the complete hatch date (Table 3). When hatching was complete, surviving fry were impartially reduced to four groups of 20 fry each per growth chamber. Survival was monitored at least weekly by visually inspecting each growth chamber, and behavioral or physical changes were recorded.

From the beginning of hatch until day 30 of growth, all fry were fed live brine shrimp nauplii in combination with a standard commercial fish food (Rangen's®) 3 to 4 times a day ad libitum. All aquaria were siphoned daily to remove fecal material, excess food and any biological growth on the glass or stainless steel screen. At 30 days post hatch all surviving fish were killed in ice water and immediately measured for standard length, blotted and weighed.

VI. Test Procedure - Chemical and Physical

Nominal concentrations were determined by placing a known stock solution of Acrylonitrile in the Mariotte bottle and allowing the diluter to equilibrate for twenty-four hours. The high concentration was then analytically confirmed. Concentrations were

corrected by adjusting the amount of the toxicant aliquot by raising or lowering the standpipe in the Mariotte bottle. Because the diluter was designed to have a 50 percent dilution factor, serial dilutions of the high concentration were used to estimate all lower nominal concentrations.

Water quality parameters of dissolved oxygen, pH and ammonia were measured on water residue days 0, 1, 5, 10, 15, 20, 30 and 35 during the study. Two hundred milliliter test water samples, collected in 250 ml glass beakers, were taken from the control, low concentration and highest concentration with surviving eggs or fry. Measurements of these selected parameters were determined with a Corning pH-millivolt meter and selective ion probes. Temperature in control chamber 3D was monitored continuously using a remote platinum-resistance sensor and strip chart recorder.

Analysis of all water samples for Acrylonitrile (AN) were accomplished by gas-liquid chromatography as outlined in the Monsanto Industrial Chemicals Company procedure entitled, "Determination of Acrylonitrile in Water for Environmental Fate Studies."

Representative 100 ml volumes of water samples were collected in 100 ml volumetric flasks directly from the test aquaria. If necessary, dilutions were made by adding an appropriate aliquot of the solution to a 100 ml volumetric flask and diluting to volume with deionized water. For an internal standard, 50 µl of a 1.00 mg/ml Propionitrile (PN) stock solution was added to each 100 ml sample solution for GC analysis. GC standards were also prepared in this manner to contain 0.5 µg/ml PN.

Direct aqueous injections of samples and standards were done within 6 hours of sampling. Between sampling and injection, all samples and standards were stored in a refrigerator at 4-10°C. A Varian Model 3700 gas chromatograph equipped with a Thermionic Specific Detector (TSD) was employed with the following parameters:

Column: 20" X 2 mm I.D. stainless steel
Packing: 100/120 mesh Porapak Q (Waters Associates)
Carrier Gas: Helium, 30 ml/minute
Column Temperature: 135°C
Injector Temperature: 170°C
Detector Temperature: 250°C
Detector Settings: Bias Voltage -4
Bead Current 480
H_2 Pressure 10 psi

Linearity was established for each day's injections by calculating a relative response factor (R) for each standard injected using the equation

$$R = \frac{(C_A)(A_p)}{(A_A)(C_p)}$$

where, C_A = concentration of AN in µg/ml,
C_p^A = concentration of PN in µg/ml,
A_p^p = height of AN peak,
and A_p^A = height of PN peak.

Example calculation of R for 0.1 µg/ml AN standard, injected October 6, 1980, 4:10 p.m.:

$$R = \frac{(0.1)(146)}{(46)(0.5)} = 0.635$$

An average relative response factor (R_{ave}) was calculated for each set of standards by averaging the R obtained for all standards in that set. The concentrations of AN in the samples were calculated by using the equation:

$$C_A = \frac{(R_{ave})(A_A)(C_p)(\text{Dilution Factor})}{(A_p)}$$

where, C_a = concentration of AN in the sample in µg/ml,
R_{ave} = average relative response factor,
A_A^A = peak height of AN,
C_p^A = concentration of PN in the sample in µg/ml
and A_p^p = peak height of PN.

Example calculation of C_A for Treatment Level 5, Day 5, 10/11/80, 2:09 p.m. injection:

$$C_A = \frac{(0.589)(73)(0.5)(20)}{(116)} = 3.71 \text{ µg/ml AN.}$$

Accuracy of the method was determined by recoveries of AN spiked into control aquarium water. Analysis of spiking levels of 0.25 µg/ml AN and 4.0 µg/ml AN, and blind spikes yielded average recoveries of 106%, 104% and 92.5%, respectively.

Precision of the method was determined by analyzing triplicates of the high fortification and treatment level 3 for each sample day. Precision results are included in the raw data.

Because AN residues were present in some control samples, a reagent blank of well water was included with each set. The concentration of AN in the controls were calculated although it should be noted that the response for these residues were lower than that of the standard range.

RESULTS

The test concentrations of Acrylonitrile were determined on water residue days 0, 1, 5, 10, 20, 30 and 35 through the use of gas chromatographic methods outlined in the text of this report. The results are summarized in Table 4. The mean measured concentrations of Acrylonitrile were: 0.34 mg/l, 0.44 mg/l, 0.86 mg/l, 1.8 mg/l and

3.6 mg/l. These values represented 136%, 88%, 86%, 90% and 90% of the expected nominal values, respectively. The Acrylonitrile stock was analyzed at each water residue sample day and results tabulated in Table 5. The mean stock concentration was 70% of the expected nominal concentration. The difference was attributed to the volatility of Acrylonitrile. The measured stock concentration remained consistent throughout the study.

Low levels of Acrylonitrile were found to be present in the control water throughout the first ten days of the study. Again, the volatility of the compound resulted in contamination of the control water due to agitation during the dilution cycle. To remedy this situation, the control water portion of the diluter face was separated from the toxicant mixing area of the proportional diluter and the flow splitting box was covered with polyethylene plastic. As a result, levels of Acrylonitrile in the control water steadily declined from water residue days 1 to 20. Levels of Acrylonitrile in the control water remained below the detectable limit from water residue day 20 until termination of the study.

Water quality parameters of dissolved oxygen, pH and ammonia were measured in the control, low concentration and highest concentration with surviving fish on water residue days 0, 1, 5, 10, 15, 20, 25, 30 and 35 during the study (Table 6). The dissolved oxygen concentrations ranged from 5.4 mg/l to 8.3 mg/l which represents 64 to 99% saturation and was considered adequate for testing (11). The ammonia concentrations remained constant and consistent with the control. Temperature, which was monitored continuously in growth chamber 3D, did not fluctuate by more than ±1°C in any 24 hour period.

Mean percentage hatch in the control aquaria was 69 while the percentages in the exposure aquaria ranged between 46 and 87 (Table 7 and Figure 3). There was no apparent difference between the appearance of the control eggs and those continuously exposed to Acrylonitrile. A multiple means comparison was used for the parameter of percentage hatch but, because the incidence of fungus in various egg cups was not uniform in frequency, hatchability would not be a reliable indicator of toxicant effect.

The survivability of fathead minnow fry exposed to 0.86 mg/l, 1.8 mg/l and 3.6 mg/l of Acrylonitrile was significantly lower than the control after 30 days (Table 7 and Figure 3). There was no abnormal behavior observed prior to death of the fry which is an unusual characteristic which may be peculiar to Acrylonitrile. Survival of fathead minnows at concentrations less than 0.86 mg/l was not significantly affected but scattered mortality in all concentrations except the control was observed during the study. To eliminate disease as a cause of observed mortality, one fathead minnow fry was removed on November 6, 1980, from the 0.86 mg/l exposure for disease investigation. Pathological examination revealed that no disease was present. Results of this investigation have been included in the raw data.

As seen in Figure 2, each test tanks is composed of four chambers, A, B, C and D with A and B (AB) being distinct from C and D (CD). In the comparison of growth, as measured by length and weight between the

control and treated aquaria, a significant difference was revealed. The analysis of duplicate chambers AB showed that all concentrations significantly decreased growth, as measured by standard length and weight, of the fathead minnow fry after 30 days of exposure (Table 7 and Figures 5, 6). However, the analysis of duplicate chambers CD showed that 0.34 mg/l of Acrylonitrile did not significantly decrease growth (Table 7 and Figures 5, 6). Neither of the above programs included the concentrations 0.86 mg/l, 1.8 mg/l or 3.6 mg/l in the analyses because the percent survival at these levels was found to be significantly reduced.

Subsequent to the typical analysis of variance mentioned above, the same programs were run with one major change. The analysis was run comparing the exposure concentrations from one duplicate to the control of the other duplicate. For example, an analysis of growth variance as measured by standard length, was done comparing exposure concentrations of duplicate CD to the AB control. It turned out that this variation made little difference in the results as already presented.

Based on the data for this 30 day fathead minnow early life stage study the Maximum Acceptable Toxicant Concentration (MATC) limits as defined cannot be estimated for Acrylonitrile. But the results of growth and length analysis for the low concentration of 0.34 mg/l gave a significant difference in the AB chambers and no significant difference in the CD chambers. It is our opinion that the low concentration of 0.34 mg/l would be an estimate of the upper limit of the MATC at 30 days of exposure to Acrylonitrile.

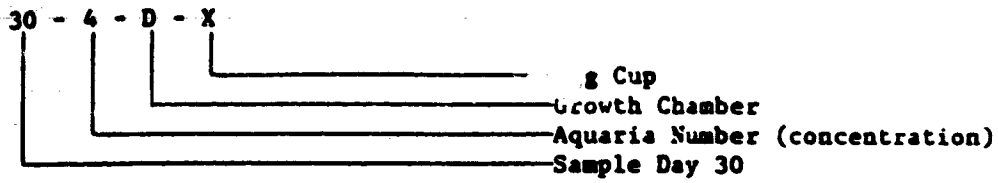
The study was conducted following the intent of the Good Laboratory Practice Regulations (13) and the final report was reviewed by Analytical BioChemistry Laboratories' Quality Assurance Unit. Original raw data was provided to Monsanto Chemical Company, with a copy retained at Analytical BioChemistry Laboratories.

