MASTER FILE
Project No. [redacted] BB-79-135

ANALYTICAL BIO CHEMISTRY LABORATORIES, INC.
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Static Acute Bioassay Report
#23246

Submitted To:

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

Acute Toxicity of Therminol 66 (AB-79-1384353-1a) to Fathead Minnows (Pimephales promelas)

May 25, 1979

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
Carl M. Thompson — Date
Aquatic Biologist

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

Approved By:

James A. Ault — 6/29/79
James A. Ault — Date
Quality Assurance Officer

Cyle D. Johnson — 7-10-79
Cyle D. Johnson — Date
Laboratory Manager

SUMMARY

The acute toxicity of Therminol 66 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.

Compound	96-hour LC_{50} (95% C.I.)
Therminol 66	>1,000 mg/l
Antimycin A	0.000032 mg/l (0.000025-0.000040 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 May 21 to May 25, 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 LC_{50} levels for Therminol 66 to fathead minnows (Pimephales promelas). A preliminary range-finding study was conducted from May 2 to May 4, 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).

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 Osage Catfisheries, Inc. in Osage Beach, Missouri. 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 0.46 g and a mean standard length of 30.4 mm. Weight and length measurements were made a representative group of fish prior to the test and are included in Appendix I.

The static fish bioassay was conducted in 40 liter glass aquaria containing 30 liters of laboratory well water. The water parameters of this dilution water are 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. The use of 30 liters of test solution was authorized by the customer in a letter included in the appendix.

A 48 hour range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentration was set at 1,000 mg/l. The range-finding test produced no mortality at 1,000 mg/l. At the customer's request (9), the definitive test was conducted with a control versus a test chamber at 1,000 mg/l. The letter authorizing this protocol deviation is included in Appendix I. Ten fish were added to each test chamber by random assignment within 30 minutes after addition of toxicant aliquots. A single mortality observed after 96 hours of exposure in the 1,000 mg/l test chamber was attributed to a fin-rot infection of the dead individual and not related to compound toxicity.

The Therminol 66 standard was received on February 22, 1979, in good condition. The sample upon receipt was observed to be a yellow liquid and was stored at 1°C. Sample purity was not provided; therefore, the 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 prepared by transferring direct weights of the compound to the test chambers. An oily film was observed on the surface of test chambers following toxicant additions. The concentrations of Therminol 66 tested were above the solubility of the compound in water as reported in the letter authorizing the study (Appendix I). 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 Therminol 66 and the reference test against Antimycin A, a piscicide. These values were obtained by employing the statistical method described by Litchfield and Wilcoxon (6). Survival rates, concentration 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 controls throughout the test. 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.

Parameter	Concentration
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

TABLE 2

Acute Toxicity of Therminol 66 and a Reference Compound (Antimycin A) to Fathead Minnows (Pimephales promelas)*

Compound	LC_{50} in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
Therminol 66	>1,000	>1,000	>1,000
Antimycin A***	0.000047 (0.000039-0.000057)**	0.000037 (0.000030-0.000045)**	0.000032 (0.000025-0.000040)**

*Bioassay as conducted at 22°C (±1.0), mean weight and length, 0.46 g and 30.4 mm.

**95% confidence interval.

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

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TABLE 3

Test Material - Therminol 66

Four Day Static Acute Toxicity Test with Fathead Minnows

mg/l Concentration	Percent Mortality Hours			Water Quality											
				24 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	8.3	7.3	<0.10	22	7.6	8.2	<0.10	22	5.4	8.0	1.0
1,000	0	0	10	22	8.5	7.4	<0.10	22	7.8	8.3	<0.10	22	5.2	8.0	1.2

*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 8001-8) used with an Extech Model 671 pH and mV meter.

MONS 017480

LITERATURE CITED

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

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

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

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

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

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

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

(8) Food and Drug Administration. Regulations for Good Laboratory Practice. Federal Register, Vol. 43, No. 247, 59986-60025, December 22, 1978.

