FYI - 0198 - 1328

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)

[107-13-1]

FYI-98-001328

ORIGINAL

June 18, 1980

84980000030

Submitted By: Analytical BioChemistry Laboratories, Inc.
7200 East ADC 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

Contains No CBI

Approved By:

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

Dale D. Johnson 6-20-80
Dale 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 5o 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)[A]

Compound	LC_{50} in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
Acrylonitrile	64 (32-100)[AA]	42 (32-56)	24 (18-32)
Antimycin A[AAA]	0.000070 (0.000041-0.000097)	0.000032 (0.000027-0.000038)	0.000018 (0.000014-0.000022)

[A]Bioassay as conducted at 12°C (±1.0). Mean weight and length, 0.41 g and 32.9 mm.

[AA]95% Confidence Interval

[AAA]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.

(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 an 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 Pa 58.

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

(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 #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
Quality Assurance Officer

Date

APPENDIX I

RAW DATA

3.2

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant Acrylonitrile Test Species Rainbow Trout (Lot #2160) Study No. 255535-
Date Initiated 6/12/80 Time 10 am Date Terminated 6/16/80
Dilution Water Recon 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			
13.0	0	N	0	N	0	N	0	2SUR		
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	[illegible]		CT		[illegible]		[illegible]			
Date	6/13		6/14		6/15		6/16			

Remarks: N= Normal, LOE= Loss of Equilibrium, SUR= Surfacing
DIS = Dissociation
SOUN = Sounding

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

0 0 1 4

Test Acrylonitrile 96 hr Static

Species Rainbow trout (Lot # 2180)

Prepared By [signature]

Checked By [signature]

Water Quality

Concentration mg/l	JB 6/12 Day 0 Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	JB ~~Day~~ 48 hr Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	JB 6/16 Day 4 Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l	Day Temp. °C	D.O.* mg/l	pH**	NH_3*** mg/l
Control	12	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	8.8	7.1	–	12	8.5	6.9	<.1				
56.0	12	~~8.7~~	~~7.2~~													
100.0	12	8.7	7.2	<.1												

* JB entry error 6/12   * entry error JB 6/16

*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.

ANALYTICAL BIOCHEMISTRY LABS - AQUATIC BIOASSAY LAB

COMPOUND PREPARATIONS

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

Preparation of Concentrated Working Standard

Date 6/12/80 Chemist [signature]

Final Gross Weight 3.75 g Dilution Volume 250 (DI 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]

Accounted for purity of primary standard.

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Preliminary

Toxicant Acrylonitrile Test Species Rainbow Trout (Lot # 2180) Study No. 25555

Date Initiated 6/10/80 Time 4:00 pm Date Terminated 6/12/80

Dilution Water Recon H_2O No./Vessel 9 Vessel Size 5 l

Test Conc. mg/l (ppm)	MORTALITY AND BEHAVIORAL OBSERVATIONS 24 hr. Dead	24 hr. Obs.	48 hr. Dead	48 hr. Obs.	72 hr. Dead	72 hr. Obs.	96 hr. Dead	96 hr. Obs.	Dead	Obs.
Control										
56	1	220 E some spine curvature	5							
Observer	[signature]	[signature]	[signature]							
Date	6/11	6/11	6/12							

Remarks:

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

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant Preliminary Acrylonitrile Test Species Rainbow Trout (Lot # 2180) Study No. 25555

Da . Initiated 6/9/80 Time 3:30pm Date Terminated 6/12/80

Dilution Water Recon H.O No./Vessel 5 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										
~~0.32~~										
~~9.20~~										
3.2	0	N	0	N	0	N				
32	0	N	0	5 LOE some spine curvature	3	2 LOE				
Observer	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]				
Date	6/10	6/10	6/11	6/11	6/12	6/12				

Remarks:

Prepared By: [illegible] Checked By: [illegible]

ANALYTICAL BIOCHEMISTRY LABS - AQUATIC BIOASSAY LAB

COMPOUND PREPARATIONS

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

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	56	15	56
2.				
3.				
4.				
5.				
6.				
7.				

Remarks:

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

Correct for [illegible] of primary standard.

Abc 42

LC_{50} COMPUTER ANALYSIS DATA SHEET
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}.

