

EE-20
AR 726-0115

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, DEA salt; may also be referred to as PFOS DEA salt, FC-99, or 3M Sample No. 2. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, compd. with 2,2'-iminobis[ethanol] (1:1), CAS # 70225-14-8)

Remarks: Test sample is a mixture of the test substance in water (approximately 25% test substance and 75% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. Standard Methods.

Type: Static acute

GLP: Yes

Year completed: 1979

Species: *Lepomis macrochirus*

Supplier: Osage Catfisheries, Inc. in Osage Beach, Missouri.

Analytical monitoring: pH and DO / ammonia content

Exposure period: 96-hours

Statistical methods: Probit analysis.

Test fish age: Not noted.

Length and weight: Average length = 28.6 ± 2.17 mm.
Average weight = 0.60 ± 0.15 g

Loading: 0.2 g fish / L

Pretreatment: None

Test conditions:

Dilution water: Laboratory well water

Dilution water chemistry:

Dissolved Oxygen:	9.3 mg/L
hardness:	255 mg/L as CaCO ₃
alkalinity:	368 mg/L as CaCO ₃
pH:	7.8
Conductivity:	50 μ mhos/cm

Stock and test solution preparation: Primary stock prepared in deionized water at a concentration of 150 mg/mL. The test concentrations were prepared by transferring appropriate aliquots

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of the stock standard directly to the test chambers. The test solutions were noted to foam when stirring in toxicant aliquots. Test concentrations were prepared based on total sample, not on percent concentration of the test substance in the test sample.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted

Exposure vessels: 40 liter glass aquaria containing 30L of test solution.

Number of replicates: one

Number of fish per replicate: ten

Number of concentrations: six plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

6.0 - 8.4 mg/L (control exposure)

5.8 - 8.3 mg/L (18 mg/L exposure)

pH range (0 – 96 hours)

8.2 - 8.3 (control exposure)

8.3 - 8.3 (18 mg/L exposure)

Test temperature: Temperature held constant at 22°C through use of a water bath for test vessels.

RESULTS

Nominal concentrations: Bk control, 18, 37, 75, 160, 320, 650 mg/L

Element value: 24-hour LC_{50} = 460 (370-580) mg/L
48-hour LC_{50} = 370 (290-470) mg/L
96-hour LC_{50} = 31 (22-43) mg/L
96-hour NOEC = 18 mg/L (C.I. not calculated)

All element values based on nominal concentrations

Statistical Evaluation of Mortality:

Probit analysis was used to calculate LC_{50} values and the corresponding confidence limits.

Quality Check for Test Organism Health:

The bluegill sunfish 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, indicating that the fish were in good condition.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
18	0	0	0	0
37	0	0	10	80
75	0	0	30	90
160	0	0	70	100
320	0	20	100	100
650	100	100	100	100

CONCLUSIONS

The test sample 96-hour LC₅₀ for bluegill sunfish was determined to be 31 mg/L with a 95% confidence interval of 22-43 mg/L. The 96-hour no observed effect concentration was 18 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for determination of the test substance concentration in the test solutions and determination of the sample purity. There were also gaps in the measurement of water quality criteria for a number of the concentrations at given time intervals.

REFERENCES

This study was conducted by Analytical BioChemistry Laboratories, Inc. of Columbia, Missouri on behalf of the 3M Company.

OTHER

Last changed: 5/2/00

001568

Submitted By: Analytical BioChemistry Laboratories, Inc.
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Prepared By:

Carl M. Thompson 7/13/79
Carl M. Thompson Date
Aquatic Biologist

Alan D. Forbis 7/13/79
Alan D. Forbis Date
Aquatic Supervisor

Approved By:

James A. Ault 7/13/79
James A. Ault Date
Quality Assurance Officer

Lyle D. Johnson 7-13-79
Lyle D. Johnson Date
Laboratory Manager

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SUMMARY

The acute toxicity of 3M Sample No. 2 to bluegill sunfish (Lepomis macrochirus) 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 bluegill sunfish 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 bluegill sunfish are summarized below.