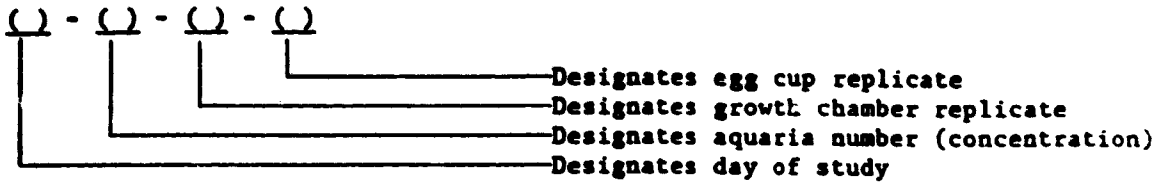
TABLE 1: Chemical characteristics of well water at ABC's Aquatic Bioassay Laboratory.

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	0.0157 ppm
Zinc	<0.01 ppm
Measured organophosphorus pesticides	[a]
Measured organochlorine pesticides plus PCB's	[a]

[a] See Raw Data Report for individual analyses.

TABLE 2: Tank code example.





Day of Study

0, 1, 15, 30, 60
(These numbers are not in the code for daily observations.)

Aquaria Identification

Control (1), 2, 3, 4, 5, 6

Growth Chamber

A, B, C, D

Egg Cups

X, Y
(These letters are not in the code after the eggs are removed from cups.)

TABLE 3: Hatching success of fathead minnow eggs continuously exposed to Acrylonitrile.

Egg Cup Code	No. eggs per cup	Day 1 Cumulative Hatch	Day 2 Cumulative Hatch	Day 3 Cumulative Hatch (Approximate %)	Day 4 Cumulative Hatch (Approximate %)	Day 5 Cumulative Hatch (Approximate %)
Control-B-X	50	--	--	5	95	100[b]
B-Y	50	--	--	10	90	100
D-X	50	--	--	2	95	100
D-Y	50	--	--	6	95	100
2-A-X	50	--	--	2	90	100
A-Y	50	--	--	2	90	100
C-X	50	--	--	--	99	100
C-Y	50	--	--	--	95	100
3-A-X	50	--	--	--	95	100
A-Y	50	--	--	--	60	100
C-X	50	--	--	2	90	100
C-Y	50	--	--	10	95	100
4-A-X	50	--	--	6	95	100
A-Y	50	--	--	--	90	100
C-X	50	--	--	20	90	100
C-Y	50	--	--	2	80	100
5-B-X	50	--	--	--	90	100
B-Y	50	--	--	4	80	100
D-X	50	--	--	2	60	100
D-Y	50	--	--	1	95	100
6-B-X	50	--	--	0	60	100
B-Y	50	--	--	2	60	100
D-X	50	--	--	2	60	100
D-Y	50	--	--	0	50	100

[a]Day 5 is the date of complete hatch.

[b]100 percent hatch values indicate that all of the eggs which are capable of hatching have hatched.

NOTE: See Table 2 for description of egg cup and tank code.

TABLE 4: Concentrations of Acrylonitrile during the 30 day early life stage study with fathead minnows.

Aquaria No.	Nominal Concentration (mg/l)	Measured Concentration (mg/l) Day 0	Day 1A	Day 1B	Day 5	Day 10	Day 20	Day 30	Day 30	Mean (±S.D.) or (% recovery)
Control	---	0.045	0.037	0.041	0.023	0.016	<0.015	<0.012	0.020	<0.026 (±0.013)
Level #1	0.25	0.32	0.38	0.21	0.21	0.38	0.40	0.40	0.39	0.34 (±0.082)
Level #2	0.5	0.39	0.45	0.45	0.45	0.44	0.50	0.41	0.41	0.44 (±0.034)
Level #3a	1.0	0.79	0.94	0.77	0.80	0.87	1.0	0.74	0.94	0.86 (±0.095)
b	1.0	0.82	0.94	0.78	0.80	0.89	1.0	0.58	0.96	0.85 (±0.13)
c	1.0	0.82	0.93	0.78	0.82	0.89	1.1	0.75	0.95	0.88 (±0.11)
Level #4	2.0	1.4	1.6	1.8	2.1	1.3	2.4	2.0	2.1	1.8 (±0.38)
Level #5	4.0	3.0[b]	3.4	3.5	3.7	3.3	3.3	4.0	4.3	3.6 (±0.42)
Low Spike	0.25	0.26 (104%)	0.27 (108%)	0.27 (108%)	0.26 (104%)	0.27 (108%)	0.26 (104%)	0.26 (104%)	0.27 (108%)	0.27 (±0.0053)
Blind Spike	[a]	0.89 (89%)	0.38 (84%)	0.82 (90%)	0.60 (100%)	0.49 (82%)	0.34 (97%)	1.1 (110%)	2.3 (92%)	---
High Spike a	4.0	4.1 (103%)	4.0 (100%)	4.1 (103%)	4.2 (105%)	4.1 (103%)	4.0 (100%)	3.8 (95%)	5.5 (138%)	4.2 (105%)
b	4.0	4.1 (103%)	4.0 (100%)	4.1 (103%)	4.0 (100%)	4.1 (103%)	4.1 (103%)	3.9 (98%)	4.2 (105%)	4.2 (103%)
c	4.0	4.3 (108%)	4.1 (103%)	4.2 (105%)	4.1 (103%)	4.1 (103%)	4.2 (105%)	4.2 (105%)	4.4 (110%)	4.2 (105%)
Reagent Blank	---	<0.011	<0.011	<0.010	<0.013	<0.013	<0.015	<0.012	<0.013	<0.012

[a] The following nominal concentrations were used for blind spikes; 1.0, 0.45, 0.91, 0.60, 0.60, 0.35, 1.0 and 25 mg/l, respectively for the above sample days.

[b] Percent recovery are included in the parenthesis. The average recoveries for the high, low and blind spike was 104%, 108% and 93%, respectively.

TABLE 5: Concentrations of Acrylonitrile in stock solutions during the 30 day early life stage study with fathead minnows.

Water Residue Day/Date	Acrylonitrile (mg/ml)	Percent of Nominal Concentration
0 (10/6/80)	5.21	54
1A (10/7/80)	6.56	68
1B (10/7/80)	6.64	69
5 (10/11/80)	6.80	71
10 (10/16/80)	6.39	67
20 (10/26/80)	7.14	74
30 (11/5/80)	7.91	82
35 (11/10/80)	7.28	76
Mean	6.74	70
Standard Deviation	0.79	8.2

Nominal Stock Concentration = 9.6 mg/ml.

TABLE 6: Water quality measurements during the Acrylonitrile early life stage study with fathead minnows.

	Water Quality											
	Control				Low Concentration				High Concentration[e]			
Study Day	Temp.[a] °C	D.O.[b] mg/l	pH[c]	NH_3[d] mg/l	Temp.[a] °C	D.O.[b] mg/l	pH[c]	NH_3[d] mg/l	Temp.[a] °C	D.O.[b] mg/l	pH[c]	NH_3[d] mg/l
0	25	7.9	7.9	0.39	25	7.8	7.9	0.40	25	7.9	8.0	0.38
1	24	8.2	7.9	0.40	24	8.2	7.9	0.40	24	8.3	8.0	0.38
5	25	8.0	8.0	0.45	25	8.0	8.0	0.50	25	8.1	8.0	0.40
10	24	6.2	7.9	0.36	24	7.2	8.0	0.40	24	7.4	8.1	0.38
15	25	7.5	7.9	0.35	25	7.5	7.9	0.40	25	7.9	8.0	0.45
20	25	7.3	8.0	0.25	25	7.0	8.0	0.20	25	7.0	8.0	0.54
25	25	6.8	7.9	0.22	25	6.6	7.9	0.28	25	6.7	7.9	0.30
30	25	6.3	7.8	1.0	25	6.0	7.8	0.70	25	5.4	7.8	1.0
35	24	7.9	7.8	0.45	24	7.6	7.8	0.39	24	7.3	7.8	0.64

[a]Temperature - Monitored continuously using a remote platinum resistance sensor and strip chart recorder.

[b]Dissolved oxygen concentrations - Dissolved Oxygen Probe used with a YSI Model 54A Dissolved Oyxgen System.

[c]pH - pH Probe (Corning Model 476022) used with a Corning Model 125 pH and mV meter.

[d]Ammonia concentrations - Ammonia Probe (Extech Model 8002-8) used with a Corning Model 125 pH and mV meter.

[e]Highest concentration with surviving fry.

TABLE 7: Mean percentage hatch of eggs, mean survival, standard lengths and wet weights of fathead minnow fry continuously exposed to Acrylonitrile.

