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MONSANTO CO		
Contractor		
Document Title		
INITIAL SUBMISSION: LETTER FROM MONSANTO CO TO USEPA REGARDING ENCLOSED PROTOCOLS AND TEST RESULTS FOR 2-MERCAPTOBENZOTHIAZOLE WITH ATTACHMENTS, DATED 03/29/84		
Chemical Category		
2-MERCAPTOBENZOTHIAZOLE		

741-0794-001075



741-0794-001075
INIT 87/14/94

Monsanto



84948888164

MONSANTO POLYMER PRODUCTS CO.
800 N. Lindbergh Boulevard
St. Louis, Missouri 63187
Phone: (314) 884-1000

March 29, 1984

Contains No CBI

Dr. Louis Borghi
Staff Scientist
Dyna-Mac Corporation
11140 Rockville Pike
Rockville, MD 20852

Dear Dr. Borghi:

Enclosed are the protocols and test results for aquatic toxicity of 2-mercaptobenzothiazole, trade named THIOTAX® accelerator, that were referenced in an ITC submittal dated 7/16/82 by J. R. Condray, and that were requested by you in your February 7, 1984 letter. I am still awaiting a copy of an environmental study also referenced in the ITC submittal, but I thought these would be helpful to you now.

You also asked if Monsanto had any unpublished test data on biodegradation of MBT. A short study using an incubation technique showed little biodegradation of MBT, but photo transformation studies indicated an aqueous solution half-life of 3.7 hours in sunlight and a half-life of about 100 hours in the dark. MBT is thus quite susceptible to chemical degradation under environmental conditions. A summary of the degradation studies is enclosed.

Sincerely,

B. J. Hill
Product Acceptability Manager
Rubber Chemicals

Enclosure

cc: J. R. Condray

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

**Static Acute Bioassay Report
#23809**

Submitted To:

Monsanto Chemical Company NIB
Attn: Dr. William J. Adams
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

AB-79-315

(MBT)

Acute Toxicity of Thiotax (AB-79-1384365-1a)
to Fathead Minnows (Pimephales promelas)

August 27, 1979

0 0 0 4

Submitted By: Analytical BioChemistry Laboratories, Inc.
7206 East ABC Lane
P. O. Box 1097
Columbia, Missouri 65205

Prepared By:

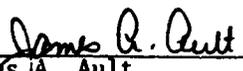

Carl M. Thompson
Aquatic Biologist

8/30/79
Date

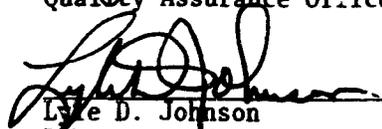

Alan D. Forbis
Aquatic Supervisor

5/30/79
Date

Approved By:


James A. Ault
Quality Assurance Officer

8/30/79
Date


Lyle D. Johnson
Laboratory Manager

8-30-79
Date

SUMMARY

The acute toxicity of Thiotax 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 LC₅₀ 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 fat-head minnows are summarized below.

<u>Compound</u>	<u>96-hour LC₅₀ (95% C.I.)</u>
Thiotax	11 mg/l (8.3-15 mg/l)
Antimycin A	0.000028 mg/l (0.000023-0.000034 mg/l)

Also, the results indicated a 96 hour, no observed effect concentration of 4.2 mg/l.

INTRODUCTION

This static bioassay was performed at the aquatic bioassay laboratory of Analytical BioChemistry Laboratories, Inc., Columbia, Missouri, for Monsanto Chemical Company, from August 22 to August 26, 1979, as authorized in a letter from Monsanto Chemical Company on February 1, 1979 (Appendix I). The purpose of this test was to determine the 24, 48 and 96 hour LC₅₀ levels for Thiotax to fathead minnows (Pimephales promelas). A preliminary range-finding test was conducted from August 7 to August 11, 1979, 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 Dr. William J. Adams, Monsanto Chemical Company, on August 17, 1979.

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 0.20 g and a mean standard length of 22.9 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 laboratory well water with 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 96 hour range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 0.1, 1, 10 and 100 mg/l. Based on the results of preliminary testing, six concentrations of the test compound, ranging in a logarithmic series from 4.2 to 75 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 Thiotax standard was received on May 21, 1979, in good condition. The sample upon receipt was observed to be a yellow powder and

was stored at 1°C. Sample purity was listed as 99+% active ingredients. Test concentrations were prepared based on the total compound. Before the test concentrations were prepared, the standard was allowed to warm to room temperature (22°C). The test concentrations were obtained by transferring appropriate aliquots of a working solution directly to the test chambers. The working solution was prepared in hancegrade acetone. The compound was observed to form a yellow precipitate in all test solutions. It was noted that the concentrations tested were above the solubility of Thiotax in water as reported in the protocol for this study (Appendix I). All standard weights and dilution values are listed in Appendix I.

RESULTS

Table 2 presents the predicted LC₅₀ values and 95% confidence intervals for Thiotax and the reference test against Antimycin A, a piscicide. These values were obtained by employing the statistical method described by Litchfield and Wilcoxon (6) or Stephan (9). 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 Chemical Company, with a copy retained at Analytical BioChemistry Laboratories.

TABLE 1

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

<u>Parameter</u>	<u>Concentration</u>
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
DDVP	<40 ng/l
Diazinon	<20 ng/l
Disyston	<20 ng/l
Methyl Parathion	<80 ng/l
Malathion	<110 ng/l
Ethyl Parathion	<80 ng/l

TABLE 2

The Acute Toxicity of Thiotax and Antimycin A
to Fathead Minnows (Pimephales promelas)

Compound	LC ₅₀ in milligrams/liter (ppm)			
	24 hours	48 hours	96 hours	
Thiotax	18 (14-23)**	13 (10-17)**	11 (8.3-15)**	
Antimycin A***	0.000048 (0.000040-0.000057)**	0.000032 (0.000026-0.000039)**	0.000028 (0.000023-0.000034)**	

*Bioassay as conducted at 22°C (±1.0), mean weight and length, 0.20 g and 22.9 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 Thiotax to Fathead Minnows (Pimephales promelas)

		Water Quality													
mg/l Concentration	Percent Mortality Hours		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	22	9.9	8.3	0.17	22	7.3	8.2	0.23	22	4.3	8.3	0.4
4.2	0	0	0	22	9.7	8.2	0.14	22	7.2	8.2	0.22	22	4.0	8.1	0.5
7.5	0	10	10												
14	20	60	80					22	7.0	8.2	0.22	22	4.4	8.0	0.3
24	100	100	100												
42	100	100	100												
75	100	100	100	22	9.5	8.0	0.15								

*Dissolved oxygen concentrations - Dissolved Oxygen Probe (Extech Model 8012) used with an Extech Model 671 pH and mV meter.

**pH - pH Probe (Fisher Model 13-639-108) 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 Antinycin 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. Federal Register, Vol. 43, No. 247, 59986-60025, December 22, 1978.
- (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.

Quality Assurance Statement for final report #23809 entitled, "Acute Toxicity of Thiotax to Fathead Minnows (Pimephales promelas)," for Dr. William J. Adams, 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 Thiotax 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 August 28, 1979, 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
Quality Assurance Officer

8/30/79
Date

APPENDIX I
RAW DATA

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division
ACUTE TOXICITY BIOASSAY

Toxicant Thiobax Test Species Fathead Minnow (Lot # 2775) Study No. 23909
 Date Initiated 8/22/79 Time 11:30 am Date Terminated 8/26/79
 Dilution Water Well H₂O No./Vessel 10 Vessel Size 150

MORTALITY AND BEHAVIORAL OBSERVATIONS

Test Conc. mg/l (ppm)	24 hr.		48 hr.		72 hr.		96 hr.		Dead	Obs.
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.		
Control	0		0		0		0			
4.2	0		0		0		6			
7.5	0		1		1		1			
14	2		6		7		8			
24	10		10		10		10			
42	10		10		10		10			
75	10		10		10		10			
Observer	JG		CT		WAGM		WAGM			
Date	8/23		8/24		8-25		8-26			

Remarks: Yellow precipitate formed at all concentrations

Prepared By: [Signature] Checked By: [Signature]

Test Thiostens

Species Fathead Minnow

Prepared By [Signature]

Checked By [Signature]

Water Quality

Concentration mg/l	0 hour		48 hours		96 hours		Temp. C	D.O.* mg/l	NH ₃ ** mg/l	pH**	pH**	pH**	D.O.* mg/l	Temp. C	D.O.* mg/l	pH**
	Temp. C	D.O.* mg/l	Temp. C	D.O.* mg/l	Temp. C	D.O.* mg/l										
Control	22	7.90	8.30	1.17	22	7.3	8.2	0.23	22	4.3	8.3	0.44	22	4.3	8.1	0.51
4.2	22	9.70	8.20	1.14	22	7.2	8.2	0.22	22	4.0	8.1	0.51	22	4.0	8.1	0.51
7.5																
14					22	7.0	8.2	0.22	22	4.4	8.0	0.30	22	4.4	8.0	0.30
24																
42																
75	22	9.50	8.00	1.15												

*Dissolved oxygen concentrations - Dissolved Oxygen Probe (Extech Model 8012) used with an Extech Model 671 pH and mV meter.

**pH - pH Probe (Fisher Model 13-639-10H) 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.

COMPOUND PREPARATIONS

Compound Thiazol Lot No. --- Purity 99+% Lab No. 23809

Preparation of Concentrated Working Standard

Date 8/17/79 Chemist Jung Duffner
 Final Gross Weight 0.75 g Dilution Volume 50 (acetone)
 Tare Weight 0.00 g Concentration 15 mg/l
 Net Weight 0.75 g Balance calibrated with class S weights
 Adj. Net Weight 0.75 g* $\frac{10.00}{(\text{class S})} \text{ g} + \frac{0.00}{(\text{tare})} \text{ g} = \frac{10.00}{(\text{final wt.})} \text{ g}$

Preparation of Test Concentrations

Test Preliminary FHM Date 8/17/79 Chemist Jung Duffner

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control				
1.	<u>15</u>	<u>1</u>	<u>15</u>	<u>1</u>
2.	<u>15</u>	<u>1</u>	<u>15</u>	<u>1</u>
3.	<u>15</u>	<u>10</u>	<u>15</u>	<u>10</u>
4.				
5.				
6.				
7.				

Preparation of Concentrated Working Standard

Date 8/22/79 Chemist Jung Duffner
 Final Gross Weight 7.50 g Dilution Volume 50 (acetone)
 Tare Weight 0.00 g Concentration 150 mg/l
 Net Weight 7.50 g Balance calibrated with class S weights
 Adj. Net Weight 7.50 g* $\frac{5.00}{(\text{class S})} \text{ g} + \frac{0.00}{(\text{tare})} \text{ g} = \frac{5.00}{(\text{final wt.})} \text{ g}$

Preparation of Test Concentrations

Test Definitive FHM Date 8/22/79 Chemist Jung Duffner

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control				
1.	<u>150</u>	<u>1.2</u>	<u>15</u>	<u>4.2</u>
2.	<u>150</u>	<u>2.5</u>	<u>15</u>	<u>7.5</u>
3.	<u>150</u>	<u>1.4</u>	<u>15</u>	<u>1.4</u>
4.	<u>150</u>	<u>2.4</u>	<u>15</u>	<u>2.4</u>
5.	<u>150</u>	<u>4.2</u>	<u>15</u>	<u>4.2</u>
6.	<u>150</u>	<u>2.5</u>	<u>15</u>	<u>7.5</u>
7.				

Remarks: _____

Prepared By: Jung DuffnerChecked By: Carl Hansen

*corrected for purity of primary standard.

