abc

...STER FILE
Project No. ~~AB-80-~~539 FYI - 0198 - 1327

FYI-98-001327

Static Acute Bioassay Report
#25554

Submitted To:

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

AB-80-539

84980000029

[107-13-1] Acute value

Acute Toxicity of Acrylonitrile to
Fathead Minnow (Pimephales promelas)

=15 mg/L

ORIGINAL

Contains No CBI

Chemic = 0.034

June 20, 1980

.034 | 15000 = 447
137
160
136
240

or
use 30 day LC50 &
compare to 28 day FELS
A.C. ratio 47.5 times

A.C. ratio is 44.5

.034 | 2600 = 361
238
220

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

Prepared By:

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

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

Approved By:

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

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

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 2.

| Compound | 96-hour $LC_{50}$ (95% C.I.) |
|---|---|
| Acrylonitrile | 15 mg/l (12-19 mg/l) |
| Antimycin A | 0.000012 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 24.3 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 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 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 |
| Acrylonitrile | 42 (32-56)** | 22 (10-32) |
| Antimycin A*** | 0.000050 (0.000024-0.000075) | 0.000037 (0.000024-0.000075) |

*Bioassay as 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, Lo

TABLE 2

Acute Toxicity of Acryloritrile and Antimycin A to Fathead Minnows (*Pimephales promelas*)*

| $LC_{50}$ in milligrams/liter (ppm) | | |
|---|---|---|
| hours | 48 hours | 96 hours |
| 42<br>-56)** | 22<br>(10-32) | 15<br>(12-19) |
| 00050<br>4-0.000075) | 0.000037<br>(0.000024-0.000075) | 0.000032<br>(0.000024-0.000042) |

0), mean weight and length, 0.21 g and 24.8 mm.

on Sigma Chemical Company, Type III, crystalline, Lot 125C-0152.

TABLE 3

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

| mg/l Concentration | Percent Mortality Hours | | | Water Quality | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 hours | | | | 48 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/ |
| Control | 0 | 0 | 0 | 21 | 9.0 | 6.6 | <0.10 | 21 | 7.5 | 6.6 | -- |
| 5.6 | 0 | 0 | 0 | 21 | 8.9 | 6.6 | <0.10 | 21 | 7.6 | 6.6 | -- |
| 10 | 0 | 0 | 10 | | | | | | | | |
| 18 | 0 | 20 | 70 | | | | | 21 | 7.9 | 6.6 | -- |
| 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 Mod

TABLE 3

Water Quality Measurements During the Acute Toxicity
nitrile to Fathead Minnows (Pimephales promelas)

| Water Quality | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| hours | | | 48 hours | | | | 96 hours | | | |
| * | 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 |
| | 6.6 | <0.10 | 21 | 7.5 | 6.6 | --- | 21 | 4.5 | 7.0 | 0.20 |
| | 6.6 | <0.10 | 21 | 7.6 | 6.6 | --- | 21 | 6.0 | 7.1 | 0.32 |
| | | | 21 | 7.9 | 6.6 | --- | 21 | 6.5 | 6.9 | 0.26 |
| | 6.6 | <0.10 | | | | | | | | |

xygen Probe (YSI Model 54).

an Extech Model 671 pH and mV meter.

e (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 [illegible] and F. A. Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection [illegible] Research Series EPA-660/3-[illegible] May, 1975. [illegible]

(6) Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A Simplified Method of Evaluating Dose Effect Experiments. Jour. Pharm. Exp. Ther. 96. 9113.

(7) National Academy of Sciences. 1971 Water Quality Criteria, 1972. U. S. Department of Commerce, PB-236 139. 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 6-23-80

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 #2440) Study No. 2555-4
Date Initiated 6/16/80 Time 2:30pm Date Terminated 6/20/80
Dilution Water Recon $H_2O$ No./Vessel 10 Vessel Size 15-D

| 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 | 6LOE | 7 | 3LOE | | |
| 32.0 | 0 | 2LOE 8SUR | 10 | | 10 | | 10 | | | |
| 56.0 | 10 | | 10 | | 10 | | 10 | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| Observer Date | [illegible] | | [illegible] 6/16 | | [illegible] 6/16 | | CT 6/20 | | | |

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

[illegible] CT 6/20/80

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

Test: Acrylonitrile 96 hr Static

Species: Fathead Minnows (Lot # 2450)

Prepared By: [illegible]

Checked By: [illegible]

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 Temp. °C | D.O.* mg/l | pH** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 21 | 5.0 | 6.6 | <.1 | 21 | 7.5 | 6.6 | – | 21 | 4.5 | 7.0 |
| 5.6 | 21 | 5.9 | 6.6 | <.1 | 21 | 7.6 | 6.6 | – | 21 | 6.0 | 7.1 |
| 10.0 | | | | | | | | | | | |
| 18.0 | | | | | 21 | 7.9 | 6.6 | – | 21 | 6.5 | 6.9 |
| 32.0 | | | | | | | | | | | |
| 56.0 | 21 | 5.9 | 6.6 | <.1 | | | ~~6.6~~ | | | | |

*Dissolved oxygen concentrations - Dissolved Oxygen Probe (YSI Dissolved Oxygen [illegible]

*pH - pH Probe (Orion Model 91-06) used with an Extech Model 671 pH and mV mete[illegible]

***Ammonia concentrations - Ammonia Probe (Extech Model 8002-8) used with an Ext[illegible]ter.

Prepared By: [illegible]