(9) Personal communication, Bill Adams, Ph.D., Monsanto Chemical Company, March 15, 1979.

Quality Assurance Statement for final report #23246 entitled, "Acute Toxicity of Therminol 66 (AB-79-1384353-1a) to Fathead Minnows (Pimephales promelas)," 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 Therminol 66 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. It was also noted that since the purity of the compound being tested had not been supplied, the LC_{50} 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 6/29/79

James A. Ault Date
Quality Assurance Officer

APPENDIX I
RAW DATA

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Bioassay Lab
Acute Toxicity Bioassay

Date: 5/21/79
Time: 3:30 pm
Tank:
Purity:
Solvent: Acetone

Definitive

Toxicant: Therminol 66 Lab No.: 23246
Formulator: [illegible] Date Made: 5/21/79

Stock Standards * See remarks

Final Gross Wt. _____ g
Tare wt. _____ g
Net wt. _____ g
Adj. net wt. _____ g*
Dilution vol. _____ ml
Concentration _____ mg/ml (Balance Calibration ___ **)

Working Standards

Aliquot of concentrated stock _____ m
Dilution Vol. _____ m
Final Concentration _____ mg/m

0.46 g [illegible]

Test Species: Fathead Minnows Lot No. 1179 Temp.: 22°C
Source: Osage Catfisheries Condition: good Weight: [illegible] Length.: [illegible]
No./Vessel: 10 Vessel Size: 30 l Void: [illegible]
Water Chemistry: Well H_2O pH: 7.8 Alkalinity: 310 mg/l $CaCO_3$ Hardness: 240 mg/l $CaCO_3$

Concentration				24 hr		48 hr		72 hr		96 hr			
mg/l	[illegible]	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	20 ml acetone			0		0		0		0			
1000 A	30 ~~75~~	(not [illegible] JG [illegible])		0		0		0		1(1)			
~~1000 B~~ (not used JG [illegible])	~~75~~ 30	(not JG [illegible])											
Observer				JG		JG		JG		CT			
Date				5/22		5/23		5/24		5/25			

Remarks: (1) mortality probably due to fin rot, as this individual had signs of the fin rot problem [illegible] 5/25/79
Direct weight of toxicant was added to each test chamber
This was followed by a 20 ml Acetone rinse of the sample container

Prepared by: [illegible] Checked by: [illegible]

*Corrected for purity of primary standard
**Checked with class C weights

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Definitive
Thermimel 66 FHM ~~BLC~~ (error 5/21/79 JG)

[illegible] 5/25/79

Concentration mg/l	Water Quality															
					24 hours				48 hours				96 hours			
	Temp. °C	D.O.* mg/l	pH**	NH$_3$*** mg^3/l	Temp. °C	D.O.* mg/l	pH**	NH$_3$*** mg^3/l	Temp. °C	D.O.* mg/l	pH**	NH$_3$*** mg^3/l	Temp. °C	D.O.* mg/l	pH**	NH$_3$*** mg^3/l
Control					22	8.3	7.3	<.1	22	7.6	8.2	<.1	22	5.4	8.0	1.0
1000%					22	8.5	7.4	<.1	22	7.8	8.3	<.1	22	5.2	8.0	1.2
~~1000 B~~ (1) entered in error C.T. 5/25/79																

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

**pH - pH Probe (Extech Model 200C) 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.

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ANALYTICAL BIOCHEMISTRY LABS
Aquatic Bioassay Lab
Acute Toxicity Bioassay

Date: 5-2-79
Time: 1:00 pm

Preliminary

Toxicant: Thermino 66 Lab No.: 23246 Tank:
Formulator: Jerry Dunham Date Made: Purity:
Solvent: Acetone