Aquaria Identification	Mean Measured Concentration of Acrylonitrile (mg/l)	Mean Hatch (%)	Survival (%)	Mean Standard Length[a] (mm) AB	Mean Standard Length (mm) CD	Mean Wet Weight (g) AB	Mean Wet Weight (g) CD
Control	<0.026	69	81	22.2[b] (±1.98)	21.2 (±1.5)	0.21 (±0.052)	0.19 (±0.038)
Level #1	0.34	87*	75	20.5 (±1.72)*	21.3 (±1.5)	0.16 (±0.042)*	0.19 (±0.040)
Level #2	0.44	62	85	20.8 (±1.33)*	20.1 (±1.6)*	0.17 (±0.36)*	0.15 (±0.039)*
Level #3	0.8	69	54*	19.8[c] (±0.85)	20.1[c] (±1.1)	0.12[c] (±0.018)	0.15[c] (±0.027)
Level #4	1.8	62	15*	18.2[c] (±1.59)	---	0.098[c] (±0.034)	--
Level #5	3.6	46*	0*	---	---	---	---

[a]Standard length is measured from tip of head to the base of the caudal fin.

[b]Standard deviations are shown in parentheses.

[c]Values not included in multiple means comparison.

*Denotes values significantly different (P=0.05) from the control using one way analysis of variance (ANOVA) and Fisher's protected Least Significant Difference (LSD) multiple means comparison.

FIGURE 1: Photographs of test system with egg incubation cups and growth chambers.

A

B

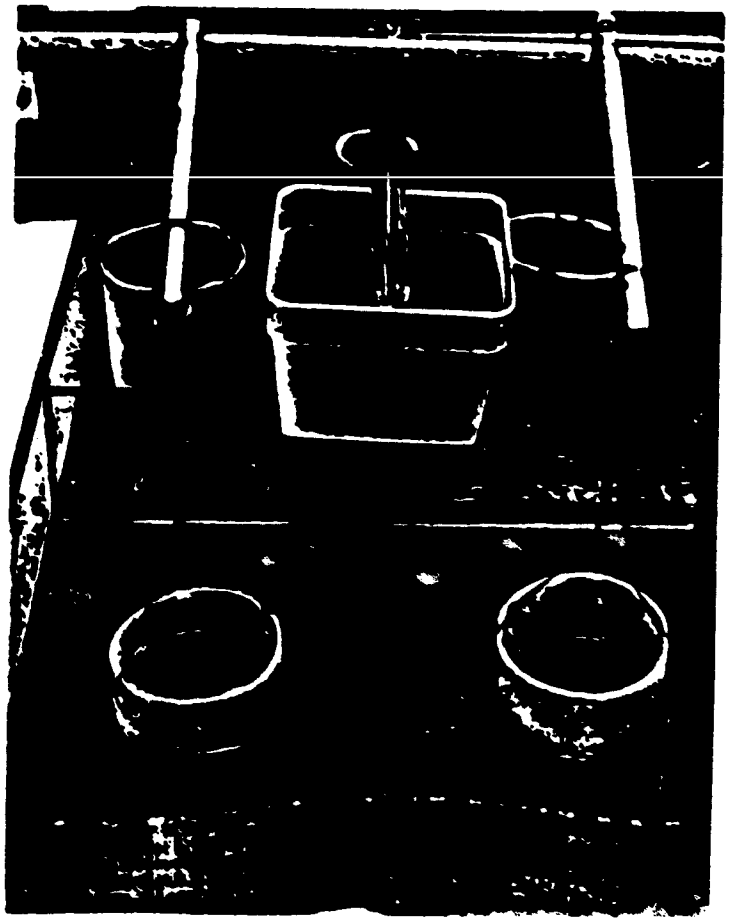


FIGURE 2: Early life stage toxicity testing system schematic and terminology.

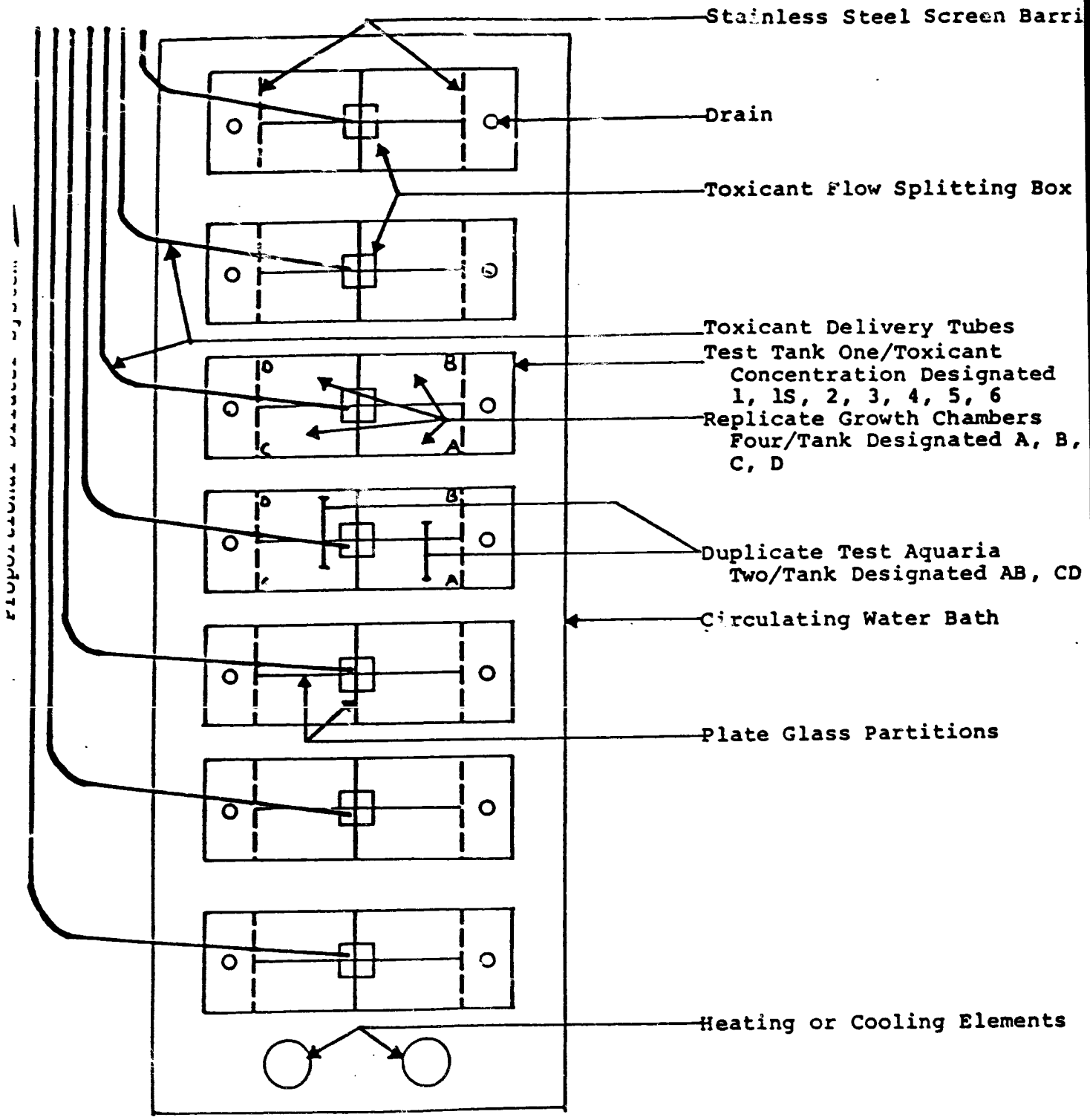


FIGURE 3: Mean percent egg hatch and mean percent survival of fathead minnows exposed to Acrylonitrile.

▨ Denotes values significantly different (P=0.05) from the control.