Checked By: [illegible]

Water Quality

| *** /l | Day 2 6/15 Temp. °C | D.O.* mg/l | pH** | $NH_3$*** mg/l | Day 4 6/20 Temp. °C | D.O.* mg/l | pH** | $NH_3$*** mg/l | Day Temp. °C | D.O.* mg/l | pH** | $NH_3$*** mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| .1 | 21 | 7.5 | 6.6 | – | 21 | 4.5 | 7.0 | 0.20 | | | | |
| 1 | 21 | 7.6 | 6.6 | – | 21 | 6.0 | 7.1 | 0.32 | | | | |
| | | | | | | | | | | | | |
| | 21 | 7.9 | 6.6 | – | 21 | 6.5 | 6.9 | 0.26 | | | | |
| | | | | | | | | | | | | |
| .1 | | | 6.6 | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |

· Dissolved Oxygen Probe (YSI Dissolved Oxygen System Model 54).

) used with an Extech Model 671 pH and mV meter.

.a Probe (Extech Model 8002-8) used with an Extech Model 671 pH and mV

2.

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

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

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

Dilution Water Recon $H_2O$ No./Vessel 5 Vessel Size 15G

| 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~~ 56 | 0 | N | 0 | N | | | | | | |
| 56 ~~5.6~~ | 5- | | 5- | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| Observer | [illegible] | [illegible] | [illegible] | [illegible] | | | | | | |
| Date | 6/12 | 6/12 | 6/13 | 6/13 | | | | | | |

Remarks: entry error CT 6/20/80

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

bc 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 | .1128356202303 | 16.812848165756 | 12.667550702863 | 21.910067920265 |
| 3 | .1137364580698 | 16.881936021608 | 13.556483077578 | 22.299667747999 |
| 2 | .1677540585244 | 17.762112389045 | 14.137515085873 | 22.224427558341 |

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

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 7 | .2856070526639 | 1 | .6729623435 39 |

SLOPE = 6.586246140383
95 PERCENT CONFIDENCE LIMITS = 3.066410205 1327 AND 10.1060420756 33

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, 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: [illegible] Checked by: [signature] Date: [illegible]

DC 42

# LC50 COMPUTER ANALYSIS DATA SHEET
## ACUTE TOXICITY BIOASSAY

Test Material: Acrylonitrile

Test Species: Fathead minnow

Lab No.: 25554 Exposure Period: 96 hrs

Laboratory:
Analytical BioChemistry Labs
Aquatic Toxicology Dept
7200 ABC Lane, P.O. Box
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 | | 10 | 1.07421875 |
| 5.6 | 10 | | 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 14.2821066898

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

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE | |
|---|---|---|---|---|
| 4 | .11358720692715 | 14.8076076835 7 | 10.86780166666 | 19.17307370347 |
| 3 | .11370435178181 | 14.85147892998 | 11.8987590887 01 | 19.05709568816 |
| 2 | .16775085452000 | 15.2073126105 | 11.56605960 2333 | 19.7000791 01846 |
| 1 | .50888097163150 | 14.2821066566 69 | 11.95068175 3971 | 23.61205845 0133 |

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

| ITERATIONS | G | W | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 7 | .72857884571820 1 | | .99608282627 |

SLOPE = 7.501036663 29507

95 PERCENT CONFIDENCE LIMITS = 3.23972023778 AND 11.2075588 8101

LC50 = 14.09576509 3716

95 PERCENT CONFIDENCE LIMITS = 11.86253759 397 AND 19.35121061870

LC1 = 7.42841581581 6

95 PERCENT CONFIDENCE LIMITS = 2.70676390 08756 AND 10.35207706463

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., M.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: 6/20/80 Checked by: [signature] Date: 6/20/80

Doc 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 | .00745625 |
| 32 | 10 | 10 | 100 | .00745625 |
| 18 | 10 | 2 | 20 | 5.46875 |
| 10 | 10 | 0 | 0 | .00745625 |
| 5.6 | 10 | 0 | 0 | .00745625 |

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 99.846875 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.

**********

Method Reported:

☑ Binomial ☐ Moving Average ☐ Probit

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

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

epared By: [signature] Date: [redacted] Checked by: [signature] Date: 6/20/80

Be 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.

**********

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., L.A. Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for alculating an $LC_{50}$. U.S. Environmental Protection Agency, Duluth, Minnesota, re-publication manuscript, August 1978.

Prepared By: [signature] Date: [illegible] Checked by: [signature] Date: 4/20/80

ANALYTICAL BIOCHEMISTRY LABS - AQUATIC BIOASSAY LAB

COMPOUND PREPARATIONS

Compound Acrylonitrile Lot No. _____ Purity _____ % Lab No. 25554

## Preparation of Concentrated Working Standard

Date 6/11/80 Chemist [illegible]

Final Gross Weight 1.50 g Dilution Volume 100 (DI $H_2O$) ml

Tare Weight 0.00 g Concentration 15 mg/ml

Net Weight 1.50 g Balance calibrated with class S weights:

Adj. Net Weight 1.50 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 [illegible]

| | 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 [illegible]

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 [illegible]

| | 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 | 22.6 | 15 | 22.6 |
| 5. | 15 | 27.0 | 15 | 27.0 |
| 6. | | | | |
| 7. | | | | |

Remarks: _____

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

*corrected for purity of primary standard.

ORIGINAL