<u>Compound</u>	<u>96-hour LC₅₀ (95% C.I.)</u>
3M Sample No. 2	31 mg/l (22-43 mg/l)
Antimycin A	0.000029 mg/l (0.000021-0.000040 mg/l)

Also, the results indicated a 96 hour, no observed effect concentration of 18 mg/l.

INTRODUCTION

This static bioassay was performed at the aquatic bioassay laboratory of Analytical BioChemistry Laboratories, Inc., Columbia, Missouri, for 3M Company, from June 12 to June 16, 1979, as authorized in a letter from 3M Company on June 6, 1979 (Appendix I). The purpose of this test was to determine the 24, 48 and 96 hour LC₅₀ levels for 3M Sample No. 2 to bluegill sunfish (Lepomis macrochirus). 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 bluegill sunfish 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 day-light 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 bluegill sunfish used for this experiment had a mean weight of 0.60 g and a mean standard length of 28.6 mm. Weight and length measurements were made on 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. Characteristics 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.

Based on the results of preliminary testing with fathead minnows (Pimephales promelas) by 3M Company, six concentrations of the test compound, ranging in a logarithmic series for 18 to 650 mg/l, with ten fish per concentration were selected for definitive bioassay. The fish were added to the test chambers by stratified random assignment within 30 minutes after addition of toxicant aliquots. The use of 40 liter glass aquaria for this study was authorized by 3M Company (Appendix I).

The 3M Sample No. 2 standard was received on June 8, 1979, in good condition. The sample upon receipt was observed to be a yellow liquid and was stored at room temperature (22°C). Sample purity was not provided; therefore, the test concentrations were prepared based on the total compound. The test concentrations were prepared by transferring appropriate aliquots of a stock standard directly to the test chambers.

The stock standard was prepared in deionized water. The test solutions were noted to foam when stirring in toxicant aliquots. 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 3M Sample No. 2 and the reference test against Antimycin A, a piscicide. These values were obtained by employing the statistical method described by Litchfield and Wilcoxon (6). 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 3M 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 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

The Acute Toxicity of 3M Sample No. 2
and Antimycin A to Bluegill Sunfish (Lepomis macrochirus)

Compound	LC ₅₀ in milligrams/liter (ppm)		
	24 hours	48 hours	96 hours
3M Sample No. 2	460 (370-580)**	370 (290-470)**	31 (22-43)**
Antimycin A***	0.000070 (0.000056-0.000088)**	0.000040 (0.000032-0.000050)**	0.000029 (0.000021-0.000040)**

*Bioassay as conducted at 22°C (±1.0), mean weight and length, 0.60 g and 28.6 mm.

**95% confidence interval.

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

TABLE 3

The mortality rates and water quality parameters during the acute toxicity test of 3M Sample No. 2 with bluegill sunfish (Lepomis macrochirus).

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	8.4	8.2	0.86	22	7.6	8.3	1.3	22	6.0	8.3	1.2
18	0	0	0	22	8.3	8.3	0.78	22	7.8	8.3	1.3	22	5.8	8.3	1.2
37	0	0	80												
75	0	0	90												
160	0	0	100									22	4.8	8.3	1.5
320	0	20	100	22	8.1	8.1	0.90	22	7.4	8.3	1.6				
650	100	100	100	22	8.0	8.1	0.78								

*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.

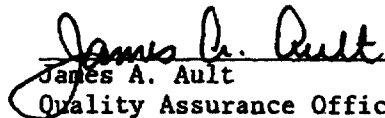
LITERATURE CITED

- (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 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.

Quality Assurance Statement for final report #23893 entitled, "Acute Toxicity of 3M Sample No. 2 to Bluegill Sunfish (Lepomis macrochirus)," for Dale Bacon, 3M Company, St. Paul, Minnesota.