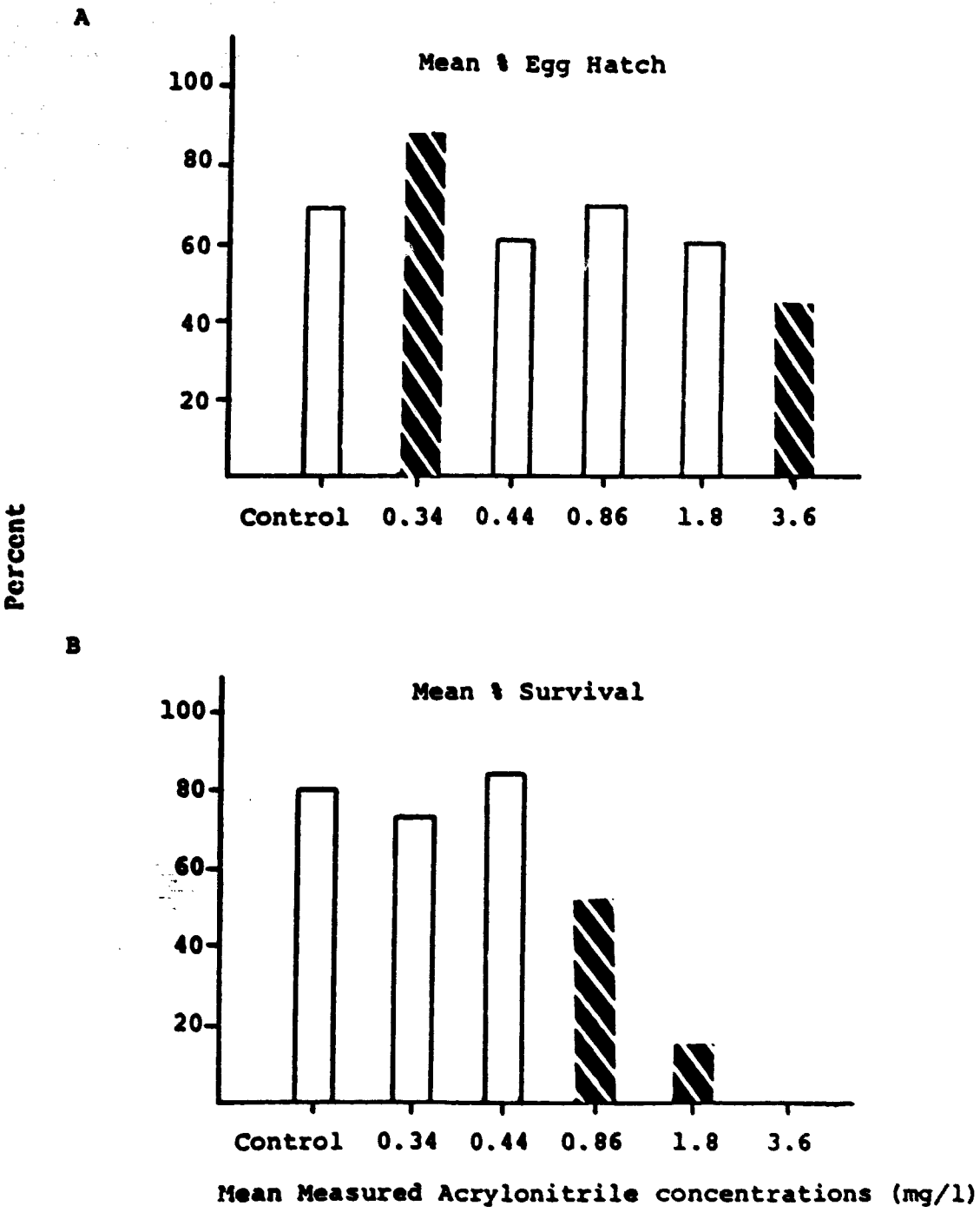
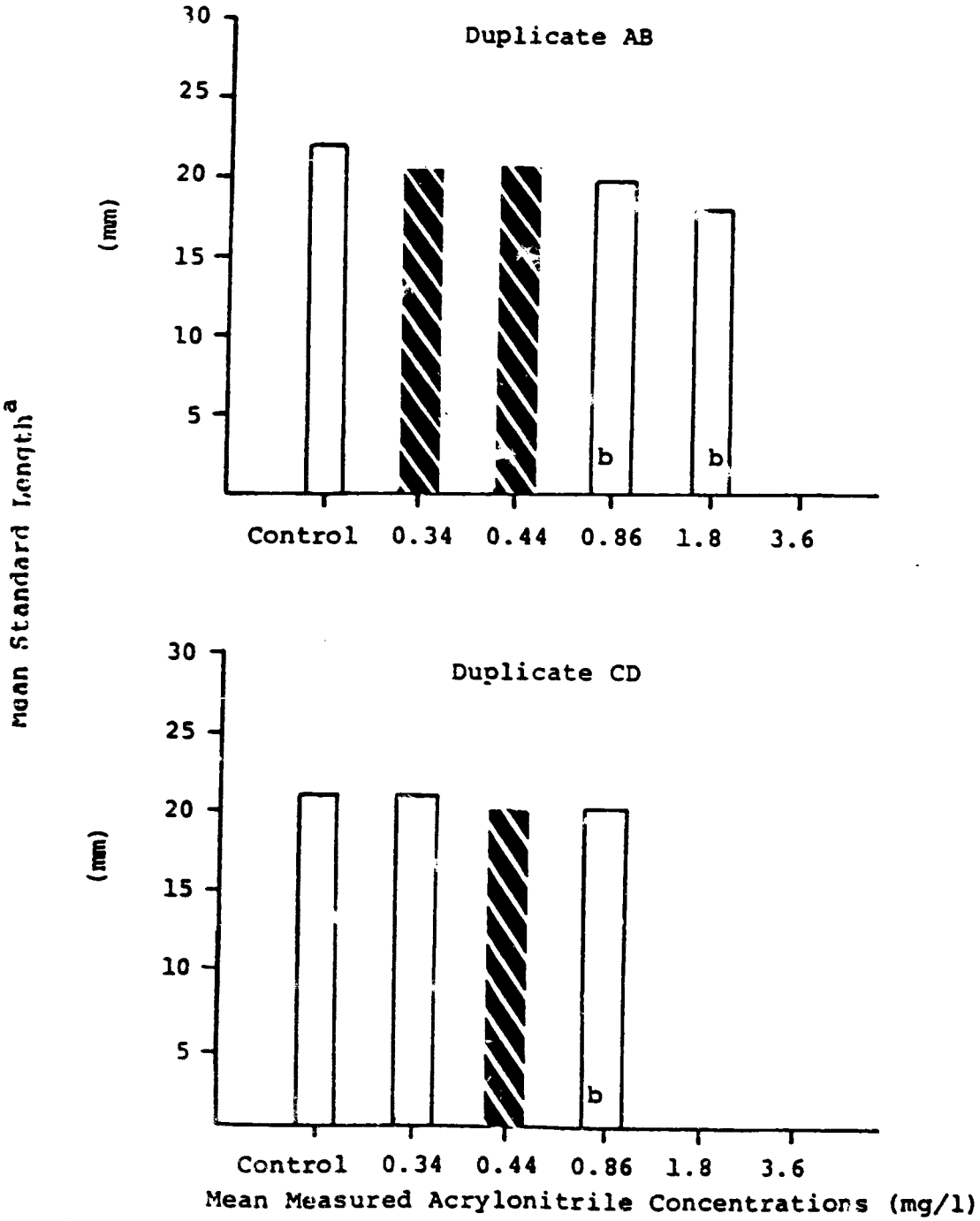


FIGURE 4: Mean standard length of fathead minnow frv after 30 days of exposure to Acrylonitrile.

Denotes values significantly (P=0.05) different from the control.

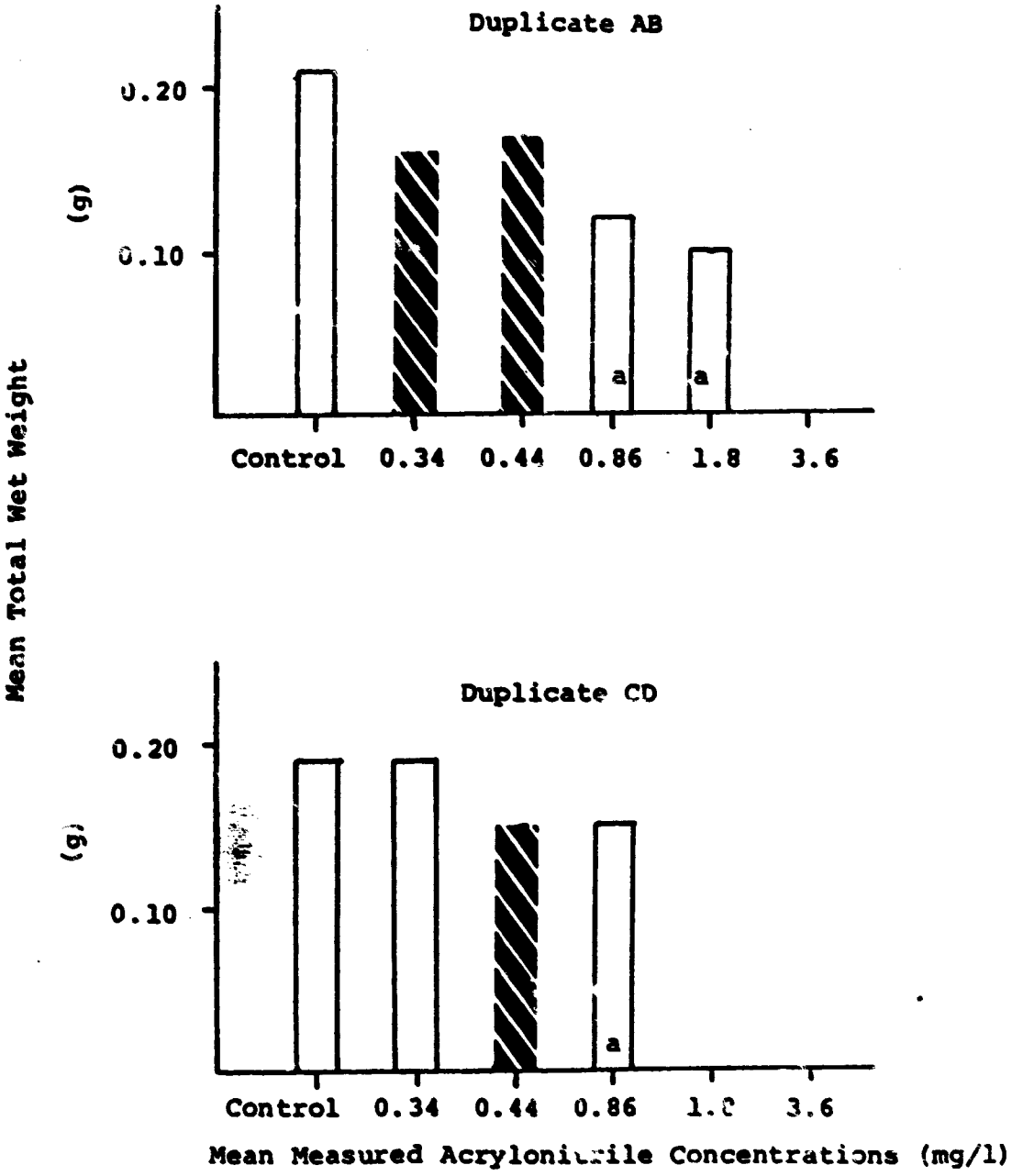


[a]Standard length is from the apex of the head to the caudal peduncle.

[b]Values not included in multiple means comparison.