ANALYTICAL BIOCHEMISTRY LABS

Aquatic Toxicology Division

ACUTE TOXICITY BIOASSAY

Toxicant Thiostax Test Species Fathel Minam (Lot #2079) Study No. 23609
 Date Initiated 8/2/79 Time 2:30 pm Date Terminated 8/9/79
 Dilution Water Well H₂O No./Vessel 5 Vessel Size 15L

MORTALITY AND BEHAVIORAL OBSERVATIONS

Test Conc. mg/l (ppm)	24 hr.		48 hr.		72 hr.		96 hr.		Dead	Obs.
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.		
Control										
.1	0		0							
1	0		0							
10	1		2							
100	5		5							
	% 100		% 100							
Observer	JG		JG							
Date	8/8		8/9							

Remarks: Precipitate formed at 100 mg/l concentration

Prepared By: [Signature] Checked By: [Signature]

ANALYTICAL BIOCHEMISTRY LABORATORIES, INC.

Aquatic Toxicology Division

PREPARATION OF TEST CONCENTRATIONS (weight/volume)

Compound Thioteax Lab No. 23809 ^{analyzed in error of 8/27/79} Test Prelim FHM
 Date 6/9/79 Prepared By J. Duffin Checked By C. H. H. H.
 Compound Purity 99+% Balance Calibration: 1.00 ^{g+} 0.00 ^{g-} 1.00 ^g
 (Class S wt.) (Tare) (Final wt.)

Sample Number	Gross Weight(g)	Tare Weight(g)	Net Weight(g)	Purity Correction	Dilution Volume(l)	Final Conc.(mg/l)
1	<u>1.5</u>	<u>0.00</u>	<u>1.5</u>	<u>-</u>	<u>15</u>	<u>100</u>
2	_____	_____	_____	_____	_____	_____
3	_____	_____	_____	_____	_____	_____
4	_____	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	_____	_____
6	_____	_____	_____	_____	_____	_____
7	_____	_____	_____	_____	_____	_____
8	_____	_____	_____	_____	_____	_____

Compound _____ Lab No. _____ Test _____
 Date _____ Prepared By _____ Checked By _____
 Compound Purity _____ Balance Calibration: _____ ^{g+} _____ ^{g-} _____ ^g
 (Class S wt.) (Tare) (Final wt.)

Sample Number	Gross Weight(g)	Tare Weight(g)	Net Weight(g)	Purity Correction	Dilution Volume(l)	Final Conc.(mg/l)
1	_____	_____	_____	_____	_____	_____
2	_____	_____	_____	_____	_____	_____
3	_____	_____	_____	_____	_____	_____
4	_____	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	_____	_____
6	_____	_____	_____	_____	_____	_____
7	_____	_____	_____	_____	_____	_____
8	_____	_____	_____	_____	_____	_____

11114

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Thiotax Date tested 8/22-8/26/79
 Test Species Fedhead minnow ^{LOT} 279 Date reported 8/27/79
 Lab Number 23809 Temperature 22°C
 Exposure period 24 hrs Water quality Well #120

Concentration mg/L	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
Control	0/10	0	—	—	—
7.5	0/10	0 (0.1)	0.1	0	0.000
14	2/10	20	20	0	0.000
24	10/10	100 (95.6)	86	9.6	0.080
	1				
	1				
	1				

Total animals = 30 Total contribution to Chi 0.080
 K = 3 Chi²- contribution X total animals = 0.80
 to Chi K
 Chi² (p=.05) for (K-2) 1 deg. of freedom = 3.84

LC₈₄ = 23
 LC₅₀ = 18
 LC₁₆ = 13

$$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2} = \frac{23/18 + 18/13}{2} = 1.33$$

Confidence limits (.05) for LC₅₀

$$f LC_{50} = \frac{10}{S^{2.77} / \sqrt{N}} = 1.28$$
 24 hr LC₅₀ = 18 mg/L (14-23 mg/L)
 95% C.I.

LC₅₀ / f LC₅₀ = lower limit = 14
 LC₅₀ X f LC₅₀ = upper limit = 23

Analysis by: Constance Hoxen Aquatic Biologist 8/27/79
 (Name) (Title) (Date)

Prepared By: Ala D. Foh Date: 8/27/79

ANALYTICAL BIOCHEMISTRY LABORATORIES
 Aquatic Bioassay Laboratory
 Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Thiatax Date tested 8/22-8/26/79
 Test Species Fathead minnow LOT 279 Date reported 8/27/79
 Lab Number 23809 Temperature 22°C
 Exposure period 48 hrs Water quality well H₂O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
control	0/10	0	-	-	-
4.2	0/10	0 (.2)	.3	.1	0.000
7.5	1/10	10	10	0	0.000
14	6/10	60	60	0	0.000
24	10/10	100 (98.4)	95	3.4	0.025
	1				
	1				

Total animals = 40 Total contribution to Chi 0.025
 K = 4 Chi²- contribution X total animals = 0.25
 to Chi K
 Chi² (p=.05) for (K-2) 2 deg. of freedom = 5.99

LC₈₄ = 19
 LC₅₀ = 13
 LC₁₆ = 5.4

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$
 $S = \frac{19/13 + 13/5.4}{2} = 1.50$

Confidence limits (.05) for LC₅₀

$N' = \frac{20}{f LC_{50} = S^{2.77} / \sqrt{N'}} = 1.29$

48 hr LC₅₀ = 13 mg/l (10-17 mg/l)
 95% C.I.

LC₅₀ / f LC₅₀ = lower limit = 10
 LC₅₀ x f LC₅₀ = upper limit = 17

Analysis by: Carolyn Thorsen Aquatic Biologist 8/27/79
 (Name) (Title) (Date)

Checked by: Alan D. Fodis Date: 8/27/79

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Thiatax Date tested 8/22-8/26/79
 Test Species Fathead minnow ^{LOT} 279 Date reported 8/27/79
 Lab Number 23809 Temperature 22°C
 Exposure period 96 hrs Water quality well H₂O

Concentration	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
<u>1 mg/l</u> <u>CONTROL</u>	<u>0/10</u>	<u>0</u>	<u>-</u>	<u>-</u>	<u>-</u>
<u>4.2</u>	<u>0/10</u>	<u>0 (.2)</u>	<u>.3</u>	<u>.1</u>	<u>0.000</u>
<u>7.5</u>	<u>1/10</u>	<u>10</u>	<u>13</u>	<u>3</u>	<u>0.008</u>
<u>14</u>	<u>8/10</u>	<u>80</u>	<u>80</u>	<u>0</u>	<u>0.000</u>
<u>24</u>	<u>10/10</u>	<u>100 (9.0)</u>	<u>99.5</u>	<u>.5</u>	<u>0.002</u>
	<u>1</u>				
	<u>1</u>				

Total animals = 40
 K = 4

Total contribution to Chi 0.010
 Chi² - contribution X total animals = 0.10
 to Chi K
 Chi² (p-.05) for (K-2) 2 deg. of freedom = 5.99

LC₈₄ = 15
 LC₅₀ = 11
 LC₁₆ = 7.8

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$
 $S = \frac{15/11 + 11/7.8}{2} = 1.39$

Confidence limits (.05) for LC₅₀

$H' = \frac{10}{f LC_{50} = S^{2.77} / \sqrt{H'}} = 1.33$

96 hr LC₅₀ = 11 mg/l (8.3 - 15 mg/l)
 95% C.I.

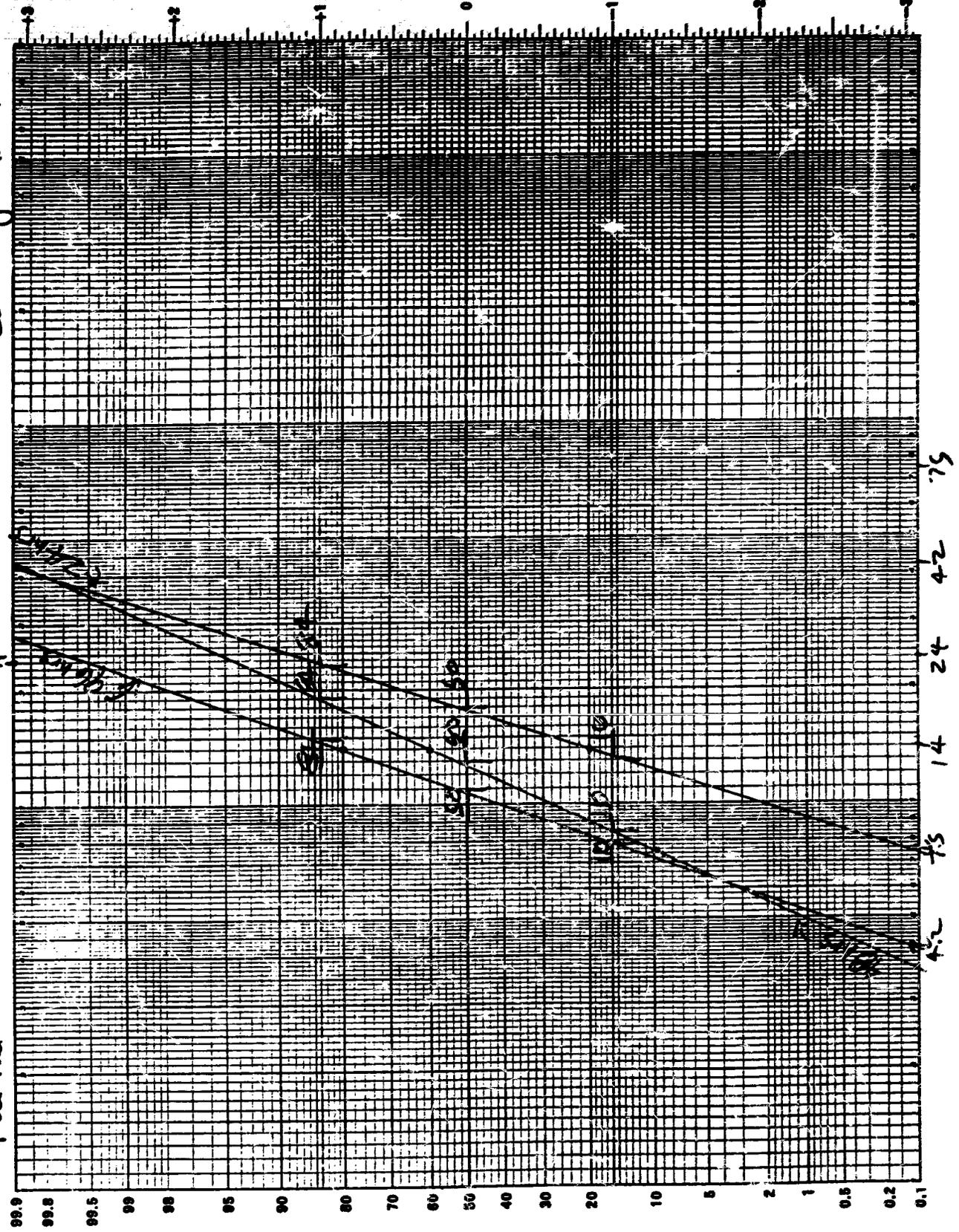
LC₅₀ / f LC₅₀ = lower limit = 8.3
 LC₅₀ x f LC₅₀ = upper limit = 15

Analysis by: Carl J. Hansen Aquatic Biologist (Name) 8/27/79 (Date)

Checked by: Ala. D. John Date: 8/27/79

Thiotax
Fatthead minnow

Casey



% mortality

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Toxicology Division
ACUTE TOXICITY BIOASSAY

Toxicant: Aluminum A Test Species: Freshwater Minnow (Lot # 475) Study No. Reference
 Date Initiated: 8/16/79 Time: 4:00pm Date Terminated: 8/20/79
 Dilution Water: Recap H₂O No./Vessel: 10 Vessel Size: 150

MORTALITY AND BEHAVIORAL OBSERVATIONS

Test Conc. mg/l (ppm)	24 hr.		48 hr.		72 hr.		96 hr.		Dead	Obs
	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.		
Control	0		0		0		0			
0.00024	0		2		3		3			
0.00037	2		7		8		8			
0.00056	7	ALOE	10		10		10			
0.00087	10		10		10		10			
0.0014	10		10		10		10			
Observer	JG		JG		JG		JG			
Date	8/17		8/18		8/19		8/20			

LOE - loss of equilibrium

Remarks: mean wt = 0.30 g , mean standard length = 22.2 mm

Prepared By: Greg Duffner Checked By: Carl [Signature]

ANALYTICAL BIOCHEMISTRY
COMPOUND PREPARATIONS

Reference
Toxicant

Compound Antimony A Lot No. Reference Purity 100 % Lab No. Toxicant

Preparation of Concentrated Working Standard

Date 5/28/79 Chemist Judy Duffner Allquot 1.5
 Final Gross Weight 0.030 g Dilution Volume 100 (acetone)
 Tare Weight 0.000 g Concentration 0.0015 mg/
 Net Weight 0.030 g Balance calibrated with class S weight
 Adj. Net Weight 0.030 g* $\frac{1.000 \text{ g}}{\text{(class S)}} + \frac{0.000 \text{ g}}{\text{(tare)}} = \frac{1.000 \text{ g}}{\text{(final wt.)}}$
 Dilution 100ml acetone

Preparation of Test Concentrations

Test FHM Anti-A Date 8/6/79 Chemist Judy Duffner

	Conc. of Work. Std. (mg/ml)	Aliq. Vol. (ml)	Dilution Vol. (l)	Final Conc. (mg/l)
Control				
1.	<u>0.0015</u>	<u>.24</u>	<u>15</u>	<u>0.000024</u>
2.	<u>0.0015</u>	<u>.37</u>	<u>15</u>	<u>0.000037</u>
3.	<u>0.0015</u>	<u>.56</u>	<u>15</u>	<u>0.000056</u>
4.	<u>0.0015</u>	<u>.87</u>	<u>15</u>	<u>0.000087</u>
5.	<u>0.0015</u>	<u>1.40</u>	<u>15</u>	<u>0.00014</u>
6.				
7.				