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 3M Sample No. 2 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 LC₅₀ would have to be calculated based on nominal concentrations of the total compound. A final inspection of all data and records on June 20, 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

7/13/79
Date

APPENDIX I
RAW DATA

001578

ANALYTICAL BIOCHEMISTRY LABS
Aquatic Bioassay Lab
Acute Toxicity Bioassay

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Date: 6/12/79
Time: 8 am
Tank: 30 l aquaria
Purity:
Solvent: deionized H₂O

Toxicant: 3M-2 Lab No.: 23893
Formulator: C. J. Thomsen Date Made: 6/11/79

Stock Standards

Final Gross Wt. 30.000 g
Tare wt. 0.000 g
Net wt. 30.000 g
Adj. net wt. 30.000 g*
Dilution vol. 200 ml
Concentration 160 mg/ml (Balance Calibration ☒ **)

Working Standards

Aliquot of concentrated stock
Dilution Vol.
Final Concentration mg

$$15.000 \text{ g} + 0.000 \text{ g} = 15.000 \text{ g} \quad \begin{matrix} (\text{class S}) \\ \text{tare} \end{matrix}$$

Test Species: Bluegill Sunfish Lot No. 1579 Temp.: 22°C
Source: Osage Catfisheries Condition: Excellent Weight: Length.:
No./Vessel: 10 Vessel Size: 30 l Void: 48 hrs
Water Chemistry: well H₂O pH: 7.8 Alkalinity: 368 mg/l Hardness: 255 mg/l
CaCO₃CaCO₃

Concentration				24 hr		48 hr		72 hr		96 hr			
mg/l	ml	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
CONTROL	-			0		0		0		0			
8	3.6			0		0		0		0			
37	7.4			0		0		1		8			
75	15			0		0		3		9			
160	32			0		0		7	2LOE	10			
320	64			0		2	1LOE	10		10			
650-560 (U) ^{collected}	130			10		10		10		10			
Observer				CT		CT		CT		16			
Date				6/13		6/14		6/15		6/16			

Remarks: (1) entered in error CT 6/13/79 LOE = loss of equilibrium

- test concentrations prepared by adding indicated aliquots of the stock standard to 30 l aquaria - test material noted to foam when stirring in toxicant aliquots

Prepared by: C. J. Thomsen

Checked by: A. C. Fink

- Corrected for purity of primary standard

**Checked with class S weights

001579

3m¹ -
Bluegill Sunfish

End of hours

concentration mg/l	Water Quality															
	0 hours				24 hours				48 hours				96 hours			
	Temp. C	D.O.* mg/l	pH**	NH ₃ *** mg /l	Temp. C	D.O.* mg/l	pH**	NH ₃ *** mg /l	Temp. C	D.O.* mg/l	pH**	NH ₃ *** mg /l	Temp. C	D.O.* mg/l	pH**	NH ₃ *** mg /l
CONTROL	22	8.7	8.2	1.3	22	8.4	8.2	0.86	22	7.6	8.3	1.3	22	6.0	8.3	1.2
18	22	8.6	8.2	1.0	22	8.3	8.3	0.78	22	7.8	8.3	1.3	22	5.8	8.3	1.2
37																
75																
160													22	4.8 6.6	8.3	4.5
320	22	8.6	8.2	1.1	22	8.4	8.1	0.90	22	7.4	8.3	1.6				
650	22	8.4	8.3	0.90	22	8.0	8.1	0.78								

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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.

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant 3m-2 Date tested 6/12 - 6/16/79
 Test Species Bluegill Sunfish ^{LOT} 1579 Date reported 6/17/79
 Lab Number 23893 Temperature 22°C
 Exposure period 24 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	—	—	—
160	0/10	0 (.1)	.1	0	0.000
320	0/10	0 (.49)	16	11.1	0.090
650	10/10	100 (95.1)	84	11.1	0.090
	1				
	1				
	1				

Total animals = 30
 K = 3

Total contribution to Chi 0.180
 Chi² - contribution X total animals = 1.80
 to Chi K
 Chi² (p-.05) for (K-2) 1 deg. of freedom = 3.84

LC₈₄ = 650
 LC₅₀ = 460
 LC₁₆ = 320

$$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2} = \frac{650/460 + 460/320}{2} = 1.43$$

Confidence limits (.05) for LC₅₀

$$N' = \frac{20}{f LC_{50} = S^{2.77} / \sqrt{N'}} = 1.25$$

$$24\text{-hr } LC_{50} = 460 \text{ mg/L } (370 - 580 \text{ mg/L})$$

95% C.I.