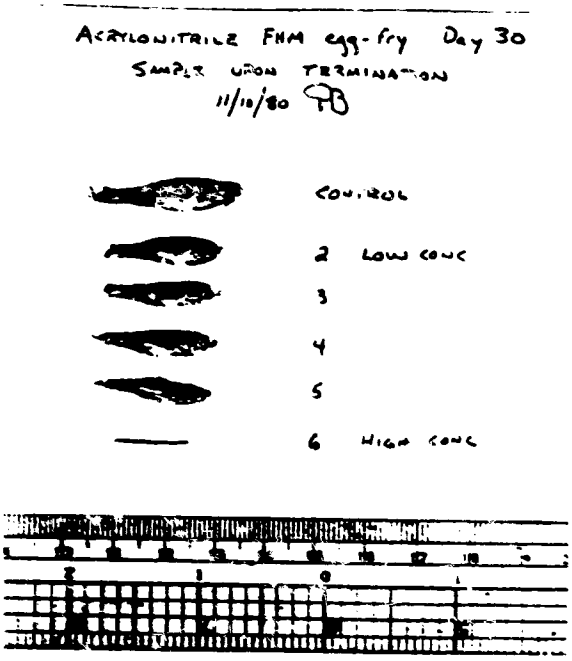
FIGURE 5: Mean total wet weight of fathead minnow fry after 30 days of exposure to Acrylonitrile.

▨ Denotes values significantly different (P=0.05) from the control.



[a]Values not included in multiple means comparison.

FIGURE 6: A comparison of growth between control and treated groups of fathead minnows after 30 days of exposure to Acrylonitrile.



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(13) Food and Drug Administration. 1978. Regulations for Good Laboratory Practice in Non-clinical Animal Studies, 21 CFR Part 58.

Quality Assurance Statement for Final Report #25673 entitled, "Early Life Stage Toxicity of Acrylonitrile to Fathead Minnows (Pimephales promelas) in a Flow-Through System," for Ms. A. F. Werner, Monsanto Chemical Company, St. Louis, Missouri.

In accordance with ABC Laboratories intent that all studies conducted by our facilities meet or exceed the criteria promulgated by the Good Laboratory Practice regulations for Non-clinical Laboratory Studies (21 CFR, Part 58) to assure adherence to protocol specifications and methods, the above named report was reviewed by a member of our Quality Assurance Unit.

In-progress study inspections were conducted on October 8, 10, 13, 17, 20, 22, 24, 27, 31 and on November 3, 1980.

A final inspection of all data and records on November 20, 1980, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by the Aquatic Toxicology Division of ABC Laboratories, Inc.

If you should have any questions concerning this statement or the function of our Quality Assurance Unit, please contact me at your convenience.

James A. Ault 12/1/80

James A. Ault — Date

Quality Assurance Officer

C930.005

JOHN D. WALKER

Static Acute Bioassay Report
#25555

Submitted To:

Monsanto Industrial Chemicals Co., N2A
Attn: Ms. A. F. Werner
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

AB-80-540

Acute Toxicity of Acrylonitrile to
Rainbow Trout (Salmo gairdneri)

June 18, 1980

Submitted By: Analytical BioChemistry Laboratories, Inc.
7200 East ABC Lane
P. O. Box 1097
Columbia, Missouri 65205

Prepared By:

Carl M. Thompson 6/20/80
Carl M. Thompson Date
Aquatic Supervisor

Jerry Griffen (CT) 6/20/80
Jerry Griffen Date
Biologist

Approved By:

James A. Ault 6/20/80
James A. Ault Date
Quality Assurance Officer

Lyle D. Johnson 6-20-80
Lyle D. Johnson Date
Laboratory Manager

SUMMARY

The acute toxicity of Acrylonitrile to rainbow trout (Salmo gairdneri) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1). Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.

As a quality check, the rainbow trout were challenged with a reference compound, Antimycin A. The observed 96 hour LC_{50} and 95% confidence limits (C.I.) were within the 95% confidence limits reported in the literature (2), indicating that the fish were in good condition.

The results of the four day static fish toxicity study using rainbow trout are summarized below. The 24 and 48 hour LC_{50} values were also determined and are given in Table 2.

Compound	96-hour LC_{50} (95% C.I.)
Acrylonitrile	24 mg/l (18-32 mg/l)
Antimycin A	0.000018 mg/l (0.000014-0.000022 mg/l)

Also, the results indicated a 96 hour, no observed effect concentration of 10 mg/l.

INTRODUCTION

This static bioassay was performed at the aquatic bioassay laboratory of Analytical BioChemistry Laboratories, Inc., Columbia, Missouri, for Monsanto Industrial Chemicals Co., from June 12 to June 16, 1980, as authorized in a letter from Monsanto Chemicals Co. on May 2, 1980 (Appendix I). The purpose of this test was to determine the 24, 48 and 96 hour LC_{50} levels for Acrylonitrile to rainbow trout (Salmo gairdneri). A preliminary range-finding study was conducted from June 9 to June 12, 1980, to determine the concentration range for the definitive bioassay. The study was performed following the procedures outlined in ABC Protocol Number 7601 (Appendix I), as approved by Ms. A. F. Werner, Monsanto Cemicals Co., on June 12, 1980.

METHODS AND MATERIALS

The procedures for static bioassay, as described in Standard Methods for Examination of Water and Wastewater (3) and Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1), were used in this experiment. The rainbow trout used in the test were obtained from Beitey's Resort in Valley, Washington. The fish were identified to species using the taxonomic keys developed by Eddy (4). All test fish were held in culture tanks on a 16 hour daylight photoperiod and observed for at least fourteen days prior to testing. Fish culture techniques used were basically those described by Brauhn et. al. (5). A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in Appendix I. During this period, the fish received a standard commercial fish food (Rangen's) daily until 48 hours prior to testing at which time feeding was discontinued. The rainbow trout used for this experiment had a mean weight of 0.43 g and a mean standard length of 32.9 mm. Weight and length measurements were made on the control group of fish at the termination of the test and are included in Appendix I.

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconsituted water composed of the following compounds in the amounts stated per liter of deionized water:

48 mg $NaHCO_3$
30 mg $CaSO_4$
30 mg $MgSO_4$
2 mg KCl

The water parameters of this dilution water were, dissolved oxygen: 8.6 mg/l; pH: 7.1; total hardness: 45 mg/l $CaCO_3$; and total alkalinity: 35 mg/l $CaCO_3$. The well water source from which this dilution water was prepared had the characteristics shown in Table 1.

These vessels were kept in a water bath at 12°C (±1.0). The test fish were acclimated to the dilution water and test temperature and held without food 48 hours prior to testing.

A 72 hour range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 3.2, 32 and 56 mg/l. Based on the results of preliminary testing, six concentrations of the test compound, ranging in a logarithmic series from 5.6 to 100 mg/l, with ten fish per concentration were selected for definitive bioassay. The fish were added to the test chambers by random assignment within 30 minutes after addition of toxicant aliquots.

The Acrylonitrile standard was received on May 15, 1980, in good condition. The sample upon receipt was observed to be a clear liquid and was stored at room temperature (20°C). Sample purity was listed as 99%. Test concentrations were prepared based on the total compound. The test concentrations were obtained by transferring appropriate aliquots of a working solution directly to the test chambers. The working solution was prepared in deionized water. All standard weights and dilution values are listed in Appendix I.

RESULTS

Table 2 presents the predicted LC_{50} values and 95% confidence intervals for Acrylonitrile and the reference test against Antimycin A, a piscicide. These values were obtained by employing a computerized LC_{50} program developed by Stephan et. al. (10). Mortality rates, test concentrations and water quality data are presented in Table 3.

The dissolved oxygen concentration which stayed between 60 and 100% saturation was considered adequate for testing. The pH values remained consistent with the control throughout the study. The ammonia concentrations were below the toxic limit (7).

The study was conducted following the intent of the Good Laboratory Practice Regulations (8) and the final report was reviewed by Analytical BioChemistry Laboratories' Quality Assurance Unit. All original raw data was provided to Monsanto Industrial Chemicals Co., 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	0.0157 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 and Antimycin A to Rainbow Trout (*Salmo gairdneri*)*

Compound	LC_{50} in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
Acrylonitrile	64 (32-100)**	42 (32-56)	24 (18-32)
Antimycin A***	0.000070 (0.000051-0.000097)	0.000032 (0.000027-0.000038)	0.000018 (0.000014-0.000022)

*Bioassay as conducted at 12°C (±1.0), mean weight and length, 0.43 g and 32.9 mm.

**95% confidence interval.

***Antimycin A standard obtained from Sigma Chemical Company, Type III, crystalline, Lot 125C-0152.

TABLE 3

Mortality Rates and Water Quality Measurements During the Acute Toxicity Test of Acrylonitrile to Rainbow Trout (Salmo gairdneri)

mg/l Concentration	Percent Mortality Hours			Water Quality											
				0 hours				48 hours				96 hours			
	24	48	96	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	0	0	0	12	8.6	7.1	<0.10	12	9.0	7.1	---	12	8.6	7.1	<0.10
5.6	0	0	0	12	8.6	7.1	<0.10	12	9.1	7.1	---	12	8.6	7.0	<0.10
10	0	0	0												
18	0	0	0												
32	0	0	100					12	8.8	7.1	---	12	8.5	6.9	<0.10
56	30	100	100												
100	100	100	100	12	8.7	7.2	<0.10								

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

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

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

LITERATURE CITED

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

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Quality Assurance Statement for final report #25555 entitled, "Acute Toxicity of Acrylonitrile to Rainbow Trout (Salmo gairdneri)," for Ms. A. F. Werner, Monsanto Industrial Chemicals Co., 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 an acceptable form by a member of our Quality Assurance Unit. An inspection of the daily mortality rate of the test organisms prior to the initiation of the study indicated they were in good health and should not bias the observed mortality in the study. A final inspection of all data and records on June 19, 1980, indicated 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 6/20/80

James A. Ault Date
Quality Assurance Officer

APPENDIX I
RAW DATA

P O Box 1097 • Columbia MO 65201 • (314) 474-8579

March 21, 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	
HC	<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
δBHC	<0.4	<0.4	<0.4
hlordane		7.3	6.8
Heptachlor	<0.4	<0.4	<0.4
Heptachlor Epoxide	<0.8	---	<0.8
αChlordane	7.7	7.3	6.8
βChlordane	<0.8	<0.8	<0.8
Tech Chlordane	<2	<2	<2
ieldrin + Aldrin	<1	<1	<1
Aldrin	<0.4	<0.4	<0.4
Dieldrin	<1	<1	<1
DT + 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
drin	<2	<2	<2
ptachlor	<0.4	<0.4	0.66
ptachlor Epoxide	<0.8	2.3	<0.8
ndane	<0.4	<0.4	<0.4
thoxychlor	<8	**	**
xaphene	<50	<50	<50
B	<30	<30	<30
Aroclor 1242	<30	<30	<30
Aroclor 1016	<30	<30	<30
Aroclor 1254	<30	<30	<30
Aroclor 1260	<30	<30	<30
B	0.37	0.76	0.73
rex	<4	<4	<4

ndicates less than, if present at all.
ry strong response observed at retention time
rresponding to diethyl hexylphthalate.
Occlu by DEHP response.