Preparation of Concentrated Working Standard

Date _____ Chemist _____
 Final Gross Weight _____ g Dilution Volume _____
 Tare Weight _____ g Concentration _____ mg/
 Net Weight _____ g Balance calibrated with class S weight
 Adj. Net Weight _____ g* $\frac{\text{g}}{\text{(class S)}} + \frac{\text{g}}{\text{(tare)}} = \frac{\text{g}}{\text{(final wt.)}}$

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: Judy Duffner Checked By: Carl Hoppe

*corrected for purity of primary standard.

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Antimony A Date tested 8/16/79 - 8/20/79
 Test Species Fathead Minnow Lot 279 Date reported 8/20/79
 Lab Number Reference Toxicant Temperature 22°C
 Exposure period 24 hrs Water quality Recon. H₂O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	U-E	Contribution to Chi
Control	0/10	0	-	-	-
0.000024	0/10	0 (0)	1	.7	0.005
0.000037	2/10	20	20	0	0.000
0.000056	7/10	70	70	0	0.000
0.000087	10/10	100 (99)	97	2	0.014
0.00014	10/10	100	-	-	-

Total animals = 40
 K = 4

Total contribution to Chi 0.019
 Chi² contribution X total animals = 0.19
 to Chi
 Chi² (p=.05) for (K-2) 2 deg. of freedom = 5.99

LC₈₄ = 0.000064
 LC₅₀ = 0.000048
 LC₁₆ = 0.000036

$$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2} = \frac{0.000064/0.000048 + 0.000048/0.000036}{2} = 1.33$$

Confidence limits (.05) for LC₅₀

$$N' = \frac{20}{f LC_{50}} = \frac{20}{S^{2.77} / \sqrt{N'}} = 1.20$$

24 hr LC₅₀ = 0.000048 mg/l (95% C.I. 0.000040 - 0.000057 mg/l)

LC₅₀ / f LC₅₀ = lower limit = 0.000040
 LC₅₀ X f LC₅₀ = upper limit = 0.000057

Analysis by: Carlton Trahan (Name) Aquatic Biologist (Title) 8/20/79 (Date)

Checked By: Alan J. Fochi Date: 8/22/79

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Aerimycin A Date tested 8/16 - 8/20/79
 Test Species Fathead minnow ^{LOT} 219 Date reported 8/20/79
 Lab Number Reference Toxicant Temperature 22°C
 Exposure period 48 hrs Water quality Recon. H₂O

Concentration <i>mg/l</i>	No. dead / total no.	Observed % mortality	Expected % mortality	D-E	Contribution to Chi
CONTROL	0/10	0	—	—	—
0.00024	2/10	20	20	0	0.000
0.00037	7/10	70	70	0	0.000
0.00056	10/10	100 (100)	96	2.7	0.020
	1				
	1				
	1				

Total animals = 30
 K = 3

Total contribution to Chi 0.020
 Chi²- contribution X total animals = 0.20
 to Chi K
 Chi² (p-.05) for (K-2) 1 deg. of freedom = 3.84

LC₈₄ = 0.00043
 LC₅₀ = 0.00032
 LC₁₆ = 0.00023

$$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2} = \frac{0.00043/0.00032 + 0.00032/0.00023}{2} = 1.37$$

Confidence limits (.05) for LC₅₀

$$N = \frac{20}{f LC_{50} = 5^{2.77} / \sqrt{N}} = 1.21 \quad 48 \text{ hr } LC_{50} = 0.00032 \text{ mg/l} \quad \left(\begin{array}{l} 0.00026 \\ 0.00039 \text{ mg/l} \end{array} \right) \quad 95\% \text{ C.I.}$$

LC₅₀ / f LC₅₀ = lower limit = 0.00026
 LC₅₀ x f LC₅₀ = upper limit = 0.00039

Analysis by: Carl S. Thompson (Name) Aquatic Biologist (Title) 8/20/79 (Date)

Checked By: Ala. D. Forbes Date: 8/22/79

ANALYTICAL BIOCHEMISTRY LABORATORIES

Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Aquatic A Date tested 8/16 - 8/20/79
 Test Species Fathead minnow ^{LC50} ~~LC50~~ Date reported 8/20/79
 Test Number Reference Toxicant Temperature 22°C
 Exposure period 96 hrs Water quality Fresh H₂O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	D-E	Contribution to Chi
CONTROL	0/10	0	-	-	-
0.00024	3/10	30	30	0	0.000
0.00037	8/10	80	80	0	0.000
0.00056	10/10	100 (100)	95.0	1	0.007
	1				
	1				
	1				

Total animals = 30
 K = 3

Total contribution to Chi 0.007
 Chi² - contribution X total animals = 0.07
 to Chi K
 Chi² (p-.05) for (K-2) 1 deg. of freedom = 3.84

LC₉₄ = 0.00039
 LC₅₀ = 0.00028
 LC₁₆ = 0.00021

$S = \frac{LC_{94}/LC_{50} + LC_{50}/LC_{16}}{2}$
 $S = \frac{0.00039}{0.00028} + \frac{0.00028}{0.00021} = 1.36$

Confidence limits (.05) for LC₅₀

$N' = \frac{20}{f}$
 $f LC_{50} = S^{2.77} / \sqrt{N'} = 1.21$

96 hr LC₅₀ = 0.00028 mg/l ^(0.00023 - 0.00034 mg/l)
 95% C.I.

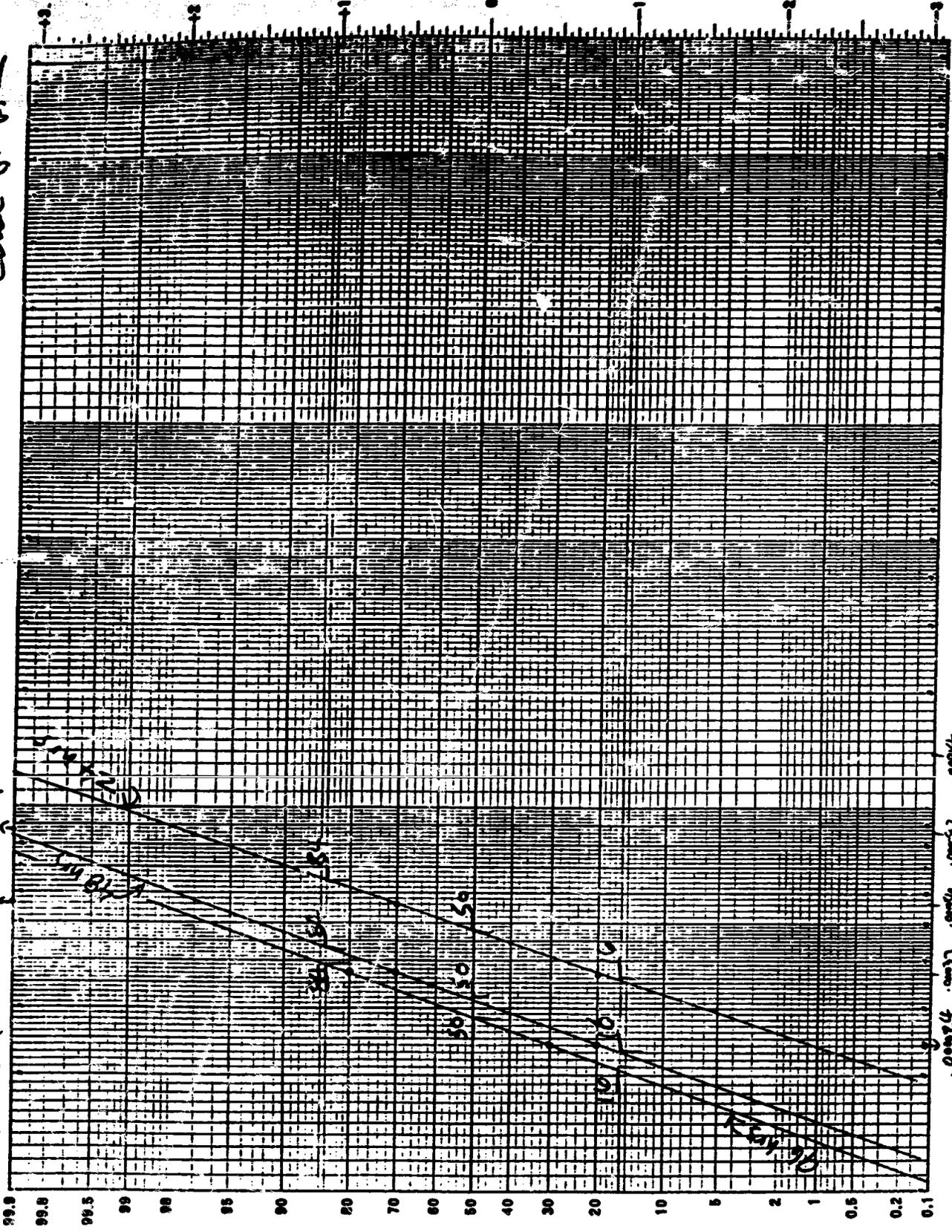
LC₅₀ / f LC₅₀ = lower limit = 0.00023
 LC₅₀ X f LC₅₀ = upper limit = 0.00034

Analysis by: Conroy, J. P. Aquatic Biologist 8/20/79
 (Name) (Title) (Date)

Checked By: W. D. Forbi Date: 8/20/79

CASE NUMBER

Aptingsein A
Fatigue Minimum Lot 2179



Hours: 1000, 500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1

Anticipation of cost 100000

% Mortality

ANALYTICAL BIOCHEMISTRY LABS

AQUATIC BIOASSAY LAB

TEST FISH MEASUREMENTS

Test: ThiotaxTest Species: Fathead MinnowLot No. 2779Source: Fetting Fish HatcheryDate Measured 8/27/79Group Measured: Control group at test termination

Fish No.	Standard Length (mm)	Weight (g)
1	24	0.20
2	26	0.24
3	22	0.20
4	23	0.22
5	24	0.22
6	18	0.16
7	20	0.16
8	24	0.20
9	22	0.20
10	26	0.24
Mean Standard Length (mm)	22.9 (S.D. = 2.5)	
Mean Weight (g)		0.20 (S.D. = 0.03)

Remarks: _____

Prepared By: Carl J. HoplerChecked By: Ab. D. John

Fish Requisition and Disposition Record

Species: Emthead minnow Date Rec: 8/13/79 Lot #: 2779
 Source: Fetting Fish Hatchery No. Rec: 2000 Tank #: 3
Bradley, Nebraska Designated Use: Bionology