LC₅₀ / f LC₅₀ = lower limit = 370

LC₅₀ X f LC₅₀ = upper limit = 580

Analysis by: Curtis J. Thoren Aquatic Biologist 4/17/79
 (Name) (Title) (Date)

Checked By: Al. E. Tolbr Date: 6/19/79

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant 3m-2

Date tested 6/12 - 6/16/79

Test Species Bluegill Sunfish ^{LOT 1579}

Date reported 6/17/79

Lab Number 23893

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	—	—	—
160	0/10	0 (.1)	.1	0	0.000
320	2/10	20	30	10	0.050
650	10/10	100 (99.3)	98	1.3	0.009
	1				
	1				
	1				

Total animals = 30
K = 3

Total contribution to Chi 0.059

Chi² - contribution X total animals = 0.59
to Chi K

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

LC₈₄ = 490

LC₅₀ = 370

LC₁₆ = 280

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

S = $\frac{490/370 + 370/280}{2}$ = 1.32

Confidence limits (.05) for LC₅₀

N' = 10

f LC₅₀ = $S^{2.77} / \sqrt{N'}$ = 1.28

48 hr LC₅₀ = 370 mg/l (290-470 mg/l)
95% C.I.

LC₅₀ / f LC₅₀ = lower limit = 290

LC₅₀ X f LC₅₀ = upper limit = 470

Analysis by: Cathy E. Hagan (Name) Aquatic Biologist (Title) 6/17/79 (Date)

Checked By: Shad. Fohn

Date: 6/17/79

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant 3m-2 Date tested 6/12 - 6/16/79
 Test Species Bluegill Sunfish ^{Lot} 1579 Date reported 6/17/79
 Lab Number 23893 Temperature 22°C
 Exposure period 96 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	—	—	—
18	0/10	0 (4A)	16	11.1	0.090
37	8/10	80	64	16	0.120
75	9/10	90	95	5	0.050
160	10/10	100 (99.9)	99.9	0	0.000
	1				
	1				

Total animals = 40
 K = 4

Total contribution to Chi 0.260

Chi² - contribution X total animals = 2.60
 to Chi K

Chi² (p=.05) for (K-2) 2 deg. of freedom = 5.99
3.84

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 CT 6/17/79

LC₈₄ = 52

LC₅₀ = 31

LC₁₆ = 18

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

S = $\frac{52/31 + 31/18}{2}$ = 1.70

Confidence limits (.05) for LC₅₀

N' = 20

f LC₅₀ = $S^{2.77} / \sqrt{N'}$ = 1.39

96 hr LC₅₀ = 31 mg/l (22-43 mg/l)
 95% C.I.

LC₅₀ / f LC₅₀ = lower limit = 22

LC₅₀ X f LC₅₀ = upper limit = 43

Analysis by: Carl A. Johnson Aquatic Biologist 6/17/79
 (Name) (Title) (Date)