Gary Brockhart
Residue Supervisor

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 Brookhart
Gary L. Brookhart
Residue Supervisor

<indicates minimum detectable amount.

ANALYTICAL BIOCHEMISTRY LABS

AQUATIC BIOASSAY LAB

TEST FISH MEASUREMENTS

Test: Acrylonitrile 96 hr Static

Test Species: Rainbow Trout Lot No. 2180

Source: Beiter's Resort Date Measured 6/16/80

Group Measured: Control group at test termination

Fish No.	Standard Length (mm)	Weight (g)
1	32	0.41
2	33	0.46
3	31	0.40
4	35	0.48
5	32	0.39
6	36	0.56
7	33	0.44
8	33	0.41
9	32	0.35
10	32	0.36
Mean Standard Length (mm)	32.9 ± 1.52	
Mean Weight (g)		0.43 ± 0.06

Remarks:

Prepared By: [signature] Checked By: [signature]

Species: Rainbow trout Date Rec'd: 5/1/80 Time:

Source: Beiter's Resort No. Rec.: 2000 Tank#: 1

Designated Use: Brassay

Comments:

Daily Record

Date	#Dead	Comments	Name	Date	#Dead	Comments	Name
4/4	0	Fed Feed					
5/5	0	"					
6/6	0	"					
7/7	0	"					
8/8	0	"					
9/9	0	"					
10/10	0	"					
11/11	1	"					
12/12	0	"					
13/13	0	"					
14/14	0	"					
15/15	0	"					
16/16	0	"					
17/17	0	"					

Checked by Carl G. Hunter

Species: Rainbow trout Eggs Date Rec: 5/1/80 Lot #:

Source: Deltegs Resort No. Rec: 20000 Tank #:

Designated Use: Bioassay

Comments:

Size and Disposition Record

Date	Length (mm)	Weight (gm)	# Used	Name	Date	Length (mm)	Weight (gm)	# Used	Name

Treatment Record

Date	Drug Used	Conc.	Time	Results	Name

Daily Record

Date	#Dead	Comments	Name	Date	#Dead	Comments	Name
5/2	212	11 C° from 12°		5/19	0	Fed 4 x	
3	641			5/20	16	"	
4	57	13°		5/21	2	"	
5	63	Starting to hatch		5/22	14	"	
6	42	75% hatched		5/23	16	"	
7	49			5/24	3	"	
8	31			5/26	0	"	
9	20			5/27	8	"	
10	18			5/28	0	"	
11	54			5/29	0	"	
12	43			5/30	2	"	
13	42			5/31	0	Fed 4, 2, 4	
14	20			6/1	0	Fed 4 x 4	
15	24			6/2	0	"	
17	12			6/3	0	"	

5/16 Moved to aquaria

Checked by

Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant: Acrylonitrile Test Species Rainbow Trout (Lot # 2160) Study No. 25535-

Date Initiated 6/12/80 Time 10 a.m. Date Terminated 6/16/80

Dilution Water Reconstituted H_2O No./Vessel 10 Vessel Size 15 L

Test Conc. mg/l (ppm)	MORTALITY AND BEHAVIORAL OBSERVATIONS									
	24 hr.		48 hr.		72 hr.		96 hr.			
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	0	N	0	N	0	N	0			
5.6	0	N	0	N	0	N	0			
10.0	0	N	0	N	0	N	0			
18.0	0	N	0	N	0	N	0	2 SUR		
32.0	0	N	0	8 SOUN 8 DIS	4	6 LOE	10			
56.0	3	LOE	10		10		10			
100.0	10		10		10		10			
Observer			CT				CT			
Date	6/13		6/14		6/15		6/16			

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

DIS = discoloration

SOUN = Sounding

Prepared By: [signature] Checked By: [signature]

Acrylonitrile 96 hr Static

Species Rainbow Trout (Lot # 2160)

Prepared By [signature]

Checked By [signature]

Water Quality

Concentration mg/l	6/12 Day 0 Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	PB Day 48hr Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	6/14 Day 2 Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Day Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l
Control	R	8.6	7.1	<.1	12	9.0	7.1	–	12	8.6	~~6.7~~ 7.1	<.1				
5.6	12	8.6	7.1	<.1	12	9.1	7.1	–	12	8.6	7.00	<.1				
10.0																
18.0																
32.0					12	4.8	7.1	–	12	5.5	6.9	<.1				
56.0	12	5.7	7.2													
100.0	12	5.7	7.2	<.1												

* PB entry error 6/12

* entry error PB 6/14

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

H - 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 671 pH and mV
er.

Compound: Acrylonitrile Lot No. _____ Purity 3 Lab No. 25[illegible]

Preparation of Concentrated Working Standard

Date 6/12/80 Chemist [signature]

Final Gross Weight 3.75 g Dilution Volume 250 (DW H_2O) ml

Tare Weight 0.00 g Concentration 15 mg/ml

Net Weight 3.75 g Balance calibrated with class S weights:

Adj. Net Weight 3.75 g* 1.00 g (class S) + 0.00 g (tare) = 1.00 g (final wt.)

Preparation of Test Concentrations

Test Definitive RBT Date 6/12/80 Chemist [signature]

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control		-	15	-
1.	15	5.6	15	5.6
2.	15	10.0	15	10.0
3.	15	18.0	15	18.0
4.	15	32.0	15	32.0
5.	15	56.0	15	56.0
6.	15	100.0	15	100.0
7.				

Preparation of Concentrated Working Standard

Date _____ Chemist _____

Final Gross Weight _____ g Dilution Volume _____ ml

Tare Weight _____ g Concentration _____ mg/ml

Net Weight _____ g Balance calibrated with class S weights:

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

Preparation of Test Concentrations

Test _____ Date _____ Chemist _____

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control				
1.				
2.				
3.				
4.				
5.				
6.				
7.				

Remarks: _____

Prepared By: [signature] Checked By: [signature]

*corrected for purity of primary standard.

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

Preliminary

ACUTE TOXICITY BIOASSAY

Toxicant Acrylonitrile Test Species Rainbow Trout (Lot # 2160) Study No. 25-5-53

Date Initiated 6/10/80 Time 4:00 pm Date Terminated 6/18/80

Dilution Water Recon H2O No./Vessel 9 Vessel Size 5-2

Test Conc. mg/l (ppm)	MORTALITY AND BEHAVIORAL OBSERVATIONS									
	24 hr.		48 hr.		72 hr.		96 hr.			
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control										
56	1	NOE some spinal curvature	5-							
Observer	[illegible]	[illegible]	[illegible]							
Date	6/11	6/11	6/12							

Remarks: _____

Prepared By: [signature] Checked By: [signature]

Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Preliminary

Toxicant Perylenimide Test Species Rainbow Trout (Lot # 2160) Study No. 25-5-5-5

Date Initiated 6/8/90 Time 3:30 pm Date Terminated 6/12/90

Dilution Water Recon H2O No./Vessel 5 Vessel Size 15 L

Test Conc. g/l (ppm)	MORTALITY AND BEHAVIORAL OBSERVATIONS									
	24 hr.		48 hr.		72 hr.		96 hr.			
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control										
~~0.32~~										
~~9.20~~										
3.2	0	N	0	N	0	N				
32	0	N	0	5 LOE some spin curvature	3	2 LOE				
Observer	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]				
Date	6/9	6/10	6/11	6/11	6/12	6/12				

Remarks: _____

Prepared By: [signature] Checked By: [signature]

COMPOUND PREPARATION

Compound: Acrylonitrile Lot No. _____ Purity _____ % Lab No. 25333

Preparation of Concentrated Working Standard

Date 6/9/80 Chemist [signature]

Final Gross Weight 0.75 g Dilution Volume 50 (DI H_2O) ml

Tare Weight 0.00 g Concentration 15 mg/ml

Net Weight 0.75 g Balance calibrated with class S weights

Adj. Net Weight 0.75 g* 1.00 g (class S) + 0.00 g (tare) = 1.00 g (final wt.)

Preparation of Test Concentrations

Test Prelim RBT Date 6/9/80 Chemist [signature]

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	-	-	-	
1.	15	3.2	15	3.2
2.	15	32	15	32
3.				
4.				
5.				
6.				
7.				