Comments: Shipped by air - arrived good condition

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
16	<u>Combiotic</u>	<u>25 ppm</u>	<u>1 hr</u>		<u>[Signature]</u>
17	<u>Combiotic</u>	<u>25 ppm</u>	<u>1 hr</u>		<u>[Signature]</u>
18	<u>Combiotic</u>	<u>25 ppm</u>	<u>1 hr</u>		<u>[Signature]</u>

Daily Record

Date	#Dead	Comments	Name	Date	#Dead	Comments	Name
8/19	15	Shipping loss	<u>[Signature]</u>	8/21	0	Fed 8	<u>[Signature]</u>
8/22	0	Fed 8	<u>[Signature]</u>	8/22	0	"	<u>[Signature]</u>
8/23	0	"	<u>[Signature]</u>	8/23	0	"	<u>[Signature]</u>
8/24	0	"	<u>[Signature]</u>	8/24	0	"	<u>[Signature]</u>
8/25	0	"	<u>[Signature]</u>	8-25	0	Fed 2	<u>[Signature]</u>
8/26	0	"	<u>[Signature]</u>	8/26	0	Fed 8	<u>[Signature]</u>
8/27	0	"	<u>[Signature]</u>	8/27	0	"	<u>[Signature]</u>
8/28	0	"	<u>[Signature]</u>				
8/29	0	"	<u>[Signature]</u>				
8/30	2	"	<u>[Signature]</u>				
8/31	0	"	<u>[Signature]</u>				
9/1	0	"	<u>[Signature]</u>				
9/2	0	"	<u>[Signature]</u>				
9/3	0	"	<u>[Signature]</u>				
9/4	0	"	<u>[Signature]</u>				
9/5	0	"	<u>[Signature]</u>				
9/6	0	"	<u>[Signature]</u>				
9/7	0	"	<u>[Signature]</u>				
9/8	0	"	<u>[Signature]</u>				
9/9	0	"	<u>[Signature]</u>				
9/10	0	"	<u>[Signature]</u>				
9/11	0	"	<u>[Signature]</u>				
9/12	0	"	<u>[Signature]</u>				
9/13	0	"	<u>[Signature]</u>				
9/14	0	"	<u>[Signature]</u>				
9/15	0	"	<u>[Signature]</u>				
9/16	0	"	<u>[Signature]</u>				
9/17	0	"	<u>[Signature]</u>				
9/18	0	"	<u>[Signature]</u>				
9/19	0	"	<u>[Signature]</u>				
9/20	0	"	<u>[Signature]</u>				
9/21	0	"	<u>[Signature]</u>				
9/22	0	"	<u>[Signature]</u>				
9/23	0	"	<u>[Signature]</u>				
9/24	0	"	<u>[Signature]</u>				
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9/29	0	"	<u>[Signature]</u>				
9/30	0	"	<u>[Signature]</u>				
10/1	0	"	<u>[Signature]</u>				
10/2	0	"	<u>[Signature]</u>				
10/3	0	"	<u>[Signature]</u>				
10/4	0	"	<u>[Signature]</u>				
10/5	0	"	<u>[Signature]</u>				
10/6	0	"	<u>[Signature]</u>				
10/7	0	"	<u>[Signature]</u>				
10/8	0	"	<u>[Signature]</u>				
10/9	0	"	<u>[Signature]</u>				
10/10	0	"	<u>[Signature]</u>				
10/11	0	"	<u>[Signature]</u>				
10/12	0	"	<u>[Signature]</u>				
10/13	0	"	<u>[Signature]</u>				
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10/15	0	"	<u>[Signature]</u>				
10/16	0	"	<u>[Signature]</u>				
10/17	0	"	<u>[Signature]</u>				
10/18	0	"	<u>[Signature]</u>				
10/19	0	"	<u>[Signature]</u>				
10/20	0	"	<u>[Signature]</u>				
10/21	0	"	<u>[Signature]</u>				
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10/26	0	"	<u>[Signature]</u>				
10/27	0	"	<u>[Signature]</u>				
10/28	0	"	<u>[Signature]</u>				
10/29	0	"	<u>[Signature]</u>				
10/30	0	"	<u>[Signature]</u>				
10/31	0	"	<u>[Signature]</u>				

Checked by [Signature]



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

ABC PROTOCOL NO. 7601
(Revised June 21, 1979)

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

ABC Study Number 23809

Test Material Thiotax (Study No. AB-79-1384365-1a)

Test Species Fathead Minnows (Pimephales promelas)

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 LC₅₀ levels of the toxicant during a 96 hour exposure period. An LC₅₀ 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 LC₅₀ 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 ABC Lane, P. O. Box 1097, Columbia, Missouri 65205.

4.0 RANGE-FINDING STUDY

4.1 General. For most chemical substances, the approximate toxic level to aquatic organisms is not known. Because this information is essential before a definitive toxicity test can be conducted, ABC routinely performs range-finding tests for static bioassays with 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) 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 $\pm 1^{\circ}\text{C}$ of the desired test temperature (Table 1). For temperature control in the water bath, thermostatically controlled heating elements will be used for warmwater tests ($22 \pm 1^{\circ}\text{C}$) and refrigeration units (MinoOCool[®]) for coldwater bioassays ($12 \pm 1^{\circ}\text{C}$). 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 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 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 8.3. The test concentrations will be prepared on a weight/volume basis unless otherwise specified. A record of all sample weights and dilutions will be kept, checked by a second party, and furnished in the final report.

4.5 Test Procedure. The rangefinding 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 size will conform to the maximum loading limitation of 0.8 grams of fish per liter of solution (5). One of the following types of glass 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 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 LC₅₀ values and their corresponding 95 percent confidence limits. For data sets with one or more partial mortalities, in addition to 0 and 100 percent response levels, the statistical method described by Litchfield and Wilcoxon (11) will be employed. If no partial mortalities are represented, i.e. if the test yields only 0 and 100 percent mortalities at any observation period, a binomial test (12) will be applied for the LC₅₀ estimation.

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 CHANGES

In the event that modifications of this protocol are deemed necessary, a written statement of any changes and reason(s) proposed by the study sponsor or ABC will be submitted to the other party. All agreed changes will be expressed in writing, signed and dated by the sponsor's study director. The signed changes will be appended to the protocol and included with the final report.

7.0 SPONSOR AUTHORIZATIONS DURING THE STUDY

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

8.0 TEST-SPECIFIC INFORMATION

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

8.2 Study Sponsor:

8.2.1 Company Monsanto Chemical Company

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

8.2.3 Study Director (Coordinator)

<u>Dr. William J. Adams</u>	<u>Study Director</u>
Name	Title

8.3 Test Material:

8.3.1 Name Thiotax (Study No. AB-79-1384365-1a)

8.3.2 Code Number AB-79-1384365-1a

8.3.3 Physical Description yellow powder

8.3.4 Purity 99%+ active Thiotax

8.3.5 Stability Stable to heat & light

8.3.6 Recommended Solvent Acetone

8.3.7 Water Solubilit, ~ 5 ppm

8.3.8 Handling Precautions No special handling required

8.4 Test Concentrations:

8.4.1 Definitive Concentrations 4.2, 7.5, 14, 24, 42 and
75 mg/l

8.5 Test Fish:

8.5.1 Species Fathead Minnows (Pimephales promelas)

8.5.2 Supplier Fattig Fish Hatchery
Brady, Nebraska

8.6 Study Dates:

8.6.1 Proposed starting date of definitive study 8/22/79

8.6.2 Proposed completion date of definitive study 3/26/79

8.7 ABC Study Personnel:

8.7.1 Study Director

Carl M. Thompson Aquatic Biologist
Name Title

8.7.2 Principle Investigator

Jerry R. 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

William J. Adams Dr. Bill Adams 8-17-79
Name Title Date

8.8.2 ABC's Study Director

Carl M. Thompson Study Director 8/15/79
Name Title Date

8.8.3 ABC's Laboratory Director

[Signature] Lab Manager 8/15/79
Name Title Date

TABLE 1: RECOMMENDED SPECIES AND TEST TEMPERATURES^a

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

^aAdapted from (5).

^bRecommended for compounds used in Japan.

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

<u>Col. 1</u>	<u>Col. 2</u>	<u>Col. 3</u>	<u>Col. 4</u>	<u>Col. 5</u>
10.0	10.0	10.0	10.0	10.0
			7.5	8.7
				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.

<u>Parameters</u>	<u>Concentration</u>
Dissolved Oxygen	9.3 ppm
pH	8.2
Hardness (CaCO ₃)	255 ppm
Alkalinity (CaCO ₃)	268 ppm
Conductivity	50 umhos/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
DDVP	<40 ng/l
Diazinon	<20 ng/l
Disyston	<20 ng/l
Methyl Parathion	<80 ng/l
Malathion	<110 ng/l
Ethyl Parathion	<80 ng/l

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.



(MBS)

Toxicity of Thiotax (BN-79-1384365-1e)
to the freshwater alga Selenastrum
capricornutum

BN-79-313

1/77 ~~BP 77-7-107~~

BIONOMICS



Toxicity of Thiotax (BN-79-1384365-1e)
to the freshwater alga Selenastrum
capricornutum

BN-79-313

~~BP 77-17~~

Toxicity Test Report

Submitted to

Monsanto Industrial Chemicals Company

St. Louis, Missouri

Project Number H97-500

Report Number BP-79-7-107

EG&G, Bionomics
Marine Research Laboratory
Route 6, Box 1002
Pensacola, Florida
July 1979

A phytotoxicity test was performed at Bionomics Marine Research Laboratory (BMRL), Pensacola, Florida, to determine the effect of Thiotax (BN-79-1384365-1a) on the freshwater alga Selenastrum capricornutum. Results of the test are reported as 24-, 48-, 72-, and 96-hour EC50's (the concentrations of the test material estimated to cause a 50% decrease of in vivo chlorophyll α in exposed cultures as compared to the control at the specified times). Cell numbers in exposed and control cultures were also determined after 96 hours of exposure and another 96-hour EC50 was calculated (the concentration of the test material estimated to cause a 50% decrease of cell numbers in exposed cultures as compared to the control). Confirmation of effect by measurement of two different growth factors is important, in our opinion, because of the various alga responses in the presence of toxicants (Hall, 1973).

All raw data related to this test is stored at BMRL.

MATERIALS AND METHODS

Test material

The sample was received at BMRL on 22 May 1979. The sample was contained in a clear-glass bottle labeled "Thiotax (lot NL01-007); BN-79-1384365-1a." The test material was a pale yellow powder. Concentrations are reported here as micrograms (μg) of test material per liter (l) of algal growth medium or parts per billion (ppb).

Test alga

The freshwater alga tested was the unicellular green alga, Selenastrum capricornutum. The culture was obtained from the U.S. Environmental Protection Agency's Environmental Research Laboratory, Corvallis, Oregon, and maintained in stock culture at BMRL.

Test conditions

Culture and test procedures followed those of U.S. Environmental Protection Agency (1971) except as noted. Beginning cell numbers in the test flasks were 20×10^3 cells/milliliter (ml). Cultures were incubated at 24 ± 1 degrees Celsius ($^{\circ}\text{C}$) under $\sim 3,800$ lux illumination. Triplicate cultures were employed for each of the test concentrations and the controls. Test containers were 125-ml flasks each containing 50 ml of test medium. Concentrations for the definitive test were based on the results of a 96-hour range-finding test. Test concentrations were prepared by adding appropriate volumes of a stock solution of test material to each flask. A primary stock solution was prepared by adding a weighed amount of test material to reagent grade dimethylformamide (DMF) and secondary stock solutions were prepared by serial dilution for the range-finding and definitive tests. A solvent control was also maintained to which was added 0.05 ml of DMF, the maximum volume added to a test flask for the definitive test.

Measurements of in vivo chlorophyll a in cultures were performed by using a Turner Model 111 fluorometer. Cell counts were made by using a hemacytometer and a Zeiss Standard 14 compound microscope.

The definitive test was conducted 4-8 June 1979.