LC_{50} analysis performed by a computerized LC_{50} program developed by Stephan, C.E., W.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: Carl M. Snapper Date: 6/17/80 Checked by: [signature] Date: 6/19/80

LC50 COMPUTER ANALYSIS DATA SHEET
ACUTE TOXICITY BIOASSAY

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

Laboratory:
Analytical BioChemistry Labs
Aquatic Toxicology Division
7200 ABC Lane, P.O. Box 1097
Columbia, MO 65[illegible]

****

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 42.332027348798

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.

****

☐ [illegible] ☐ [illegible] ☐ Probit

[illegible] is that which gives the narrowest confidence limits for [illegible]

[illegible] performed by a computerized LC50 program developed by Stephan, C.E., [illegible] Burke and R.W. Andrew. 1973. A computer program for [illegible] Environmental Protection Agency, Duluth, Minnesota, [illegible] August 1973.

Prepared By: [signature] Date: 6/17/80 Checked by: [signature] Date: 6/18/80

ibc 42 LC_{50} COMPUTER ANALYSIS DATA SHEET

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 1[illegible]
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 99.8046875 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.A. Busch, R. Smith, J. Burke and P.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

ACUTE TOXICITY BIOASSAY

Test Material: [illegible]
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 24.000003061888

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 Required:

☒ Binomial ☐ Moving Average ☐ Probit

[illegible] the narrowest confidence limits for [illegible]

[illegible] a computerized LC50 program developed by Stephan, C.E., [illegible] and R.W. Andrew. 1978. A computer program for [illegible] Environmental Protection Agency, Duluth, Minnesota. [illegible] 1978.

Prepared By [signature] Date 6/17/80 Checked by: [signature] Date: 6/16/[illegible]

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant Antimycin A Test Species Rainbow Trout (Lot #2460) Study No. _____

Date Initiated 6/5/80 Time 4:00 pm Date Terminated 6/9/80

Dilution Water Recon H_2O No./Vessel 10 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	N	0	N	0	N	0	N		
0.000014	0	N	0	N	1	8N 1 LOE	2	8N		
0.000021	0	N	1	8N 1 LOE	4 B4	4N 2SUR	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		PB 6/7		6/8 PB					

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

Length = 32.4mm

Weight = 0.35g

Prepared By: _____ Checked By: _____

ANALYTICAL BIOCHEMISTRY LABS - AQUATIC BIOASSAY LAB

COMPOUND PREPARATIONS

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

Preparation of Concentrated Working Standard

Date _____ Chemist [signature]

Final Gross Weight 0.030 g Dilution Volume 5 ml/100 (acetone) ml

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	0.00015
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.				

[signature] Checked by [signature]

ANALYTICAL BIOCHEMISTRY LABORATORIES
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 Reon. 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
	/				
	/				
	/				

Tot. animals = 40

K = 4

Total contribution to Chi 0.538

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

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

LC_{84} = 0.00012

LC_{50} = 0.000070

LC_{16} = 0.000042

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

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

Confidence limits (.05) for LC_{50}

$W' = \frac{20}{}$

$f\,LC_{50} = S^{(2-77/\sqrt{W'})}$ = 1.38

LC_{50} = [illegible]

$LC_{50} / f\,LC_{50}$ = lower limit = 0.000051

$LC_{50} \times f\,LC_{50}$ = upper limit = 0.00097

Analysis by: [signature] (Name) [signature] (Title) [illegible] (Date)

Checked By: [signature] Date: [illegible]

ANALYTICAL BIOCHEMISTRY LABORATORIES
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 48 hrs — Water quality Recon H_2O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	Q=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 (999)	84	15.9	0.180
	/				
	/				
	/				
	/				

Total animals = 30

K = 3

Total contribution to Chi 0.210

Chi2- contribution to Chi X $\frac{\text{total animals}}{K}$ = 2.10

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

LC_{84} = 0.000042

LC_{50} = 0.000032

LC_{16} = 0.000024

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

$S = \frac{\frac{0.000042}{0.000032} + \frac{0.000032}{0.000024}}{2} = 1.32$

Confidence limits (.05) for LC_{50}

V^1 = 2.0

$F\ LC_{50} = S^{\frac{1}{2}} \times V^1$ = 1.19

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

LC_{50} / $F\ LC_{50}$ = [illegible] limit = 0.000027

LC_{50} X $F\ LC_{50}$ = [illegible] limit = 0.000038

[illegible] Aquatic Biologist 6/10/80
(Title) (Date)