Preparation of Concentrated Working Standard

Date 6/10/80 Chemist [signature]

Final Gross Weight 1.5 g Dilution Volume 100 (DI H_2O) ml

Tare Weight 0.0 g Concentration 15 mg/ml

Net Weight 1.5 g Balance calibrated with class S weights:

Adj. Net Weight 1.5 g* 1.00 g (class S) + 0.00 g (tare) = 1.00 g (final wt.)

Preparation of Test Concentrations

Test Prelim RBT Date 6/10/80 Chemist [signature]

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	-	-	-	
1.	15	5.6	15	5.6
2.				
3.				
4.				
5.				
6.				
7.				

Remarks: _____

Prepared By: [signature] Checked By: [signature]

*corrected for purity of primary standard.

ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile
Test Species: Rainbow Trout
Lab No.: 25555 Exposure Period: 24hrs

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)
100	10	10	100	.09765625
56	10	3	30	17.1875
32	10	0	0	.09765625
18	10	0	0	.09765625
10	10	0	0	.09765625
5.6	10	0	0	.09765625

THE BINOMIAL TEST SHOWS THAT 32 AND 100 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 99.8046875 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 63.92498950562

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
****

Method Reported:

☑ Binomial ☐ Moving Average ☐ Probit

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

---

0 analysis performed by a computerized LC_{50} program developed by Stephan, C.E., . Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for culating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, -publication manuscript, August 1978.

pared By:

ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile
Test Species: Rainbow Trout
Lab No.: 25555 Exposure Period: 48 hrs

Laboratory:
Analytical BioChemistry Lab
Aquatic Toxicology Division
7200 ABC Lane, P.O. Box 103
Columbia, MO 65205

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	10	10	100	.09765625
56	10	10	100	.09765625
32	10	0	0	.09765625
18	10	0	0	.09765625
10	10	0	0	.09765625
5.6	10	0	0	.09765625

THE BINOMIAL TEST SHOWS THAT 32 AND 56 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.8046875 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 42.33282734879B

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

****

Method Reported:

☑ Binomial ☐ Moving Average ☐ Probit

NOTE: Method selected is that which gives the narrowest confidence limits for th 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.

ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile
Test Species: Rainbow Trout
Lab No.: 25555 Exposure Period: 72 hrs

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)
100	10	10	100	.09765625
56	10	10	100	.09765625
32	10	4	40	37.6953125
18	10	0	0	.09765625
10	10	0	0	.09765625
5.6	10	0	0	.09765625

THE BINOMIAL TEST SHOWS THAT 18 AND 56 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 99.804687 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 34.353276006825

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
****

Method Reported:

☑ Binomial ☐ Moving Average ☐ Probit

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

LC_{50} analysis performed by a computerized LC_{50} program developed by Stephan, C.E., D. 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, In-publication manuscript, August 1978.

Prepared By: [illegible]

Test Material: Acrylonitrile
Test Species: Rainbow Trout
Lab No.: 25555 Exposure Period: 96 hrs

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)
100	10	10	100	.09765625
56	10	10	100	.09765625
32	10	10	100	.09765625
18	10	0	0	.09765625
10	10	0	0	.09765625
5.6	10	0	0	.09765625

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 99.8046875 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 24.000000306188

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

****

Method Reported:

☑ Binomial ☐ Moving Average ☐ Probit

NOTE: Method selected is that which gives the narrowest confidence limits for the 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: 6/17/80 Checked by: [signature] Date: 6/18/80

Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant Avermectin A Test Species Rainbow Trout (Lot #2450) Study No. _____

Date Initiated 6/5/90 Time 4:00 p.m. Date Terminated 6/9/90

Dilution Water Recon H_2O No./Vessel 10 Vessel Size 15 l

MORTALITY AND BEHAVIORAL OBSERVATIONS

Test Conc. µl/l (ppm)	24 hr.		48 hr.		72 hr.		96 hr.			
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	0	N	0	N	0	N	0	N		
0.000014	0	N	0	N	1	8 N 1 LOE	2	8 N		
0.000021	0	N	1	8 N 1 LOE	4	4 N 2 SUR	10			
0.000042	1	N	10		10		10			
0.000075	9	N	10		10		10			
0.00014	9	N	10		10		10			
Observer										
Date	6/6		6/7 PB		6/8 PB		6/9			

Remarks: N= Normal, LOE= Loss of Equilibrium, SUR= Surfacing Entry error PB

Length - 30.1 mm

Weight - 0.35 g

Prepared By: _____ Checked By: _____ 6/11/90

COMPOUND PREPARATIONS

Compound Avilamycin A Lot No. _____ Purity _____ % Lab No. _____

Preparation of Concentrated Working Standard

Date _____ Chemist [signature]

Final Gross Weight 0.030 g Dilution Volume 3 ml/100 (acetone)

Tare Weight 0.000 g Concentration 0.0015 mg/ml

Net Weight 0.030 g Balance calibrated with class S weights

Adj. Net Weight 0.030 (100 ml acetone) g* 1.000 g (class S) + 0.000 g (tare) = 1.000 g (final wt.)

Preparation of Test Concentrations

Test RBT Date 6/5/80 Chemist [signature]

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	—	1.40 (acetone)	15	—
1.	0.0015	0.14	15	0.000014
2.	0.0015	0.24	15	0.000024
3.	0.0015	0.42	15	0.000042
4.	0.0015	0.75	15	0.000075
5.	0.0015	1.40	15	0.00014
6.				
7.				

Preparation of Concentrated Working Standard

Date _____ Chemist _____

Final Gross Weight _____ g Dilution Volume _____ ml

Tare Weight _____ g Concentration _____ mg/ml

Net Weight _____ g Balance calibrated with class S weights

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

Preparation of Test Concentrations

Test _____ Date _____ Chemist _____

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control				
1.				
2.				
3.				
4.				
5.				
6.				
7.				

Remarks: _____

Prepared By: [signature] Checked By: [signature]

*corrected for purity of primary standard.

Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Antimycin A — Date tested 6/5 – 6/9/80

Test Species Rainbow Trout — Date reported 6/10/80

Lab Number Lot # 2180 — Temperature 12°C

Exposure period 24 hrs — Water quality Recon. H_2O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
0.000024	0/10	0 (.6)	1.8	1.2	0.008
0.000042	1/10	10	16	6	0.03
0.000075	9/10	90	56	34	0.500
0.00014	9/10	90	90	0	0.000
	/				
	/				
	/				

Total animals = 40 — Total contribution to Chi 0.538

K = 4 — Chi²- contribution to Chi X total animals / K = 5.38

LC_{84} = 0.00012

Chi² (p-.05) for (K-2) 2 deg. of freedom = 5.99

LC_{50} = 0.000070

LC_{16} = 0.000042

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2} = 1.69$

$S = \frac{\frac{.00012}{.000070} + \frac{.000070}{.000042}}{2} =$

Confidence limits (.05) for LC_{50}

N' = 20

f $LC_{50} = S^{(2.77/\sqrt{N'})} = 1.38$

LC_{50} = 0.000070 mg/l (0.000051 – 0.000097) mg/l 95% C.I.

LC_{50} / f LC_{50} = lower limit = 0.000051

LC_{50} X f LC_{50} = upper limit = 0.000097

Analysis by: [signature] (Name) Aquatic Biologist (Title) 6/10/80 (Date)

Checked By: [signature] Date: 6/18/80

ANALYTICAL BIOPHYSICS LABORATORIES

Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

oxicant Antimycin A — Date tested 6/5-6/9/80

est Species Rainbow Trout — Date reported 6/10/80

ab Number Lot # 2180 — Temperature 12°C

xposure period 48 hrs — Water quality Recon H_2O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
0.000014	0/10	0 (.1)	.1	0	0.000
0.000024	1/10	10	16	6	0.030
0.000042	10/10	100 (99)	84	15.9	0.180
	/				
	/				
	/				
	/				

Total animals = 30

K = 3

Total contribution to Chi 0.210

Chi²- contribution to Chi X total animals / K = 2.10

Chi² (p-.05) for (K-2) 1 deg. of freedom = 3.84

LC_{84} = 0.000042

LC_{50} = 0.000032

LC_{16} = 0.000024

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ =

$S = \frac{.000042/.000032 + .000032/.000024}{2} = 1.32$

Confidence limits (.05) for LC_{50}

N' = 20

f $LC_{50} = S^{2.77}/\sqrt{N'}$ = 1.19

LC_{50} = 0.000032 mg/l (0.000027 – 0.000038 mg/l) 95% C.I.

LC_{50} / f LC_{50} = lower limit = 0.000027

LC_{50} × f LC_{50} = upper limit = 0.000038

Analysis by: Calvin Thayer (Name) Aquatic Biologist (Title) 6/10/80 (Date)

Checked By: Alan D. Fuhr Date: 6/18/80

Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

oxicant Antimycin A — Date tested 6/5-6/9/80

est Species Rainbow Trout — Date reported 6/10/80

ab Number Lot # 2180 — Temperature 12°C

xposure period 96 hrs — Water quality Reron H_2O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
Control	0/10	0	—	—	—
0.000014	2/10	20	20	0	0.000
0.000024	10/10	100 (95.1)	84	11.1	0.090
0.000042	10/10	100 (99.9)	99.9	0	0.000
	/				
	/				
	/				

Total animals = 30 — Total contribution to Chi 0.090

K = 3 — Chi2- contribution to Chi X total animals / K = 0.90

LC_{84} = 0.000024 — Chi^2 (p-.05) for (K-2) 1 deg. of freedom = 3.84

LC_{50} = 0.000018

LC_{16} = 0.000013

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ =

$S = \frac{\frac{.000024}{.000018} + \frac{.000018}{.000013}}{2} = 1.36$

Confidence limits (.05) for LC_{50}

N' = 20

f $LC_{50} = S^{2.77} / \sqrt{N'} = 1.21$

LC_{50} = 0.000018 mg/l (0.000014 - 0.000022 mg/l) 95% C.I.