Checked By: Ala D. Jorda Date: 6/19/79

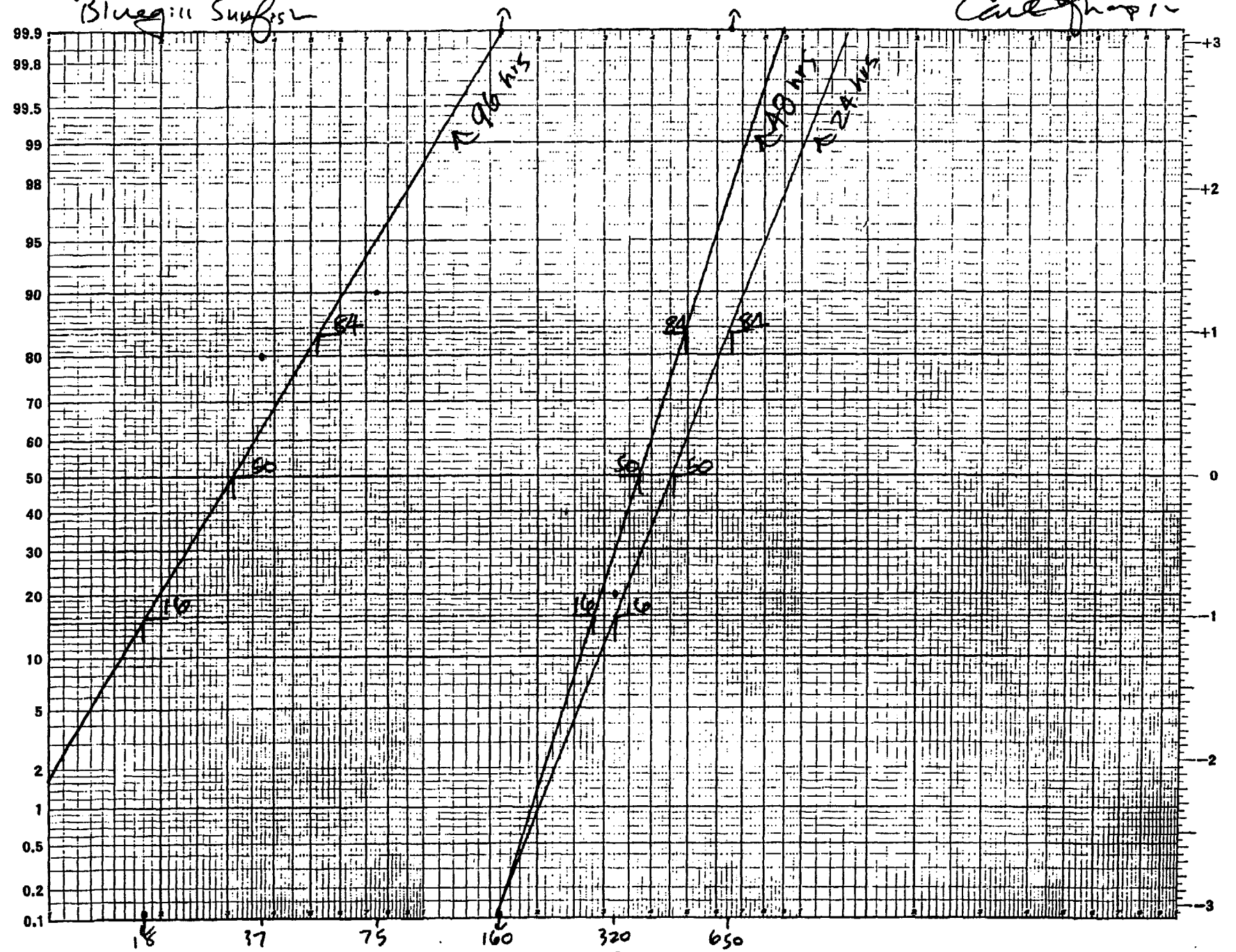
001583

3m-2
Blue: Surface

Carbograph

% mortality

001584



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ANALYTICAL BIOCHEMISTRY LABS
Aquatic Bioassay Lab
Acute Toxicity Bioassay

Date: 4/1/79Time: 4:00 pm

Tank: _____

Purity: _____

Solvent: AcetoneToxicant: Antimycin ALab No.: Reference ToxicantFormulator: Jimmy DriffenDate Made: 5/28/79Stock StandardsFinal Gross Wt. 30 gTare wt. 0.00 gNet wt. 30 mgAdj. net wt. 30 mg*Dilution vol. 100 mlConcentration 0.30 mg/ml (Balance Calibration ☒ **)Working StandardsAliquot of concentrated stock .5Dilution Vol. 100Final Concentration 0.0015 mgTest Species: Bluegill Sunfish Lot No. 6579Temp.: 22°CSource: Duke Cattfisheries Condition: goodWeight: 0.53g Length: 249No./Vessel: 5 (duplicate)Vessel Size: 15 lVoid: 48 hrWater Chemistry: Recon H₂O pH: 7.1Alkalinity: 35 mg/l Hardness: 45 mg/lCaCO₃ CaCO₃