Date: [illegible]

ANALYTICAL BIOCHEMISTRY 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 Lc # 2180 — Temperature 12°C

xposure period 96 hrs — Water quality Recon 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)	999	0	0.000
	/				
	/				
	/				

Tot. animals = 30 — Total contribution to Chi 0.090

K = 3 — Chi^2 - contribution to Chi X total animals / K = 0.90

LC_{84} = 0.000024

LC_{50} = 0.000018

LC_{16} = 0.000013

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2} = \frac{\frac{.000024}{.000018} + \frac{.000018}{.000013}}{2} = 1.36$

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

Confidence limits (.05) for LC_{50}

N' = 20

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

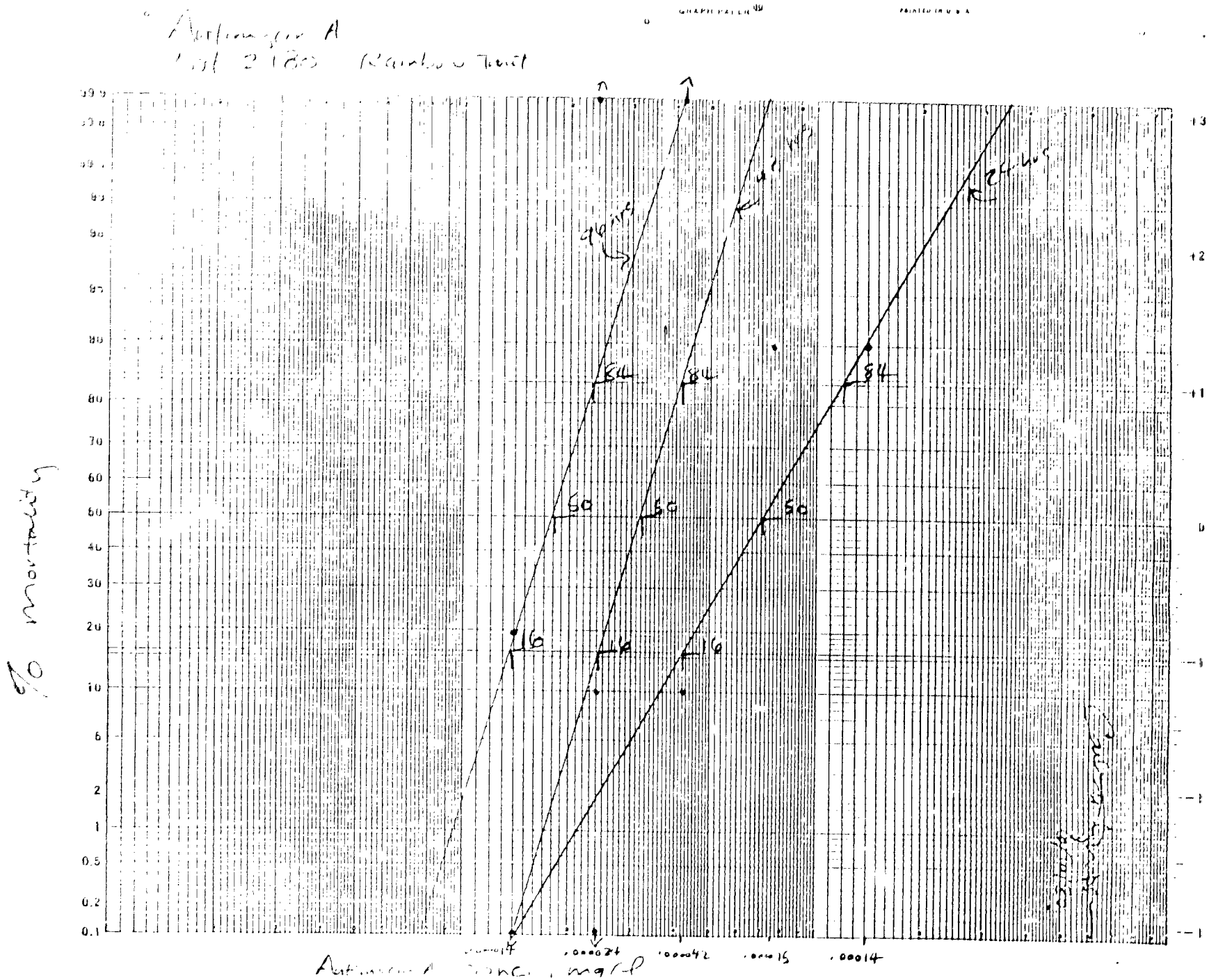
LC_{50} = 0.000018 with 95% C.L. 0.000012 - 0.000022