Stock Standards
Final Gross Wt. g
Tare wt. g
Net wt. g
Adj. net wt. g*
Dilution vol. ml
Concentration mg/ml (Balance Calibration **)

see remarks

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

Test Species: Fathead Minnows Lot No. 179 Temp.: 22°C
Source: Osage Catfisheries Condition: good Weight: 0.4 gm Length: 3.0 cm
No./Vessel: 5 Vessel Size: 15Q Void: 24 hr
Water Chemistry: well H2O pH: 7.8 Alkalinity: 160 mg/l CaCO3 Hardness: 200 mg/l CaCO3

Concentration ppm	ml	Dead	Obs.	24 hr Dead	24 hr Obs.	48 hr Dead	48 hr Obs.	72 hr Dead	72 hr Obs.	96 hr Dead	96 hr Obs.	Dead	Obs
1000	15			0		0							

Aquatic Bioassay Lab
Acute Toxicity Bioassay

Date: 4-30-79

Time: 10:30 am

Definitive

Tank:

Toxicant: Antimycin A Lab No.: Reference Toxicant Purity:

Formulator: Carl Thompson Date Made: 10-16-78 Solvent: Acetone

Stock Standards

Final Gross Wt. 900 mg
Tare wt. 0.00 g
Net wt. 30.8 g
Adj. net wt. 30.8 g*
Dilution vol. 100 ml
Concentration .30 mg/ml (Balance Calibration ✓ **)

Working Standards

Aliquot of concentrated stock .5
Dilution Vol. 100
Final Concentration 0.0015 mg

Test Species: Fathead Minnows Lot No. 1179 Temp.: 22°C
Source Aqua Culture Fisheries Condition: good Weight: 0.58 g Length: 2.7
No./Vessel 10 Vessel Size: 150 Void: 45 hr
Water Chemistry: Recon H2O pH: 7.4 Alkalinity: 35 mg/l CaCO3 Hardness: 45 mg/l CaCO3

Concentration				24 hr		48 hr		72 hr		96 hr			
ppm	ml	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs
Control	0 ml Acetone			0		0		0		0			
0.000004	.14			0		0		0		0			
0.000024	.24			0		0		0		1			
0.000042	.42			3		7		7		8			
0.000075	.75			10		10		10		10			
0.000A	.14			10		10		10		10			
Observer				JG		JG		JG		JG			
Date				5-1		5-2		5/3		5/4			

Remarks:

Prepared by: [signature] Checked by: [signature]

*Corrected for purity of primary standard
**Checked with class S weights

MONS 017487

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

xicant Antimycin A — Date tested 4/30-5/4/79
st Species Fathead Minnows LOT 1179 — Date reported 5/22/79
b Number Reference Toxicant — Temperature 22°C
posure period 24 hrs — Water quality Reron H_2O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	O-E	Contribution to Chi
CONTROL	0/10	0	—	—	—
0.000024	0/10	0 (.1)	.1	0	0.000
0.000042	3/10	30	30	0	0.000
0.000075	10/10	100 (99.5)	98.4	1.1	0.008
	/				
	/				
	/				

Total animals = 30 — Total contribution to Chi 0.008
K = 3 — Chi^2- contribution to Chi X total animals / K = 0.08
Chi^2 (p-.05) for (K-2) 1 deg. of freedom = 3.84

LC_{84} = 0.000058
LC_{50} = 0.000047
LC_{16} = 0.000039

$i = LC_{84}/LC_{50} + LC_{50}/LC_{16}$ =

$i = \frac{(.000058/.000047)^2 + .000047/.000039}{2} = 1.24$

Confidence limits (.05) for LC_{50}

$n' = 10$
$f\ LC_{50} = S^{2.77}/\sqrt{n'} = 1.20$

24 hr LC_{50} = 0.000047 mg/l (0.000039 - 0.000057 mg/l) 95% C.I.