Statistical analyses

Each test concentration was converted to a logarithm and the corresponding percentage decrease of in vivo chlorophyll a or cell numbers was converted to a probit (Finney, 1971). The 48-, 72-, and 96-hour EC50's and 95% confidence limits were then calculated by linear regression.

To determine whether growth of the solvent control differed from that of the culture medium control, data were analyzed by "Student's" t-test (Steel and Torrie, 1960). Differences were considered significant at the 95% confidence level ($P < 0.05$).

11048

RESULTS AND DISCUSSION

The toxicity of Thiotax to Selenastrum capricornutum appeared to increase slightly throughout the 96 hours of exposure. Based on decrease of in vivo chlorophyll *a*, the estimated 24-, 48-, and 72-hour EC50's were >300<600 ppb while the calculated 96-hour EC50 was 230 ppb with 95% confidence limits of 60-980 ppb. The calculated 96-hour EC50, based on cell number decrease, was 250 ppb with 95% confidence limits of 60-980 ppb (Table 1). After 96 hours of exposure, decrease of in vivo chlorophyll *a* was from 12% in cultures exposed to 100 ppb to 89% in those exposed to 600 ppb. Decrease of cell numbers was from 8% in cultures exposed to 60 ppb to 87% in those exposed to 600 ppb after 96 hours. The pH was from 7.4-7.5 after 96 hours of exposure (Table 2).

There was no significant difference between growth of the control and solvent control cultures after 96 hours of exposure, based on both in vivo chlorophyll *a* and cell numbers.

REFERENCES

- Finney, D. J. 1971. Probit Analysis. Cambridge University Press, London. 333 p.
- Hall, R. H. 1973. An Algal Toxicity Test Used in the Safety Assessment of Detergent Components. Presented before the Thirty-Sixth Annual Meeting of the American Society of Limnology and Oceanography, Inc., Salt Lake City, Utah. 23 p.
- Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York. 481 p.
- U.S. Environmental Protection Agency. 1971. Algal Assay Procedures: Bottle Test. National Eutrophication Research Program, Pacific Northwest Water Laboratory, Corvallis, OR. 82 p.

TABLE 1. Estimated or calculated EC50's for the freshwater alga *Selenastrum capricornutum* exposed to Thiotax (BN-79-1384365-1a). The criterion for effect was decrease of *in vivo* chlorophyll *a* in exposed cultures as compared to the control at 24, 48, 72, and 96 hours or decrease of cell numbers in exposed cultures as compared to the control at 96 hours. Calculations were based on nominal concentrations of Thiotax algal growth medium.

Effect Criterion	Hour	EC50 ($\mu\text{g}/\text{l}; \text{ppb}$)	95% confidence limits ($\mu\text{g}/\text{l}; \text{ppb}$)
<u>In vivo</u> chlorophyll <i>a</i>	24	>300<600	---
	48	>300<600	---
	72	>300<600	---
	96	230	60-980

Cell number	96	250	60-980

TABLE 2. Results of a 96-hour exposure of the freshwater alga Selenastrum capricornutum to Thictax (BN-79-1384365-1e). Percentage change is increase or decrease of in vivo chlorophyll a in exposed cultures as compared to the control at 24, 48, 72, and 96 hours and decrease of cell numbers in exposed cultures as compared to the control at 96 hours.

Nominal concentration ($\mu\text{g}/\text{l}$; ppb)	pH		Percentage change				Cell no. 96 h
	0 h	96 h	Chlorophyll <u>a</u>				
			24 h	48 h	72 h	96 h	
Control	7.3	7.5	---	---	---	---	---
Sol. control	7.3	7.5	+5	0	0	+1	-1
30	7.3	7.5	+8	+16	+5	+3	+2
60	7.3	7.5	-5	+6	+1	-13	-8
100	7.3	7.5	-2	+3	+1	-12	-12
300	7.3	7.5	-26	-39	-47	-51	-53
600	7.3	7.4	-51	-78	-86	-89	-87

PREPARED BY:

Terry Hollister

Terry Hollister
Study Director

AUDITED BY:

G. Scott Ward

G. Scott Ward
Quality Assurance Unit

REVIEWED BY:

Peter J. Shuba, Ph.D.

Peter J. Shuba
Project Coordinator

APPROVED BY:

Rod Parrish

Rod Parrish
Director

Project No.

BN-76-169

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
December, 1976

(MST)

Test Material: Thiotax (N008-008) CP-1975
Description: Yellow-colored powder
Report No.: _____ Other Codes: BN-76-169
Submitted by: Dr. James Laveglia, Monsanto Company, St. Louis, Missouri

ACUTE (96-HOUR) TOXICITY OF THIOTAX
TO RAINBOW TROUT AND BLUEGILL

PROCEDURES: Rainbow trout and bluegill toxicity testing procedures are on the reverse side.

SUMMARY: LC50* and 95% confidence interval (mg/l)

Rainbow trout		Bluegill	
24-hour	0.92 (0.72-1.2)	24-hour	3.4 (2.2-5.5)
48-hour	0.75 (0.55-1.0)	48-hour	2.1 (1.8-2.5)
96-hour	0.75 (0.55-1.0)	96-hour	1.5 (1.2-1.9)

*LC50 = The concentration which is lethal to 50% of a population of test organisms during the specified time period.

RESULTS:

Nominal test concentration (mg/l)	Rainbow trout Observed percentage mortality		
	24-hour	48-hour	96-hour
1.8	100	100	100
1.0	80	100	100
0.75	10	20	20
0.56	0	0	0
0.42	0	0	0
control (acetone)	0	0	0
control	0	0	0

Nominal test concentration (mg/l)	Bluegill Observed percentage mortality		
	24-hour	48-hour	96-hour
5.6	100	100	100
3.2	40	100	100
2.4	0	70	90
1.8	0	10	50
1.4	0	0	10
1.0	0	0	40
0.75	0	0	0
control (acetone)	0	0	0
control	0	0	0

PROCEDURES:

Testing was conducted according to the protocol submitted to Monsanto Company in October, 1976.

Rainbow trout: The test material, in reagent-grade acetone, was introduced into 15 l of diluent water in all-glass vessels. To each test vessel, 10 rainbow trout (*Salmo gairdneri*, 3.7 cm standard length) were then added. Fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at $12 \pm 1.0^\circ\text{C}$. Dissolved oxygen ranged from 9.9 mg/l (93% of saturation) to 2.0 mg/l (19% of saturation) at the beginning and end of exposure, respectively. pH values ranged from 7.4 initially to 6.9 at the end of the test. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure.

Bluegill: The test material, in reagent-grade acetone, was introduced into 15 l of diluent water in all-glass vessels. To each test vessel, 10 bluegill (*Lepomis macrochirus*, 3.8 cm standard length) were then added. Fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at $22 \pm 1.0^\circ\text{C}$. Dissolved oxygen ranged from 8.8 mg/l (100% of saturation) to 0.3 mg/l (3% of saturation) at the beginning and end of exposure, respectively. pH values ranged from 7.3 initially to 6.7 at the end of the test. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure.

STATISTICS:

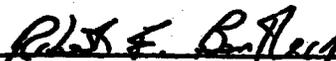
Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50's and the 95% confidence intervals were calculated from the regression equation.

Reported by:



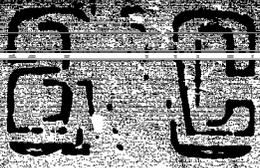
Robert J. Buccafusco
Aquatic Biologist

Approved by:



Robert E. Bentley
Aquatic Toxicologist





MASTER FILE AR-79-214

ANALYTICAL BIO-CHEMISTRY LABORATORIES, INC.
P.O. Box 1007 · Columbia, MO 65205 · (314) 474-8579

**Static Acute Bioassay Report
#23810**

Submitted To:

**Monsanto Chemical Company NIB
Attn: Bill Adams
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166**

(MBT)

**Acute Toxicity of Thiofax (AB-79-1384365-1d)
to Daphnia magna**

May 31, 1979

0056

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

Prepared By:

Carl M. Thompson 7/10/79
Date
Carl M. Thompson
Aquatic Biologist

Alan D. Forbis 7/10/79
Date
Alan D. Forbis
Aquatic Supervisor

Approved By:

James A. Ault 7/10/79
Date
James A. Ault
Quality Assurance Officer

Lyle D. Johnson 7-10-79
Date
Lyle D. Johnson
Laboratory Manager

0057

SUMMARY

The acute toxicity of ^{Thiofax} ~~Thiofax~~ to Daphnia magna was assessed using the methods outlined by the Committee 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.

<u>Compound</u>	<u>48-hour LC₅₀</u> <u>(95% C.I.)</u>
Thiofax	4.1 (3.6-4.7) mg/l

The no effect level observed for Thiofax was 1.8 mg/l after 48 hours.

0058

INTRODUCTION

This definitive static bioassay was performed at the aquatic bioassay laboratory of Analytical BioChemistry Laboratories, Inc., Columbia, Missouri, for Monsanto Chemical Company, from May 29 to May 31, 1979. The purpose of this test was to determine the 24 and 48 hour LC₅₀ levels for Thiofax to Daphnia magna. The study was performed using ABC Protocol #7806.

METHODS AND MATERIALS

The procedure 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 2. 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 10 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 bioassays.

The Thiofax standard was received on May 21, 1979, as a yellow powder and was stored at 1°C. Sample purity was not provided; therefore, test concentrations were prepared based on total compound received. All standard weights and dilutions can be found in Appendix I. Acetone was used in the preparation of all working stock solutions.

RESULTS

Table 1 presents the predicted LC₅₀ (3) values and 95% confidence intervals for Thiofax.

The dissolved oxygen was 8.8 mg/l and the pH was 7.4 at the termination of the test.

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
Acute Toxicity of Thiofax
to Daphnia magna

<u>Compound</u>	<u>LC₅₀ (mg/l)</u>	
	<u>24 hours</u>	<u>48 hours</u>
Thiofax	7.0 (6.0-8.1)*	4.1 (3.6-4.7)*

***95% confidence interval (3).**

TABLE 2

**Chemical characteristics of well water at ABC's
Aquatic Bioassay Laboratory**

<u>Parameter</u>	<u>Concentration</u>
Dissolved Oxygen	9.3 ppm
pH	7.8
Hardness (CaCO ₃)	255 ppm
Alkalinity (CaCO ₃)	368 ppm
Conductivity	50 umhos/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
DDVP	<40 ng/l
Diazinon	<20 ng/l
Disyston	<20 ng/l
Methyl Parathion	<80 ng/l
Malathion	<110 ng/l
Ethyl Parathion	<80 ng/l

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. 660/3-750/9.
- (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. Federal Register, Vol. 43, No. 247, 59986-60025, December 22, 1978.