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

$LC_{50} \times f\ LC_{50}$ = upper limit = 0.000022

Analysis by: [signature] (Name) Aquatic Biologist (Title) [date] (Date)

Checked By: [signature] Date: [date]



Antimycin A
Rainbow Trout
% mortality
Antimycin A conc., mg/l
.000014
.000024
.000042
.00015
.00014
96 hrs
24 hrs
84
50
16

ANALYTICAL BIOCHEMISTRY LABS

AQUATIC BIOASSAY LAB

TEST FISH MEASUREMENTS

Test: Acrylonitrile 96 hr Static

Test Species: Rainbow Trout Lot No. 2180

Source: Beiley'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]

Fish Requisition and Disposition Record

Species: [illegible] Trout Eggs Date Rec: [illegible] Lot #: [illegible]

Source: Berkeys Resort No. Rec: 2000 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
[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]		[illegible]	5/[illegible]	[illegible]		[illegible]
[illegible]	57	15°	[illegible]	5/21	[illegible]		[illegible]
[illegible]	63	Starting to hatch	[illegible]	5/22	[illegible]		[illegible]
[illegible]	42	75% hatched	[illegible]	5/23	[illegible]		[illegible]
[illegible]	[illegible]		[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]		[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]		[illegible]	[illegible]	8		[illegible]
[illegible]	[illegible]		[illegible]	[illegible]	0		[illegible]
[illegible]	54		[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]		[illegible]	5/30	2		[illegible]
[illegible]	[illegible]		[illegible]	5/3	0	[illegible]	[illegible]
[illegible]	[illegible]		[illegible]	[illegible]	0	[illegible]	[illegible]
[illegible]	[illegible]		[illegible]	[illegible]	0		[illegible]
[illegible]	12		[illegible]	[illegible]	0		[illegible]

[illegible]

Checked by [illegible]

Fish Requisition and Disposition Record

Species: Rainbow Trout Date Rec: 5/1/80 Lot#: [illegible]

Source: Beiteys Resort No. Rec.: 2000 Tank#: 1

Designated Use: [illegible]

Comments:

Daily Record

Date	#Dead	Comments	Name	Date	#Dead	Comments	Name
/4	0	Fed 8 & 4	[illegible]				
/5	0	"	[illegible]				
6/6	0	"	[illegible]				
/7	0	"	[illegible]				
/8	0	"	[illegible]				
/9	0	"	[illegible]				
/10	0	"	[illegible]				
/11	1	"	[illegible]				
/12	0	"	[illegible]				
/13	0	"	[illegible]				
/14	0	"	[illegible]				
/16	0	"	[illegible]				
6/17	0	"	[illegible]				

Checked by [illegible]

ANALYTICAL BIO CHEMISTRY LABORATORIES INC
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	
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
lordane	<0.8	<0.8	<0.8
Tech Chlordane	<2	<2	<2
Dieldrin + Aldrin	<1	<1	<1
Aldrin	<0.4	<0.4	<0.4
Dieldrin	<1	<1	<1
DDT + Metabolites	<2	<2	<2
o,p'-DDE	<1	<1	<1
p,p'-DDE	<1	<1	<1
o,p'-DDT	<2	<2	<2
p,p'-DDE	<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 [illegible]	<30	<30	<30
Aroclor [illegible]	<30	<30	<30
Aroclor [illegible]	<30	<30	<30
[illegible]	0.37	0.76	0.73
[illegible]	<4	<4	<4

*Indicates less than, if present at all.
[illegible] response observed at retention time [illegible] pending to diethyl hexylphthalate.
** Occlu[illegible] 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
Dursban:	<10	<20
Methyl Parathion:	<30	<80
Malathion:	<30	<110
Ethyl Parathion:	<30	<80

Gary Brookhart
Gary L. Brookhart
Residue Supervisor

<indicates minimum detectable amount.