Concentration			24 hr		48 hr		72 hr		96 hr			
mg/l	ml		Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.	Dead	Obs.
Control	A	acetone 1.8			0		0		0			
	B	acetone 1.8			0		0		0			
0.000018	A	.18			0		0		1			
	B	.18			0		0		1			
0.000032	A	.32			0		2		2			
	B	.32			0		0		3			
0.000056	A	.56			1		5		5			
	B	.56			1		5		5			
0.00010	A	1.0			5		5		5			
	B	1.0			5		5		5			
0.00018	A	1.8			5		5		5			
	B	1.8			5		5		5			
Observer			JG		CT		CT		CT			
Date			6/2		6/13		6/14		6/15			

Remarks: (1) entered in error CT 6/14

Prepared by: Jimmy Driffen
 *Corrected for purity of primary standard
 **Checked with class S weights

Checked by: Carl M. [signature]

001585

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Atrazine A Date tested 6/11 - 6/15/79
Test Species Bluegill Sunfish LST-79 Date reported 6/15/79
Lab Number Reference Toxicant Temperature 22°C
Exposure period 24 hrs Water quality Deion H₂O

Concentration mg/L	No. dead / total no.	Observed % mortality	Expected % mortality	D-E	Contribution to Chi
CONTROL	0/10	0	—	—	—
0.00032	0/10	0 (.1)	.1	0	0.000
0.00096	2/10	20	20	0	0.000
0.00210	10/10	100 (97.4)	92	5.4	0.045
	1				
	1				
	1				

Total animals = 30
K = 3

Total contribution to Chi 0.045

Chi² - contribution X total animals = 0.45
to Chi K

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

LC₈₄ = 0.00096

LC₅₀ = 0.00070

LC₁₆ = 0.00054

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

$$S = \frac{\frac{0.00096}{0.00070} + \frac{0.00070}{0.00054}}{2} = 1.29$$

Confidence limits (.05) for LC₅₀

N' = 10

$\pm LC_{50} = S^{2.77} / \sqrt{N'}$ = 1.25

24 hr LC₅₀ = 0.00070 mg/L (0.00096 - 0.00054) mg
95% C.I.

LC₅₀ / f LC₅₀ = lower limit = 0.00056

LC₅₀ X f LC₅₀ = upper limit = 0.00088

Analysis by: Carl J. Fox Aquatic Biologist 6/15/79
(Name) (Title) (Date)

Checked By: John D. Foster Date: 6/15/79

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Antimycin A Date tested 6/11-6/15/79
 Test Species Bluegill Sunfish ^{LOT} 1579 Date reported 6/15/79
 Lab Number Reference Toxicant Temperature 22°C
 Exposure period 48 hrs Water quality Decon H₂O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	D-E	Contribution to Chi
CONTROL	0/10	0	—	—	—
0.000018	0/10	0 (.1)	.1	0	0.000
0.000032	2/10	20	20	0	0.000
0.000056	10/10	100 (91.4)	92	8	0.045
	1				
	1				
	1				

Total animals = 30
 K = 3

Total contribution to Chi 0.045

Chi² - contribution X total animals = 0.45
 to Chi K

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

$$LC_{84} = 0.000052$$

$$LC_{50} = 0.000040$$

$$LC_{16} = 0.000031$$

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

$$S = \frac{1000052^2}{.000040 + .000031} = 1.30$$

Confidence limits (.05) for LC₅₀

$$N' = 10$$

$$LC_{50} = S^{2.77} / \sqrt{N'} = 1.25$$

$$48 \text{ hr } LC_{50} = 0.000040 \text{ mg/l } (0.000032 - 0.000056) \text{ 95\% C.I.}$$

$$LC_{50} / f LC_{50} = \text{lower limit} = 0.000032$$

$$LC_{50} \times f LC_{50} = \text{upper limit} = 0.000050$$

Analysis by: Cathy J. Henson Aquatic Biologist 6/15/79
 (Name) (Title) (Date)

Checked By: Al D. Tolin Date: 6/17/79

ANALYTICAL BIOCHEMISTRY LABORATORIES
Aquatic Bioassay Laboratory
Acute Toxicity Bioassay