$LC_{50} / f\ LC_{50}$ = lower limit = .000039
$LC_{50} \times f\ LC_{50}$ = upper limit = .000057

Analysis by: Carl Stephan (Name) Aquatic Biologist (Title) 5/22/79 (Date)

Checked by: [signature] Date: 5/31/79

MONS 017488

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

icant Antimycin A — Date tested 4/30-5/4/79
st Species Fathead Minnows LOT 1179 — Date reported 5/22/79
o Number Reference Toxicant — Temperature 22°C
posure period 48 hrs — Water quality Tap con H₂O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	D=E	Contribution to Chi
CONTROL	0/10	0	—	—	—
0.000024	0/10	0 (0.7)	2	1.3	0.009
0.000042	7/10	70	70	0	0.000
0.000075	10/10	100 (99.9)	99.9	0	0.000
	/				
	/				
	/				

otal animals = 30 — Total contribution to Chi 0.009
K = 3 — Chi²- contribution to Chi X total animals / K = 0.09

LC_{84} = .000047
LC_{50} = .000037
LC_{16} = .000030
Chi² (p-.05) for (K-2) 1 deg. of freedom = 3.84

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

$= \frac{.000047/.000037 + .000037/.000030}{2} = 1.25$

Confidence limits (.05) for LC_{50}

$N' = 10$
$f\ LC_{50} = S^{2.77}/\sqrt{N'} = 1.22$

48 hr LC_{50} = 0.000037 mg/l (0.000030 – 0.000045 mg/l) 95% C.I.

$LC_{50} / f\ LC_{50}$ = lower limit = .000030
$LC_{50} \times f\ LC_{50}$ = upper limit = .000045

Analysis by: Carl M. Thompson (Name) Aquatic Biologist (Title) 5/22/79 (Date)

Checked By: Alan D. Fowler Date: 5/31/79

MONS 017489

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Antimycin A Date tested 4/30 – 5/4/79
Test Species Fathead Minnows LOT 1179 Date reported 5/22/79
Lab Number Reference Toxicant Temperature 22°C
Exposure period 96 hrs Water quality Recon H_2O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	D-E	Contribution to Chi
CONTROL	0/10	0	—	-	—
0.000014	0/10	0 (.1)	.1	0	0.000
0.000024	1/10	10	14	4	0.014
0.000042	8/10	80	84	4	0.012
0.000075	10/10	100 (99.9)	99.9	0	0.000
	/				
	/				

Total animals = 40 Total contribution to Chi 0.026
K = 4 Chi²- contribution to Chi X total animals / K = 0.26
Chi² (p-.05) for (K-2) 2 deg. of freedom = 5.99

LC_{84} = 0.000042
LC_{50} = 0.000032
LC_{16} = 0.000025

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

$$S = \frac{\frac{.000042}{.000032} + \frac{.000032}{.000025}}{2} = 1.30$$

Confidence limits (.05) for LC_{50}

N' = 10
$f\ LC_{50} = S^{2.77} / \sqrt{N'} = 1.26$

96 hr LC_{50} = 0.000032 mg/l (0.000025 – 0.000040 mg/l) 95% C.I.