Monsanto

MONSANTO INDUSTRIAL CHEMICALS CO.
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

May 2, 1980

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

Dear Alan:

I would like to authorize the studies outlined on Page 1 of your proposal 3216 for acrylonitrile. This will include 96 hour acute statics on bluegill and rainbow trout (Protocol #7601), a 48 hour acute static using daphnids (Protocol #7806) and a time independent flow-through study using bluegill (Protocol #7807).

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

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

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

Sincerely,

Fran Werner

A. F. Werner

AFW:bn

cc: W. B. Wilson - Monsanto

ABC
[illegible]
P.O. Box [illegible] • Columbia, MO [illegible] • [illegible]

ABC PROTOCOL NO. 7601

(Revised November 6, 1979)

STATIC BIOASSAY PROCEDURE FOR DETERMINING THE ACUTE TOXICITY OF CHEMICAL SUBSTANCES TO FRESHWATER FISH

ABC Study Number 25555

Test Material Acrylonitrile

Test Species Rainbow Trout (Salmo gairdneri)

1.0 INTRODUCTION

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

2.0 OBJECTIVES

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

3.0 TESTING FACILITY

The study will be conducted by the Aquatic Toxicology Division of Analytical BioChemistry Laboratories, Inc., 7200 East 'BC Lane, P. O. Box 1097, Columbia, Missouri 65205.

4.0 RANGE-FINDING STUDY

4.1 General. For most chemical substances, the approximate toxic level to aquatic organisms is not known. Because this information is essential before a definitive toxicity test can be conducted, ABC routinely performs range-finding tests for static bioassays with fish. The information derived from this preliminary test will be used to set concentration levels for the definitive bioassay described in section 5.0.

4.2 Test Fish. The fish species to be used should be selected by the study sponsor from the list of recommended species in Table 1. The most common species used for toxicity testing includes bluegill sunfish (Lepomis macrochirus), rainbow trout (Salmo gairdneri), and fathead minnows (Pimephales promelas). Fish ranging in size from actively-feeding fry (~0.2 g) to 5.0 g will be used as test organisms. The fish standard length of the largest fish will be no more than twice that of the shortest fish. The fish will be obtained from an established commercial hatchery or in-house cultures. The particular source to be used is dependent upon the seasonal availability of test fish and will be listed at the time of protocol approval in the test-specific information of section 8.5, along with the species selected by the study sponsor.

The test fish lot will be from the same year class and will be identified to species using the taxonomic keys developed by Eddy (8) and cultured following the techniques described by Brauhn et al. (9). All test fish will be held on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, will be kept and included with the final report. During the holding period, they will receive a standard commercial fish food (Rangen's[3]) at a maintenance rate of 3 to 5 percent of their body weight per day. The test lot will be held without food and acclimated to the test temperature for 48 hours prior to testing. If mortality of the test lot exceeds 10 percent in the 48 hours previous to testing, the fish will not be used. Previous to or concurrent with the test, the fish lot will be challenged against a reference compound, Antimycin A*, to determine their general health and suitability as test organisms. The results of the Antimycin A test will be compared against published toxicity values (10).

4.3 Test System. The range finding test will be conducted in five gallon widemouth glass jars containing 15 liters of test solution and five fish per concentration. These test vessels will be immersed in a circulating water bath with temperatures maintained within ±1°C of the desired test temperature (Table 1). For temperature control in the water bath, thermostatically controlled heating elements will be used for warmwater tests (22 ±1°C) and refrigeration units (Min-O-Cool[3]) for coldwater bioassays (12 ±1°C). The dilution water used will be a soft reconstituted water of the following makeup in deionized water: 48 mg/l $NaHCO_3$, 30 mg/l $CaSO_4 \cdot 2H_2O$, 30 mg/l $MgSO_4$, and 2.0 mg/l KCl. The water quality parameters of this dilution water are: pH: 7.2-7.6; total hardness: 40-48 mg/l $CaCO_3$; and total alkalinity: 30-35 mg/l $CaCO_3$. Also available, at the sponsor's request, is an alternate dilution water from a deep well source with chemical and physical characteristics shown in Table 3.