Probit Analysis Work Sheet

Toxicant Antimycin A Date tested 6/11 - 6/15/79
 Test Species Bluegill Sunfish ^{LOT} 1579 Date reported 6/15/79
 Lab Number Reference Toxicant Temperature 22°C
 Exposure period 96 hrs Water quality Decon H₂O

Concentration mg/l	No. dead / total no.	Observed % mortality	Expected % mortality	D-E	Contribution to Chi
CONTROL	0/10	0	—	—	—
0.000018	2/10	20	10	10	0.120
0.000032	5/10	50	60	10	0.040
0.000056	10/10	100 (98.7)	96	2.7	0.020
	1				
	1				
	1				

Total animals = 30
 K = 3

Total contribution to Chi 0.180

Chi² - contribution X total animals = 1.80
 to Chi K

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

$$LC_{84} = 0.000042$$

$$LC_{50} = 0.000029$$

$$LC_{16} = 0.000020$$

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

$$S = \frac{\frac{0.000042}{0.000029} + \frac{0.000029}{0.000020}}{2} = 1.45$$

Confidence limits (.05) for LC₅₀

$$N' = \frac{10}{1.38} = 7.25$$

$$f LC_{50} = S^{2-77} / \sqrt{N'} = 1.25$$

$$96 \text{ hr } LC_{50} = 0.000029 \text{ mg/l } \left(\frac{0.000021 - 0.000040 \text{ mg/l}}{95\% \text{ C.I.}} \right)$$

$$LC_{50} / f LC_{50} = \text{lower limit} = 0.000021$$

$$LC_{50} \times f LC_{50} = \text{upper limit} = 0.000040$$

Analysis by: Carl A. Johnson Aquatic Biologist 6/15/79
 (Name) (Title) (Date)