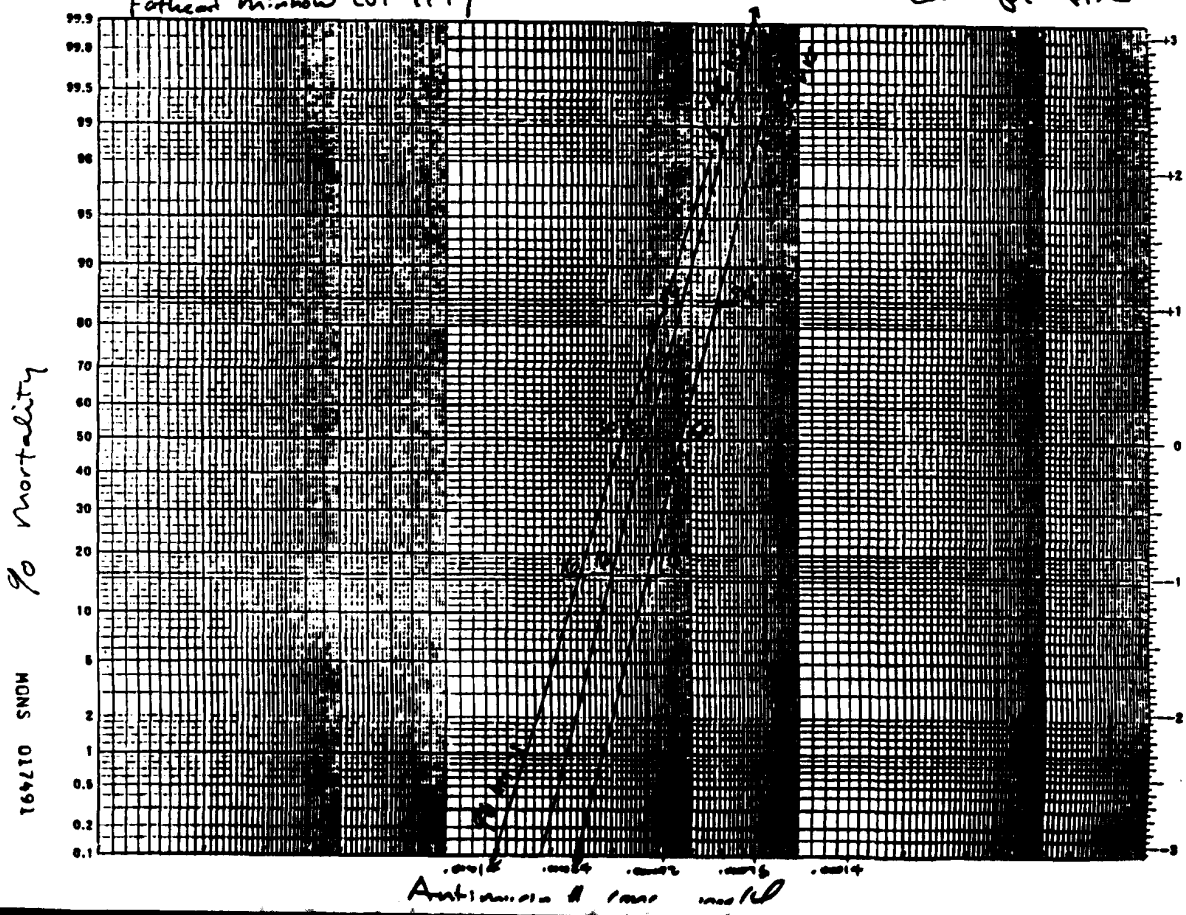
LC_{50} / f LC_{50} = lower limit = .000025
LC_{50} X f LC_{50} = upper limit = .000040

Analysis by: [signature] (Name) Aquatic Biologist (Title) 5/22/79 (Date)

Checked By: [signature] Date: 5/31/79

MONS 017490

Antimycin A
Fathead minnow Lot 1179



MONS 017491

ANALYTICAL BIOCHEMISTRY LABS

AQUATIC BIOASSAY LAB

TEST FISH MEASUREMENTS

Test: Therminal 66

Test Species: Fathead minnows Lot No. 1179

Source: Osage Catfisheries, Inc. Date Measured 5/21/79

Group Measured: representative group at test initiation

Fish No.	Standard Length (mm)	Weight (g)
1	32	0.48
2	32	0.50
3	30	0.39
4	28	0.31
5	30	0.49
6	29	0.41
7	35	0.83
8	29	0.31
9	31	0.52
10	28	0.40
Mean Standard Length (mm)	30.4 (S.D. = 2.2)	
Mean Weight (g)		0.46 (S.D. = 0.15)

Remarks:

MONS 017492

Prepared By: Carlos Thapia Checked By: [illegible]