4.4 Test Material. Specific information regarding the test material is to be supplied by the sponsor and will be addressed at the time of protocol approval in section 3.3. The test concentrations will be prepared on a weight/volume basis unless otherwise specified. A record of all sample weights and dilutions will be kept, checked by a second party, and furnished in the final report.

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

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

4.5.2 The range-finding test will be initiated by exposing groups of five fish to at least three toxicant concentrations

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

spaced by a factor of 10. The test fish will be placed in the test chambers by stratified random assignment within 30 minutes after solution preparations. The initial toxicant concentrations most often used are 1, 10 and 100 mg/l. Numerous static tests by ABC have shown that a significant percentage of the compounds tested have aquatic toxicities which fall within this range.

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

4.5.4 The preliminary test will be conducted for a period of 24 to 96 hours - the exact duration dependent upon the results of the initial concentrations tested. In most cases, a preliminary test for 48 hours at 3 toxicant concentrations is sufficient to determine the toxic range.

4.5.5 Results of the range-finding study will be used to set the concentration range of the definitive study described in section 5.0. At least five toxicant concentrations selected from the logarithmic scale presented in Table 2, which fall within the preliminary test range, have proven to be adequate in assessing most compounds.

5.0 DEFINITIVE STUDY

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

5.2 Test Fish. Aspects concerning the acquisition, culture and acclimation of test fish will be the same as discussed in section 4.2.

5.3 Test System. For all definitive testing, the test vessel [illegible] conform to the maximum loading limitation of 0.8 grams of [illegible] per liter of solution (5). One of the following types of [illegible] test chambers will be used. (a) 5 gallon glass jars containing 15 liters of solution, (b) 40 liter aquaria containing 30

liters of solution, or (c) 100 liter aquaria containing 75 liters of solution. ABC will select the type of chamber to be used. In most instances, the test chambers used will be 40 liter glass aquaria containing 30 liters of solution, which is a recommended test vessel for static bioassays (5). The water bath system described in section 4.3 will be used to control test temperatures. The test dilution water will be the same as discussed in section 4.3.

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

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

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

5.5.2 The definitive test will be initiated by exposing groups of 10 organisms to at least 5 toxicant concentrations and a dilution water control. The test concentrations used will be based upon the results of the range-finding test and will be selected from one of the logarithmic series presented in Table 2. The exact concentrations to be used will be addressed in section 8.4 at the time of protocol approval. If a solvent is used in the preparation of test solutions, the control chamber will receive an aliquot of the solvent equivalent to the highest amount used in the test chambers. The test organisms will be placed in the test chambers by stratified random assignment within 30 minutes after solution preparations.

5.5.3 As an alternate test design, duplicate 5 gallon jars per concentration containing 15 liters of solution and 5 fish each may be used, if so authorized by the sponsor.

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

5.5.5 Length and weight measurements will be made on either a representative group of the test fish prior to testing or on the control group at test termination.

5.6 Test Procedure-Chemical and Physical. Water quality parameters of temperature, dissolved oxygen, pH and ammonia will be monitored throughout the test. Measurements of temperature, dissolved oxygen and pH will be made at 0, 48 and 96 hours of testing in the control, low concentration and highest concentration with surviving fish. Ammonia levels will be determined in the same test chambers at 0 and 96 hours. If at any point in the study dissolved oxygen levels are observed to be below or approaching 40 percent

saturation, ABC will contact the sponsor's study director for authorization to artificially aerate the test chambers with compressed air for the duration of the study. The authorization procedure used will be that described in section 7.0.

5.7 Analysis of Results. The results of the definitive study will be statistically analyzed for 24, 48 and 96 hour LC_{50} values and their corresponding 95 percent confidence limits. These values will be determined by an LC_{50} computer program developed by Stephan et. al. (13) or by manual methods described by Litchfield and Wilcoxon (11) and Stephan (12).