Quality Assurance Statement for final report #23810 entitled, "Acute Toxicity of Thiofax (AB-79-1384365-1d) to Daphnia magna," for Bill Adams, 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 Thiofax was conducted and found to be in acceptable form by a member of our Quality Assurance Unit. It was noted that since the purity of the compound being treated had not been supplied, the LC₅₀ would have to be calculated based on nominal concentrations of the total compound. A final inspection of all data and records on May 31, 1979, 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 7/10/79
James A. Ault Date
Quality Assurance Officer

APPENDIX I
RAW DATA

ANALYTICAL BIOCHEMISTRY Lab
Aquatic Bioassay Lab
Acute Toxicity Bioassay

Date: (5/29/79) - (5/31/79)

Time: 8:00

Tank: _____

Purity: _____

Solvent: Acetone

Toxicant: Piclor

Lab No.: 23910

Formulator: AF

Date Made: 5/29/79

Stock Standards
 Final Gross Wt. 0.200 g
 Tare wt. 0.000 g
 Net wt. 0.200 g
 Adj. net wt. _____ g*
 Dilution vol. 10 ml
 Concentration 20 mg/ml

Working Standards
 Aliquot of concentrated stock _____
 Dilution Vol. _____
 Final Concentration _____ mg

(Balance Calibration **)

Test Species: Daphnia magna Lot No. _____ Temp.: 20°C
 Source AAC Condition: EXC. Weight: _____ Length: _____
 No./Vessel: 10 Vessel Size: 250 (200) Void: _____
 Water Chemistry: AAC well pH: 7.8 Alkalinity: 368 mg/l Hardness: 255
 (CaCO₃) (CaCO₃)

Concentration mg/l	ml	24 hr		48 hr		72 hr		96 hr		Dead	Obs
		Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.		
<u>etidine</u>											
<u>5/26/79 AF</u>											
<u>10</u>				<u>4/9</u>		<u>10</u>					
<u>100</u>				<u>10</u>		<u>10</u>					
<u>Reference</u>											
<u>Testis AF</u>											
<u>Control</u>				<u>0</u>		<u>0</u>					
<u>Control/stock</u>				<u>0</u>		<u>0</u>					
<u>10</u>	<u>0.10</u>			<u>8</u>	<u>7</u>	<u>10</u>	<u>10</u>				
<u>5.0</u>	<u>0.050</u>			<u>3</u>	<u>2</u>	<u>9</u>	<u>8</u>				
<u>3.2</u>	<u>0.032</u>			<u>0</u>	<u>0</u>	<u>2</u>	<u>2</u>				
<u>1.8</u>	<u>0.018</u>			<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>				
<u>1.0</u>	<u>0.010</u>			<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>				
<u>Observer</u>				<u>AF</u>		<u>AF</u>					
<u>Date</u>				<u>5/29/79</u>		<u>5/29/79</u>					
<u>Observer</u>				<u>JG</u>		<u>JG</u>					
<u>Date</u>				<u>5/27</u>		<u>5/27</u>					

Remarks: 1 essay 5/27/79 JG

0.0 - 8.8 mg/l 3.2 mg/l beaker at
pH - 7.4) termination of study

Prepared by: AF
 Checked by: Bill McAllister

COMPOUND PREPARATIONS

Compound Aspirin Lot No. _____ Purity _____ Lab No. 23910

Preparation of Concentrated Working Standard Acetone

Date 5/20/79 Chemist A. Korbis
 Initial Gross Weight 0.200 g Dilution Volume 10 ml
 Tare Weight 0.000 g Concentration 20 mg/ml
 Net Weight 0.200 g Balance calibrated with class S weights:
 Adj. Net Weight _____ g $\frac{1.000}{\text{(class S)}} \text{ g} + \frac{0.000}{\text{(tare)}} \text{ g} = \frac{1.000}{\text{(final wt.)}} \text{ g}$

Preparation of Test Concentrations

Std.	Conc. of Work. Std. (mg/ml)	Date <u>5/25/79</u> Aliq. Vol. (ml)	Chemist <u>A. Korbis</u> Dilution Vol. (l)	Final Conc. (mg/l)
Control	_____	_____	_____	_____
1.	<u>20</u>	<u>1.0</u>	<u>0.2</u>	<u>100</u>
2.	<u>20</u>	<u>0.1</u>	<u>0.2</u>	<u>10</u>
3.	_____	_____	_____	_____
4.	_____	_____	_____	_____
5.	_____	_____	_____	_____
6.	_____	_____	_____	_____
7.	_____	_____	_____	_____

Preparation of Concentrated Working Standard

Date _____ Chemist _____
 Initial 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 $\frac{\text{g}}{\text{(class S)}} + \frac{\text{g}}{\text{(tare)}} = \frac{\text{g}}{\text{(final wt.)}}$

Preparation of Test Concentrations

Std.	Conc. of Work. Std. (mg/ml)	Date <u>5/29/79</u> Aliq. Vol. (ml)	Chemist <u>A. Korbis</u> Dilution Vol. (l)	Final Conc. (mg/l)
Control	_____	_____	_____	_____
1.	<u>Acetone</u>	<u>0.10</u>	<u>0.2</u>	<u>Control</u>
2.	<u>20</u>	<u>0.10</u>	<u>0.2</u>	<u>Control</u>
3.	<u>20</u>	<u>0.056</u>	<u>0.2</u>	<u>10</u>
4.	<u>20</u>	<u>0.032</u>	<u>0.2</u>	<u>5.6</u>
5.	<u>20</u>	<u>0.018</u>	<u>0.2</u>	<u>2.2</u>
6.	<u>20</u>	<u>0.010</u>	<u>0.2</u>	<u>1.8</u>
7.	_____	_____	_____	<u>1.0</u>

Remarks: _____

Prepared By: A. D. Korbis Checked By: Will M. White
 corrected for purity of primary standard.

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Testicant Thiotar Date tested (5/28/79)-(5/31/79)
 Test Species Daphnia magna Date reported 5/31/79
 Test Number 23810 Temperature 20°C
 Exposure period 24hr Water quality well H₂O

mg/L Concentration	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
Control	0/20				
Control + acetone	0/20				
10	17/20	85	84	1	0.000
5.6	5/20	25	25	0	0.000
3.2	0/20	0 (0.3)	1	0.7	0.005
1.8	0/20				
1.0	0/20				

Total animals = 60 Total contribution to Chi 0.005
 K = 3 Chi²- contribution X total animals = 0.10
 to Chi K
 Chi² (p=.05) for (K-2) 1 deg. of freedom = 3.84

$z_{.84} = \underline{1.0}$
 $z_{.50} = \underline{0}$
 $z_{.16} = \underline{-0.98}$

$$\frac{LC_{.84}/LC_{.50} + LC_{.50}/LC_{.16}}{2} = \underline{1.41}$$

Confidence limits (.05) for LC₅₀

$$LC_{50} = \frac{40}{5^{2.77} / \sqrt{N}} = \underline{1.16}$$

$$LC_{50} = \frac{7.0(6.0 - 8.1)ms/l}{95\% C.I.}$$

C₅₀ / f LC₅₀ = lower limit = 6.0
 C₅₀ X f LC₅₀ = upper limit = 8.1

Analysis by: John D. Folan (Name) Agentic Supervisor (Title) 7/1/79 (Date)

Checked By: Bill McAllister Date: 5-31-79

**Aquatic Bioassay Laboratory
Acute Toxicity Bioassay**

Probit Analysis Work Sheet

Test Species Thiara Date tested (5/29/79) - (5/31/79)
 Test Species Raphia Date reported 5/31/79
 Test Number 23816 Temperature 20°C
 Exposure period 48h Water quality well H₂O

Concentration ^{mg/L}	No. dead / total no.	Observed % mortality	Expected % mortality	D-E	Contribution to Chi
Control	0/20				
Control + Thiara	0/20				
1.0	20/20	100 (99.9)	99.8	0.1	0.000
5.6	17/20	85	84	1	0.000
3.2	4/20	20	20	0	0.000
1.8	6/20	0 (0.12)	0.4	0.28	0.002
1.0	0/20				

Total animals = 80
 K = 4

Total contribution to Chi 0.002
 Chi² - contribution X total animals = 0.04
 to Chi K
 Chi² (p=.05) for (K-2) 2 deg. of freedom = 5.99

$C_{84} = \underline{5.6}$
 $C_{50} = \underline{4.1}$
 $C_{16} = \underline{3.0}$

$$\frac{C_{84}/C_{50} + C_{50}/C_{16}}{2} = 1.37$$

Confidence limits (.05) for LC₅₀

$$f = \frac{40}{52.77 / \sqrt{N}} = 1.15$$

$$LC_{50} = \frac{4.1 (3.6 - 4.7) \text{ mg/L}}{95\% \text{ C.I.}}$$

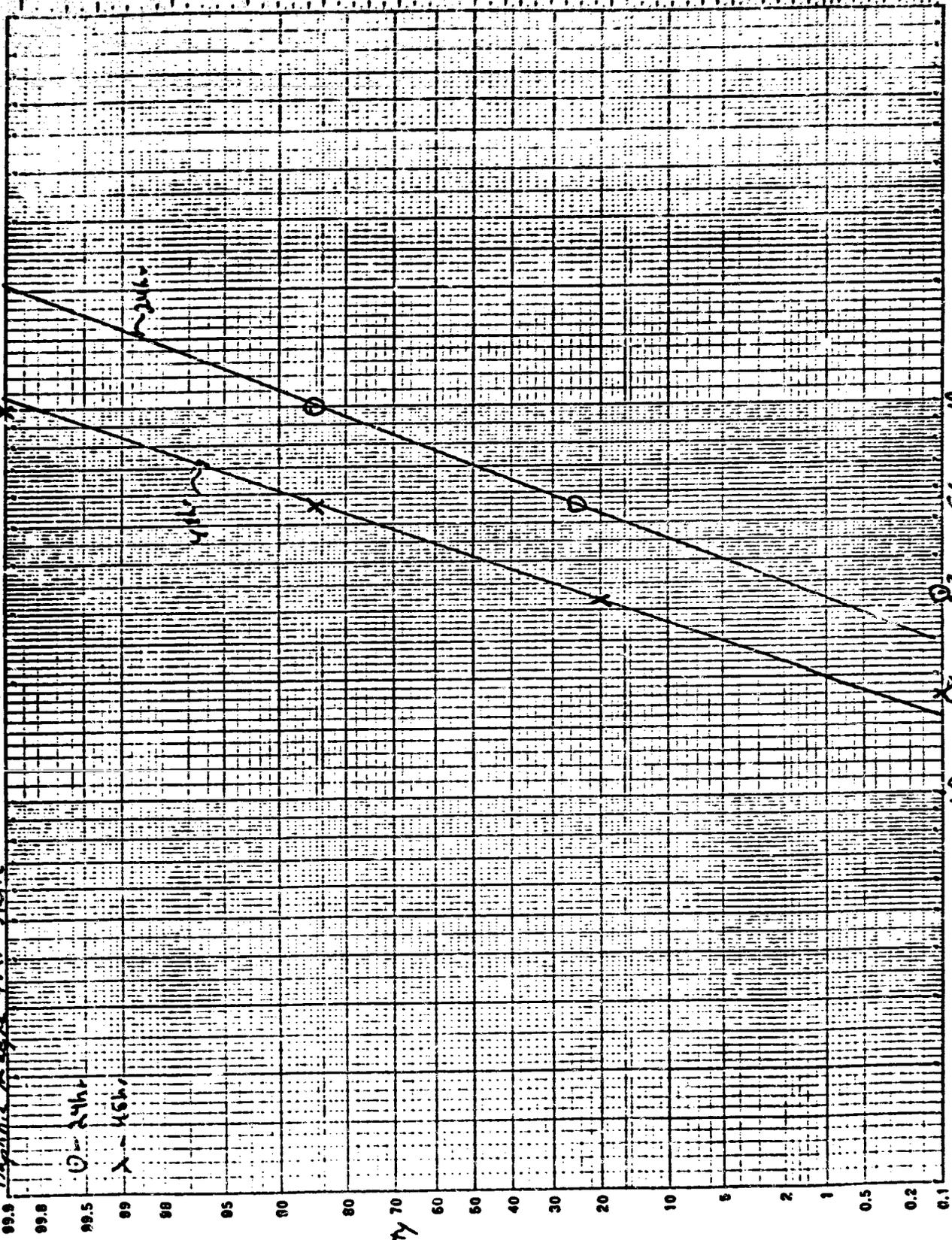
LC₅₀ / f LC₅₀ = lower limit = 3.6
 LC₅₀ X f LC₅₀ = upper limit = 4.7

Analysis by: John D. Fodis (Name) Aquatic's Impression (Title) 5/31/79 (Date)

Checked By: Bill Maltz Date: 5-31-79

Thio tax
Daphnia magna 4hr static

○ - 24hr
x - 45hr



9
mortality

mg/L

C. Daphnia Food

All daphnia will be fed food prepared in the following manner.

Place 12 g PR-11, 3 g active dry yeast and 400 ml dilution water in a blender and blend for five minutes at high speed. Pour into a 1 liter beaker. Rinse the blender with 600 ml dilution water and add to beaker. Mix well and let settle for ten minutes. Siphon 800 ml into a 1000 ml graduated cylinder. Pour into closed container.