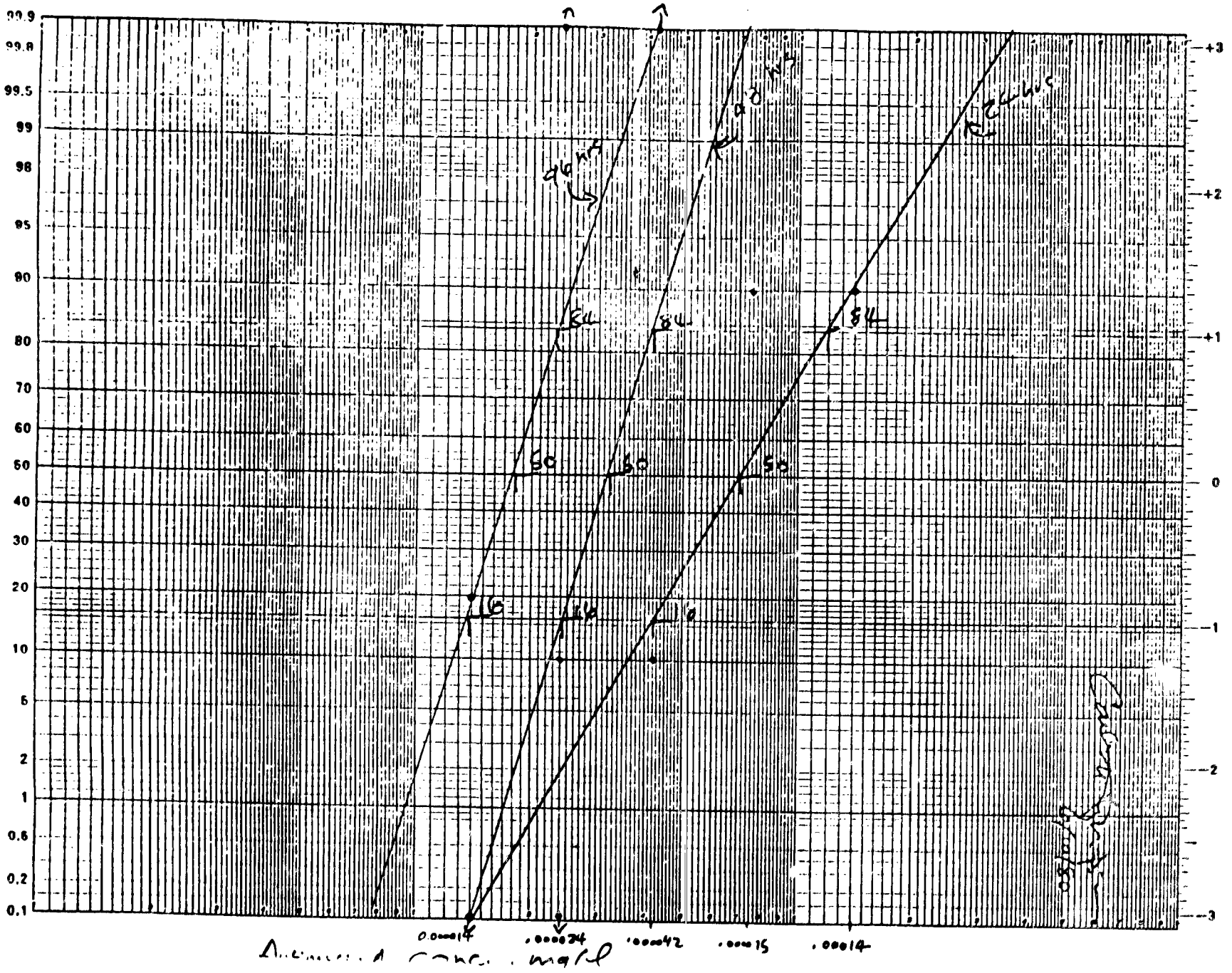
LC_{50} / f LC_{50} = lower limit = 0.000014

LC_{50} X f LC_{50} = upper limit = 0.000022

Analysis by: Carl A. Mayer (Name) Aquatic Biologist (Title) 6/10/80 (Date)

Checked By: Ken D. Fisher Date: 6/16/80

Aurintricarboxylic Acid
Lot 2180 -- Rainbow Trout



C930.006

Project No. AB-80-541

JOHN D. WALKER

Static Acute Bioassay Report
#25556

Submitted To:

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

AB-80-541

Acute Toxicity of Acrylonitrile
to Daphnia magna

June 12, 1980

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

Prepared By:

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

Paul Boudreau 6/13/80
Paul Boudreau Date
Biologist

Approved By:

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

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

SUMMARY

The acute toxicity of Acrylonitrile to Daphnia magna was assessed using the method 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 (±1.0). 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 5.6 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	0.0157 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	---
3.2	0	0					19	8.6	8.0	---
5.6	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 671 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 6/16/80

James A. Ault Date
Quality Assurance Officer

APPENDIX I

RAW DATA

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant Endothall Test Species Daphnia magna (Lot #) Study No. 2556
Date Initiated 6/3/80 Time 10:45 am Date Terminated 6/9/80
Dilution Water well H₂O No./Vessel 10 Vessel Size 250 (200) ml

Test Conc. mg/l (ppm)		MORTALITY AND BEHAVIORAL OBSERVATIONS									
		24 hr.		48 hr.		72 hr.		96 hr.			
		Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	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		6/6		6/9							

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

Prepared By: [signature] Checked By: [signature]

Acrylonitrile

Species Daphnia magna

Prepared By [signature]

Checked By [signature]

Water Quality

ntration 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	-								

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

H - 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 ...r.

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

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

Date Initiated 5/18/90 Time 4:30pm Date Terminated 5/20/90

Dilution Water well water No./Vessel 10 Vessel Size .25l (2l)

Test Conc. mg/l (ppm)	MORTALITY AND BEHAVIORAL OBSERVATIONS									
	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 5/20/90 10.00	0		5							
Observer	PB		PB							
Date	5/19		5/20							

Remarks: _____

Prepared By: Paul Boudreau Checked By: Lisa D. Foster

Compound: Acrylonitrile Lot No. _____ Purity _____ % Lab No. _____

Preparation of Concentrated Working Standard

Date 5/16/80 Chemist Paul Boudreaux

Final Gross Weight 0.100 g Dilution Volume 50 H2O

Tare Weight 0.000 g Concentration 2 mg/

Net Weight 0.100 g Balance calibrated with class S weight

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

Preparation of Test Concentrations

Test Acrylonitrile Prelim Date 5/16/80 Chemist Paul Boudreaux

	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
ADDED 5/20/80 3.	2.0	1.000	.2	10.000
4.				
5.				
6.				
7.				

Preparation of Concentrated Working Standard

Date 6/7/80 Chemist Paul Boudreaux

Final Gross Weight 0.200 g Dilution Volume 10 m

Tare Weight 0.000 g Concentration 20 mg/m

Net Weight 0.200 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 Acrylonitrile Confirm Date 6/7/80 Chemist Paul Boudreaux

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control	—	—	.2	CONTROL
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 Boudreaux Checked By: Hbd Forb

*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 La
Aquatic Toxicology Divisio
7200 ABC Lane, P.O. Box 10
Columbia, MO 65205

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
32	20	5	25	2.0694732666016
18	20	0	0	9.536743164062 5E-05
10	20	0	0	9.536743164062 5E-05
5.6	20	0	0	9.536743164062 5E-05
3.2	20	0	0	9.536743164062 5E-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.

****

ethod Reported:

☒ Binomial ☐ Moving Average ☐ Probit

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

0 analysis performed by a computerized LC_{50} program developed by Stephan, C.E., . Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for culating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, -publication manuscript, August 1978.

pared By: [illegible] Date: [illegible]

Test Material: Amphotericin

Test Species: Daphnia magna

Lab No.: 25556 Exposure Period: 48hr

Laboratory:
Analytical BioChemistry, I
Aquatic Toxicology Divisio
7201 ABC Lane, P.O. Box 10
Columbia, MO 65205

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
32	20	20	100	9.536743164062SE-05
18	20	4	20	.5908966064453 1
10	20	0	0	9.536743164062SE-05
5.6	20	1	5	2.0027160644531E-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	8.3884417307513	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 = -10.676381276801 AND 21.936719933085

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

LC1 = 7.7682494315488
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Method Reported:

☑ Binomial ☐ Moving Average ☐ Probit

NOTE: Method selected is that which gives the narrowest confidence limits for the 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, Re-publication manuscript, August 1978.

Prepared By: [signature] Date: 6/2/80 Checked by: [signature] Date: 6/2/80

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 Brookhart
Gary L. Brookhart
Residue Supervisor

<indicates minimum detectable amount.

P O Box [illegible]

March 21, 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
δBHC	<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
βChlordane	<0.8	<0.8	<0.8
Tecn Chlordane	<2	<2	<2
Dieldrin + Aldrin	<1	<1	<1
Aldrin	<0.4	<0.4	<0
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

Lucania magna Culture 4

Date	#Dead	#Alive	#1st star	#Young Adult/Day	Study ID	Comments	J
6/4/80	–	43	–	–	—	fed & changed	
6/6/80	–	43	–	–	–	fed + changed	
6/7/80	–	43	496	H.S	25556 - 25561 DV	fed + changed	
6/9/80	–	42	–	–	–	fed + changed	
6/11/80	–	42	–	–		fed + [illegible]	