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

5.8.1 Study dates of both preliminary and definitive phases.

5.8.2 Objectives and test methods.

5.8.3 Reference to the statistical methods used for data analysis.

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

5.8.5 Description of test design.

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

5.8.7 Location of raw data.

5.8.8 List and signatures of study personnel.

5.8.9 Statement by ABC's Quality Assurance Unit.

5.8.10 The report appendix will contain the original raw data for mortality observations and water quality, results of the Antimycin A reference test, records of fish size and daily holding log, letter of test authorization, letters of authorized protocol changes, and a copy of the approved protocol.

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

6.0 PROTOCOL AMENDMENTS

In the event that modifications of this protocol are deemed necessary, a written statement of any changes and reason(s) pro-

0 0 9

posed by the study sponsor or ABC will be submitted to the other party. All agreed changes will be expressed in writing, signed and dated by the sponsor's study director. The signed changes will be appended to the protocol and included with the final report.

7.0 SPONSOR AUTHORIZATIONS DURING THE STUDY

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

8.0 TEST-SPECIFIC INFORMATION

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

8.2 Study Sponsor:

8.2.1 Company Monsanto Chemical Company, N2A

8.2.2 Address 800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

8.2.3 Study Director (Coordinator)

Ms. A. F. Werner — Name
Study Director — Title

8.3 Test Material:

8.3.1 Name Acrylonitrile

8.3.2 Code Number ——

8.3.3 Physical Description clear liquid

8.3.4 Purity 99+%

8.3.5 Stability

8.3.6 Recommended Solvent H_2O

8.3.7 Water Solubility 7%

8.3.8 Handling Precautions Avoid breathing vapors
Normal handling precautions

8.4 Test Concentrations:

8.4.1 Definitive Concentrations 5.6, 10, 18, 32, 56 and 100 mg/l

8.5 Test Fish:

8.5.1 Species Rainbow Trout (Salmo gairdneri)

8.5.2 Supplier Beitey's Resort
Valley, Washington

8.6 Study Dates:

8.6.1 Proposed starting date of definitive study 6/12/80

8.6.2 Proposed completion date of definitive study 6/16/80

8.7 ABC Study Personnel:

8.7.1 Study Director

Carl M. Thompson	Aquatic Supervisor
Name	Title

8.7.2 Principal Investigator

Jerry Griffen	Biologist
Name	Title

8.7.3 Quality Assurance Officer

James A. Ault	Quality Assurance Officer
Name	Title

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

8.8.1 Sponsor's Study Director

A F Werner	Environmental Biologist	6/12/80
Name	Title	Date

8.8.2 ABC's Study Director

[signature]	Aquatic Supervisor	6/12/80
Name	Title	Date

8.8.3 ABC's Laboratory Director

[signature]	Laboratory Manager	6/12/80
Name	Title	Date

TABLE 1: RECOMMENDED SPECIES AND TEST TEMPERATURES[a]

Recommended Species	Recommended Test Temperature (°C)
Rainbow Trout, Salmo gairdneri	12
Brook trout, Salvelinus fontinalis	12
Bluegill, Lepomis macrochirus	22
Fathead minnow, Pimephales promelas	22
Channel catfish, Ictalurus punctatus	22
Carp, Cyprinus carpio[b]	22

[a]Adapted from (5).

[b]Recommended for compounds used in Japan.

TABLE 2: Guide to selection of experimental concentrations based on progressive bisection of intervals on logarithmic scale.

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

TABLE 3: Chemical characteristics of well water at ABC's Aquatic Bioassay Laboratory.

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

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

9.0 REFERENCES

(1) U.S. Congress. 1976. Toxic Substances Control Act. Public Law 94-469. Federal Register, October 11, 1976. 2003-2051.

(2) U.S. Environmental Protection Agency. 1978. Registration of pesticides in the United States, proposed guidelines. Federal Register, July 10, 1978: 29696-29741.

(3) U.S. Congress. 1977. Clean Water Act of 1977. Public Law 95-217. Federal Register, December 27, 1977: 1566-1611.

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

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

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

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

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

(9) 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.

(10) 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.

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

(12) American Society for Testing and Materials. 1977. Aquatic Toxicology and Hazard Evaluation. ASTM-STP 634. 315 p.

(13) 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.