Mix the 800 ml of food mixture well, place 25 ml in a tared aluminum weighing pan, dry at 103°C and weigh. Dilute food mixture to 5 mg dry solids per ml of mixture by adding Y ml of dilution water to food mixture in the container, where

$$Y \text{ ml} = \frac{(775 \text{ ml}) \times (\text{mg of solids in weighing pan})}{(5 \text{ mg/ml}) \times (25 \text{ ml of food mixture in weighing pan})} - 775 \text{ ml.}$$

Mix well, cover and store in refrigerator for up to 14 days. Mix thoroughly before each use. Food concentration for the cultures will be 30 mg/l.

D. Test Compound

Enough chemical substance will be supplied by the contracting company in sufficient amounts to perform any preliminary and definitive toxicity testing. Also, the contracting company will make available to ABC Labs any solubility, toxicity and human hazard data.

E. Test Procedure-Biological

When necessary a preliminary test will be conducted in order to define the general toxicity level of the test material. It would be composed of 2 to 5 widely spaced concentrations of test material containing 5 to 10 daphnia per beaker. After this level has been determined, at least five or more concentrations of test material, ranging in a logarithmic series, with 10 daphnia per beaker will be selected for their respective bioassays. Generally duplicate beakers will be used for each concentration but this can be reduced or expanded to meet the needs of individual companies. The daphnia will be added to the test by random assignment within 15 minutes after the addition of test material. Mortality data will be recorded at 24 hours and 48 hours. Mortality will be defined as no observed movement by the Daphnia magna.





#7806

Static Bioassay Procedure for Determining Toxicity
of Chemical Substances to Daphnia magna

I. Introduction

Daphnids are an ecologically significant macroinvertebrate found in most fresh water habitats. Chemical substances in the aquatic environment may or may not produce toxicity of the Daphnids. The primary objective of the Daphnia magna static bioassay is to define the 48 hour toxicity of chemical substances by determining the median lethal concentrations (LC₅₀'s) during the course of the test. This protocol describes the technical approach used to meet this objective.

II. Methods and Materials

The biological methods used for the 48 hour static toxicity test are basically those described in Standard Methods for Examination of Water and Wastewater (1) and Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (2).

A. Daphnia Culture

The Daphnia magna used in the test will be cultured at Analytical BioChemistry Laboratories. All daphnia will be held in culture tanks on a 16-hour daylight photoperiod. Subcultures containing adult daphnia are maintained for first instar daphnia production. Only first instar daphnia less than 24 hours old are used in the toxicity testing.

B. Test System

The test system will be composed of 250 ml beakers which will contain 200 ml of ABC well water containing various concentrations of test material. The beakers will be maintained at 20°C ± 1°C during the study.

F. Test Procedure-Chemical

Dissolved oxygen and pH measurements will be taken from the highest concentration with five daphnia remaining and recorded on raw data sheets.

III. Results

By employing the statistical method described by Litchfield and Wilcoxon (3), LC₅₀ values and 95% confidence intervals will be calculated for 24 and 48 hours and reported in table form.

The study will be conducted following the intent of the Good Laboratory Practice regulations (4) and the final report will be reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data will be provided to the contracting company with a copy being retained by Analytical BioChemistry Laboratories.

IV. 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) American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed. Washington, DC 1193 p.
- (3) Litchfield, J. T., Jr. and F. Wilcoxon. 1949. A Simplified Method of Evaluating Dose-Effect Experiments. Jour. Pharm. Exp. Ther. 96:99-113.
- (4) Food and Drug Administration. 1976. Proposed Regulations for Good Laboratory Practice. Federal Register 41 (225):51205-51230.

THIOTAX (TX)

Summary

Solubility:	At pH 5, 51 ppm (25°C) At pH 7, 118 ppm (25°C) At pH 9, 900 ppm (25°C)
Vapor pressure:	< 1.9×10^{-6} torr (25°C)
Log $P_{o/w}$:	2.42
Photodegradation:	$t_{1/2}$ = 3.7 hr at midday in August
Dark control:	$t_{1/2}$ = 100 hr
Biodegradation:	Not biodegraded in 8 weeks

Solubility and Vapor Pressure

Methods

Solubility of TX at pH 5, 7, and 9 was measured by the Campbell method. The temperature of the Campbell apparatus was regulated by a temperature control bath at 25°C. Since TX degrades rapidly when exposed to sunlight, the Campbell apparatus was wrapped with aluminum foil. One sample at each pH was equilibrated while being stirred for about 10 hours before analysis. A second sample at each pH was equilibrated for about 14 hours. There was no significant difference in solubility between the 10-hour and 14-hour samples.

The vapor pressure of TX was determined by the gas saturation method. Conditions for these measurements were:

Flow rate:	6.20 ml/min
Sampling time:	10,050 min
Room temperature:	20°C
Volume at room temperature:	62,266 liters
Volume at 25°C:	63,241 liters

Recovery of approximately 50 µg Thiotax spiked onto Tenax GC was measured. The spiked samples were stored for about 3 weeks before being desorbed with 1 ml of acetonitrile and analyzed by HPLC. The results of these recovery measurements are:

<u>Sample No.</u>	<u>TX Spiked (µg)</u>	<u>TX Recovered (µg)</u>	<u>Recovery %</u>
1	49.9	49.1	98.4
2	49.9	40.1	80.5

The HPLC conditions were:

Instrument: DuPont Model 848 HPLC with a 254-µm detector
Column: µ Bondapak C₁₈ (Water's Associates)
Guard column: Co-Pell (Whatman) (5 cm x 4.4 mm ID)
Solvent: 60% acetonitrile/40% water
Pressure: 2000 psig
Sample loop: 10 µl

Results and Discussion

The HPLC analysis of TX indicates that it contains only one major peak (see Figure 29). The solubility of TX increased about twofold between pH 5 and 7 and by about an order of magnitude between pH 7 and 9. Results of these measurements are:

<u>pH</u>	<u>Solubility (g/ml)</u>
5	51 ± 10 (25°C)
7	118 ± 38 (25°C)
9	905 (25°C)

No measurable amount of TX was collected during the vapor pressure measurements. Recovery from Tenax-GC, the sorbent used to collect TX, ranged from 80 to 98%. The vapor pressure of TX is estimated to be $< 1.9 \times 10^{-6}$ torr.

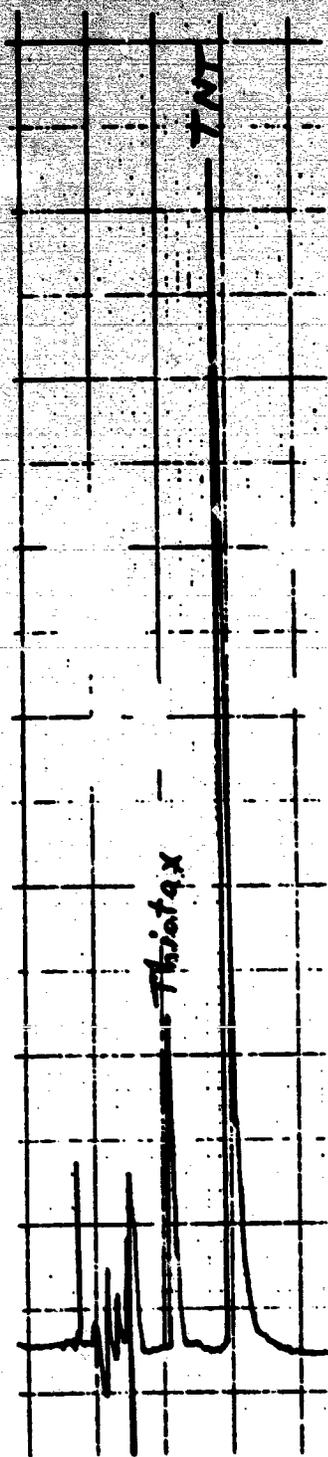


FIGURE 29. HPLC CHROMATOGRAPHIC PROFILE OF THIOTAX

n-Octanol/Water Partition Coefficient

Methods

TX (1-3 mg) was dissolved in 2 ml of octanol. Water (20 ml) was added and the biphasic system was inverted 400 times by hand. The solutions were centrifuged at 2000 rpm for 15 minutes and the phases were separated. The octanol was diluted 1000-fold with the HPLC mobile phase prior to analysis.

TX was analyzed by HPLC under the following conditions:

Column:	250 mm x 4.6 mm LiChrosorb ODS-C ₁₈ column
Solvent:	Acetonitrile/water (50/50)
Flow rate:	2 ml/min
Detection:	UV at 254 nm
Quantitation:	Internal standard, 2,4,6-trinitrotoluene (TNT)
Retention time:	TX, 3.7 min; TNT, 4.6 min.

The HPLC profile of TX appears in Figure 29.

The log P value for thiotax was found to be 2.42 ± 0.11 , based on two determinations, shown below:

Log P Values

1	2.34
2	2.50

Phototransformation

Summary

Sunlight photolysis of a 1.1-ppm TX solution indicated that this compound has a half-life of ^{3.7}~~2.7~~ hours at midday in August. In the dark, the half-life of TX in aqueous solution was about 100 hours.

Method

Solutions of 1.1 ppm (6.7 M) TX* were prepared in Milli-Q water,

* Concentration calculated assuming pure material used.

using 1X acetonitrile by volume as a cosolvent.* The solutions were placed in borosilicate tubes (11 mm ID) in a rack at a 60° angle to the sun and photolyzed on August 31, 1979. Dark controls were maintained at 23°C. Photolyzed solutions were either analyzed immediately after photolysis or placed in ice until HPLC analysis was performed.

Aqueous samples were analyzed by direct injection onto the HPLC column. The conditions were:

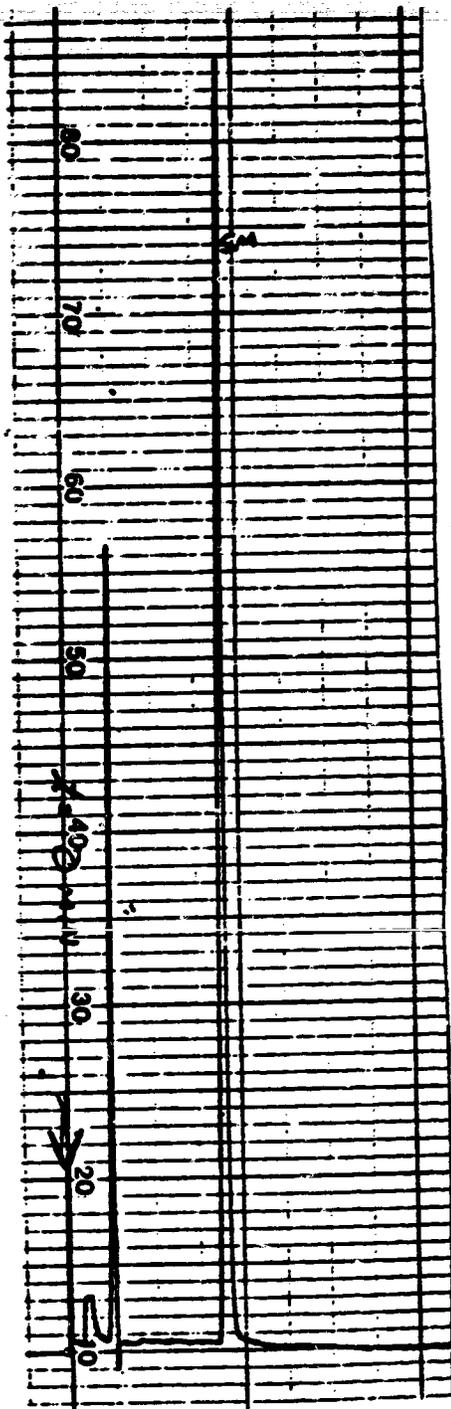
Instrument:	Waters Associates Model 60000A pumps, Model M440 UV detector at 254 nm
Column:	30 cm x 4 mm μ Bondapak C ₁₈ (Waters Associates)
Mobile phase:	35% acetonitrile:65% water, isocratic
Eluting times:	Starting material, 7.0 min, products A and E, approximately 1 min, product C, ~ 4.7 min, product D, ~ 6.2 min (see Figure 30).