ABC Aquatic Research Laboratory
Fish Requisition and Disposition Record

Species: Fathead minnows Date Rec: 4/4/79 Lot #: 1179
Source: Osage Catfisheries, Inc. No. Rec.: 1500 Tank #: 7
Osage Beach, MO Designated Use: Bioassay

Comments: arrived good condition

Size and Disposition Record

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

Treatment Record

Date	Drug Used	Conc.	Time	Results
4/19	Formalin + mal. grn	2.5 ppm + 0.1 ppm	8-9 am	[illegible]

MONS 017493

Daily Record

Date	#Dead	Comments	Date	#Dead	Comments	Date	#Dead	Comment
4/19	2	Shipping loss [illegible]	4/19	0	Fed 8A [illegible]	5/3	0	[illegible]
5	0	Fed 8A [illegible]	4/20	0	" [illegible]	5/4	0	[illegible]
6	0	" [illegible]	4/21	0	" [illegible]	5/6	1	[illegible]
7-9	0	" [illegible]	4/22	0	" [illegible]	5/7	0	[illegible]
4/10	0	" [illegible]	4/23	0	" [illegible]	5/8	0	[illegible]
11	0	" [illegible]	4/24	1	" [illegible]	5/9	0	[illegible]
12	0	" [illegible]	4/25	1	" [illegible]	5/10	0	[illegible]
13	0	" [illegible]	4/26	0	" [illegible]	5/11	0	[illegible]
14	0	Fed 8A [illegible]	4/27	0	" [illegible]	5-12	—	[illegible]
15	0	" [illegible]	4/28	0	" [illegible]	5-13	—	[illegible]
16	1	Fed 8A [illegible]	4/29 5/1	0	" [illegible]	5/14	0	[illegible]
17	0	" [illegible]	5/2	0	" [illegible]	5/15	0	[illegible]
18	0	" [illegible]						

ABC Aquatic Research Laboratory
Fish Requisition and Disposition Record

Fathead minnows — Date Rec: 4/4/79 — Lot#: 1179

ce: — No. Rec.: 1500 — Tank#: 7

Designated Use: Biomassay

ments:

Daily Record

te	#Dead	Comments	Name	Date	#Dead	Comments	Name
7/6	0	Fed 5dy					
17	0	Fed 6d 4					
18	0	"					
21	0	"					
22	0	"					
23	1	"					
24	0	"					

MONS 017494

Checked by

Monsanto

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

February 1, 1979

Mr. Alan Forbis
ABC Laboratories
7200 East ABC Lane
P.O. Box 1097
Columbia, MO 65201

Dear Alan:

I would like you to conduct several aquatic toxicity tests as described in the attached toxicity agreement form.

These products should not present any special problem which would require special handling other than good hygienic practices. The solubility of these materials is 1-5 ppm, except for EMA and the Dequest products which are quite water soluble. I suggest you use acetone when a solvent carrier is required. I prefer well water in place of reconstituted water for all studies. Please conduct the Daphnia magna studies using duplicate concentrations with 10 Daphnia per beaker. The required protocol for all studies is the EPA Methods For Acute Toxicity Tests With Fish, Macroinvertebrates And Amphibians (1972).

Our previous confidentiality agreement with ABC Laboratories will be considered binding.

Please send me four copies of the final report, all of which should contain copies of the raw data. Each report should contain the project number for the corresponding chemical and test as shown on the attached toxicity agreement form. The chemicals will be shipped in 1-3 weeks.

Please sign both copies of the toxicity agreement form and return the original to me. If you have any questions please call me.

Sincerely,

William J. Adams

/sf

MONSANTO INDUSTRIAL CHEMICALS COMPANY
ENVIRONMENTAL SCIENCES SECTION

OUTSIDE TOXICITY STUDIES AGREEMENT

Study Request

This is a request and the authorization for ABC Laboratories to conduct several aquatic toxicity tests as indicated below.