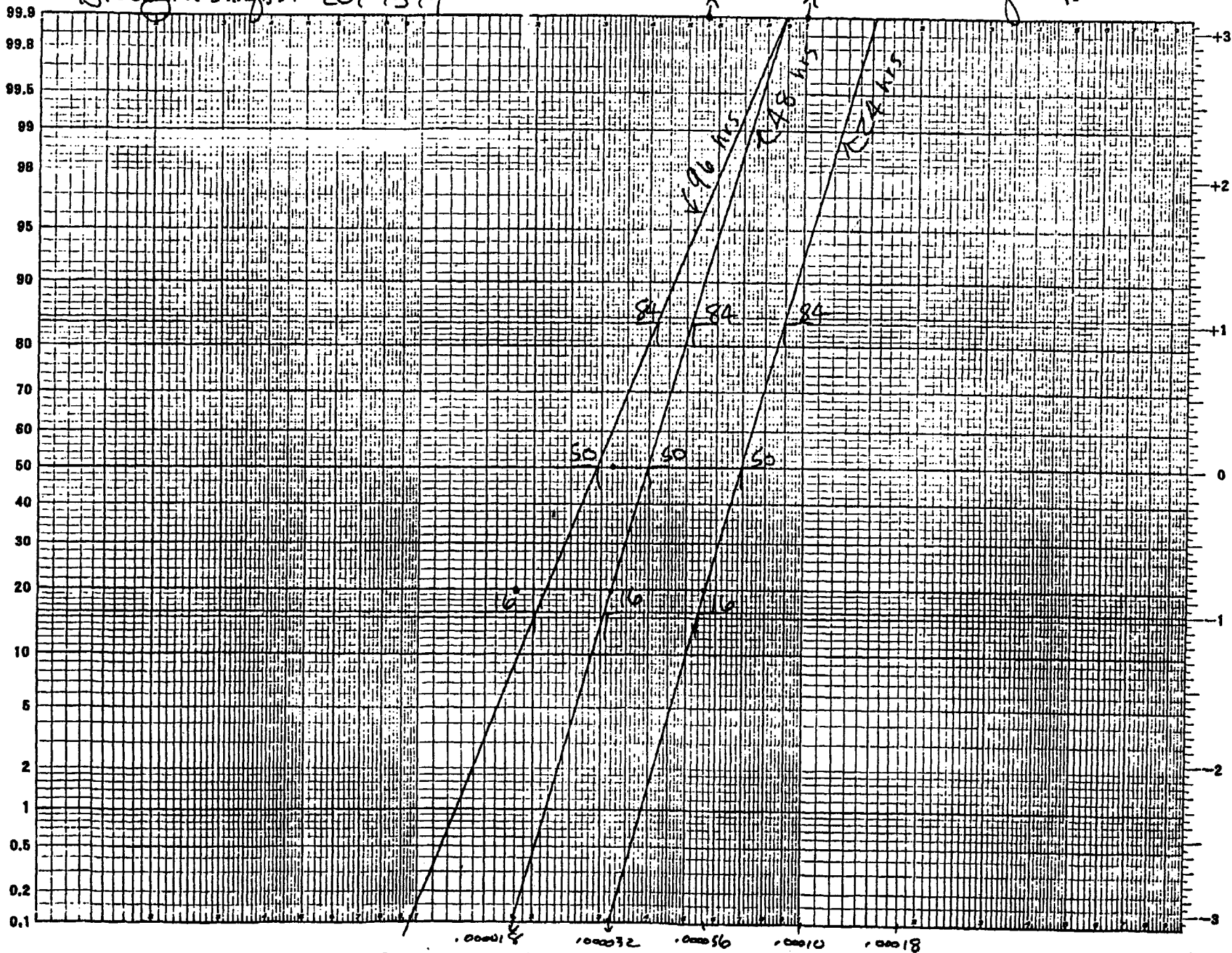
Checked By: Alan J. John Date: 6/12/79

Intimycin A
Bluegill Sunfish LOT 1579

Carbonyl

% mortality

001589



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AQUATIC BIOASSAY LAB

TEST FISH MEASUREMENTS

Test: 3m Samples 1-9, BLG StaticsTest Species: Bluegill SunfishLot No. 1579Source: Osage Catfisheries, Inc.Date Measured 6/12/79Group Measured: representative group prior to testing

Fish No.	Standard Length (mm)	Weight (g)
1	29	0.68
2	27	0.42
3	30	0.80
4	24	0.40
5	30	0.58
6	30	0.62
7	30	0.68
8	30	0.72
9	30	0.72
10	26	0.40
Mean Standard Length (mm)	28.6 (S.D. = 2.17)	
Mean Weight (g)	0.60 (S.D. = 0.15)	

Remarks: _____

Prepared By: Cathy JonesChecked By: Alan J. Jordan

001590

BEST COPY AVAILABLEABC Aquatic Research Laboratory
Fish Requisition and Disposition Record

Species: Bluegill Sunfish Date Rec: 5/25/79 Lot #: 4 1579
Source: Osego Catfisheries No. Rec.: 1000 Tank #: 4
Designated Use: Bioassay

Comments: _____

_____Size and Disposition Record

<u>Date</u>	<u>Length (mm)</u>	<u>Weight (gm)</u>	<u># Used</u>	<u>Date</u>	<u>Length (mm)</u>	<u>Weight (gm)</u>	<u># Us</u>
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Treatment Record

<u>Date</u>	<u>Drug Used</u>	<u>Conc.</u>	<u>Time</u>	<u>Result</u>
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Daily Record

<u>Date</u>	<u>#Dead</u>	<u>Comments</u>	<u>Date</u>	<u>#Dead</u>	<u>Comments</u>	<u>Date</u>	<u>#Dead</u>	<u>Commen</u>
5/25/79	0	Fed 8 x 4 AM	6/14	0	Fed 8 x 4 AM			
5/26/79	0	Fed 8 x 2 AM	6/15	0	"			
5/27/79	0	Fed 8 x 1 AM	6/16	0	"			
5/28/79	1	"	6/17	0	"			
5/29/79	0	Fed 8 x 1 AM						
5/30/79	0	"						
5/31/79	0	"						
6/1/79	0	Fed 8 x 1 AM						
6/2/79	0	"						
6/3/79	0	"						
6/4/79	0	"						
6/5/79	0	"						
6/6/79	0	"						
6/7/79	0	"						
6/8/79	0	"						
6/9/79	0	"						
6/10/79	0	"						
6/11/79	0	"						
6/12/79	0	"						
6/13/79	0	"						

001591