Results and Discussion

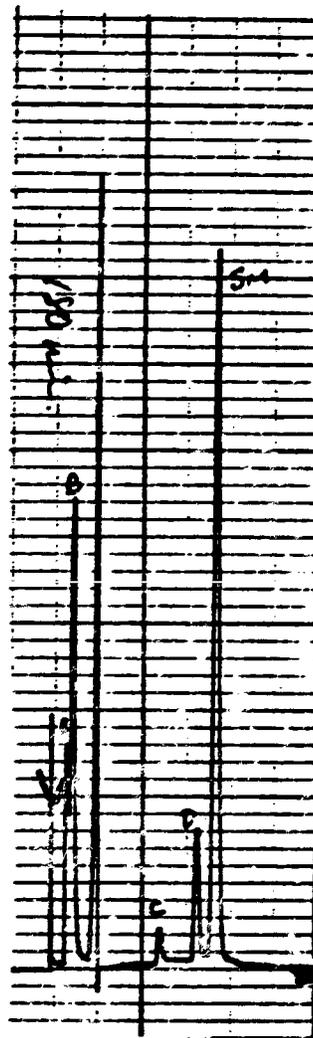
The UV absorption spectrum of TX (Figure 31) shows that it absorbs strongly into the solar spectral region. The measurements for the sunlight photolysis of a 1.1-ppm sample of TX indicated a photolysis rate constant of $5.2 (\pm 0.3) \times 10^{-5} \text{ sec}^{-1}$ (see Figure 32). This corresponds to a half-life of 3.7 hours at midday. The dark reaction measurements indicated a half-life of approximately 100 hours at 23°C.

Four photolysis products, # A + D were observed; two polar products elute with the solvent front, and two products elute before TX (Figure 33).

* Acetonitrile was used to solubilize TX. Previous work at SRI International shows acetonitrile to be inert in aqueous photolysis reactions.



INITIAL TRACE



AFTER 180 MINUTES IN SUNLIGHT

241465

FIGURE 30. HPLC ANALYSIS OF THIOTAX IN 35% ACETONITRILE-WATER, ISOCHRATIC

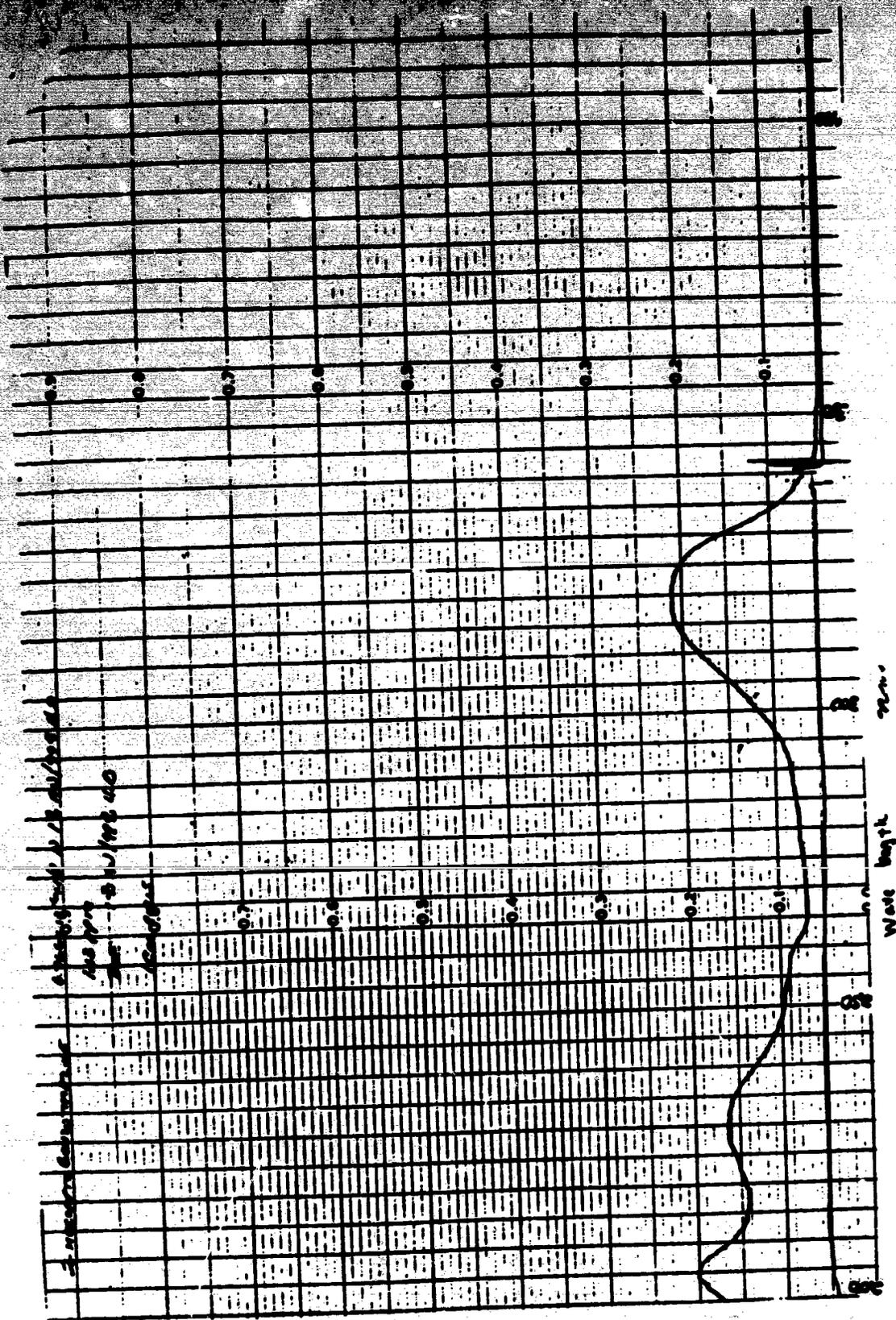


FIGURE 31. UV ABSORPTION SPECTRUM OF THIOTAX [6.8 x 10⁻⁶ M (1.1 ppm) in 1% ACETONITRILE-WATER, 1-CM CELL]

241466

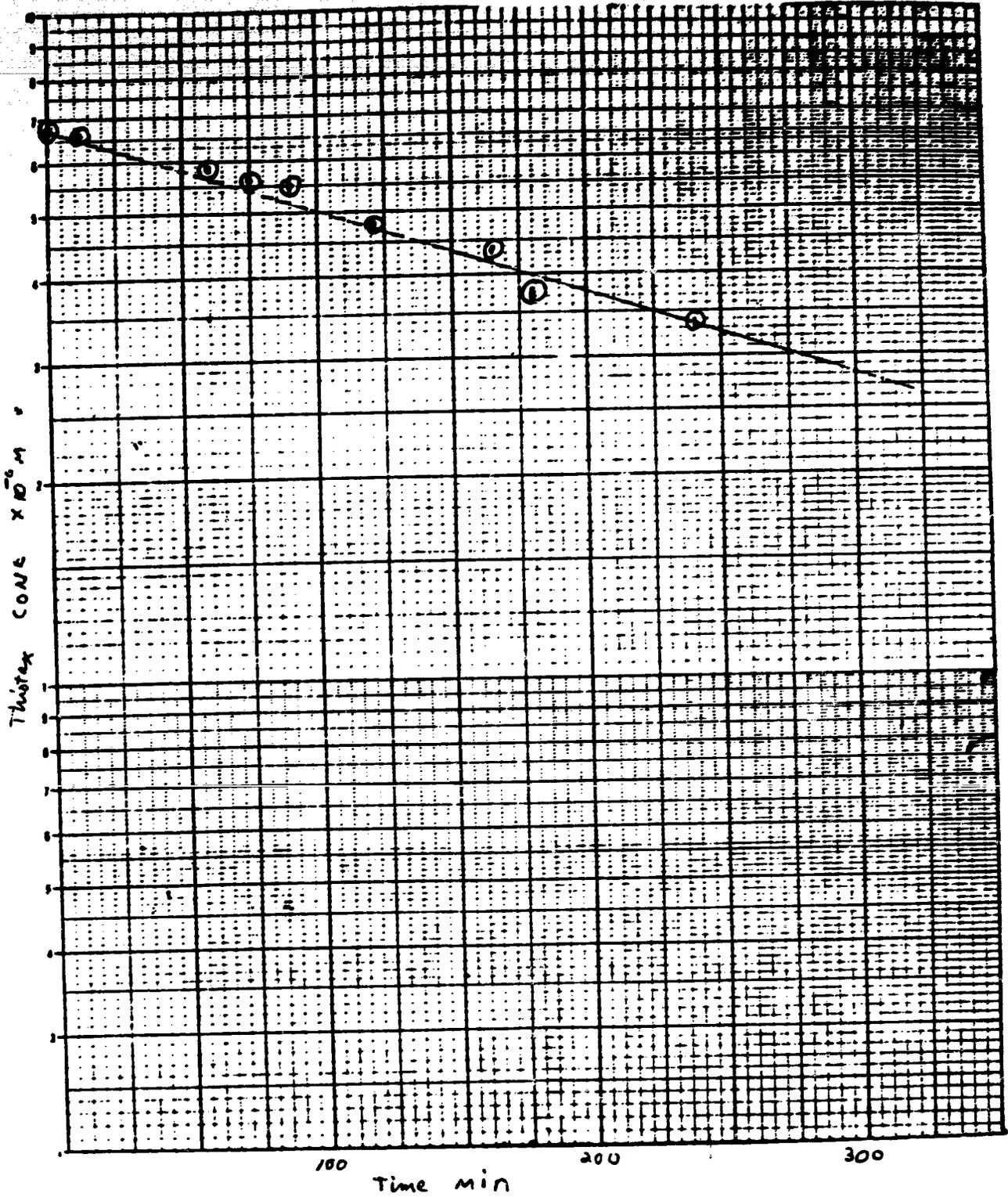


FIGURE 32. THIOTAX SUNLIGHT PHOTOLYSIS

Biodegradation

Summary

TX was not biodegraded during eight weeks of incubation. Although the TX concentration in the test water decreased, the compound was also chemically degraded in the sterile control. Therefore, the reaction was not biological.

Method

To 2 liters of screen-filtered river water in a 4-liter glass bottle were added 20 ml of 10% potassium phosphate buffer (pH 7.5) and 50 μ l of a stock solution of TX (40 mg/ml of DMSO). The final concentrations were 1 ppm of TX, 25 μ l/liter of DMSO, and 1 g/liter of buffer. The sterile control was autoclaved river water. The biodegradability test control was prepared by adding 4 ml of a stock solution of quinoline (2 mg/ml of Milli-Q water) to the buffered river water. The test samples, in duplicate, were incubated in the dark at 21-25°C.

To 2 ml of water sample, 2 ml of 1.09 μ g/ml trinitrotoluene in acetonitrile was added as internal standard, and TX was analyzed by HPLC under the following conditions:

Column: 250 mm \times 4.6 mm LiChrosorb ODS-C₁₈ column

Solvent: 50% acetonitrile:50% water

Flow rate: 2 ml/min

Detection: UV at 254 nm

Results and Discussion

Average residues (in ppm) in the water at various incubation times were:

<u>Incubation Time</u> <u>(weeks)</u>	<u>Sterile</u> <u>Control</u> <u>(ppm)</u>	<u>Biodegradation</u> <u>Sample</u> <u>(ppm)</u>
0	0.85	0.94
1	0.36	0.77
2	0.39	0.70
3	0.29	0.40
4	0.28	0.34
5	0.24	0.24
6	0.28	0.23
7	0.28	0.24
8	0.23	0.19

Quinoline was not degraded in 3 days but disappeared within a week.

TX was not biodegraded. Although there was a decrease in TX concentration, TX in the sterile control was also transformed; hence, the decrease in biodegradation test water may be due to a chemical process. The photolysis control showed that TX was transformed in a 1% acetonitrile in aqueous solution with a half-life of about 100 hours in the dark. The biodegradation test result confirmed that finding. Some components in natural water or sterile river water may act as catalysts or inhibitors of chemical degradation and therefore different degradation rates may be observed in various water bodies. Thiotax may be biodegraded if some other compounds can act as cometabolic substrates.