Chemical	Type of Test	Species	Project Number	Cost ($)
Santosol 100	Static Acute	fathead minnow	AB-79-1384344-1b	300
Santosol 100	Static Acute	rainbow trout	AB-79-1384344-1c	300
Santosol 100	Static Acute	bluegill sunfish	AB-79-1384344-1d	300
EMA 31,91,1103	Static Acute	fathead minnow	AB-79-1384352-1a	300
EMA 31,91,1103	Static Acute	rainbow trout	AB-79-1384352-1b	300
EMA 31,91,1103	Static Acute	Daphnia magna	AB-79-1384352-1c	300
Therminol 66	Static Acute	fathead minnow	AB-79-1384353-1a	300
Therminol 66	Static Acute	rainbow trout	AB-79-1384353-1b	300
Therminol 66	Static Acute	Daphnia magna	AB-79-1384353-1c	300
Therminol 88	Static Acute	fathead minnow	AB-79-1384354-1a	300
Therminol 88	Static Acute	rainbow trout	AB-79-1384354-1b	300
Therminol 88	Static Acute	Daphnia magna	AB-79-1384354-1c	300
Therminol VP1	Static Acute	fathead minnow	AB-79-1384355-1a	300
Therminol VP1	Static Acute	rainbow trout	AB-79-1384355-1b	300
Therminol VP1	Static Acute	Daphnia magna	AB-79-1384355-1c	300
WCM	Static Acute	fathead minnow	AB-79-1384356-1a	300
WCM	Static Acute	rainbow trout	AB-79-1384356-1b	300
WCM	Static Acute	Daphnia magna	AB-79-1384356-1c	300
DBPP	Time Independent (14-day flow-through)	~~fathead minnow~~ rainbow trout w/A	AB-79-1384336-3	1,995
Dequest 2000	Time Independent (14-day flow-through)	rainbow trout	AB-79-1384338-1	1,995
Dequest 2010	Time Independent (14-day flow-through)	rainbow trout	AB-79-1384339-1	1,995
Dequest 2041	Time Independent (14-day flow-through)	rainbow trout	AB-79-1384340-1	1,995
Dequest 2051	Time Independent (14-day flow-through)	rainbow trout	AB-79-1384341-1	1,995

To Be Performed By:	ABC Laboratory
Assigned Project Number:	See Above
Expected Date of Report:	Santosol 100 reports to be completed by 3-1-79, DBPP report by 4-1-79 and the remainder as time allows.
Cost:	
Manner of Payment:	Direct payment by check upon receipt of final report.

William J. Adams 2-5-79
William J. Adams — Date
Project Director

J. Coleman Weber 2/19/79
J. Coleman Weber — Date
Manager, Product Acceptability
Specialty Chemicals Division

2-20-79
Contract Laboratory Director — Date

MONS 017497

Monsanto

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

April 3, 1979

Mr. Carl Thompson
ABC Laboratories
7200 East ABC Lane
P. O. Box 1097
Columbia, MO 65201

Dear Carl:

This letter is to establish a standard operating test procedure regarding test concentrations for static acute toxicity tests contracted by the Environmental Sciences Division of Monsanto Industrial Chemicals with ABC Laboratories.

Chemicals which are not readily soluble in water and require a carrier solvent should be tested up to 1,000 mg/ℓ. Chemicals which are water soluble should be tested up to 5,000 mg/ℓ provided the solubility of the material permits.

Sincerely,

William J. Adams
Senior Research Biologist

/sp

APR 09 1979

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

May 22, 1979

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

RE: The acute toxicities of EMA-31, WCM and Therminol 66 to fathead minnows (Pimephales promelas).

Dear Bill:

For the above cited studies, we conducted the tests with 10 fish in 40 liter aquaria containing 30 liters of solution. This was a deviation from ABC Protocol #7601 which outlines the use of 5 gallon jars containing 15 liters of solution. This change was felt necessary for the above cited tests to reduce biomass loading.

This was discussed with you by telephone and met with your verbal approval. However, before sending the final reports to you, we need your written approval to be in compliance with the FDA's Good Laboratory Practice regulations. Please sign both copies of this letter. Return the original to us and retain the copy for your files. If you have any questions, please give me a call.

Sincerely,

Carl M. Thompson
Aquatic Biologist

CMT/gsl

APPROVED BY:

MAY 24 1979

5-24-79
William J. Adams, PhD
Monsanto Chemical Company

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

#7601

(Revised January 29, 1979)

Static Bioassay Procedure for Determining Acute Toxicity of Chemical Substances to Fresh Water Fish

The bioassay is used primarily for the evaluation of toxicity of chemical substances. However, it is also used extensively in determining acceptable effluent discharge rates, establishing sensitivity levels of specific fish species and identifying effects of other variables such as temperature, pH and water hardness on toxicity. The methods used by Analytical BioChemistry Laboratories are patterned after those presented by the American Public Health Association (1) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (2).

BIOASSAY PROCEDURE

Fish representative of both warm and cold water species with an average weight between 0.5 and 5.0 g are used in the bioassay. Fish species most commonly used are bluegill sunfish, fathead minnows and rainbow trout, with other species available upon request. All fish are purchased from reliable private hatcheries and observed for general health and suitability for a period not less than 14 days prior to experimental use. Just after arrival of test fish at the lab, they are given a prophylactic treatment with formalin plus malachite green. Fish are held in stock tanks on a 16-hour daylight photoperiod in well water that is relatively hard and has a temperature of 16°C (±4°C, seasonal variation). They are fed a well balanced commercial fish food that is low in contaminates and confirmed by analytical testing at ABC Labs. If mortality of any particular lot exceeds 10% in the 48 hours previous to testing, the fish are not used.

Preliminary tests are conducted in order to find the general toxic level of the test material if needed. After this level has been determined, at least five concentrations of the test material in a logarithmic series are selected for the definitive bioassay. Bioassay vessels, five gallon (wide mouth) glass jars, are filled with 15 liters of reconstituted water with the following make up in deionized water:

30 mg calcium sulfate/liter
30 mg magnesium sulfate/liter
48 mg sodium bicarbonate/liter
2 mg potassium chloride/liter

Tests will be conducted at other hardness levels if so requested or in effluent solutions provided by the customer.

After a 48 hour acclimation and food voidance period, 10 test fish are added to each of the concentrations of test material and control. The test temperature is 12°C for cold water species and 22°C for warm water species, with the actual test temperature not varying more than 1°C from the selected temperature. The test solutions are prepared within one hour of the start of the bioassay and the fish added randomly to the test chambers. A dissolved oxygen concentration in each test chamber of between 40% and 100% saturation for warm water species and 60% to 100% for cold water species is considered adequate for testing and assures reliable results. Measurements of temperature, dissolved oxygen, pH and ammonia in the control and high/low concentrations are reported at 24, 48 and 96 hours.

The fish are observed for a 96-hour period with mortalities and/or unusual behaviorial reactions recorded every 24 hours. Dead organisms are removed at each 24 hour observation period.

In order to ensure quality bioassay data, each experimental lot of fish is challenged with a reference piscicide, according to the above procedure. At the end of the test period, usually 96 hours, a determination of LC_{50} (medial lethal concentration) values are made for the 24, 48 and 96 hour observation periods, employing the technique of Litchfield and Wilcoxon (3).

The study is conducted following the intent of the Good Laboratory Practice Regulations (4) and the final report is reviewed by Analytical BioChemistry Laboratories' Quality Assurance Unit.

REFERENCES

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

(2) 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.

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

(4) Food and Drug Administration. Regulations for Good Laboratory Practice. Federal Register, Vol. 43, No. 247, 59986-60025, December 22, 1978.